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Challenges due to bacterial infections of the honey bees and contributions to manage pest problems

Muhammad Sarwar

Nuclear Institute for Food & Agriculture (NIFA), Tarnab, Peshawar, Pakistan.

Abstract
This publication contains information concerning bacterial infections American foulbrood (irregular capped and uncapped comb cells, larvae change from pearly white to dark brown), European foulbrood (discolored capping, larvae change from glistening white to faint yellow), Septicaemia **(**mildly shriveled abdomen, hemolymph milky-white**),** Parathypus (swollen abdomen, liquid intestinal contents), Powdery Scale (scales result from dead larvae) and Spiroplasmosis (pathogens reach hemolymph and kill bee) rapidly deteriorating to honeybees. Recognizing disease symptoms in honeybee colonies is an essential part of good beekeeping management. Peoples unexperienced in handling bee's equipment and collecting samples should first read the agricultural message on safe beekeeping practices. Early detection allows for prompt remedial action and helps in preventing serious disease outbreak and economic losses. Thorough inspections of brood should be conducted in early spring, during the main honey producing season and in autumn when hives are prepared for winter. Look for any unusual cell caps and brood, especially larvae that are off-color or abnormally positioned in the cell. Make routine inspections for treatment of colonies with lower levels of infection by shaking adult bees onto clean comb, destroying the infected brood, feeding the colony with antibiotic oxytetracycline and by destruction of heavily infected colonies to enforce break in infections cycle.

Keywords: Bacteria, Honey bee disease, Honey bee disorder, honey bee parasite, Honey bee pest

1. Introduction

Honeybee hives have long been providing to humans with honey and bees wax, and such commercial uses have spawned a large beekeeping industry, though many species still occur in the wild. All honeybees are social and cooperative insects, and live on stored honey and pollen all winter, and cluster into a ball to conserve warmth. Larvae are fed from the stores during this season, and by spring, the hive is swarming with a new generation of bees. Bee diseases are present throughout the globe and are responsible for large annual losses in bees, honey and equipment, and add greatly to the cost of production. Also, the loss of pollinating bees results in a lower yield of seed and fruit. Bee diseases spread quickly within a colony, and the crowding of colonies increases the possibility of the spread of infections from hive to hive. When searching for disease symptoms beware of that a colony may have more than one disease. There are two major bacterial infections affecting honey bee colonies, American foulbrood (AFB) caused by *Paenibacillus larvae* and European foulbrood (EFB) caused by *Melissococcus plutonius.* Both diseases cause the death of infected brood, but AFB is far more virulent and can ultimately result in colony death if uncontrolled. The EFB is a far more sporadic disease from which generally only weak colony succumbs. In the case of AFB, larvae become infected by consuming the spores in food, which then germinate in the mid-gut, invading the tissues and killing the larvae usually after pupation. Once a colony is infected with AFB, the disease can usually progress until the colony dies. Similarly, for EFB, it is the larvae that become infected by ingesting contaminated food; the bacteria multiply within the midgut of the infected larvae and compete with the larvae for food. Infected larvae usually die due to starvation rather than invasion of the body tissues by the bacterium. In some cases the larvae survive to pupation, thus producing undersized adults. It is especially important that the two most serious brood diseases American foulbrood and European foulbrood can be detected early. Make routine inspections for these diseases and should be detected in their early stages for prompt treatment to prevent their spread. The AFB and EFB are notifiable diseases throughout many states and the only method of control in is destruction of infected colonies. This article describes laboratory techniques used to diagnose diseases and other abnormalities of the honey bee, identifies parasites and pests, and. also included are directions for control of infections [1, 2, 3, 4, 5, 6].

1.1. American Foulbrood (AFB

American foulbrood (AFB), caused by the spore-forming *Paenibacillus larvae* (formerly classified as *Bacillus larvae*, *Paenibacillus larvae* and species *larvae/ pulvifaciens*), is the most widespread and destructive of the bee brood diseases. The *P. larvae* is a rod-shaped bacterium and larvae up to three days old become infected by ingesting spores present in their food, while young larvae less than 24 hours old are most susceptible to infection. Spores germinate in the gut of the larva and the vegetative bacteria begin to grow by taking nourishment from the larva. Spores cannot germinate in larvae over three days old. Infected larvae normally die after their cell is sealed. The vegetative form of the bacterium will die, but not before it produces many millions of spores. American foulbrood spores are extremely resistant to desiccation and can remain viable for more than 40 years in honey and beekeeping equipment. Each dead larva may contain as many as 100 million spores. This disease only affects the bee larvae, but is highly infectious and deadly to bee brood, and infected larvae darken and die. At first, AFB is slow to establish and only a few larvae may be affected and in advanced cases the brood pattern can be irregular. The

absence of an irregular brood pattern does not mean the disease is absent [7, 8, 9].

1.1.1. Symptoms of American Foulbrood

Infected larvae and pupae die of bee after their cells have been capped. The capping of an infected cell may be slightly sunken and darker than healthy ones around it. Adult bees often puncture the capping of infected cells and may remove them entirely. Adult bees may later partly or totally remove the caps, and as a result the caps may be perforated. They may also be sunken, concave, dark and at times greasy-looking. Diseased larvae and pupae always lie stretched out on their backs on the lower wall of their cells. The color of dead larvae and pupae is at first dull white, then light brown, later coffee-brown and finally black. As it continues to decay and become dried, it turns dark brown and finally it turns into a black dried scale on the lower side of the cell. These scales are difficult to remove and remain a site for constant re-infection. A single scale can contain one billion spores, and it takes as few as 35 spores to trigger the disease. These scales are difficult to see and can easily be missed when purchasing used equipment. Other characteristic symptoms of American foulbrood are the somewhat glossy, uniform color of the dead larva or pupa, and the melted look as the body and the body wall rot. Sometimes the bacteria make the pupal tongue stick to the top of the cell. When this happens, the tongue looks like a smooth and fine thread extending vertically across the cell. However, many advanced cases of American foulbrood do not show this symptom. The bacteria rot the skin of the developing bee and turn the body into a slimy mass that becomes stickier as it dries. This condition is the basis for the 'ropiness' test that can be used to aid in diagnosing the disease [10, 11].

1.1.2. Diagnosis of American Foulbrood

Odor or smell of American foulbrood is not a reliable diagnostic tool because some cases of AFB have no discernible smell at all. The odor of disease is distinctive, but is not a reliable indicator because people's sensitivities to odors vary so widely, and the odor may be strong or weak. The odor may be similar to that of old fashioned animal glues that are now rarely used. However, it is better to rely on eyes to diagnose the disease. Colonies with high levels of AFB can also have a foul odor similar to a chicken house. As more and more brood becomes infected and dies, the colony dwindles and eventually collapses. For diagnosis**, s**elect a sample of brood comb about 5 inches square that contains large numbers of affected cells. Mail it to laboratory in a strong cardboard or wooden box without an airtight wrapping. Samples that are crushed or moldy because of improper packaging make diagnosis impossible [12, 13].

1.1.2.1. Field Diagnosis

i. Infected hive may show less than normal bee flight with dead bees on the bottom board. The colony may appear weak after opening the hive.
ii. The capped brood is uneven with puncture holes in the caps of brood cells and colonies with heavy infestation often display irritable behavior.
iii. The AFB has a distinct foul odor that can help in alerting the beekeepers to a disease problem.
iv. With a toothpick, lift punctured cap and remove content of brood cell. The larval remnant may be a light brown mass sunk onto the bottom side of the brood cell. If the mass is ropy when withdrawing the toothpick from the cell, there is a strong indication of American Foulbrood disease.
v. Place toothpick in a small plastic bag or plastic wrap and mail to the Apiculture office for confirmation.
vi. Over time, the larval remains in the cell will dry and harden into a dark brown leathery scale on the bottom side of the brood cell. A single scale contains millions of spores that remain viable for decades and bees cannot remove scales from cells.
vii. The AFB scales can be readily detected in the field by holding the brood frame at an angle of approximately 15-20 degrees.

1.1.2.2. Laboratory Diagnosis

i. The AFB is caused by *Paenibacillus larvae*, a spore-forming bacterium and a microscope slide can be prepared by dissolving a small part of an AFB scale. Stir the scale with a toothpick in a droplet of water placed on a slide and apply a cover slip.
ii. Under 400 X magnification, the AFB spores are readily visible, which are characterized by being very slightly oblong, uniform in size and shape.
iii. The *P. larvae*, is competitive and does not tolerate growth of other bacteria in the parasitized bee larva. As a result, most microscopic slides can show a predominance of *P. larvae* spores. This is not always the case with poor samples or those left in the collection bag for too long and in such case, secondary invaders such as molds will appear.

1.1.3. Methods to Control American Foulbrood

Disease control is primarily the responsibility of each beekeeper, who must learn the symptoms of the diseases and inspect the colonies carefully for the presence of American foulbrood. Most state laws require the burning of colonies of bees infected with American foulbrood. The infected colony must be killed and all the contents of the hive burned, including bees, combs, frames and honey. The fire should be built in a pit and the ashes might be covered afterwards. The cover, bottom board and hive bodies should also be scraped and then scorched. Inspection and prevention are the best methods of control, and any medicinal agents or mixtures should be applied to colony only after inspection in the spring and at least 4 weeks before the main nectar flow. They may be used again after the honey is removed in late summer or during the fall. Use them with care at the proper dosages, and follow the directions and precautions on the labels. The only approved medicinal agent for preventive feeding for American foulbrood is oxytetratycline HCl (Terramycin). It is fed as a mixture in either powdered sugar, sugar syrup, or in vegetable oil extender patties and is limited to the months of September and February. It is important to never feed Terramycin within four weeks of a nectar flow to avoid contamination. This material does not kill the diseased organism, but prevents its growth when present in low concentrations in the food fed by the workers to larvae. It is possible to breed bees that are genetically resistant to AFB and other diseases. One of the most important characteristics in bees is the so-called 'hygienic behavior', which is the ability of bees to detect and remove abnormal cells of brood from the colony. Hygienic queens are available from nationally-advertised queen breeders [14, 15, 16].

i. Beekeeper must become thoroughly familiar with visual detection of brood diseases and inspect regularly, especially

when disease has been reported in the area or after the colony has been placed in crop pollination.

ii. For frames with suspect signs of brood disease, take a sample and mail to the Apiculture office for analysis.

iii. When AFB has been confirmed, kill the bees and burn all the equipment or shake bees onto foundation and burn all the old equipment. Feed the bees with medicated sugar syrup at two week intervals until foundation has been drawn out.

iv. Reduce the exchange of hive equipment between hives and apiaries and replace 20% of all brood frames each year so that after a few years, no brood frame is older than five years.

v. Do not barrel feed or leave used hive equipment exposed to foraging bees and apply hygienic management practices, including clean clothing, hive tools and gloves.

vi. Antibiotic-resistant AFB (r-AFB) has become established, so, antibiotics must be used for treatment purposes only and do not use antibiotics as a prophylactic (preventive) measure.

vii. Use antibiotics only as recommended, never use the product after its expiry date, and follow preparation instructions carefully. There is strong evidence of the development of resistance to antibiotics in *P. larvae*e as they act merely to stop the bacteria from reproducing without killing them. Also antibiotics do not affect the bacterial spores which are the primary mode of transmission [17].

viii. Also, treat along the margins of the brood chamber according to the label on Tylan 100 Soluble (Tylosin) with powdered sugar mix. Be careful not to put powdered mix directly on to open brood. Colonies should receive three treatments administered as a dust in confectioners/powdered sugar. The 200 mg dose is applied (dusted) over the top bars of the brood chamber once weekly for three weeks. Disinfect contaminated empty hive parts using Gamma irradiation. Supers with frames without bees should be placed in containers that are inaccessible to honey bees (bee tight) for irradiation using 1.2 m rads to completely sterilize the combs and hive parts. Shaking of bees onto fresh comb has been demonstrated to be a control method for AFB with re-infection rates of around 5% [18].

1.2. European Foulbrood (EFB)

It is caused by a bacterium, *Streptococcus pluton* that does not always kill the infected larva but sometimes may kill large numbers of larvae very rapidly. But, *Melissococcus plutonius* is a bacterium that infects the midgut of the bee larvae. European foulbrood is considered less serious than American foulbrood. *M. plutonius* is not a spore-forming bacterium, but bacterial cells can survive several months on wax foundation. European foulbrood is often considered a stress disease that is dangerous only if the colony is already under stress for other reasons, while, an otherwise healthy colony can usually survive European foulbrood. Worker, drone and queen larvae of bee are all susceptible to this foulbrood [19].

1.2.1. Symptoms of European Foulbrood

A laboratory diagnosis may be needed to confirm which disease is present as the disease and its symptoms are highly variable, probably because of the presence of several other organisms in the dead and dying larvae. Symptoms include dead and dying larvae which can appear curled upwards, brown or yellow, melted or deflated with tracheal tubes more apparent, or dried out and rubbery. Larvae infected with European foulbrood are mostly affected while they are curled and before the cell is capped. In cases where larvae die after their cell has been sealed, the cap may be perforated, sunken, concave and dark. Dead larvae are twisted in their cells and may be positioned on the upper, lower or side walls of the cell. Their color turns to off-white, yellow, then brown and sometimes black. Freshly dead larvae have a soft and watery consistency, which soon becomes pasty. The scales are tough and rubbery, but may become brittle. They are found in any position and unlike AFB, are easily removed from the cells. Often the disease spreads quickly in the hive and an irregular brood pattern may soon be noticed in advanced cases. Older larvae killed by this disease lie stretched out on the lower walls of their cells very similar to those killed by AFB. When the matchstick test is used, the remains can stretch sometimes to about 18 mm, but usually much less. The remains can usually only stretch once or twice and thereafter cannot be drawn out from the cell [20, 21].

The EFB does not usually kill the colony, but a heavy infection will seriously reduce honey production. It is not necessary for beekeepers to kill colonies infected with EFB, but it is essential to be able to distinguish European from American foulbrood disease. Larvae infected with EFB usually die while still coiled in the bottom of the unsealed cell. This is distinctly different from what occurs with AFB. In some instances the disease may also affect sealed larvae and rarely pupae. When this happens, the larvae usually die in a partially curled or distorted position, only rarely lying straight on the lower side of the cell as these do when infected with American foulbrood. Affected larvae are not always the same color as with AFB, but may be yellow, gray, or brown, or a mixture of these colors. The air tubes, or tracheae, often remain visible in the larvae infected with EFB. Their presence helps to distinguish the disease from AFB, in which no tracheae can be seen in the decaying brood. The odor of European foulbrood may be described as being sour or similar to the odor of rotting fish. As with AFB, it is best not to use odor for diagnosis because of its variability and the differences in the ability of peoples to distinguish odors. The typical consistency of EFB-infected larvae is dough like, and the remains may be somewhat ropy, but less slimy and elastic than those of AFB-infected bees. When pulled out of the cell, the material reacts like dough or taffy when the pieces separate. Dried scales in comb may appear similar to those of American foulbrood if lying straight in the cells. However, most of them are turned or twisted in the cell and can be easily removed, whereas the scales of AFB are difficult to remove [22, 23].

1.2.2. Diagnosis of European Foulbrood

As with AFB, odor is not a reliable method of disease diagnosis because often there is no odor present this foulbrood [24].

1.2.2.1. Field Diagnosis

i. European Foulbrood is much less serious than AFB, but EFB shows up when the colonies have been under stress due to other diseases, colonies nearby, poor management and weather.

ii. The EFB is easily controlled with standard antibiotic treatments and affects bee brood much the same as AFB except that the disease affects open brood i.e., the larvae are affected before they are capped.

iii. An affected cells show discolored larva often in twisted positions with visible tracheal tubes and the brood has a sour odor, distinctly different from AFB.
iv. The EFB scales are easily removed from the cell a compared to AFB scales and when scales are detected, collect samples for laboratory examination. Although field analysis is often correct, accurate distinction between AFB and EFB can only be made through microscopic examination.

1.2.2.2 Laboratory Diagnosis

i. The EFB is primarily caused by *Melissococcus pluton,* but the secondary invader *Bacillus alvei* is mostly observed when samples are examined microscopically.
ii. Samples are prepared the same way as AFB samples, but unlike AFB, EFB microscopic samples generally display a wide variety of microbes.
iii. At 400 X, *B. alvei* is readily visible by its long spindle shaped spores and the spores do not jiggle, but float by in the solution.

1.2.3. Control and Treatment

European foulbrood may be controlled by use of Terramycin in the same way as American foulbrood. This dual control exerted by the antibiotic makes it a good choice for preventive feeding where both diseases are a threat. Honey bee strains vary in their resistance to European foulbrood. When only one or a few colonies are affected, they should be requeened with a different strain of bees. The organisms associated with European foulbrood are usually present even in hives that do not show symptoms of disease. The susceptibility of the particular strain of bees, and perhaps nutritional factors bring about the appearance of the disease at damaging levels. The sanitation precautions recommended in the section on AFB apply also to EFB. Likewise, bee stocks selected for hygienic behavior can be expected to minimize outbreaks of EFB. The disease sometimes goes away on its own at the onset of a strong nectar flow. The beekeeper may be able to control the disease by simulating a nectar flow (by feeding sugar syrup) and by requeening the colony [25].

Preventive biennial treatments with Terramycin antibiotic can also prevent EFB and as with AFB, and it is important to consider antibiotic treatments as a preventive measure, but not a cure. Terramycin treatments in EFB-infected colonies may actually be counterproductive because the medication permits those infected larvae to survive which would otherwise perish. These survivors then persist in the colony as a source of contamination. If the infected larvae are instead permitted to die, the house bees eject them from the hive and with them go the source of infection. The bacterium does not form long-lived spores that persist on hive surfaces [26].

i. For preventive treatments, inspect brood frames regularly, be familiar with field symptoms and remove all frames with significant numbers of affected cells.
ii. Spray or sprinkle antibiotics (oxytetracycline) dissolved in 250 ml of sugar syrup over the colony every 3-4 days for 10 days.
iii. Requeening provides a distinct break in the brood cycle of the colony, allowing the bees to clean up existing disease. It may also provide new bees with better cleaning behavior i.e., less susceptible to disease.
iv. Minimize robbing by preventing sugar spillage and do not barrel feed.
v. Apply hygienic management practices, clean hive tools, smoker and gloves after inspection of each apiary, clean clothes regularly, and replace brood frames after five years.

It is a serious disease, but with careful management and thought, its incidence can be reduced. If colonies need treatment, apply the antibiotic to the brood nest under the queen excluder at least eight weeks before anticipating honey extraction [27].

1.3. Septicemia

Septicemia is a bacterial disease of adult honey bees that is rarely encountered; it is caused by *Pseudomonas apiseptica* Burnside, which is gram negative bacteria, with oval or ellipsoid shape and can stay active in a bee carcass for 1 month. It is quite a common disease within a hive, but it poses a real threat only if the natural immune system of the hive inhabitants is weakened or has been subjected to a stress of some kind. The bacteria, by some unknown method, make their way to the hemolymph, bees may become infected via their tracheal system multiply rapidly, and ultimately cause the death of the host. Conditions for of disease include swarming and building honeycomb, and it affects drones and worker bees. When the hive individuals are too weak, the bacillus enters the bee's respiratory system and from here it arrives in the hemlymph where it multiples and leads to the individual's death through septicemia. This disease typical can outburst any time throughout a year, especially in condition of high hive humidity. The disease does not cause too many casualties and the healing might be spontaneous if the causing agents disappear. The dead bees decompose quickly and become very fragile with the body parts detaching at the most gentle touch. Serious outburst are quite rare, however the disease should not be treated with superficiality [28, 29].

1.3.1. Symptoms and Diagnosis:

Bee colonies are rapidly deteriorating, bees are crawling with spread wings, they cannot fly, are falling on the ground with mildly shriveled abdomen and infected bees survive one day at most. The infected individuals present a milk-like hemlymph and they are very slow in movement. They also present abdominal contractions and before dying they crawl at the hive entrance. In case of severe infestation they die in quite a large number. Bees that die from septicemia appear to have no connective tissues and dismember easily. The legs, wings, head, thorax and abdomen separate-even by the slightest handling. Hemolymph of infected bees also may be milky white in color. The isolation and identification of *Ps. apiseptica* bacteria from the hemolymph may be necessary for verification**.** Diagnose by isolating pathogens from hemolymph on a nutrient medium (agar, broth), coloring (aniline colors) and then by microscopic examination of an isolated pathogen. The material for laboratory examinations are live bees and also by biochemical examinations (liquefaction of gelatin, milk coagulation) [30].

1.3.2. Treatment

There is no available chemical treatment for honey bees infested with *Bacillus Apisepticus*. It has been observed that antibiotic treatment prevents complications, but does not cure septicemia. Hygiene is the best prevention measure against this honey bee disease. Bee colonies are to be destroyed on the spot and honeycomb molded into wax [31].

1.4. Parathypus

Parathypus is caused by bacterium *patatyphi alvei* that is a round shaped, short gram negative stick, does not create spores,

mobile, and breeds well on usual nutrient mediums (agar, broth). Bees become infected by bacterium gy ingestion of infected food and water. Other conditions are also important to cause the infection including weather conditions during winter, lack of water and weak population. The spread of contagion is done via drones and other bees from different hives, beekeepers and honeycomb frames. Parathypus usually occurs in February and March, but period from April to September is not excluded either. There can also be various other causes of disease like over-population, outside temperature too high, honey chamber completely full and some other form of trouble. In the first three cases, one observes many fanners at the hive entrance, whilst in the last case the hive entrance is cold and empty [32].

1.4.1. Symptoms and Diagnosis

Clinical manifestation of this disease includes the bees become weak and cannot fly. They get liquid, light-brown feces (can be spotted at the bottom of the hive) with swollen abdomen and pathohystologycal changes on intestinum. The disease itself can last from one to a few days normally 8-15. The bee mortality rate can vary depending on weather conditions in that the weather conditions can lower or even sometimes remove the disease, especially if hygiene measures are implemented. Diagnosis can be done by isolating pathogens from the hemolymph and antibiogram is also required. Differential diagnoses include nosemosis [excluded by microscopic examination of intestinal contents (absence of spores of *Nosema apis*)] and dysentery [examination of intestinal contents (absence of bacteria and undigested pollen) [33, 34].

1.4.2. Treatment

If there is no natural recovery (due to weather and good pasture), the subsequent steps are taken, sugar syrup treatment, antibiotic therapy (based on antibiogram), disinfection of all equipment, and removing of frames with honeycombs stained with feces or moving bees to clean hives. Prevention steps are destroying insects which can transfer microorganisms to bees and replacing of the low quality honey with sugar syrup for better nutrition [35].

1.5. Powdery Scale

Powdery scale disease is believed to be caused by the bacterium *Bacillus pulvijaciens* and is of no economic significance. Spores of *B. pulvijaciens* have been isolated from honey, but there is still some question as to whether this bacterium is indeed the cause of powdery scale disease. But, *Paenibacillus larvae* subsp., *pulvifaciens* (*Bacillus pulvifaciens*) is the bacterium suspected of causing powdery scale disease. This disease is seldom reported because the incidence is low or perhaps because the average beekeeper is unable to identify it. A useful diagnostic characteristic is the scale that results from the dead larva. The scale is light brown to yellow and extends from the base to the top of the cell. The scale is powdery; when handled it crumbles into a dust. The *P. pulvifaciens* vegetative cells measure 0.3-0.6 to 1.5-3.0 mμ and the spores are 1.0 to 1.3-1.5 mμ. The bacterium can be isolated on nutrient agar, but growth is more luxuriant on glucose agar. When first isolated, the organism produces a reddish-brown pigment that can be lost by subculturing. The *P. pulvifaciens* closely resembles *P. larvae*, but the spores do not exhibit Brownian movement in the modified hanging drop technique. Also, *P. pulvifaciens* is distinguished by its ability to grow at 20°C on nutrient agar [36, 37].

1.6. Spiroplasmosis

Spiroplasma species is the bacterium that causes spiroplasmosis in adult honey bees. Spiroplasma is a helical, motile, cell-wall-free prokaryote that is found in the hemolymph of infected adult honey bees. The organism is a tiny, coiled and sometimes branched filament 0.7-1.2 mμ in diameter. Its length increases with age and ranges from 2 mμ to more than 10 mμ. Spiroplasma can best be seen in the hemolymph, using dark-field microscopy and they can also be seen by using the oil-immersion objective of a phase-contrast microscope. Hemolymph can be taken from adult bees by puncturing the intersegmental membrane directly behind the first coxae, using a fine capillary tube made from the tip of a Pasteur pipet. This organism can be cultured in standard mycoplasma broth medium 14 (GIBCO) and in Singh's mosquito tissue culture medium with 20-percent fetal calf serum. Data showed a high variation of *S. melliferum* infection in honey bees with peak prevalence in May during the course of one-year study period. The colony prevalence increased from 5% in February to 68% in May and then decreased to 25% in June and 22% in July. Despite that pathogenicity of spiroplasmas in honey bee, colonies remain to be determined, and results indicated that spiroplasma infections need to be included for the consideration of the impacts on honey bee health [38, 39].

2. Conclusion

Apiary inspectors and beekeepers must be able to recognize bee diseases and parasites and to differentiate the serious infections from the less important ones. To identify brood diseases, carefully examine dead brood found in the cells. The appearance of the combs may indicate which brood disease is present, but final diagnosis depends on the symptoms shown by the dead brood. Dead brood in open cells of a comb can be seen clearly if the comb is inclined so that direct sunlight falls on the lower side of the cells. Remove sunken, discolored, or punctured capping and examine them for dead brood. When inspectors and beekeepers examine dead brood, observe its appearance and position in the cells. And also note its age, color, consistency, and odor. For instance, if the affected brood is unsealed in the comb then European foulbrood is suspected. If only the sealed brood is affected, and has collapsed into a ropy brown mass, American foulbrood is suspected. Symptoms in European foulbrood the 'pepperbox' pattern of capped and uncapped cells develops only when the disease attains serious proportions. Unlike American foulbrood, most of the larvae die before their cells are capped. However, inspectors and beekeepers can sometimes observe discolored, sunken, or punctured capping. When American foulbrood is discovered in apiary, then diseased colonies should be destroyed by burning. Before burning of diseased colonies, however, inspectors and beekeepers must dig a pit to hold the burned material. Immediately, after all the bees have been killed, place the hive on pieces of burlap or strong paper; this can make it easier to gather up and burn the bits of comb, honey, or dead bees. Do this quickly to reduce the possibility of robber bees spreading the disease to healthy colonies. It is essential that adequate protective clothing, including a bee veil, is worn and techniques for safe handling of bees are understood before opening hives and collecting samples. The federal honey bee act should be enforced to prevent the introduction and spread of diseases and parasites harmful to honey bees in the states and elsewhere.

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