Review on major Honey Bee Diseases and their Management system in Ethiopia

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Summary: Honey bees are very important social insects because the can gives us priceless honey and provision of water and syrups is critical for sustainable of honey bees. In addition we must protect them from different diseases like: bacterial bee diseases (American Foulbrood, European Foulbrood, Powdery Scale, Septicemia and Spiroplasmosis), viral bee diseases (Sacbrood Disease, Chronic Bee Paralysis, Filamentous Virus, Acute Paralysis Bee Virus and Kashmir Bee Virus), fungal bee diseases (Chalk brood and stone brood) and parasitic bee diseases (Nosemosis, Amoeba and Varroa mites).

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1. Introduction

Honey bee is the social insects that live together in groups and cooperating for different tasks. Foraging and defending purpose of their enemies (Yellow jackets insect, mites, mice, ant, honey guides, birds and spider). Bees protecting their enemy by stinging, biting and vibrating their wings to care everything in the hive like young broods, honey. The social insects live in a colony of 10,000 to 100,000 bees consisted with three castes. A queen is fertile female and produced from fertile egg. Drone is 5 or a few hundred proven males and produced from unfertile egg. Worker bees are sterile females and produced from fertile egg. The term social insect means the individual insect lives out its life in a social community known as a colony. Like other animals honey bees are infected by different diseases (Eva et al., 2012).

2. Bacteria bee diseases

Bacteria bee diseases are diseases caused by original Bacteria source of infection such as:-American Foulbrood, European Foulbrood, Powdery Scale, Septicemia and Spiroplasmosis.

2.1. American Foulbrood

Cause: American foulbrood is a disease affecting the bee larvae and is caused by Bacillus larvae White. The disease is prevalent in many tropical and sub-tropical countries. It is the most

destructive of all bee diseases causing annual losses of several hundreds of thousands of dollars in US but this does not mean it is found in other countries. The pathogen is a rod-shaped, flagellate, motile bacillus highly resistant to heat, desiccation and disinfectants (Eva *et al.*, 2012).

Symptoms: The characteristic disease signs of AFB include some or all of the following: Uneven or 'Pepper-pot' brood pattern, Sunken, greasy or perforated, darkened cell capping, Roping, sticky larval remains when drawn out with a matchstick, Dark "scales", which are difficult to remove from cells

Frames smell rotten, or foul, Colony may show spotty brood (unevenly scattered empty cells), the diseased larvae are generally moist, discoloured and sunken, Decaying larvae are soft, sticky, Dead larvae are brown or black coloured, Larvae that die from AFB lay in upright position after capping, Scales on bottom walls of open cells, AFB has a characteristic smell in the advanced stage, A pupa that has died in capped cell shows a fine threadlike tongue or mouthparts projecting in the center of the cell and honey and pollen may be stored within the brood area (Eva *et al.*, 2012).

Transmission: The most common method is through the beekeeper, The spores can easily transferred, if frames of honey or brood are moved between hives, or if other contaminated equipment is used, Robbing by adult bees of dead or dying infected colonies is also an important mode of transmission and If left to run its course, all colonies infected with AFB will eventually die from the disease.

Pathogenesis: Infection occurs when bee larvae ingest P. Larvae spores in contaminated food given to them by nurse bees. The spores germinate in the larval mid gut into the vegetative forms (rod stage) a day after ingestion by the larvae, becoming bacteria. The rods penetrate the gut wall entering the tissues where they proliferate rapidly and at an enormous rate, feeding at the expense of the tissues, and continuing to proliferate until larval death. New spores form after the larva dies. The infected larvae die after their cell is sealed over. These spores are a potential source of infection. Once inside the larval gut again, the cycle will repeat (Eva *et al.*, 2012).

Diagnosis: Smears are preferred for the diagnosis of the bacterial diseases AFB and EFB. Smears are prepared from larvae, which are showing signs of disease. The technique is simple and the smears are ready for laboratory examination.

What to send to the lab for the diagnosis? Table 1 is the samples that are taken to laboratory.

Suspected disease	Sample
American foulbrood (AFB)	Larval smear (preferred) or comb sample containing diseased brood
European foulbrood (EFB)	Larval smear (preferred) or comb sample containing diseased brood
Chalkbrood	'Mummies' in or from comb
Sacbrood	Comb sample containing diseased brood
Nosema	Adult bees
Unknown disease	Contact an apiary officer immediately

Table 1. The samples that are taken to laboratory.

Treatment: Terramycin (oxytetracycline) is the only drug approved for use against American Foul Brood. Foulbrood is contagious... practice safe beekeeping. Avoid used equipment. Strong hives resist bacteria. You can try Tylon (antibiotic) for mild cases. For severe cases kill the bees and burn the hive. Bees can be killed by spraying with soapy water (Johan *et al.*, 2014).

2.2. European Foulbrood

Cause: Bacterium Melissococcus pluton, and has become one of the most serious bee diseases EFB can cause extensive losses in both amateur and commercial apiaries. Beekeepers should avoid severe outbreaks through sound management practices,

regular checks for disease and early treatment when disease is confirmed.

Pathways of infection: The disease remains in a vegetative cell state all the time and can remain viable for up to 3 years. Only when the disease is multiplying in the bee larvae is the bacterium susceptible to antibiotics. EFB is highly contagious with all stages of larvae development susceptible to infection (Johan et al., 2014).

Incidences of the disease are strongly correlated with climatic and nutritional stress factors. Cooler wet weather and poor nutrition will promote the incidence of this disease. **Signs of the disease:** May have a mottled, peppered appearance, with healthy brood cells intermingled with dead or dying ones. Affected larvae are: unsealed, curled up stage, although in severe cases brood of all ages may be affected. Diseased larvae collapse and become dislodged from their normal position in the cells. Their colour changes from pearly white to yellow and finally, yellowish brown and after two to four weeks, larvae dry up to form a brown scale, Appearance with scattered sunken and perforated capping.

Pupae have a similar appearance with AFB. The odour of infected brood varies from odorless to sour. Dead brood probed has a watery consistency and

although the sealed brown pupae has a slightly ropy consistency.

Transmission: EFB is highly contagious but infection may remain without visible signs for a long period. Sudden outbreaks of disease can occurthese probably result from a change of seasonal conditions and other stress related factors such as: Nutritional deficiencies, Shifting the bees and domination by older worker bees, especially in early spring.

Diagnosis: Diagnosis solely on the basis of the signs described above is not always reliable. EFB can be easily confused with a number of non-disease conditions and viral diseases. The only accurate diagnostic method is laboratory examination, particularly where the stages resemble signs of AFB. With both diseases the brood appears mottled, peppered. Both EFB and AFB can result in diseased larvae under sealed cells exhibiting a sunken, dark appearance with perforated capping. Dead brood probed with a matchstick may show signs of a brown **ropy consistency** in infections of both EFB and AFB.

Treatment: The only antibiotic recommended for the treatment of EFB is Oxytetracycline hydrochloride (OTC). The protocols for an Apiary Officer to issue an order to supply OTC include: The officer or an inspector must sight samples of diseased brood or the regional Veterinary Laboratory has confirmed the disease, The quantity of OTC prescribed must not exceed the dose rate to treat all the hives infected in the apiary or apiaries, The order can only be issued if the disease has been diagnosed within the past 8 weeks, The quantity of OTC for which the order is made will not exceed the number of hives the beekeeper has registered and The issuing of an authority to purchase OTC is at the discretion of the Apiary Officer (Johan *et al.*, 2014). **2.3. Powdery Scale**

Cause: 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 Diagnosis: 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/grinds into a dust. P. pulvifaciens vegetative cells measure 0.3–0.6 1.5–3.0 um. The spores are 1.0-1.3-1.5um. 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 sub culturing. P. pulvifaciens closely resembles P. larvae, but the spores do not exhibit Brownian movement in the modified hanging drop technique. P. pulvifaciens is distinguished by its ability to grow at 20 degree centigrade on nutrient agar.

2.4. Septicemia

Cause: Pseudomonas aeruginosa (= Pseudomonas apiseptica) is the bacterium that causes septicemia in adult honey bees. This disease results in the destruction of connective tissues of the thorax, legs, wings, and antennae. Consequently, the affected bees fall apart when handled. Dead or dying bees may have a putrid odor.

Diagnosis: P. aeruginosa rods measure 0.5-0.8 to 1.5-3.0 um. They are gram-negative and occur singly, in pairs, or in short chains. A bacterial smear and Gram stain can easily be prepared after removing a wing from the thorax. To isolate this organism, streak the base of a wing across Difco Pseudomonas isolation agar or Pseudomonas agar F. The optimum temperature for growth is 370 C. P. aeruginosa in culture is characterized by the excretion of diffusible yellow-green pigments. It can diagnose by reproducing the disease symptoms in healthy, caged bees. Bees with septicemia die within 24 hours; they exhibit the typical odor and "break apart" symptom after about 48 hours.

2.5. Spiroplasmosis

Cause: 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 um in diameter. Its length increases with age and ranges from 2 um to more than 10 um.

Diagnosis: Spiroplasma can best be seen in the hemolymph, using dark-field microscopy.

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 (GIBCO) and in Singh's mosquito tissue culture medium with 20 percent fetal calf serum (Johan *et al.*, 2014).

3. Viral bee diseases

Viral bee diseases are diseases caused by original virus source of infection such as:- Sacbrood Disease, Chronic Bee Paralysis, Filamentous Virus, Acute Paralysis Bee Virus and Kashmir Bee Virus.

3.1. Sacbrood Disease:

Is a disease of honey bees in many parts of the World and Infection can vary in hives from a few cells per frame to 90% the brood. Colony of infection can range from 0 to 100 per cent.

Cause: SBV or Sacbrood Virus (Morator aetatulas) often appears during spring or colony buildup and causes larval death. The virus is common in hives but only causes disease in bees that are genetically susceptible. Is caused by an increased larva to adult ratio; however, as the colony expands and develops sufficient strength the nurse bees.

Symptoms: Affected Larvae die after the cell has been capped. The raised mouth parts of the larvae are a sign of sac brood. The mouth parts from white through to yellow and dark brown. The larvae develop a sac of liquid at the anal end. The brown larvae may dry to form wrinkled, brittle scales which are easily removed from the cell. Drone brood may also be affected with sac brood disease. Sac brood is most commonly seen in the early spring and the signs

of the disease disappear with warmer weather and a good honey flow. The first appearance of sackbrood should not be confused with American foulbrood disease.

The distinguishing characteristics are that the brown larvae will not 'rope' as with American foulbrood disease and the beekeeper should test for 'rope' effect. Scales caused by sacbrood are easily removed while those caused by American foulbrood disease adhere to the cell wall and cannot be removed in one piece. Where American foulbrood and sacbrood diseases occur in a hive at the same time, the visual signs and 'rope' effect of American foulbrood disease may become less obvious.

Transmission: The spread of viruses is cross contamination by the beekeeper could spread the disease. Bees 'drifting' from hive to hive, contaminated drinking water, contaminated equipment and wind may also transmit the virus. Honey bees act as a reservoir for the sac brood virus. Viruses appear to accumulate in the hypopharangeal gland of worker bees, and these bees may transmit the infection to larvae through feeding.

Diagnosis: Diagnosis of sacbrood disease is difficult because special reagents are required to identify the virus. However, material collected from affected apiaries can be examined for the presence of a virus in the Electron Microscope. The presence of virus in colonies showing sackbrood is enough to diagnosis.

Control and Prevention: Because sac brood disease is caused by a virus, there is no method of treating affected bees. Re-queening hives showing persistent or severe signs of the disease is recommended. New queens should come from hives that show resistance to the disease. Hygiene is important in limiting or preventing. Hygiene may be outlined as: 1) Avoid introduction of bees and equipment from unknown sources 2) Avoid exposing honeycombs and equipment for robbing. 3) Spare equipment must be stored and fumigated away from robber bees. 4) Watch for the signs of the disease and report immediately when you saw any.5) Submit smears to Animal Health Laboratories.

3.2. Chronic Bee Paralysis:

Adult bees affected by chronic bee paralysis are usually found on the top bars of the combs. They appear to tremble uncontrollably and are unable to fly. In severe cases, large numbers of bees are found crawling out the hive entrance. Individual bees are frequently black, hairless, and shiny. In some cases, paralysis like symptoms can be caused by toxic chemical.

Diagnosis: Ideally, the diagnosis of this disease is made using serological techniques. Since this is beyond the capability of most laboratories, diagnosis is usually made by observing symptoms in individual bees and, when possible, colony behavior. Chronic paralysis can be diagnosed by reproducing the disease symptoms in caged bees. This can be done by spraying, feeding, or injecting a water extract made from suspect bees.

The extract is prepared by macerating the equivalent of one suspect bee in 1 ml water. This is then centrifuged to eliminate large suspended matter and passed through a 0.45um filter to remove bacteria. To feed up to 20 caged bees, mix 2 ml of the extract with an equal volume of sugar syrup. For inoculation, each bee receives 1.0 - 1.3 of the extract through a dorsal abdominal intersegmental membrane. The symptoms of paralysis should be visible after 6 days. Control bees should be treated with extracts made from healthy bees.

3.3. Filamentous Virus

Filamentous virus is also known as F-virus and bee rickettsiosis. Can be diagnosed by examining the hemolymph of infected adult bees using dark field or phase contrast microscopes. The hemolymph of infected honey bees is milky white and contains many spherical to rod shaped viral particles of a size close to the limit of resolution for light microscopy. The viral particles consist of a folded nucleocapsid.

3.4. Acute Paralysis Bee Virus and Kashmir Bee Virus

Acute paralysis bee virus (APBV) and Kashmir bee virus (KBV) are two serologically related viruses, and the antiserum produced from one virus will cross-react with the other virus. These viruses commonly occur in apparently healthy adult bees. No specific gross symptoms have been attributed to either virus. Whereas APBV is a disease of adults, KBV is reported to cause mortality in brood and adult honey bees. APBV and KBV diseases can be diagnosed using immunodiffusion tests. Recently, molecular methods were developed for detecting both diseases. However, immunodiffusion and molecular methods are not routinely used in our laboratory.

4. Fungal bee diseases

Fungal bee diseases are diseases caused by original fungus source of infection such as: -Chalk brood Disease and Stone brood Diseases.

4.1. Chalk brood Disease

Cause: Chalk brood is an invasive mycosis in honey bees (Apis mellifera L.) produced by Ascosphaera apis. Chalk brood is now found in honey bee colonies around the world. The incidence of chalk brood has increased in recent years. Most members of the genus Ascosphaera are associated with social and solitary bees. Some of them are saprophytes. Chalk brood disease was recognized in the early1900s. Epidemiologically, the disease is found in Ethiopia. A better understanding of chalk brood epidemiology will lead to improved management tactics of this highly prevalent disease.

Pathogenesis: Sexually produced A. apis spores (ascospores) are historically considered the primary source of brood infection. Two avenues of infection by ascospores are possible: Through **ingestion** and through **cuticle**. A. apis spores cannot germinate on the larval cuticle, therefore they must be consumed to infect larvae. A. apis can infect brood of any caste (workers, drones, or queens).

According to Bailey (1963, 1981), larvae are most susceptible at 3–4 days of age, while others report that 1 and 2 day-old larvae are highly susceptible as well.

Condition	Symptoms
Chalk brood	White and mouldy Hard larvae White or grey/black mummies in cells, on the floor, or out in front of the hive
American foulbrood	Discoloured through to dark brown Unsealed or with perforated sunken discoloured cappings Ropey larvae Hard to remove scales
European foulbrood	Twisted around cell wall White through to discoloured Yellow to dark brown Watery, granular larvae occasionally ropey
Sacbrood	Discoloured yellow through to black, gondola shaped in capped cells or under perforated caps, easily removed

Symptoms: table 2. Shows the symptoms of chalk brood and other Disease.

Diagnosis: Cut a piece of brood comb approximately 10 cm x 10 cm square containing suspect larvae, or place a matchstick in the cell of suspect larva, and obtain larval material on one end of the stick. Place the matchstick in a small vial ready to send to the laboratory.

Control and Management

Control: With no registered chemicals available for chalk brood control. The only means of controlling the disease is through management practices and use of disease resistant bees.

Chemicals: There is no effective chemical agent effective for use against chalk brood fungus. Therefore, chemicals are not recommended (Myrsini *et al.*, 2015).

Management Practice: Management practices that reduce the stress on hives also reduce the number of chalk brood spores. Maintaining strong healthy colonies. Management practices which may reduce the effects of chalk brood disease are: Removing 'mummies' around the entrance, destroying mummies, supplying new combs, providing good ventilation in hives, adding young adult bees to hives, Feeding sugar syrup, fresh uncontaminated pollen or supplement and not using the same site each year so change apiary site.

4.2. Stone brood Diseases

It attacks the brood and transforms the larva into a hard, stone-like coloured object which is found

lying in open cells. Adult bees may also be attacked and are also killed in the process. The disease has not yet been reported in Africa, but beekeepers must keep alert.

Cause: It is considered that the fungus named *Aspergillus Flavus* is the pathogen agent causing the stone brood disease. The spores of *Aspergillus Flavus* might be present within a bee family without causing damage to it.

Transmission: The disease is spread outside the hive by: drifting, robbing or swarming honey bees. Beekeepers also transmit the disease through their beekeeping tools or by moving combs that contain *Aspergillus Flavus* in hives inhabited by healthy families.

Symptoms: Larvae that died because of this disease are mummified like those that have died because of chalk brood, However, the stone brood disease makes the infected individuals green or whitish yellow. The spores are more numerous in the vicinity of the head. The fungus forms a sort of green ring near the larva head. The mummies are solid; hard to crush that is they do not have the sponge appearance typical for the chalk brood disease. In the end the pathogen fungus comes out from the integument of its host and creates a fake skin.

Diagnosis: The disease is not difficult to identify. Diagnosis can be put by inspecting the frames where the brood lives and the debris find on

the hive floor. If the honeybees are gently pushed aside it is easy to spot the cell containing larva that died due to stone brood infestation.

Zoonosis: The fungi that cause the disease might affect humans. For this reason it is advisable to destroy the heavily infected combs. So honey that comes from infected hives should not be sold for human consumption. It is believed that the fungus causing stone brood can trigger respiratory diseases in both humans and animals.

Treatment and Control: There is no chemical treatment for Stone brood. Thus, prevention is the only solution to have healthy, stone brood-free colonies. The hives and all the beekeeping tools must be clean so as to prevent infestation. The dead larvae must be removed. The hives have to be well ventilated and equipped with new frames that also have a new foundation. Hygiene is the key for having healthy bee families and the only available method to fight Stone brood and other bee diseases.

5. Parasitic bee diseases (protozoan and external) 5.1. Nosemosis

Cause: Nosema apis and Nosema ceranae. Nosema species are obligate, fungus-like, intra cellular parasites that are limited to specific host's species. Nosema apis and N. ceranae cannot be reared in laboratory culture, as is possible with most bacteria and other fungi. They can multiply in living honey bee midgut, and perhaps other, cells (Myrsini *et al.*, 2015).

Life Cycle: 1) Bee ingests Nosema spores 2) Spores are filtered out of the honey sac by the proventricular valve 3) Spores released into the midgut 4) Mid gut stimulate germination 5) The organism penetrates a midgut cell and grows by absorbing nutrients from cell 6) The parasite increases in size until it is large enough to divide in half 7) That stimulus triggers sporulation 8) Depending upon the species of Nosema, approximately 100 spores can begin to develop as early as four days post-infection or up to nine days later 9) Some of the early, thin walled spores appear to germinate inside the infected cells, sending their polar filaments into adjacent cells 10) Infecting other tissues, at least in bumble bees 11) Environmentally resistant, thick walled spores are released into the midgut lumen to start the process.

Effects on Colony: Nosema infections have specific negative effects on honey bees. Worker bees that ingest spores are not capable of producing brood food secretions. Their life spans can be reduced up to 78%. Young queens that ingest Nosema apis spores normally are superseded within a month. Queenless and dwindle away in early spring. When high percentages of workers are infected and spore counts exceed ten million spores per bee. The disease may be associated with Colony Collapse Disorder (CCD).

Signs: No symptoms are specifically indicative of Nosema disease, but there is Inability of bees to fly. Excreta on combs or lighting boards. N. apis may cause the ventriculus of heavily infected bees to become white, soft and swollen while N. ceranae infections do not. A microscopic examination is the only reliable test for the presence of this disease (Myrsini *et al.*, 2015).

Diagnosis: The only accurate method is microscopic examination of the gut of infected bees. Gathering 30 live or freshly dead bees from the hive entrance. Place live specimens in a cage with a small supply of freeze or alternatively, place 30 bees in a jar containing methylated spirits. Transport to the veterinary laboratory. Abdomens nearby are homogenized, using a mortar and pestle. The homogenate is sieved through two layers of cheesecloth into calibrated centrifuge tubes. The tubes are spun in a clinical centrifuge at 600 rpm for six minutes to drive the spores to the bottom of the tubes. The liquid (supernatant) is poured off (decanted) and the plug (pellet) at the bottom is resuspended in a specific volume of water (final calculation is spores in one ml water per bee). The plug is broken up well (resuspended) by sucking the water using pipette Then a small droplet of the suspension is placed on a blood cell counting chamber (hemocytometer). The number of spores counted over certain areas of the chamber grid can be converted to millions of spores per bee. If infection levels are below 10,000 spores per bee, no spores will be seen over the entire grid and the diagnosis is determined to be "not. detected" or "ND." That does not mean that there is no infection.

Treatment: Fumagillin B antibiotic is the only important drug to be applied. Gamma irradiation of hive equipment will kill all nosema spores present,

as well as killing all other microbial disease pathogens (Robin *et al.*, 2010).

Heat will decontaminate equipment affected by nosema. Dry equipment should be heated to 49°C and held for 24 hours at this temperature to destroy nosema spores. Combs must not contain honey or pollen, and heat must not exceed this temperature, as damage to combs may result.

5.2. Amoeba Diseases

Cause: The causative agent is Malpighamoeba mellificae. There are no outward symptoms of the infection and a positive diagnosis of M. mellificae can only be made by microscopic examination to identify the amoebic cysts. M. mellificae infections are associated with spring dwindling, dysentery and shortening the lifespan of infected bees. M. mellificae infections are very often found in association with nosemosis and it is likely that a dual infection will be more damaging to the health of the honey bee.

Treatment: Hygiene and good management is the key to controlling spread of the organism, as with Nosema and other infections. There are currently no approved proprietary products registered for the control of M. mellificae (Robin *et al.*, 2010).

5.3. Varroa mites (Varroatosis or Varroosis)

Causes: varroa destructor is the mite responsible of Varroatosis (or Varroosis), an external parasitic disease that attacks honeybee colonies (adult bees and especially the brood). Varroa destructor causes the major economic losses to the beekeeping sector because it is widespread and it has a strong adaptability to the treatments.

Symptoms: In bees' colonies contaminated by varroa it is possible to observe: the parasites on the body of adult bees, scattered brood (index of high mortality of larvae), a typical stench of dead brood, smaller bees, bees with deformed wings, clusters of bees restless and unable to fly, weakening of the colony as it becomes less populated and due to the reduced capacity of the bees in the collection and storage of supplies and abnormal swarming (especially at the end of the season) and replacement of the queen (Robin *et al.*, 2010).

Transmission: This parasitic disease is transmitted very easily by direct contact from infested to healthy bees (e.g. during the visit of a flower, by drones who can freely enter different hives, during robbing of infested hives, as effect of drifting of infested worker bees among adjacent hives, etc.). But the transmission may also occur by the direct action of the beekeeper for example by transferring parasitized brood combs from one colony to another or by the migratory beekeeping practice.

Monitoring: Since the evolution of the disease is not very evident, the monitoring of the number of parasites in each hive through periodic inspections is very important. The diagnosis of infestation can be carried out by: checking the number of parasites that fall on the hive bottom, checking the number of varroa mites affecting the male brood (which is the most affected), checking if the parasites are visible to the naked eye on adult bees, meaning that there are high levels of infestation, applying the World Organization for Animal Health (OIE)-endorsed method by which adult bees are dipped in alcohol and stirred in order to separate the varroa mites from the bees, applying the powder sugar empirical method, which entails sprinkling powder sugar on bees collected in a jar and shaking it to cause the varroa to fall through a mesh as this allows to count the mites easily (Robin et al., 2010).

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