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Good beekeeping practices

Practical manual on how to identify and control the main diseases of the honeybee *(Apis mellifera)*

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Practical manual on how to identify and control the main diseases of the honeybee *(Apis mellifera)*

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Preparation of this document

This manual serves as a practical tool for beekeepers, veterinarians and beekeeping advisory services, to identify and control main honeybee diseases in the apiary. It follows from the manual "Main beekeeping diseases: Good beekeeping practices" published in 2018.

The Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) "M. Aleandri" developed this document in collaboration with the Research and Extension Unit of the Food and Agriculture Organization of the United Nations (FAO), the International Federation of Beekeepers' Associations (APIMONDIA), and FAO's Animal Health Service. Definitions and good beekeeping practices reported in this manual, were adopted from the validation activities undertaken in collaboration with the BPRACTICES project.

This manual is part of the Technologies and practices for small agricultural producers (TECA) series of the FAO's Research and Extension Unit (AGDR). Additional details about the TECA platform can be found here: http://www.fao.org/teca.

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Acronyms and abbreviations

ABPV	Acute bee paralysis virus
ABV	Arkansas bee virus
AFB	American foulbrood
AIV	Apis iridescent virus
BBPV	Berkley bee picornavirus
BMBs	Biosecurity measures in beekeeping
BQCV	Black queen cell virus
BVX	Bee virus x
BVY	Bee virus y
CBPV	Chronic bee paralysis virus
CWV	Cloudy wing virus
DWV	Deformed wing virus
EBV	Egypt bee virus
EFB	European foulbrood
FV	Filamentous virus
GBP	Good beekeeping practice
IAPV	Israeli acute paralysis virus
IAS	Invasive alien species
KBV	Kashmir bee virus
PCR	Polymerase chain reaction
SBV	Sacbrood virus
SDG	Sustainable development goal
SHB	Small hive beetle
SPV	Slow paralysis virus


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1 Good beekeeping practices and biosecurity measures in beekeeping

Tools in support of a healthy and sustainable beekeeping sector

Good beekeeping practices and biosecurity measures in beekeeping are useful tools that the beekeeper can adopt in the apiary to guarantee the health of bees, humans (e.g. consumers) and the environment.

1.1 INTRODUCTION

Apiculture (or beekeeping) is the practice of honeybee management in hives for pollination and the production of honey and other products, such as wax, royal jelly, propolis and pollen. In addition, the production of live material, such as bees and queens, may represent other outputs of beekeeping. Finally, bees may support and provide other services, such as apitourism, apitherapy and monitoring of environmental pollution.

Beekeeping includes activities concerned with the practical management of the social honeybee species. The bee species used in beekeeping are:

- dwarf honeybees such as *Apis florea* and *Apis andreniformis*;
- giant honeybees such as *Apis laboriosa* and *Apis dorsata;*
- domestic honeybees and close relatives such as *Apis cerana*, *Apis mellifera*, *Apis koschevnikovi*, *Apis nigrocincta* and *Apis nuluensis*;
- stingless bees (Melipona);
- bumble bees (genus *Bombus*).

The above-mentioned bees may provide livelihoods and/or a source of income for many households in rural areas and small farms through:

- the production and sale of bee products, such as live bees (providing queen bees or swarms to other beekeepers), honey, pollen, wax, propolis, royal jelly, and venom. Bee products can be used as food for humans, feed for animals, in cosmetics, in medicines (used in conventional medicine, e.g. to treat allergies to bee venom, or in apitherapy) or as a component in industrial products such as polishes and sweeteners;
- the services provided by the bees, such as pollination, monitoring of environmental pollution, apitherapy, apitourism, and genetic preservation apiaries.

At the same time, the value of bees for the environment is often underestimated.

In recent decades, the beekeeping sector has been facing a growing number of external factors that affect honeybee health and productivity. These include, among others, global warming, the spread of emerging pathogens, urbanization, and losses in plant biodiversity. These factors are largely beyond the control of beekeepers, who therefore need to adopt proper beekeeping management practices and measures that help to limit the impacts of the changing environment in which they operate.

A modern approach to apiculture is moving towards a farming system that tends to be increasingly sustainable. However, sustainable apiculture always requires good knowledge on the proper management of the bees in order to optimize the natural systems and resources that beekeepers rely on.

The good beekeeping practices (GBPs) and biosecurity measures in beekeeping (BMBs) described in this chapter focus on *Apis mellifera* and aim at providing beekeepers with tools that contribute to maintaining healthy and strong colonies.

1.2 GOOD BEEKEEPING PRACTICES

Good beekeeping practices are those integrative activities that beekeepers apply to obtain optimal health for humans, honeybees and the environment. Therefore, the implementation of GBPs would have a positive effect on colony health and society, and at the same time could favour high production standards. Such practices are general measures valid for beekeeping activities and are globally accepted. They are not disease-specific and are meant to be implemented by beekeepers in primary production of hive products. They are a tool for beekeepers to successfully address the challenges they face in day-to-day apiary management.

Good beekeeping practices are the basis for a sustainable and resilient beekeeping sector. Daily implementation of GBPs in apiary management results in multiple positive impacts:

- economic benefits, such as cost reduction, larger production per unit, and higher income for beekeepers;
- better safety measures due to safer handling of veterinary medicines;
- positive impact on consumers through better quality of honeybee products;
- positive impact on public health because of reduced residues of veterinary products in honeybee products due to a safer use of medicines;
- positive impact on general honeybee performance such as improved honeybee health and productivity, and higher efficacy of treatments;
- positive environmental health benefits such as environmental protection through the use of organic treatments and biodiversity preservation.

Some relevant GBPs are mentioned below (Annex 1 provides a more extensive list).

1. Ensure proper selection of apiary sites. Suitable sites are away from sources of pollution such as intensive agriculture and industries, and provide sufficient bee fodder all year round. In addition, beehives should be sheltered from humidity and cold winds (Figure 1).
2. Ensure careful selection of suppliers, of bees and beekeeping equipment, and verification of the health status of newly acquired swarms, colonies and queen bees. For specific diseases, quarantine measures should be adopted to prevent their introduction into the apiary.
3. Identify each hive with a unique numerical code (Figure 2).
4. Keep a record of each hive visit, colony productivity and resistance to illness.
5. Verify the health status of the colonies regularly during the year (Figure 3). The frequency of hive inspections will depend upon the time of the year. During the winter season and under unfavourable weather conditions, opening the hives should be limited to a strict minimum.
6. Renew honeycombs regularly (every 2 years) and replace queens regularly (every 1–2 years). Preference should be given to queens showing disease resistance, hygienic behaviour, docility, low tendency to swarm, and high productivity.
7. Maintain balanced colony strength within the same apiary; ensure that hive capacity is sufficient to discourage swarming; and prevent robbing by removing heavily diseased or weakened colonies from the apiary, as these are more liable to be attacked and "sacked."

Figure 1: **Apiary located in a suitable location.**

Figure 2: Each hive is identified by its own unique numerical code.

Figure 3: Verify the health status of the colonies regularly during the year.

8. Undertake regular maintenance of hives to maintain them in good condition.
9. Adopt appropriate techniques to ensure the welfare of colonies, especially those that are younger/weaker; feeding of colonies with no food stocks or in case of unfavourable weather conditions as in autumn, winter and excessively cold or rainy springs; ensure good wintering; provide adequate water supplies, particularly in hot periods.
10. Avoid the use of honey to feed bees. Provide candy or glucose/fructose syrup. Verify origin and wholesomeness of feed provided to the bees.
11. Use the bee smoker appropriately, respecting the bees' welfare, and avoid using toxic material that may contaminate the honey and harm the bees.
12. Avoid the use of toxic substances such as disinfectants or chemical treatments for wood and toxic paints for hives.
13. Avoid transferring honeycombs from one colony to another if the health status of the colony is unknown. Diseased colonies should be removed from the apiary and destroyed, if necessary.
14. Ensure exclusive application of drugs registered for use in honeybees. Instructions for the use of the drugs should be strictly observed, and their use recorded in a logbook. Improper and untimely use of chemicals during honey production may lead to its contamination.
15. Undertake regular maintenance of the apiary, for example, mowing the grass in front of the hives.
16. Keep beekeeping equipment clean and in good order. When necessary, renew the materials.
17. Consult an expert in the event of anomalies.

Note that the application of GBPs in the apiary does not mean the bees will not become sick, but the incidence of diseases will decrease.

1.3 BIOSECURITY MEASURES IN BEEKEEPING

Biosecurity measures in beekeeping are all the operational activities implemented by beekeepers to reduce the risk of introduction and spread of specific honeybee disease agents. However, BMBs can only bring benefits if GBPs are systematically implemented as a prerequisite. Biosecurity measures can differ between different regions due to local factors such as climatic conditions, beekeeping equipment used, or bee races, and the prevalence, virulence and importance of the honeybee diseases.

The adoption of BMBs is the foundation of all disease control programmes, irrespective of the animal species. If biosecurity measures are properly implemented, it is possible to reduce the incidence of disease and, hence the need to apply treatments, to an absolute minimum.

Annex 2 provides an extensive list of BMBs.


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2 Main bee diseases

Factors influencing the occurrence of honeybee diseases and their classification

It is very important that beekeepers be able to recognize the first signs of disease in hives and know how to proceed to contain and treat the disease.

2.1 INTRODUCTION

Honeybees are susceptible to various diseases, some of which are very contagious and can be easily transmitted. The occurrence of diseases in honeybees depends on three factors:

1. Bees (genetic): the hygienic behaviour and resistance to various diseases varies from colony to colony and is based on the genetic heritage of the queen bees.
2. Pathogens (presence, infectious load and virulence): a disease needs the presence of the agent responsible in order to manifest itself (virus, bacteria, fungus or protozoa), but the quantity and ability to spread of the pathogen are also very important. It may happen that the pathogen is present in the colony without the manifestation of any symptoms. This is the "asymptomatic" stage of the disease, or more simply, the "dormant stage" of the disease. The "symptomatic stage" or "active stage" of a disease is when symptoms specific to the disease become visible. A disease will move from the dormant to the active stage when certain conditions are present, such as an increase in the number of the pathogens (number of SHBs, spores of American foulbrood [AFB], or spores of *Nosema* spp.) or the introduction of other stressors (e.g. other honeybee diseases, chemicals [such as pesticides], nutritional stress, and thermal stress) that are able to weaken the bees' immune system.
3. Environment (temperature, relative humidity, and presence of melliferous plants): environmental conditions and seasonal factors strongly influence the onset of diseases, in many cases they are key triggers.

TABLE 1. Main diseases of honeybees classified by nature of pathogen

Disease	Causative agent	Type
Acariasis	*Acarapis woodi*	Parasitic
Aethinosis	*Aethina tumida (small hive beetle)*	Parasitic
Tropilaelapsosis	*Tropilaelaps spp.*	Parasitic
Varroosis	*Varroa destructor*	Parasitic
American foulbrood	*Paenibacillus larvae*	Bacterial
European foulbrood	*Melissococcus pluton*	Bacterial
Chalkbrood	*Ascosphera apis*	Fungal
Stonebrood	*Aspergillus flavus*	Fungal
Nosemosis	*Nosema apis – Nosema ceranae*	Fungal
Amebiasis	*Malpighamoeba mellificae*	Protozoal
Acute bee paralysis virus (ABPV)	*Dicistroviridae*	Viral
Black queen cell virus (BQCV)	*Dicistroviridae*	Viral
Chronic bee paralysis virus (CBPV)	*Cripaviridae*	Viral
Deformed wing virus (DWV)	*Iflaviridae*	Viral
Invertrebrate iridescent virus type 6	*Iridoviridae*	Viral
Israeli Acute paralisysis virus (IAPV)	*Dicistroviridae*	Viral
Kakugo Virus	*Iflaviridae*	Viral
Kashmir bee virus (KBV)	*Dicistroviridae*	Viral
Sacbrood virus (SBV)	*Virus picorna-like*	Viral
Tobacco ringspot virus	*Secoviridae*	Viral

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2.2 CLASSIFICATION OF HONEYBEE DISEASES

Honeybee diseases can be classified by:

- the nature of the agent responsible for the disease: parasitic, fungal, bacterial or viral infection;
- the function of the individuals that are affected in the hive: brood diseases and diseases of adult bees.

2.2.1 MAIN HONEYBEE DISEASES BY NATURE OF THE CAUSATIVE AGENT

Classifying diseases by pathogen type is the most universally accepted way of classifying disease in animals. The pathogenic organisms can be parasites, fungi, bacteria or viruses. Table 1 provides an overview of the main honeybee diseases based on the type of causative agent.

2.2.2 MAIN DISEASES OF HONEYBEE BROOD

Certain diseases will affect the brood of the honeybees (Table 2). For diagnosis of the disease, the beekeeper will observe symptoms in the brood combs such as scattered brood or dead larvae in the cells. In certain cases, the pathogen will also affect adult bees (e.g. varroosis and some viroses).

2.2.3 MAIN DISEASES OF ADULT HONEYBEES

The main diseases affecting adult honeybees are:

- varroosis,
- nosemosis,
- viroses.

Varroosis and viroses may also affect the brood.

TABLE 2. Main brood diseases

Main brood diseases
Varroosis
Small hive beetle
Tropilaelapsosis
American foulbrood
European foulbrood
Chalkbrood
Stonebrood
Black queen cell virus
Sacbrood virus
Other viroses


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3 Varroosis

A parasitic diseases of the brood and adult honeybees

Varroa destructor, *or simply "varroa", is an external mite of the honeybees that causes major economic losses worldwide to the beekeeping sector.*

3.1 INTRODUCTION

Varroa destructor (*V. destructor*) is the mite responsible for the disease called varroosis. It is an external parasitic mite of the Asian honeybee (*Apis cerana*) and of the European honeybee (*Apis mellifera*) and affects both the brood and the adult bee. It is present in almost all parts of the world, except in Australia and in the islands of the South West Indian Ocean, and has shown a strong adaptability to treatments.

The parasite, as it is most commonly observed in the hive, is a small, red-brown, oval-shaped mite, the size of the head of a pin (Figure 4). It attaches itself to the body of adult honeybees, larvae or pupae (Figure 5), and feeds on them. This weakens the host, making it more vulnerable to other diseases especially viroses (e.g. deformed wing virus [DWV] or acute bee paralysis virus [ABPV]) but also nosemosis. It also induces malformations in the brood and adults.

With the exception of few subspecies of honeybees, if left untreated, an infested colony is very likely to die within one or two years.

The mites reproduce inside the brood and can live for up to five days out of the hive if the environment is favourable for their survival (temperature, humidity, etc.).

In the average temperate climate, mite populations can increase 12-fold in colonies having brood half of the year, and 800-fold in colonies having brood year-round.

The average lifespan of adult bees in heavily affected colonies decreases by 25 percent to 50 percent. Varroa not only feeds from the fat bodies and the haemolymph of the larvae and adult bees, it also causes small wounds on

the body of the bees and makes them more vulnerable to other pathogens such as viroses (which may also replicate in the salivary glands of varroa), fungi and bacteria.

3.2 MORPHOLOGY OF THE VARROA MITE

It is very easy to distinguish between the female and male varroa mites. They are distinguishable by both size and colour. The female mites can be easily observed on the adult bees (Figure 5), on the hive bottom board (Figure 7) or inside the brood. The male varroa mite has only a short life and remains inside the brood cells for its entire lifespan.

3.2.1 FEMALE VARROA MITE

On the bottom board of the hive or on the body of adult bees, the female mites can be observed as tiny (1.1 mm long and 1.5 mm wide), flat, reddish-brown, elliptical-shaped parasites. The mites have four pairs of legs that enable them to move around easily inside the hive. Only the female varroa mite has a mouth able to feed on the body of the bees or brood.

3.2.1.1 Female varroa mite life cycle

Female mites living when brood is present in the colony have an average life expectancy of 27 days. In the absence of a brood, they may live for many months. Adult females undergo two phases in their life cycle: **phoretic** and **reproductive**.

Phoretic phase

During the phoretic phase, the female varroa lives on adult bees, and moves from bee to bee to feed. It can usually be found between the abdominal segments of the bees. Its flattened shape allows it to fit between the abdominal segments, and with its claws it can easily grasp the bee and remain attached.

The phoretic period may last from 4.5 days to 11 days when brood is present in the hive. When no brood is present in the hive, the phoretic period may last up to 5–6 months.

Figure 4: Dorsal (up) and ventral (down) view of a female *Varroa destructor*.

Figure 5: Female *Varroa destructor* on the back of a drone (up) and on a pupa (down).

Reproductive phase

In order to reproduce, varroa mites need brood. The phoretic female mites enter open brood cells to feed on the developing bee and lay eggs. More than one female varroa mite can enter the same cell to reproduce. In *Apis mellifera,* varroa females enter either a worker or drone cell, although they are more attracted to drone brood. In drone cells, varroa mites have more time to replicate.

Only bee larvae that are ready to be capped are attractive to the mite. Once the cell is capped, the mother varroa mite starts laying eggs. The first egg is unfertilized and develops into a male mite. The following eggs are fertilized and hatch into female mites. The mites feed on the developing pupa and mate in the capped cell. After mating, the male mite dies. The female mites and the mother varroa mite leave the cell with the emerging bee. In the first days of life, the adult female mites parasitize adult bees, preferably nurse bees. After the phoretic stage, adult mites invade brood cells that are ready to be capped, and the entire mite life cycle repeats. Generally, of all the eggs laid, only one adult female will survive when laid in a worker cell, and only two when laid in a drone cell.

3.2.2 THE MALE VARROA MITE

The main role of the male varroa mites is to fertilize the female varroa mites. For this reason, their mouthparts are built exclusively for the transfer of sperm into the genital tracts of the females (Figure 6). They cannot feed themselves and have a short lifespan of a few days, as they are not able to survive outside the capped brood. The varroa male has a spherical body shape and whitish colour. It is smaller than the female (about 0.8 mm in diameter) and has a soft body, very similar to the immature stage of the varroa female.

3.3 TRANSMISSION

Varroa mites are transmitted very easily by direct contact from infested to healthy bees. There are many common ways varroa mites can be passed between colonies:

- Drifting of infested bees into another colony. This happens frequently in managed honeybee situations where individual bee colonies are located within metres of one another.
- Drones entering a varroa-free hive, carrying varroa mites on their bodies.
- Robbing of infested hives. It is common for strong colonies to rob weaker colonies in periods of nectar scarcity.
- Mites are passed from one bee to another during visits to flowers by foragers for nectar or pollen collection.

Transmission may also occur because of the direct action of the beekeeper, for example, by transferring parasitized

Figure 6: Male and female varroa mite mating.

brood combs from one colony to another. In fact, beekeepers often aid weak colonies by adding bees or brood from a healthier colony, and this practice may help spread the mite. Varroa transmission can also be caused by migratory beekeeping. Beekeepers may transport highly infested colonies from one area to another, facilitating the spread of varroa among different geographical areas.

The delayed application of acaricide treatments may cause an increase in parasite population, which increases the possibility for the transmission of varroa among hives or among apiaries – hence the importance of simultaneous and coordinated anti-varroa treatments both within the same apiary and between apiaries located close to one another.

3.4 DIAGNOSIS

Female varroa mites are easily detectable on the hive bottom board (Figure 7). To monitor the varroa infestation level, it is good practice to count once a month the number of mites that fall onto the bottom board (natural mite fall) within 24 hours in at least 10 percent of the hives in the same apiary. Similarly, after each treatment, the number of mites that have fallen on the bottom board should be monitored. The threshold for intervention varies from area to area and depends on the time of the year and other factors. Beekeepers should keep track of the natural mite fall and its correlation with the colony mortality caused by varroa throughout the year and for different locations, and establish the threshold based on their personal experience.

For counting the natural mite fall, it is important to keep the bottom tray clean from hive debris, and possibly lined with adhesive paper, grease or petroleum jelly (e.g. Vaseline). Considering the rapid multiplication of mites within the hives, it is very important to monitor and

understand the infestation level in the hive. Moreover, during the hive inspections, it is always important to check for visual signs of varroa infestation. These are:

- the presence of varroa mites on adult bees (Figure 8), especially at the end of the productive season, when varroa populations are at their maximum levels;
- scattered brood pattern, with perforated cappings containing dead bees at the end of metamorphosis (Figure 9);
- a dominant sour smell of putrefied brood may be detected from brood frames and when opening the hive;
- dead bees at the end of the metamorphosis, unable to leave the cells, as an effect of ABPV, which increases with high varroa infestation levels (Figure 10);
- the presence of deformed bees with stunted abdomens or deformed wings (Figure 11).

The observation of one or more of the above-mentioned signs is very often an indication of a severe varroa infestation. In such cases, treatments with acaricides are recommended in order to reduce the number of varroa mites in the hive.

Autumn/winter losses of an entire apiary (or apiaries) may be a consequence of massive varroosis.

Figure 7: Varroa mites on the bottom board of the hive.

Figure 8: Varroa mite on the thorax of a drone.

Figure 9: Scattered brood pattern with perforated cappings containing dead bees.

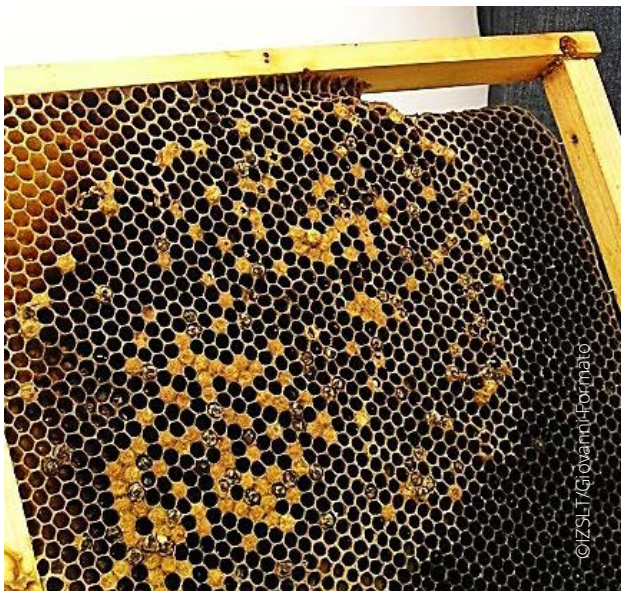
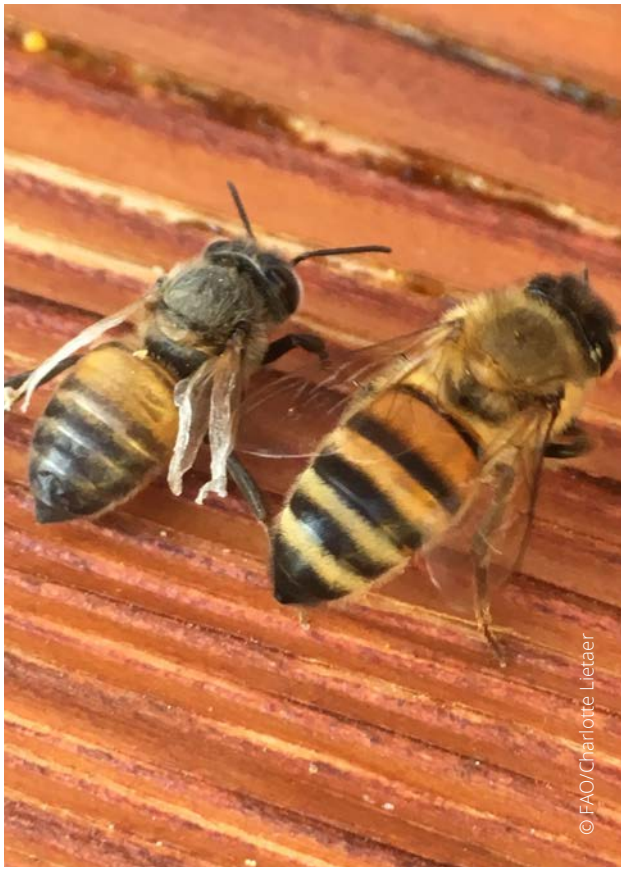


Figure 10: Dead bees not able to leave the cell at the end of the metamorphosis.

Figure 11: Stunted bee with deformed wings (left) and healthy bee (right).



3.5 MONITORING

Monitoring is a key practice to prevent severe varroa infestation. As the evolution of the disease is not very evident, monitoring of the number of parasites (varroa infestation level) in the hive through periodic inspections will be very important to understand whether acaricide treatments will be needed to keep the number of mites in the hive under control. It will be necessary to correlate the number of varroa counted with respect to other factors. These factors include the presence of signs of varroosis, the time of year, the presence of brood, the presence of supers, and the schedule of the acaricide treatments.

Within the same apiary, at least 10 percent of the hives should be sampled.

3.5.1 ON-SITE MONITORING METHODS

There are many methods to assess and monitor the level of varroa infestation in the hive. Some methods are easier and faster to conduct but are less accurate. Other methods are more labour-intensive or more invasive, requiring bees or brood to be sacrificed, but give a more accurate idea of the actual infestation level. The icing-sugar method is one of the methods most commonly adopted by beekeepers because it does not require killing bees used for testing. Particular attention should be paid to monitoring infestation levels in stronger colonies. In these hives, varroa mites have a higher possibility to replicate, considering the abundance of the brood. These hives are typically at high risk of collapse as a consequence of varroosis at the end of the active season.

3.5.1.1 Visual observation of adult bees

Observing worker bees with one or more varroa mites attached to their bodies, inside or outside the hives, is generally an indication of a high level of varroa infestation. In such cases, the recommendation is to apply one of the methods mentioned below in order to gain a clearer idea of the actual level of varroa infestation, and the adequate measures to adopt to control the varroa infestation.

3.5.1.2 Natural mite fall – sticky board method

The "natural mite fall" is the number of mites that naturally fall every day (within 24 hours) onto the bottom board of the hive. For this reason, it can also be called a "diagnostic board". It is a good indicator of the infestation level in the hive, although it may differ considering several parameters, such as the time of year, the subspecies of bee, the time elapsed since the last treatment, the altitude, and the presence of brood. It is important to keep a record of the natural mite fall observed during the year in order to compare it with the following years. Note that other insects (e.g. ants) may be able to remove the varroa mites from the bottom board, resulting in a misleading picture of the

real status of the infestation. For this reason, it is useful to line the bottom board with adhesive paper, grease or petroleum jelly (e.g. Vaseline), so that insects cannot remove fallen mites.

After each anti-varroa treatment applied in the hive, it is good practice to count the number of dead mites that fall onto the bottom board as it can provide an approximation of the actual level of infestation. Always keep in mind that part of the varroa population could have survived in the capped brood of the colony, if present.

3.5.1.3 Brood sampling

A high percentage of the mites in the colony are in capped brood cells, especially in drone brood. The female varroa mites are very visible as brown or reddish spots on the white pupae after brood decapping. Counting the number of varroa mites present on the capped brood is a good method for monitoring the level of varroa infestation. To sample the brood, with a decapping fork, remove about 200 drone or worker pupae by sliding the fork into the protruding drone cappings or slightly below the surface of the worker cell cappings. Remove the brood by pulling the decapping fork outwards. Count the number of mites on the brood, and check whether any mites were left behind in the cells.

3.5.1.4 Alcohol wash

Scoop about 300 adult bees from the brood box into a wide-mouthed transparent jar. Add 50 ml of alcohol (rubbing alcohol), close the jar and shake for at least three minutes to dislodge the mites from the bees. Remove the lid and pour the contents through a sieve to separate parts of bees and mites. Count the mites.

3.5.1.5 Soapy wash

The soapy wash is very similar to the alcohol wash, with the alcohol being replaced by a soapy water solution. Proceed as indicated above.

3.5.1.6 Icing sugar method

From the brood nest, select one external honey frame covered with a number of forager bees, and fill a 120 ml container with bees. Add 35 g of fresh icing sugar (about two tablespoons) to the jar and close. Gently rotate the jar for 60 seconds so that all bees are covered with icing sugar. Leave the closed jar in the vertical position to rest for about three minutes. Shake the contents of the jar vigorously (also with sidewall knocks) through the screen lid into a sieve that does not allow the passage of varroa mites. Shake for at least a couple of minutes. The jar can also be shaken over a container (e.g. pan) of water, the

Figure 12: Icing sugar method: (1) fill a jar with bees; (2) add icing sugar; (3 and 4) shake the jar; (5) let the jar rest in vertical position; (6) shake the icing sugar through the screen lid over a pan of water; (7) return the bees to the hive; and (8) count the fallen varroa mites.

mites will fall in the water and can be more easily counted. This method allows the survival of the bees, which can then be placed back inside the hive box (Figure 12). If icing sugar is not available locally, it can be easily made by grounding granulated sugar until it turns into power.

3.6 CONTROL

It is very likely that most colonies of *A. mellifera* in temperate climates will be damaged or even collapse within a few years if no control or inappropriate control methods are used. There exists a wide range of different methods to keep mite populations under control. A distinction can be made between biotechnical methods and treatments with different acaricides. Options and the efficiency of methods are highly dependent on location, time of year, level of infestation and honey-flow period. All these factors should be considered in order to ensure the maximum effectiveness of the treatments applied. Moreover, organic treatments should be preferred in the context of sustainable beekeeping and to prevent contamination of the honey.

3.6.1 BIOTECHNICAL METHODS TO REDUCE THE LEVEL OF VARROA INFESTATION

The level of varroa infestation can be reduced by adopting specific biotechnical methods. The most frequently used are listed below. Usually, they are more time-consuming with respect to the simple application of the chemical treatments, and require some practice.

3.6.1.1 Drone brood removal

Drone brood removal is a technique that consists of reducing the mite population by removing the drone brood, where mites prefer to reproduce. By removing capped drone brood from the hive, the beekeeper removes the varroa mites that are mating in it. The parts of the frames containing the drone brood are simply cut away with a sharp knife and disposed of. This technique is typically applied in spring when drone brood is present in the hive. Elimination of drone brood does not seem to affect colony size or honey production.

3.6.1.2 Brood interruption

"Brood interruption" consists of a number of methods that artificially stop the egg laying of the queen in the colony and consequently generate the absence of brood in the hive. This can increase the efficacy of most of the anti-varroa treatments because most of the active ingredients used for varroa control, except the formic acid-based products, are not able to penetrate the capped cells and kill the varroa mites located inside. In addition, the absence of brood interrupts the varroa life cycle. The acaricide will be applied when there is no more brood in the hive and all (female) varroa is in its phoretic phase (i.e. outside the cells, on the bees, as there is no capped brood in the hive where to reproduce). Brood interruption is mainly achieved by caging the queen in a small queen cage, or by confining the queen into a trapping comb (which must be removed from the hive before applying the acaricide).

3.6.1.3 Brood removal

Similarly to the brood interruption, the complete removal of brood ("brood removal technique"), allows to treat the bees with varroa in the phoretic phase (on the adult bees). By applying the treatment after brood removal, the beekeeper may considerably increase the acaricide efficacy of the treatment. The brood removal can be applied directly by removing all the combs containing brood (e.g. splitting a hive in two parts: one with the brood combs and nurse bees, the other with the foragers), or by removing a single "trapping comb" (the only one with brood) in which the queen has been previously confined (see 3.6.1.2).

3.6.2 ACARICIDE TREATMENT STRATEGIES

Acaricide treatment strategies use chemical products to kill varroa mites. A distinction can be made between hard and soft acaricides.

3.6.2.1 Hard acaricides

"Hard" acaricides are products that usually contain synthetic and high-environmental-impact substances to control varroa. These include pyretroids (e.g. fluvalinate, acrinathrin and flumethrin), organophosphates (e.g. coumaphos) and formamidine (e.g. amitraz). Normally, they are not approved for organic beekeeping. Most of these pesticides do not require refined knowledge of the mite's biology and are easy to apply. They are mainly used as sustained-release formulations, most often in the form of chemical-impregnated strips (Figures 13 and 14).

As lipophilic substances, they are mainly absorbed by the bees' wax, thus not directly jeopardizing the honey unless seriously misused. However, they are persistent and accumulate after repeated treatments. As a consequence, they may pollute bee products in levels exceeding permitted maximum residue limits. Other disadvantages of these acaricides are that they can be harmful to the bees and may create varroa resistance. Rotation of active ingredients is strongly recommended in order to prevent varroa resistance to acaricides.

Hard acaricide products registered for plants containing pyrethroids (e.g. fluvalinate, bifenthrin and etofenprox) or organophosphates (e.g. clorfenvinphos, chlorpyriphos, diazinon and pirimiphos-methyl) **must not be used** in beekeeping. Such use is illegal as it may harm

Figure 13: Application of hard acaricide in the form of chemical-impregnated strips.

Figure 14: Strips containing the acaricide are inserted in the hive, with the acaricide being released over a period of time inside the hive.

human health, bee health and the environment. It is extremely important to use on bees only products that are specifically registered for them and that do not put at risk the health of bees and humans.

3.6.2.2 Soft acaricides

"Soft" acaricides are low-environmental-impact acaricides. They are usually approved for organic beekeeping. They mainly include organic acids (e.g. formic acid, oxalic acid and lactic acid) and essential oils (e.g. thymol). Except for formic acid, they have proved to be effective only on phoretic varroa. This means that these products are more efficient in the absence of a capped brood. Formic acid has shown acaricide activity not only on phoretic varroa but also on varroa in the reproductive stage (e.g. within the sealed brood).

Organic compounds do not leave residual active ingredients dangerous to human health. Most of these substances are water-soluble and/or volatile and are natural honey ingredients. Therefore, contaminations that jeopardize the quality of honey or beeswax are unlikely. To date, no issues of resistance have been reported.

The miticide effects and toxicity for honeybees depend on different climatic and beekeeping conditions. These include, among others, active ingredient concentration, time of treatments, number of treatments, method of application (trickling, evaporating, spraying, sustained-release formulations, etc.), altitude, and type of hive. For this reason, the climatic conditions, within-hive conditions and the mode of application have to be carefully selected for optimal effect. Compared with "hard acaricides", the "therapeutic index" (the range between efficacy on varroa and toxicity for the bee) of soft acaricides is lower. However, the final acaricide efficacy is often more variable.
Beekeeper training is advisable.

3.6.3 INTEGRATED CONTROL

It is possible to increase the efficacy of chemical treatments (hard or soft acaricides, organic or conventional) by applying the chemical treatments in combination with biotechnical methods, such as the temporarily induced broodless stage. As discussed above, chemical treatments generally affect the mites that are outside the brood cells. When no brood is present in the hive, the varroa mites cannot reproduce. Therefore, all adult varroa mites in the hive will be attached to the bees, as there is no brood where mites can hide or replicate, and they will be affected by the chemical treatment. An example of an integrated control method is the application of oxalic acid during the queen caging process, after all the young bees have hatched and there is no longer brood in the hive.

3.6.4 SELECTIVE BREEDING OF VARROA - TOLERANT BEES

The breeding of varroa-tolerant bees is considered to be a possible long-term solution to the varroa problem. However, independent proof of "resistant lines" is lacking, and it is often difficult to make recommendations concerning the use of commercially marketed queens that are claimed to be varroa-resistant.

3.6.5 GOOD PRACTICES AND BIOSECURITY MEASURES TO PREVENT VARROA OR KEEP THE LEVEL OF VARROA IN THE HIVE UNDER CONTROL – GOLDEN RULES

The application of GBPs can help control the varroa population in the hives and limit the number of adult mites. Table 3 lists five recommendations beekeepers should adopt in their apiaries.

When using acaricides to control the number of varroa mites in the hive, there are recommendations to be observed (Table 4).

TABLE 3. Good beekeeping practices for prevention and control of varroosis

Recommendation	Advantage or reason for practice
Use hives with screened bottom boards.	Allows counting of natural mite fall.
Use nuclei and swarms originating from colonies with no clinical signs of viroses.	Low infection levels of viral diseases.
Have good knowledge of the symptoms of varroosis and viroses.	Enables early identification of high levels of varroa infestation and allows timely action to be taken.
Monitor varroa infestation levels at the beginning of the beekeeping season or before wintering.	Allows the number of varroa mites to be kept below the harmful threshold in each colony. Increases productivity, vitality and health of bees.
Select for varroa-sensitive hygiene behaviour.	Allows bees to control the level of varroa mites in the hive naturally, with less need for beekeeper intervention.

TABLE 4. General recommendations on acaricide use

Recommendation	Advantage or reason for practice
Use soft acaricides and biotechnical methods instead of hard acaricides.	Avoids post-treatment residues and is sustainable-beekeeping-oriented.
Monitor the efficacy of acaricide treatments, counting varroa mite fall after each treatment (taking into account varroa thresholds).	Promotes proper use of the most efficient product and verification of varroa resistance to any specific treatment.
Choose the most appropriate treatment, taking into account environmental conditions and conditions of the colony. For example, oxalic acid in absence of brood, and essential oils with minimum environmental temperatures needed for their proper evaporation.	Obtains the highest possible efficacy of the control method applied.
Treat simultaneously all colonies of the same apiary and, possibly, of the same area.	Reduces the transmission of varroa among different colonies.
Rotate veterinary medicines according to active ingredients. Different treatments with different modes of action should be combined.	Avoids varroa resistance and increases overall efficacy of the treatment.
Treat newly caught swarms (that have no brood) immediately.	Avoids bringing infested varroa bees into the apiary. All varroa mites are only on adult bees (as there is no brood) and treatment is therefore more efficient.
Do NOT perform chemical treatments during the nectar flow, on hives with honey supers.	Avoids residues in beehive products for human consumption.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4 Tropilaelapsosis

A parasitic disease of the brood

The tropilaelaps mite is a common natural parasite of the giant honeybees distributed throughout Asia. Although it has not spread all around the world as have other bee pathogens, it represent a potential pathogen for the western honeybee Apis mellifera *spp.*

4.1 INTRODUCTION

Tropilaelapsosis is caused by mites of the genus *Tropilaelaps*, external parasites of the honeybee that affect the bee larvae and pupae (Figure 15). The tropilaelaps mite shares some similarities with the varroa mite. It reproduces in the bee brood but, unlike the varroa mite, it cannot feed on adults because its buccal apparatus cannot penetrate the skin of adult honeybees. Therefore, it is not able to survive periods of brood interruption (natural absence of brood during the winter or artificially induced by queen caging).

Female adult tropilaelaps mites are reddish-brown in colour, about 1 mm long and 0.6 mm wide. Male tropilaelaps mites are a little smaller.

The adult mites enter the cells containing the bee larvae (of both worker bees and drones) to breed. Eggs hatch after about 12 hours, and the larvae feed on the haemolymph of bee larvae and pupae. The mites cause damage on the larvae, which results in malformation of adult bees and a high mortality rate of bee brood (up to 50 percent).

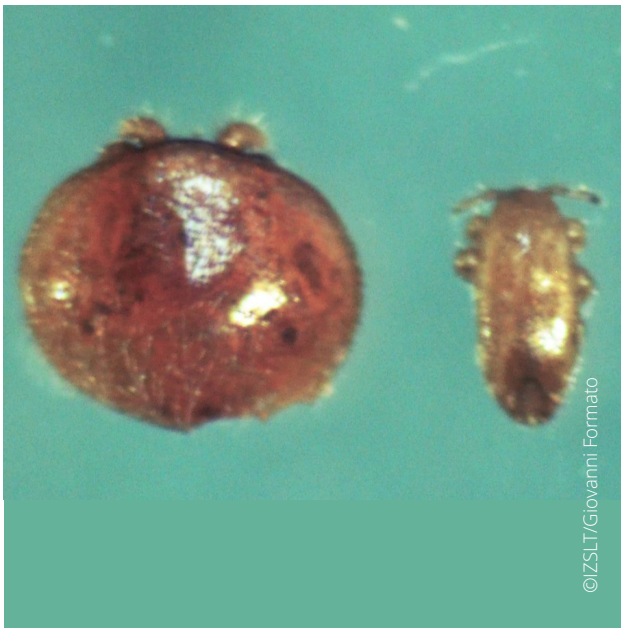
A typical characteristic of the adult tropilaelaps mite that can be observed during hive inspection is the rapid movement of the mites across the brood combs.

4.2 SYMPTOMS

In colonies heavily affected by tropilaelapsosis, the damage is very similar to varroosis, with high brood mortality, and weak adults with deformed wings and legs, and abdomens smaller than normal. Bees can also be found paralysed at the

Figure 15: ***Tropilaelaps*** **spp. adults on bee larvae and pupae.**

Figure 16: **Adults of** ***Varroa destructor*** **(left) and** ***Tropilaelaps*** **spp. (right).**



entrance of the hive. Other symptoms are an irregular brood pattern and perforated cappings, as the worker bees attempt to clean up sick or dead larvae. In heavily affected hives, up to 50 percent of the brood may die. In such cases, a bad smell of dead brood associated with the *Tropilaelaps* spp. infestation can be observed. At such levels of infestation, bees frequently swarm, contributing to the spread of the mite.

4.3 TRANSMISSION

The tropilaelaps mite can spread from hive to hive through drifting of adult honeybees carrying the mite on their bodies, looting of infested hives, and swarming. However, the spread of the parasites may occur also through common beekeeping practices, such as moving affected brood combs from one hive to another, migratory beekeeping, and the buying and selling of parasitized colonies/nucs.

4.4 DIAGNOSIS

A beekeeper could confuse tropilaelaps mites with varroa mites due to their similarity. The body of the female varroa mite is wider than it is long (measuring 1.1–1.2 mm in length and 1.5–1.6 mm in width) and it moves quite slowly, whereas the body of tropilaelaps mite is elongated and females measure about 1 mm in length and 0.6 mm in width (males are slightly smaller) (Figure 16). The tropilaelaps mite moves much faster than the varroa mite.
The diagnosis of this parasitic disease is visual, and is carried out through the observation of infected bee brood or by the observation of tropilaelaps mites that have fallen onto the bottom of the hive after a treatment with authorized acaricide products (see below).

4.4.1 ADULT HONEYBEE EXAMINATION

Adult tropilaelaps mites are usually only present in low numbers on adult bees in any given bee colony. Hence, it is usually a waste of time to try to find them on adult honeybees. Indeed, mites can be easily observed on the brood comb.

4.4.2 COMB BUMP TEST

The "comb bump" method is a rapid and simple proven method to detect tropilaelaps mites in the colony. First, remove a comb from the brood box containing capped brood. Shake all adult bees from the frame back into the colony. Then, firmly bump the frame over a white metal pan by hitting one end of the frame on the side of the pan, turning the frame, re-bumping the frame, and repeating the process once more for a total of four bumps. This process dislodges mites on the surface of the comb. The adult tropilaelaps mites can now easily be counted on the surface of the white pan.

4.4.3 BROOD EXAMINATION

When monitoring honeybee colonies for the presence of tropilaelaps mites, an examination of both drone and worker brood may provide an early indication of infestation. Mites can be observed inside capped bee brood by using a honey uncapping scratcher to pull up capped pupae. The mites are clearly visible. The younger mite stages are whitish and may be almost motionless while

feeding on their hosts' bodies, as their mouthparts and front legs are fixed to the cuticle of the bee host.

4.4.4 STICKY BOARD EXAMINATION

A precise diagnosis can be made using a sticky board on the bottom of the hive, covered with a mesh (2 mm) that prevents the bees from removing the dislodged mites. A mesh of 2 mm is large enough for mites to pass through. Make a sticky board with poster board, cardboard or other white, stiff paper coated with petroleum jelly (e.g. Vaseline) or another sticky substance, or use a sheet of sticky shelf paper. Cut the paper to fit the bottom board of the hive. Leave the bottom board under the hive for up to three days, collecting and examining the debris for mites.

For faster mite diagnosis, smoke each colony, adding 25 g of pipe tobacco to the smoker. Puff the bees between six and ten times, and close up the hive for 10–20 minutes. Pull out the sticky board after at least 10 minutes and count the mites.

The number of mites collected should be recorded and compared – different hives and different months of the year – to allow monitoring of the impact of the number of mites on the hive (e.g. losses in vitality or production).

4.5 PREVENTION AND CONTROL

Tropilaelaps spp. control can be carried out by creating a broodless stage in the hive for at least five days. This can be done by caging the queen, through artificial swarming (shaking the bees into a new hive with wax foundations), or by removing the combs containing brood.

The tropilaelaps mite is unable to feed on adult bees and cannot survive for more than two days without brood. This is a very efficient and harmless method.

The mite population can also be controlled by applying registered authorized acaricides to kill tropilaelaps mites. These products contain the same active ingredients that are able to kill varroa mite:

- soft acaricide – mainly organic acid, such as oxalic acid or formic acid; essential oils, such as thymol;
- hard acaricide – mainly pyretroids, such as fluvalinate; organophosphates, such as coumaphos.

Tropilaelapsosis prevention is possible with the adoption of GBPs and BMBs. Table 5 provides some good practices that beekeepers should adopt to prevent and/or control tropilaelapsosis.

TABLE 5. Good beekeeping practices for prevention and control of tropilaelapsosis

Recommendation	Advantage or reason for practice
Use hives with screened bottom boards.	Allows counting of natural mite fall.
Increase the efficacy of the acaricide treatments by combining them with bringing the colony into an artificial broodless state through brood removal, queen caging, or artificial swarming.	Increases the efficacy of the acaricide treatment.
Maintain the number of mites below the damage threshold by acting on signs of the disease and reduction in productivity of the colony.	Guarantees the health of the hives and limits production losses.
Adopt diagnostic tools for measuring infestation levels such as uncapping some worker or drone brood to look for mites.	Monitors the infestation levels.
Treat simultaneously all colonies of the apiary and in the same area.	Prevents the risk of re-infestation from untreated hives.
Have good knowledge of the symptoms and of the modes of transmission.	Allows optimal identification and control of the parasite.
Monitor efficacy of acaricide treatments.	Allows evaluation of the control measure adopted.
Rotate acaricides to avoid resistance.	Prevents the development of *Tropilaelaps* spp. resistance to acaricides.
Try to select and breed colonies that are tolerant and resistant.	Reduces the number of treatments needed.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5 Small hive beetle

An external parasite that may cause damage to combs and brood

The small hive beetle, or Aethina tumida, *can cause serious economic damage to the beekeeping sector, because it can destroy entire colonies as well as stored unextracted honeycombs in the honey house.*

5.1 INTRODUCTION

The small hive beetle (SHB), *Aethina tumida,* is a pest native to sub-Saharan Africa and is currently present in North America, Central America, the Caribbean, Brazil (South America), Australia, the Philippines (Asia) and Italy (Europe). Able to cause serious damage to the environment and the beekeeping economy, the SHB is considered an invasive alien species (IAS). Such species are animals and plants that are introduced accidentally or deliberately into a natural environment where they are not normally found, with serious negative consequences on their new environment. They represent a major threat to native plants and animals, causing extensive financial damage every year.

The SHB affects honeybee colonies and other pollinating insects of the Apoidea family, such as the bumblebee (genus: *Bombus*). The beetles are attracted by the smell of live bees and combs containing pollen and/or larvae. They enter beehives through the entrances or cracks in the hives. Once inside a hive, adult beetles spend the winter season feeding on pollen, honey and bee brood.

The SHB can adopt attack or defence behaviours when bees try to damage them. They assume the typical "turtle position" – retracting legs and head under their bodies. They can fool the bees through antennal contacts, which ask for food transfer by trophallaxis (a bee behaviour consisting of sharing collected nectar among worker bees through mouth-to-mouth transfer). This means that the bees will actually feed honey to the SHB. Bees can ignore the parasites, try to move them away from the hive, or try to confine them to small closed spaces in the nest with propolis (as a kind of prison).

Figure 17: Small hive beetle (*Aethina tumida)* adult.

Figure 18: Small hive beetle (*Aethina tumida*) pupa.

Adult beetles (Figure 17) mate in the colony, and the females oviposit hundreds of eggs in clutches, preferably in capped brood cells, which they perforate to deposit their eggs. Alternatively, they deposit their eggs in cracks, interstices and small gaps in the hive where it is difficult for bees to access and remove the eggs. One single female may oviposit 1 000–2 000 eggs in her lifetime (4–6 months). Many species of bees are able to identify and remove the more accessible beetle eggs from the hive. However, when eggs are laid in cracks in the hive where bees cannot reach, the bees cannot remove the eggs and the larvae can develop freely.

The SHB larvae emerge from the eggs after 1–6 days (most within 3 days), depending on the temperature and relative humidity conditions. They feed on the pollen, honey and bee brood they find in the hive. Larval development takes 1–4 weeks (usually about 2 weeks), depending on food availability and temperature. At the end of their development, when they are about 1 cm long, the mature larvae reach the "wandering phase" where they congregate at the bottom of the hive and leave the colony (usually through the hive entrance), dropping on the ground to pupate (Figure 18) in the soil surrounding the hive. Here, they penetrate into the soil down to 5–60 cm deep in order to begin the process of metamorphosis. Pupation in the ground may take 2–12 weeks (usually 3–4 weeks), depending on the temperature and the soil properties. Pupation is a stage characterized by high mortality because the SHB is very vulnerable in this phase. Soils that are too hard or too muddy greatly reduce the birth rate of adults. This is why the SHB prefers sandy soils for pupation.

Emerging adults leave the soil and fly to search for new host colonies, thereby completing their life cycle.

External factors condition the damage done by the parasite to the beehive:

- Environmental factors, especially temperature (development of any stage of SHB stops below 10 °C, and temperatures above 35 °C cause high mortality of all SHB life stages) and rainfall (the soil moisture should be above 5 percent for pupation of SHB).
- Genetics and bee behaviour (the species and race of the bees) affect the number of beetle cycles. For African honeybee subspecies, SHB is not a serious threat because they can defend themselves very well from the parasite by adopting different behavioural strategies, such as more efficient fighting and trapping of the beetles. However, European bees do not show the same aggressiveness towards SHB.

5.2 MORPHOLOGY OF THE SMALL HIVE BEETLE

5.2.1 MORPHOLOGY OF THE ADULT SMALL HIVE BEETLE

Adult SHBs are flat, oval-shaped (0.5–0.7 cm long and 0.3–0.45 cm wide) beetles ranging in colour with increasing age. The colours range from yellow-reddish, to brown, dark brown and eventually to black when they reach

sexual maturity. Their antennae are club-shaped, and their long legs enable them to move easily and rapidly inside the hives. The armour on their back and the characteristic "turtle position" (retracting head and legs under the body) they assume when attacked protect them from honeybee bites and stings. They are very good flyers, and can easily fly from one apiary to another.

5.2.2 MORPHOLOGY OF SMALL HIVE BEETLE LARVAE

The larvae of the SHB are cream-coloured and about 11 mm long at the end of their development stage. The larvae can be recognized by four rows of dorsal spikes along their backs, three pairs of legs, and two rear spines (Figure 19). These are three characteristics that distinguish SHB larvae from larvae of the wax moth (*Galleria mellonella*) (Figure 20).

5.2.3 MORPHOLOGY OF SMALL HIVE BEETLE EGGS

The eggs of the SHB are white-pearly with a shape quite similar to those of bees but smaller (about two-thirds of the size) (Figure 21). They are 1.4 mm long and 0.26 mm wide.

5.3 SYMPTOMS

The larvae of the SHB are responsible for the greater damage inside the hive. They dig tunnels among the cells of the combs (Figure 22) to feed on pollen, honey and bee brood. They can also move up to the honey super when it is not well populated. By defecating, they increase the humidity of the honey, causing its fermentation. In the worse cases, this may destroy an entire hive or induce the colony to swarm. Typically, SHB infestation leads to the death of weak colonies already affected by other diseases (such as varroa). When a massive infestation of the parasite occurs, the flight activity of bee colonies may decline, with a subsequent impact on productivity. The weakest colonies are at a greater risk of massive infestation, while stronger ones are able to ward off the larval forms of SHB by removing the beetle larvae and accessible eggs from the hive, or by containing the adults. When a massive infestation of larvae occurs, the combs become slimy and acquire a characteristic smell of rotten oranges. The fermented smell is a typical sign of infestation by SHB.

Larvae of the SHB may even cause considerable damage to stored, unextracted honeycombs, in warehouses (where combs and supers are stored for wintering) and honey houses, because bees are not present to protect the supers. In this condition of absence of bees, SHBs may replicate, reaching high levels of infestation.

Figure 19: Small hive beetle (*Aethina tumida*) larva, dorsal view (bottom) and ventral view (top). A larva of the small hive beetle can be distinguished from that of the wax moth larvae by its four rows of dorsal spikes along the back, three pairs of legs and two rear spines.

Figure 20: Wax moth (*Galleria mellonella*) larva on bottom tray of the hive.

Figure 21: The eggs of the small hive beetle are white-pearly with a shape similar to those of bees but smaller.

Figure 22: Damage caused by larvae of the small hive beetle (*Aethina tumida*) in honeycombs (top) and in the brood combs (centre). Damage caused by wax moth (*Galleria mellonella*) in a comb (bottom).

5.4 TRANSMISSION

The SHB can spread very rapidly, flying from apiary to apiary, but also through the trade of packaged bees, artificial swarms, queen bees, raw beeswax and beekeeping materials.

The adult SHB can survive several days without food. Thus, it can be easily introduced, even accidentally, in an SHB-free country through international trade. In this case, SHB is considered an IAS and may represent a strong threat not only to the economy of beekeeping, but even to the ecosystem.

5.5 DIAGNOSIS

5.5.1 ON-SITE DIAGNOSIS

Adult SHBs have a dark colour and can move very quickly. They are not easily identified among the bees within the hive, particularly when the number of adult beetles in the hive is low. Moreover, SHBs avoid light, hide in crevices or cavities of the hives, or fly away from combs when the beekeeper opens the hive for inspection. On-site diagnosis of adult SHBs can be improved both with the use of traps, or by using a divider made of wood, felt, cardboard or a similar material placed laterally between the hive wall and the external comb, to act as a refuge for SHB. The SHB present in the hive will hide between the hive wall and the inserted divider. Both the traps and the divider are tools that may facilitate detection of SHB.

5.5.2 LABORATORY METHODS FOR DIAGNOSIS

When it is possible to collect the whole body of the parasite, the best way to identify SHB is through morphological identification. This identification is not always simple (both for adults and larvae), especially because it frequently needs microscopical examination and the support of a specialized laboratory to perform a differential diagnosis (for example, differentiation between the larvae of SHB and the larvae of wax moth; or differentiation between adults of different species of nitidulid beetles).

Once the sample has been collected, it may be preserved in a closed jar or a hermetic container with 70 percent ethanol alcohol until it reaches the specialized laboratory for confirmation. The specimens collected in or near honeybee hives should be killed before submission (for example, by freezing them or putting them in ethanol).

In the event of doubt concerning the identification of the sample, or if the sample is broken, is incomplete or parts are missing, the morphological analysis is not able to provide a clear diagnosis. Instead, confirmatory testing can be done using molecular methods (e.g. polymerase chain reaction [PCR]) in specialized laboratories.

5.6 PREVENTION AND CONTROL

5.6.1 GOOD BEEKEEPING PRACTICES FOR SMALL HIVE BEETLE

The most important general rule to prevent damage by SHB is to keep only healthy and strong colonies in the apiary. Table 6 gives an overview of the most important GBPs that may be adopted to further limit the damage caused by SHB.

5.6.2 BIOSECURITY MEASURES TO PREVENT AND CONTROL SMALL HIVE BEETLE

Biosecurity measures for SHB are all of the integrated measures implemented by individual beekeepers to reduce the risk of introduction and spread of SHB. They can be adopted in areas both where the beetle is endemic and in areas where it is not present.

Carrying out periodical hive inspections to detect and eliminate the parasite (adults and larvae) can help to avoid the spread of SHB.

At the farm level, stocking combs (brood and honeycombs) in a cold chamber at a temperature below 10 °C and/or with a relative humidity below 34 percent kills SHB eggst and inhibits larval development.

In order to avoid providing a substrate for SHB reproduction, artificial nutrition should be given in small amounts so the bees can consume it in a short time.

The SHB can also cause serious damage in the honey house. Placing a fluorescent light source on the floor of the extraction room overnight attracts the SHB larvae. In this way, they may be collected and destroyed by putting them in alcohol or detergent solution. The pest control procedures listed in Table 7 should be adopted to limit the damage that SHB can cause in the honey house.

For areas where SHB is not present, it is important for beekeepers to have a good knowledge of the morphology of SHB eggs, larvae and adults, as well as hive inspection methods, and to periodically monitor the possible presence of SHB by sampling debris or honey.

Visual inspections of the hives can help regularly identify and eliminate SHB. The installation of a divider or a trap in the hives before the examination can help to locate SHB more easily during the inspection.

TABLE 6. Good beekeeping practices for prevention and control of small hive beetle

Recommendation	Advantage or reason for practice
Maintain hives in good condition, avoid using broken hives and hives with cracks.	Limits the number of places where SHB can lay eggs and where bees cannot remove them.
Avoid having beekeeping material abandoned in the apiary (in particular, built combs with honey and/or brood). Remove dead colonies (combs, food stores, boxes, etc.) as soon as possible and melt/destroy all organic materials that could attract SHB.	Abandoned colonies or beekeeping material (especially combs, food stores) are substrates for SHB in which to feed and multiply.
Balance colony strength among colonies.	Avoids having weak colonies within the apiary, in which SHB can replicate more easily.
Ensure that the bees cover all frames in the hive and that there are no empty spaces.	Reduces the areas in which SHB may “escape” or “hide” from attack by bees.
Prepare the hives properly for wintering.	Results in stronger colonies in the spring.
Reduce the size of the hive entrance, especially during colder months.	Enables the bees to better defend the hive entrance and reduce the possibility of SHB entering the hive.
Insert a divider board in the beehive, between the hive wall and the last frame.	Makes identification of SHB in the hive easier.
Reduce the volume for the hive nest and remove the empty frames.	Ensures that all combs/areas of the beehive are well populated and covered with bees, and they are therefore able to fight the parasites.
Sample on a regular basis hive debris from the bottom board for diagnosis of honeybee diseases.	May allow a pre-clinical identification of the diseases.
If in doubt about the presence of SHB, seek technical support of a veterinarian, technician or beekeeping expert.	It is extremely important to ask for the help of experts to correctly diagnose a disease, especially in case of doubt.
Attend training programmes on beekeeping and honeybee diseases to learn how to identify, prevent and control the disease.	It is fundamental that the beekeeper be able to recognize correctly honeybee diseases.
Avoid the transport of unauthorized live material at risk (hives, queens, nucleus, etc.) from areas where SHB is present.	Prevents SHB from gaining access to areas where it is not present.

TABLE 7. Biosecurity measures to be adopted in the honey house to limit damage caused by the small hive beetle

Recommendation	Advantage or reason for practice
Keep working rooms and equipment clean, tidy and in order. Apply general hygiene practices such as regular cleaning of equipment.	Reduces opportunities for the SHB to reproduce and develop, and can help limit damage caused by SHB.
Combs and honey frames should be extracted as soon as possible.	Prevents SHB from having time to multiply on them.
Extracted honey should be stored in airtight, sealed containers (drums, hobbocks) and access by bees or vermin to the stored honey should be avoided.	Prevents SHB from accessing the honey.


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6 Nosemosis

A fungal disease of adult honeybees

Nosemosis occurs worldwide and is characterized by diarrhoea in adult honeybees. It seems to have a strong impact on the beekeeping sector, especially when associated with other pathogens (varroosis, virosis, amoebiasis, etc.) or environmental pollutants (pesticides).

6.1 INTRODUCTION

Nosemosis is a disease caused by two different species of the fungus *Nosema* spp.: *N. apis* and *N. ceranae*. Both affect adult honeybees but their symptoms and prevalence differ depending on the area. The spores of *N. apis* and *N. ceranae* are hardly morphologically distinguishable (Figure 23), and represent the resistant and propagation form of the disease. Spores can remain infectious for from a few days to up to five years at low temperatures. Heat, as well as solar ultraviolet radiation, can kill them in a few hours.

The following factors influence nosemosis:

- wet and cold spells increase the chances of infection among the bees of the same hive because they force the bees indoors;
- scarcity of honey and pollen flows;
- seasonal patterns can also affect the spread of infection. During long, cold winters and cold, rainy springs, the bees may not find nectar and pollen;
- frequent hive inspections during adverse weather conditions (e.g. winter season, windy or rainy weather) can trigger the onset of the disease as well as its propagation due to the induced stress;
- the presence of other diseases (such as amoebiasis, varroosis or viruses) exacerbates the symptoms of nosemosis.

6.2 SYMPTOMS

6.2.1 SYMPTOMS OF NOSEMOSIS CAUSED BY *NOSEMA APIS*

Nosema apis is responsible for the "classic" known form of the disease, which is widespread especially in cold and

wet areas. It appears more easily during spring and in mismanaged hives during winter. It occurs mainly with a decrease in the colony population. The disease never affects the larval stages and seldom the queen.

Nosema apis spores, found in the faeces of the bees, are directly or indirectly ingested by adult honeybees and develop in the intestines of the bees, affecting their digestive functions (bees are unable to absorb nutrients from their food). Nursing bees also become unable to produce royal jelly. The spores are expelled with faeces and can be swallowed by other bees, which become infected. Eventually, the colony succumbs due to depopulation as adult bees die and no new bees are born (brood is not fed).

After contact by bees with N. apis, the following infection symptoms will appear:

- intestinal disorders, such as diarrhoea (Figure 24), which can be observed on the running board and hive entrance, and the honeycombs, which will be smeared with diarrhoeal faeces (Figure 25);
- bees become unable to produce royal jelly, and hence, unable to feed the brood;
- foraging bees reduce their activity until it stops completely;
- in the rare cases in which the queen is sick, egg-laying greatly decreases;
- some bees are no longer able to fly, they walk with their wings spread out in "K" form, paralyzed, while other bees gather in small groups;
- dead bees with swollen abdomen and legs retracted below the chest can be found on the bottom of the hive. First, there is a slow depopulation, and work then decreases while the state of restlessness of the colony increases.

6.2.2 SYMPTOMS OF NOSEMOSIS CAUSED BY *NOSEMA CERANAE*

Nosema ceranae is a new species of fungus. It was isolated for the first time in 1996 by Fries on *Apis cerana*, a bee species widespread in Southeast Asia. In 2006, it was discovered for the first time by Higes in the European honeybee (*Apis mellifera*). *Nosema ceranae* has spread across vast areas of Europe, replacing the indigenous form of *N. apis* on *Apis mellifera*, and resulting in quite different clinical signs from the diarrhoea typically associated with nosemosis caused by *N. apis*.

The disease can occur throughout the year. Typical is the absence of diarrhoea. It seems that foraging bees die away from the hive, causing a progressive depopulation of the colonies (without noticing the presence of dead bees) until the total loss of the family.

Figure 23: The microscopic spores of *N. ceranae* are hardly distinguishable from those of *N. apis* at 40x magnification.

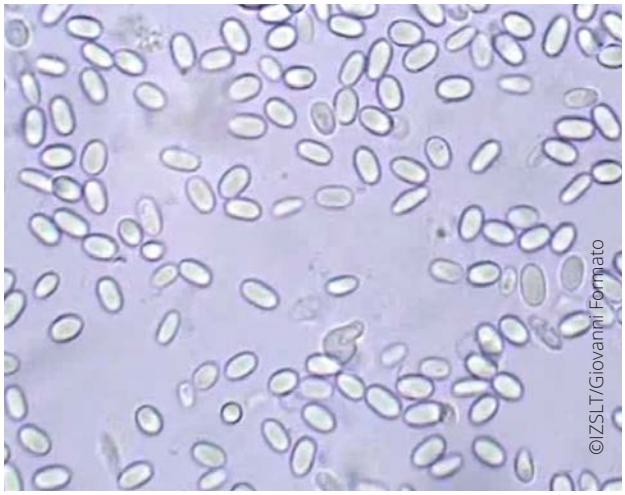
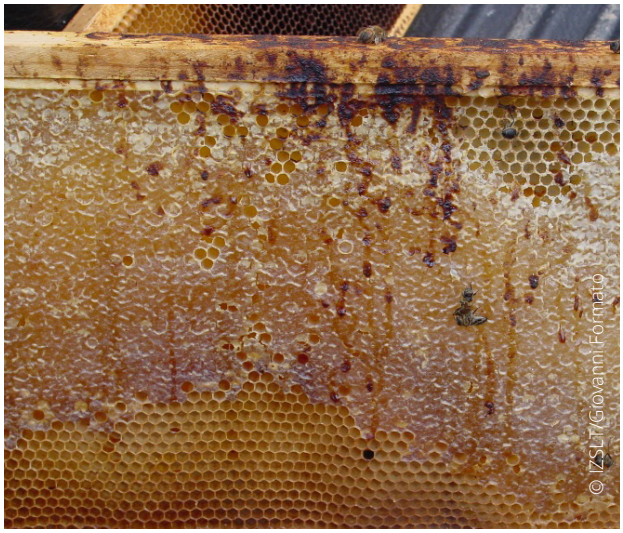


Figure 24: Diarrhoeal faeces on the hive entrance.

Figure 25: Honeycomb smeared with diarrhoeal faeces.



6.3 TRANSMISSION

Nosema apis and *Nosema ceranae* can be transmitted in the same way.

The ingestion of the fungus by the bees occurs directly, through ingestion of faeces, or indirectly, through ingestion of contaminated honey, water or food.

The spores of *N. ceranae* are very resistant in the environment and can withstand very cold or very hot temperatures. This means that the re-infection of the colonies and the recurrence of the disease after a long time is very possible.

The disease can be spread from hive to hive and apiary to apiary by the bees and by the beekeeper through:

- drifting of infected workers and drones;
- drone displacement;
- robbing of infected colonies;
- interchanging infected honeycombs from one hive to another;
- feeding of bees with contaminated honey;
- using infected tools or equipment.

6.4 DIAGNOSIS

A field test consists of examining the colour of the terminal portion of the digestive system of some bees (beekeepers may extract the gut by pulling off the stinger with tweezers or with their nails). In healthy bees, it has a reddish colour, while in sick bees it is milky white (Figures 26 and 27). However, this sign is seen only when the disease has already reached a certain severity. Only a laboratory test can make an early diagnosis – by searching with a microscope for the spores at the intestinal level or directly on faeces.

The microscopic spores of *N. ceranae* are hardly morphologically distinguishable from those of *N. apis* (Figure 23). Thus, it is possible to make a diagnosis only through PCR, which allows the sequencing of a very specific and characteristic part of the *N. ceranae* genome on the spores. The cost and availability of this exam depend on each country and laboratory.

6.5 PREVENTION AND CONTROL

The adoption of GBPs and BMBs can reduce the risk of *Nosema* spp. occurrence.

6.5.1 GOOD BEEKEEPING PRACTICES TO REDUCE THE RISK OF NOSEMOSIS OUTBREAK

Table 8 gives an overview of GBPs to adopt in order to maintain healthy colonies and prevent infection by *Nosema* spp.

Figure 26: Positive field test: milky white gut of a bee affected by nosemosis.

Figure 27: Negative field test: the gut of a healthy bee is reddish in colour.

6.5.2 BIOSECURITY MEASURES IN BEEKEEPING TO REDUCE THE RISK OF NOSEMOSIS OUTBREAK

The beekeeper can adopt the following BMBs to reduce the risk of introduction and spread of nosemosis:

- Select queen breeders with stocks that are free from *Nosema* spp.
- Select and breed honeybees that are resistant to *Nosema* spp., if possible.
- In early autumn or spring, take samples of forager honeybees for analysis in a specialized laboratory and the early diagnose of nosemosis (PCR and/or microscopic methods).
- When the infection level of adult bees is too high (>100.000 spores/bee), treat the colony against *Nosema* spp. with available and registered or permitted products in your country.

However, when *N. apis* does occur, the prognosis is frequently serious because its onset is usually unnoticed and symptoms occur only at an advanced stage. Generally, the affected colonies do not recover spontaneously; therefore, the beekeeper's intervention becomes necessary.

If the disease is well developed, particularly in weak families, the infected combs should be destroyed, and the hive should be sterilized or destroyed. The honey can be used for human consumption. To destroy a hive and avoid further contamination, a hole of at least 50 cm should be dug in the ground, the hive and combs should be burned, and the hole should be duly covered. Infected bees should be killed by asphyxia with sulphur dioxide, or treated with authorized products and moved to a new/disinfected hive with new/disinfected combs and a new foundation.

TABLE 8. Good beekeeping practices for prevention and control of nosemosis

Recommendation	Advantage or reason for practice
Select the right apiary location (non-humid, not exposed to cold winds), orient the hives preferably towards the sun, and prefer slightly ventilated areas.	Reduces the probability of fungi multiplication.
Prepare the hives for wintering using a dummy board to adjust the volume of the hive to the size of the colony. Unpopulated frames should be removed. In cold climates, keep the hives warm during wintering until late spring.	Prevents thermal stress on the bees during the cold season.
Ensure enough food is available in the hive during the winter. If necessary, provide good-quality food.	Prevents nutritional stress on the bees during the cold season.
Before wintering of the hives, apply appropriate treatments against varroa.	Guarantees the efficiency of the immune system of the bees.
Ensure enough protein-rich food is available to the bees in late summer and autumn. Locate apiaries in a location where pollen sources are available to the colony in the late summer and autumn. When possible, plant plants that provide pollen during late summer or autumn close to the apiary. If not possible, feed bees with protein-rich supplements.	Prevents nutritional stress on the bees.
Use an adequate number of honeycombs in relation to the colony population.	Prevents thermal stress on the bees.
Disturb the bees as little as possible during winter. Limit inspections to sunny days only, and the warmest hours of the day.	Avoids thermal stress on the bees.
Do not reuse combs (neither if empty or with stores of honey and/or pollen) originating from depopulated (few workers with the queen) or collapsed hives.	Reduces the probability of contamination among the bees. Depopulation of a colony is often a sign that something is wrong with the colony. In addition, small colonies are more susceptible to diseases.
Prevent the pollution of artificial water sources by faeces and by drowned or dead bees.	Reduces the probability of contamination among the bees.
Buy queens from queen breeders with stocks that are free from *Nosema* spp. Whenever possible, select and breed honeybees that are resistant to *Nosema* spp.	Bees showing genetic resistance to *Nosema* spp. are less likely to contract nosemosis.
Remove and burn combs with signs of diarrhoeal faeces.	Reduces the infection levels of diseases in the beehives.
If possible, send samples of forager honeybees (or hive debris) in early autumn or spring to a laboratory for analysis (European foulbrood, American foulbrood, nosemosis).	Early detection of diseases can avoid contamination of other bees.
Adopt a proper pathogen (e.g. *V. destructor*) control of the bee colony. Regularly monitor the level of varroa infestation (e.g. using icing sugar method).	Disease-free bees have a stronger immune system and can better fight diseases.
Strengthen and stimulate the colonies in autumn and spring with the administration of stimulant integrators or feed supplements.	Nutritional stress due to the lack of adequate food can compromise the immune system of the bees and hence make them more prone to disease.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7 Amoebiasis

A parasitic disease of adult honeybees

Amoebiasis is usually present in the temperate regions of both hemispheres, but it seems to be absent in tropical and subtropical zones. It affects a very low proportion of colonies and is rarely identified.

7.1 INTRODUCTION

Amoebiasis is caused by a protozoan called *Malpighamoeba mellificae* and affects adult honeybees. The symptoms of Amoebiasis are honeybees with a swollen abdomen and diarrhoea, very similar to the symptoms of nosemosis. In fact, amoebiasis and nosemosis are frequently observed together as a mixed infection.

Bees become infected by ingesting honey or pollen contaminated by the faeces of infected bees. The infection causes an inflammation of the intestines of the adult bees, which progressively become unable to work properly. The beekeeper can observe diarrhoeal faeces at the hive entrance and on the front of the hive, and honeybees that are unable to fly and with quivering and trembling wings.

The disease occurs mainly in spring and then disappears after a few months. In severe cases, amoebiasis kills the adult bees and, consequently, there are not enough bees in the hive to take care of the brood, which ultimately dies.

7.2 SYMPTOMS

Symptoms of amoebiasis (Figure 28) are similar to those of nosemosis (caused by *N. apis*):

- swollen abdomen;
- inability to fly;
- quivering wings;
- diarrhoeal faeces smeared on honeycombs and also located at the entrance and the front of the hive.

Amoebiasis and nosemosis are frequently observed together as a mixed infection. Diagnosis is confirmed by laboratory identification of microscopic cysts in the tubules and faeces of the bees.

7.3 TRANSMISSION

Inside the hive, cleaning worker bees become infected when removing excrement from the hives. They pass on the infection, and the disease is transmitted through ingestion of contaminated honey and pollen.

Amoebiasis is transmitted from one hive to another by drifting bees (scouting and harvesting bees returning to the wrong hive), robbing and watering stations. The beekeeper can also transmit the disease between colonies by moving infected combs from an infected hive to a healthy one, and by using infected beekeeping equipment and tools.

Long winters, cold weather, and cool and wet summer and autumn months help spread the disease. However, amoebiasis affects a very low proportion of colonies and is rarely identified. Colonies are usually able to restore their health naturally after infection when weather conditions improve.

7.4 DIAGNOSIS

It is possible to make a diagnosis in the field through the observation of the following symptoms:

- bees with swollen abdomen and diarrhoea (Figure 29);
- diarroeal faeces on honeycombs and at hive entrance (Figure 28);
- bees with trembling wings, unable to fly.

As the symptoms of amoebiasis are very similar to those of nosemosis, it is only through examination of bees in the laboratory that a final diagnosis is possible.

7.5 PREVENTION AND CONTROL

Control measures for amoebiasis are similar to those for nosemosis. The adoption of GBPs can help in preventing and controlling amoebiasis (Table 9).

Figure 28: Hive entrance spotted with diarrhoeal faeces.

Figure 29: Bee with diarrhoea.

TABLE 9. Good beekeeping practices for prevention and control of amoebiasis

Recommendation	Advantage or reason for practice
Clean and disinfect regularly beekeeping equipment and hive tools (e.g. using bleach), ideally after each use.	Reduces the bacteria population in the hive.
Ensure that the hives are located in a good place: sunny and dry places; avoid humidity and wind.	Reduces thermal stress on the bees.
Strengthen and stimulate the colonies in autumn and spring with the administration of fortified feeding with vegetal substances or vitamin supplements specific for bees.	Reduces nutritional stress on the bees.
Control other pathogens (mainly varroa) to ensure a good health status of the colony.	Other pathogens, especially varroa, cause immunosuppression in bees.
Remove combs from colonies that have signs of the disease (diarrhoea). Melt the beeswax.	Reduces the transmission of the disease to healthy bees.
Administer supplements to infected colonies.	Reduces nutritional stress on the bees.
Do not feed bees with honey or pollen taken from unhealthy colonies.	Reduces transmission of the disease to healthy bees.
Do not exchange any combs between diseased and healthy colonies.	Reduces transmission of the disease to healthy bees.


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8 American foulbrood

The worst bacterial disease of the brood

American foulbrood is considered the most widespread and destructive infectious honeybee disease, and it can cause serious economic losses to beekeeping.
The term "American" does not refer to the disease originating from the United States of America, but rather to the fact that it was first identified and studied there.

8.1 INTRODUCTION

American foulbrood (AFB) is caused by a spore-forming bacterium, *Paenibacillus larvae* (*P. larvae*). Honeybee larvae are the main target of *P. larvae* in their first 24 hours of life. The infection starts with the oral uptake of the *P. larvae* by a honeybee larvae through food. The spores become active in the digestive tract of young larvae, where they begin to proliferate massively. After seven days of infection, the infected larvae die and the *P. larvae* returns to spore form as it is unable to find suitable conditions for development.

The spores are the resistant form of this germ and can withstand a temperature of 100 °C for several minutes. In a suitable environment (e.g. in the intestine of the larvae), a single spore is able to produce 250 million new bacilli after only 24 hours. The spores can remain viable for more than 30 years in an infected hive, being able to contaminate new colonies. The long survival rate and the contagiousness of AFB are serious problems for the control of the disease. Thorough and effective sanitation measures are needed to eliminate the disease in an affected apiary.

8.2 SYMPTOMS

The onset of symptoms depends on the number of spores. There must be at least 50 million spores for the disease to appear in a bee colony. A honeybee larva that dies of AFB contains about 3 billion spores. This explains why it is so difficult to eliminate and control the spread of AFB.

In the capped cell, the infected larva loses it pearly white colour, becoming yellowish at first, and then

Figure 30: Combs affected by American foulbrood appear darker and are irregularly capped.

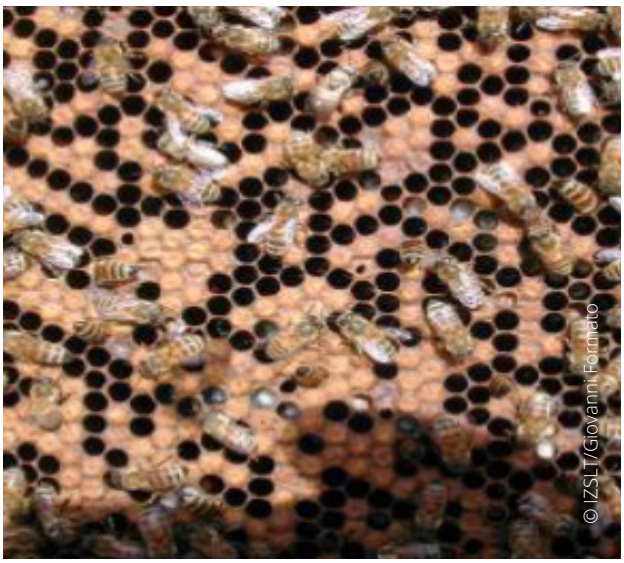

Figure 31: Cell capping are darker and sunken in cases of American foulbrood. Dead larvae appear sticky and ropy after removing a wooden stick from the cell containing a dead infected larva (toothpick test).

dark-brown, decomposing in a ropy mass. The mass then dries down to a highly infective and hard black scale, which firmly attaches to the hive cell wall. These scales, as well as the dead larvae, contain a large number of spores.

The beekeeper can observe darkened, irregularly capped combs, affected by high brood mortality (Figure 30). Some capped cells are darker, sunken or hollow in the centre (Figure 31).

A typical indicator of AFB infestation is the fetid and sour smell of the infected combs. The "toothpick test" (see point 8.4 below) can be performed to distinguish between AFB and European foulbrood (EFB). When the toothpick is pulled out of the suspect cell, it will show a trickling filament if there is AFB infection (Figure 31). In the case of EFB infection, the toothpick will not show the filament.

8.3 TRANSMISSION

Inside the hive, the contamination of larvae occurs through the food containing spores administered by the nurse bees. Worker bees clean the cells occupied by the dead brood contaminated with AFB. Thus, they are exposed and become spore carriers.

American foulbrood is spread from one hive to another by honeybees through drifting, robbing and swarming. It is also spread by the beekeeper through the transfer of infected hive material (honey, pollen, and, in particular, combs), the feeding of bees with infected honey, the use of infected beekeeping equipment, or the trade or migration of infected honeybee colonies.

American foulbrood is very contagious, and the disease can be transmitted through spore-contaminated material for a very long time. Therefore, to reduce the risk of transmission, beekeepers should notify the competent veterinary authorities as soon as the above symptoms are observed. Restrictions on colony movement and transportation of beekeeping equipment and hive products should be strictly observed in the case of presence of the disease.

8.4 DIAGNOSIS

It is possible to make a diagnosis in the field through careful examination of the brood. The first signs of AFB are:

- a scattered brood pattern;
- darker cell cappings;
- inward-sunken and sometimes punctured cell cappings;
- liquefied larvae with viscous consistency;
- a fetid and sour smell of the infected comb (and when opening the hive in cases of heavy infection);

- a black hard scale firmly attached to the lower cell wall of brood cells (remains of the dried-up decomposed larvae) is the sign of an earlier infection.

Symptoms are usually quite clear, but to confirm the suspicion of AFB outbreak, the beekeeper can perform the "toothpick test", also called "matchstick" or "ropiness test". A toothpick or fine twig is inserted into a suspect cell. In the case of a fresh infestation, a fine thread can be drawn from the cell when pulling out the toothpick (Figure 31). Where available, an AFB-diagnostic test kit for on-site diagnosis can be used (Figure 32).

When any of the above mentioned signs are present, a suspicious AFB presence must be considered. In order to confirm the presence of the disease, a sample of the affected brood comb can be sent to a laboratory that is specialized in the diagnosis of honeybee diseases.

8.5 PREVENTION AND CONTROL

The beekeeper can adopt GBPs and AFB-specific measures (BMBs) to prevent and control AFB infection. Table 10 provides a list of practices that can help to prevent AFB outbreaks.

In order to reduce the spread of the infection, it is important to take appropriate actions as soon as possible when a case of AFB is detected. Control of AFB may be carried out mainly through three different methods: (i) the shook swarm method; (ii) antibiotic treatment; and (iii) incineration of the entire colony.

8.5.1 SHOOK SWARM

The shook swarm method consists of shaking the bees from the infected combs (brood and store combs) into a clean hive with new frames and new foundation. The combs of the brood box are the ones that carry the most spores because of the dead larvae. Replacing all combs from the brood box reduces the infection level by removing the spores. Old, infected combs should be destroyed by incineration. The shook swarm method must be the preferred method for sustainable beekeeping. It gives better results in the case of strong colonies and during the honey flow, as colonies will need to build new combs starting from wax foundations..

Figure 32: American foulbrood kit for field diagnosis (test positive).

8.5.2 ANTIBIOTIC TREATMENT

In many countries, the use of antibiotics in beekeeping is not allowed. However, in some countries, there are antibiotics registered for use on honeybees against AFB. These registered medicines against AFB do not guarantee a total disinfection of the hive from the *P. larvae*, and an antibiotic treatment is able to destroy only the vegetative forms of *P. larvae* but not the spores. This increases the risk of relapses and the asymptomatic spread of the disease. In addition, the inappropriate use of antibiotics encourages the development of drug resistance and the risk of the presence of residues in the hive products. Antibiotic treatment may be effective, especially if associated with the application of the shook swarm technique. Replacing all combs will reduce the risk of antibiotic residue contamination in the hive. The persistence of antibiotic residues may vary according to the specific antibiotic used (for example, the fastest decomposition in honey is oxytetracycline, followed by streptomycin and sulphathiazole).

Antibiotic treatment in countries where antibiotics are registered may be the choice for conventional beekeeping in the case of: early stages of the disease; strong colonies; and a high prevalence of the disease in the apiary. Beekeepers should remember that antibiotics cannot protect against poor beekeeping practices.

Beekeepers should always observe the following golden rules when using antibiotics.

- Be aware of and comply with regulations on the use of veterinary medicines for honeybees. Use antimicrobials only in accordance with regulatory requirements and other veterinary and public health guidance.
- Use only registered veterinary medicines for honeybees.
- Keep detailed records of the origin and use of all medicines, including batch numbers, dates of administration, doses, treated hives and withdrawal times. Treated hives or apiaries should be clearly identified.
- Respect the required storage conditions for veterinary medicines and feeds.
- Ensure that all treatments or procedures are carried out properly, as described in the instructions (e.g. respecting the dose and method of application).
- Observe the withdrawal time of veterinary products and ensure that products from treated hives are not used for human consumption until the withdrawal periods have elapsed.

8.5.3 DESTRUCTION OF THE HIVES BY INCINERATION

The incineration (Figure 33) of combs and honeybees should occur after killing the bees (for example, by asphyxia with sulphur dioxide, or by spraying a soap-water solution [1 part of dishwashing liquid to 4 parts of water], or by pouring petrol on the frames with bees). Incineration may be chosen in the case of: weak colonies; severe AFB clinical infection; when the disease appears outside of the honey flow (colony recovery is more difficult because it is impossible to build new honeycombs and there is a high probability of relapse); and a very low prevalence of the disease in the apiary or in the geographical area. If the hive is in good condition, it may be disinfected using sodium hypochlorite and finally torching, after having first scraped off wax and propolis. If the hive is old or already damaged, it should be incinerated. To destroy a hive by incineration and avoid further contamination, a hole at least 50 cm deep should be dug in the ground. Then the combs (and the hive, if needed) should be burned, and the hole should then be covered with soil.

Figure 33: Destruction by fire of infected combs.

8.5.4 DISINFECTION OF BEEKEEPING MATERIAL

It is very important to always carefully disinfect all beekeeping equipment (boxes, boards, frames, queen excluders, feeders, etc.) and objects used for the manipulation of infected hives (hive tools, gloves, suit, etc.). Possible methods to use vary according to the substrate to disinfect. Wooden equipment can be scorched with fire and then sprayed with bleach or caustic soda. Objects can be dipped in hot paraffin or microcrystalline wax, or gamma-rayed. In order to control the disease, clean thoroughly with detergent the honey-house extraction tools/facilities (decappers, centrifuge, sieves, pumps, spins, honey extractor, etc.) and the hive-product-packaging materials (jars, tanks, barrels, etc.).

TABLE 10. Good beekeeping practices and biosecurity measures in beekeeping for prevention and control of American foulbrood

Good beekeeping practices (GBPs) and biosecurity measures in beekeeping (BMBs)	Advantage or reason for practice
Keep only strong colonies in the apiary (GBP).	Small or weak colonies are more vulnerable and susceptible to diseases.
Avoid feeding bees with honey or pollen of unknown origin (GBP).	Honey and pollen can contain spores of AFB. These spores can be present in affected hives (and honey) that do not show the symptoms of AFB yet. This is because the disease is at its early stages, or because the bacterial level is low. Through the feeding of honey containing spores of AFB to colonies, the spores can be transferred to healthy colonies.
Replace the queen at least once every two years (GBP).	Well-fertilized young queens are more productive and can guarantee stronger colonies, with many workers involved in the feeding of the brood and in the removal of the diseased brood and dead larvae.
Renew at least 30% of the old (dark) brood combs every year (GBP).	This is an effective preventive measure not only against AFB, but also for many other bee diseases, as new combs contain less bacteria compared with older ones.
Monitor the subclinical presence (e.g. before symptoms are visible) of AFB in the colonies by sending samples (e.g. adult bees, honey, icing sugar and/or hive debris) to a laboratory for analysis (BMB).	Monitoring these matrices for the presence of *P. larvae* is a good practice for diagnosing the disease in the colonies before clinical signs appear. This preventive action can be very easily performed during the inactive season through collaboration with a specialized laboratory. Moreover, in the case of sampling of hive debris, opening the hive is not needed, so avoiding cooling down the colony. It allows the beekeeper to know whether any colony contains AFB spores. In addition, where spores are present, it allows the beekeeper to know the severity of the infection, and thus, accordingly adopt specific measures (e.g. replacing older frames) to avoid the subsequent appearance of the clinical form of the disease a few months later.
Regularly clean and maintain hives to prevent robbing. Any openings in the beehive expose a colony to the risk of robbing (GBP).	Robbing is one of the ways through which AFB spores are transmitted from one hive to another.
Melt down the combs and process wax safely in order to destroy spores (GBP).	Avoids transmission of the infectious disease.

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9 European foulbrood

A bacterial disease of the brood

The propagation of European foulbrood is not limited to Europe; it can be found almost all over the world. Besides Apis mellifera*, it can also infect* Apis cerana *and* Apis dorsata.

9.1 INTRODUCTION

European foulbrood (EFB) is caused by the bacterium *Melissococcus plutonius* (*M. plutonius*), often associated with other bacteria. These include *Enterococcus faecalis*, *Achromobacter eurydice*, *Paenibacillus alvei* and *Bacillus laterosporus*. Depending on the species of bacteria associated with the bacterium *Melissococcus plutonius*, EFB can have different symptoms (presence/absence of an unpleasant acid smell, consistency of decomposed larvae, etc.).

EFB may be detected by observing uncapped brood. Infected larvae usually die when they are 4–5 days old, but may survive until the pupal stage. At this stage, the larvae faeces contain many viable cells of the pathogen. *Melissococcus plutonius* can survive and remain contagious within a seemingly healthy honeybee colony, without presenting classic EFB symptoms (subclinical presence of EFB). *Melissococcus plutonius* is quite resistant to adverse environmental conditions, and the disease can be transmitted through pollen, honey and adult bees.

Outbreaks of EFB occur more frequently under stressful conditions (food shortage, small colonies, lack of queen, etc.). Genetic predisposition is another factor influencing the hygienic behaviour of the bees. The genetic resistance to EFB of some bee species can fight the infection without causing serious damage, especially if environmental conditions are favourable. Often, diseased colonies can recover spontaneously if properly fed and/or if environmental conditions become more favourable for the bees (increase in number of flowers, sunny days, warmer temperatures, etc.), or when the affected colonies are moved to areas free of EFB.

However, although chances of recovery from EFB are higher than those from AFB, in some areas of the world EFB is more dangerous and can even seriously damage strong bee colonies.

9.2 SYMPTOMS

The bacterium develops inside the hive at the brood level. The disease is transmitted by nurse bees that are infected while attempting to clean up the cells containing dead larvae. The infected nurse bees then transmit the bacteria to the brood during feeding.

After infection, the larvae die within a few days (regardless of whether the larvae are working bees, drones or queens). *Melissococcus plutonius* kills the larvae before the cells are capped. For this reason, in affected combs, an irregular brood pattern and dead larvae in uncapped cells can be observed (Figure 34). This is one of the features that differentiates EFB from AFB, where larvae die after the capping of cells.

Another important feature useful in recognizing this disease is the changed position of the dead larvae in the cells. Healthy larvae are horizontally positioned on one side in a "C" shape, sticking to the back of the cells. Infected larvae are yellowish, contorted or curled upwards, and often lose their segmentation. The infected larvae also lose their pearly white colour and become first opaque, then yellowish and finally yellowish-brown (Figure 34). After death, the larvae become darker and decompose, turning into a slimy, soft, brown mass. But, unlike larvae infested with AFB, the mass does not appear ropey. This mass, like that of AFB, dries up, forming a dark rusty flake. However, in the case of EFB, the flake is easily removable from the cell.

Figure 34: Larvae affected by European foulbrood die in open cells before capping.

The brood appears scattered, with cells containing yellow dead larvae. Depending on the bacteria present, the dead larvae may give off smells of different intensity and typology.

Melissococcus plutonius creates a sour smell, with flabby but intact larvae. However, if the infection is associated with *Bacillus alvei*, the brood gives off a putrid smell. Larvae may appear molten, but never ropey (unlike those with AFB). There are also intermediate forms in which the combs do not give off any smell.

Some symptoms of EFB resemble those of AFB. Therefore, attention should be paid when making an on-site diagnosis. An on-site EFB test kit could be used to confirm the clinical outbreak of EFB in symptomatic hives.

Table 11 gives an overview of the main symptoms of EFB and AFB.

The disease can occur throughout the year, but it is more common in spring when there is an imbalance between the increasing number of larvae and the amount of nurse bees that survived wintering. This imbalance may trigger EFB because of the larvae's nutrition stress. For this reason, EFB is more common in cold and rainy spring seasons, when there may be food shortages, particularly in terms of low protein for the brood owing to lack of pollen.

The health status of the colony plays an important role in the development of the disease. Weak colonies or colonies that are stressed for any reason (food shortage, migratory beekeeping, pesticides, etc.), as well as genetically more sensitive colonies, are especially prone to this disease. Healthy and strong colonies will be able to recover from the disease by themselves if the season guarantees adequate food sources (e.g. pollen and nectar). When the disease is not well developed, especially if the queen is replaced and it is in a favourable time of the year (with the presence of abundant flowers providing nectar and pollen), bees may be able to clean up all of the affected cells and the disease can disappear spontaneously. Thus, the infection is prevented from spreading to the rest of the apiary.

TABLE 11. Main differences between European foulbrood and American foulbrood

European foulbrood	American foulbrood
Dead larvae in uncapped cell	Dead larvae in capped cell
Sour smell	Smell of fish gelatin
Absence of blackening of honeycombs	Dark combs, inward sunken and sometimes perforated cappings
Non-ropey dead larvae	Brown, ropey dead larvae
Removable flakes in cells	Non-removable flakes in cells

9.3 TRANSMISSION

The disease can be spread by the bees and by the beekeeper from hive to hive or apiary to apiary. Bees can transmit the disease during robbing, drifting or swarming, with the adult bees as carriers of the bacteria. In this case, food and pollen stores as well as all inner parts of the hive are contaminated. The pathogen is then transmitted by nurse bees to the young larvae during feeding. The beekeeper can also act as a vector of the disease by feeding healthy bees with infected honey, by moving diseased colonies during migratory beekeeping, through the trade of infected bees (e.g. queen bees), through the use of contaminated equipment, and by moving combs from one hive to another. The bacterium can easily spread through infected combs (through pollen, honey, brood, etc.) once placed in healthy colonies.

Asymptomatic colonies (hives that are infected but that still do not show signs of the disease) may have an important role in the spread of EFB. The migration or sale of subclinically infected colonies (colonies that do not yet show symptoms) can also lead to EFB transmission to healthy hives.

9.4 DIAGNOSIS

For an on-site diagnosis, it is sufficient to examine the brood and to look for the symptoms mentioned in section 9.2, for example:

- a scattered brood;
- contorted or upward-curled, yellow dead larvae in open cells;
- a sour smell when opening the hive (not always present).

The observation of the above symptoms can be combined with the use of a rapid diagnostic kit. Alternatively, to confirm the presence of the disease, a sample of the dead larvae can be sent to a specialized laboratory.

9.5 PREVENTION

The adoption of good practices (e.g. GBPs and BMBs) can help prevent the transmission of *Melissococcus plutonius*. Table 12 gives an overview of GBPs to adopt and how they can support healthy and strong colonies.

9.6 CONTROL

In order to reduce the spread of the infection, it is important to take appropriate action as soon as possible when EFB is detected in a colony. Controlling EFB propagation can be carried out mainly through three different methods, depending on the situation: shook swarm; incineration; and antibiotic treatment (where allowed).

9.6.1 SHOOK SWARM METHOD

The shook swarm method consists of shaking the bees from the infected combs (e.g. brood and store combs) into a clean hive with new foundations. Applying the shook swarm method reduces the level of infection. The brood and brood combs are the most contaminated elements of the hive. The old, infected combs should be destroyed immediately by incineration. This method must be the preferred option for sustainable beekeeping. It gives better results in the case of strong colonies and during honey flow, as colonies will need to build new honeycombs starting from wax foundations. When, in strong colonies, only a small number of cells are affected, it is also possible to perform a "partial shook swarm", removing only brood combs (representing the most infected material) and leaving the store combs. This will allow the colony to recover more quickly and produce honey for human consumption within a few months.

9.6.2 ANTIBIOTIC TREATMENT

In some countries, antibiotics registered for honeybees are available for the treatment of EFB, whereas in other countries the use of antibiotics against EFB is not allowed. The use of antibiotics is not allowed in organic beekeeping. Antibiotic treatment can be effective, especially if combined with the shook swarm technique, in cases of early stages of the disease, in strong colonies, and in cases of high prevalence of the disease in the apiary.

It is advisable to apply a shook swarm after antibiotic treatment to reduce the contamination of antibiotic residues in the hive. The persistence of antibiotic

TABLE 12. Good beekeeping practices for prevention and control of European foulbrood

Recommendation	Advantage or reason for practice
Ensure that colonies always have sufficient stocks of food (pollen and honey), especially at the beginning of the season.	Reduces nutritional stress on the bees.
Replace queens at least every two years.	Young, well-fertilized queens are more productive and guarantee strong colonies with many workers involved in the nutrition of the brood and in the removal of the diseased brood and dead larvae.
Replace at least 30% of brood combs every year.	Old combs contain bacteria and pollutants (pesticides, heavy metals, etc.) that have accumulated over time.
Do not move combs from one hive to another without checking the health conditions.	Prevents transmission of pathogens from diseased to healthy colonies.
Avoid feeding bees with honey and pollen cake. Only feed bees with their own honey or pollen, and if you know for sure the honey and pollen are completely safe and free of bacteria/viruses.	*Melissococcus plutonius* can be transmitted through infected honey and pollen.
When possible, sample hives for diseases by sending adult bees, honey, or other hive debris of healthy-looking colonies to a specialized laboratory for analysis, even when no symptoms are visible.	Early diagnosis of a disease can allow the beekeeper to adopt specific measures (e.g. replacing older frames) to prevent the clinical outbreak of the disease. This outbreak may occur even months later, in the presence of stress conditions. The practice also avoids economic losses caused by the reduction in production and colony mortality. In addition, it can avoid accidental spread of diseases by the beekeeper. This preventive action can be very easily performed during the inactive season by sampling different matrices of the hive such as bees, brood, honey or hive debris for analysis by a specialized laboratory. In the case of sampling hive debris, opening the hive is not needed, so avoiding cooling down the colony.
Increase the frequency of hive inspections to identify in timely fashion European foulbrood (EFB) or other brood diseases. Carry out a thorough inspection for clinical symptoms of bee diseases, at least at the beginning and at the end of the active season or after dearth or rainy periods.	Bacterial brood diseases (AFB and EFB) are more frequent when there is nutritional stress (e.g. lack of pollen and nectar). These conditions may occur especially at the beginning of the active season, when a few nurse bees are present, or at the end of the active season, when flowering is strongly reduced.
Periodic cleaning and maintenance of hives to prevent robbing.	Broken beehives with openings and crevices can attract robbing bees coming from other colonies. This condition may increase the spread of infectious diseases.
When melting down the combs, process wax safely in order to destroy bacteria and other pathogens.	To avoid the transmission of pathogens when using wax (e.g. foundation sheets) in hives, wax should be heated to at least 121 °C for at least 3 minutes, to ensure inactivation of all bacteria, including the spore-forming bacteria.
Select bees that are resistant to EFB.	Choose for reproduction those colonies that did not show EFB symptoms in the past.
Replace the queens of infected colonies.	Enables discarding of genetically sensitive queens.
Use clean beekeeping equipment and disinfect hive tools when needed.	Always use clean equipment, use disposable gloves in the case of affected hives, and disinfect (e.g. using bleach or torching) hive tools after inspecting affected colonies.

residues in the hive may vary according to the antibiotic used. The fastest decomposition in honey has been shown for oxytetracycline, followed by streptomycin and sulphathiazole.

When using antibiotics, the following rules should always be observed.

- Be aware of and comply with regulations for the use of veterinary medicines in honeybees.
- Use only registered veterinary medicines for honeybees.
- Use antimicrobials only in accordance with regulatory requirements and other veterinary and public health guidance.
- Keep detailed records of the origin and use of all medicines, including batch numbers, dates of administration, doses, treated hives and withdrawal times. Treated hives or apiaries should be clearly identified.
- Respect the required storage conditions for veterinary medicines and feeds.
- Ensure that all treatments or procedures are carried out properly, as described in the instructions (e.g. respecting dose and method of application).
- Observe the withdrawal time of veterinary products and ensure that products from treated hives are not used for human consumption until the withdrawal periods have elapsed.

9.6.3 DESTRUCTION OF THE HIVES BY INCINERATION

Incineration of honeycombs and honeybees (e.g. after killing by asphyxia with sulphur dioxide), may be the choice for several cases. These cases are: weak colonies; severe EFB clinical infection; appearance of the disease outside honey flow (colony recovery is more difficult, it is impossible to build new honeycombs and there is a high probability of relapse); and a very low prevalence of the disease in the apiary or in the geographical area.

If the beehive is in good condition, it can be disinfected. Disinfection can be done first by scraping off wax and propolis, then using sodium hypochlorite and finally torching. If the beehive is not in good condition, it should be incinerated. To avoid further contamination after the incineration, a hole of at least 50 cm deep should be dug in the ground. After killing the bees (e.g. with petrol), the hive and combs should be burned and the hole should be duly covered.

9.7 DISINFECTION PROCEDURES

After handling hives infected with EFB, all beekeeping equipment (boxes, boards, frames, queen excluders, etc.) and objects used (hive tool, gloves, suit, etc.) should be carefully disinfected. Possible methods to use vary according to the substrate to disinfect. They may include scorching wooden equipment, spraying with bleach or caustic soda, immersing objects in hot paraffin or microcrystalline wax, or using gamma-ray sterilization. Thoroughly clean the honey-house extraction tools/facilities (decappers, centrifuges, sieves, pumps, spins, honey extractors, etc.) and the hive product packaging materials (jars, tanks, barrels, etc.) with detergent in order to control the disease.

If bees are kept in areas where EFB is endemic or outbreaks occur frequently, an effective strategy for sustainable control should include the regular sampling of all hives in the apiary for the identification of subclinical diseased colonies (e.g. taking samples during in the inactive season, and analysing bees or hive debris).


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10 Chalkbrood

A fungal disease of the brood

Chalkbrood disease occurs worldwide, especially during the colony's growth in spring, when there are not enough adult bees present in the hive to keep the rapidly expanding brood warm. Chalkbrood disease is usually not a serious disease; healthy colonies are usually able to overcome it.

10.1 INTRODUCTION

Chalkbrood is a fungal disease caused by the fungus *Ascosphaera apis* and affects the gut of the brood. Bee larvae become infected by ingesting spores of *Ascosphaera apis* with food. The spores germinate in the intestines, leading to the death of the larvae, generally after capping. Each dead larva produces billions of spores and, if not removed by the worker bees, can remain infectious for several years within the hive. The brood located on the edges of the brood frames (generally the drone brood) is most affected because that is where it is the most difficult to control the temperature.

10.2 SYMPTOMS

The disease is transmitted via the ingestion of infected food containing the fungal spores. The larvae may be affected at different life stages, more frequently on the third or fourth day of larval life. They then die in the first two days after capping, so bees must uncap the cells to remove the dead larvae. Chalkbrood produces a mummification and/or calcification of the larvae (Figure 35). First, larvae appear soft, assuming the hexagonal shape of the cell. They then dry out and become hard. Most affected larvae appear white, but some turn grey or black (Figure 36). The presence of little stones (chalkbrood) on the bottom or at the entrance of the hive is typical.

Ascosphaera apis grows better in larvae situated more externally in the brood because it is colder. This phenomenon may occur especially during colony spring growth, when there are not enough adult bees to enable

Figure 35: Mummification of the larvae inside the cells.

Figure 36: Chalkbrood affected larvae appear white, but some turn grey or black.

adequate nest temperature control over the whole brood area. Less-populated and weaker colonies are more susceptible as the bees are not able to keep all brood warm. Drone larvae are usually the most affected because of their location on the margins of the brood chamber. Low temperatures, high humidity in the apiary, and poor ventilation of the hives all contribute to the disease being more severe.

10.3 TRANSMISSION

Inside the hive, chalkbrood disease is transmitted through infected food containing the fungal spores. These spores are highly infectious and can be easily spread between hives through robbing, drifting and on water sites.

In addition, the beekeeper can easily spread the disease between hives and apiaries by using contaminated equipment and transferring contaminated pollen to healthy hives. Chalkbrood spores may remain contagious for up to 15 years, or even more, in beekeeping equipment and in the soil.

Avoiding any activity that causes heat loss in the colonies can help prevent chalkbrood disease. Activities that cause heat loss include:

- too many and/or too long hive inspections during wintertime or on cold days;
- colony splits for artificial swarming;
- nest enlargement with interposition of wax combs between brood combs, especially during unfavourable periods for wax comb construction, such as early spring, autumn or winter, when the bees do not find enough food resources.

Chalkbrood may appear in hives after antibiotic treatment due to a lack of microbial competition.

10.4 DIAGNOSIS

The beekeeper can observe hard, white to grey-black, shrunken mummies in the brood combs and in and around the hive.

10.5 CONTROL

Many drugs have been tested, but the persistence of spores makes eradication of the disease impossible. The best solution seems to be the administration of sucrose syrup (1:1), acidified with lemon juice, vinegar or ascorbic acid powder until attaining a pH of 4. Prevention can also be assured with the application of GBPs in the apiary. Table 13 provides an overview of good practices to adopt in order to maintain healthy colonies and prevent chalkbrood disease.

Chalkbrood disease frequently causes spring losses, but the evolution of the disease is usually benign. Affected colonies can recover by themselves, especially if they increase their population. In particular, this happens under favourable environmental conditions, such as in sunny days of the spring and early summer periods, with the presence of abundant nutritional resources.

TABLE 13. Good beekeeping practices for the prevention and control of chalkbrood disease

Recommendation	Advantage or reason for practice
Place hives in appropriate locations, properly exposed to the sun and avoiding humid areas, with the hive entrance not exposed to main winds.	Reduces humidity and thermal stress on the bees.
Select resistant queens.	Reduces the number of hives affected by chalkbrood.
Ensure the availability of enough food reserves in the hive at all times, and provide artificial feeding when necessary.	Reduces nutritional stress in the colonies.


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11 Stonebrood (aspergillosis)

A fungal disease of the brood and adult honeybees

Stonebrood, or aspergillosis, is identified by hard larvae covered with powdery fungal spores. It is found worldwide but, unless the colony is seriously weakened by other stresses, it is usually asymptomatic.

11.1 INTRODUCTION

Stonebrood is a fungal disease caused by different species of fungi belonging to the *Aspergillus* genus. It affects adult honeybees and the brood (larvae and pupae). The main species of fungi responsible for the disease in honeybees are *Aspergillus flavus* and, less frequently, *Aspergillus fumigatus* and *Aspergillus niger*. The disease is transmitted via food or through direct contact. Green mummified larvae can be observed in the brood combs, and on the bottom board or the landing board of the hive. The mummified larvae resemble little white, yellow or green stones and are hard to crush, unlike larvae affected by chalkbrood (which are sponge-like).

The ideal temperature for the development of the fungi is between 33 °C and 37 °C, but they can also multiply at temperatures between 7 °C and 40 °C. Exposure to temperatures above 60 °C for a minimum of 30 minutes can devitalize both spores and hyphae, the long, branching, filamentous structures of fungi.

11.2 SYMPTOMS

In honeybee larvae, the infection gives rise to a characteristic ring near the head of the infected larvae. First, the dead larvae appear white and soft. Then, they become mouldy and often become covered with a sort of felt made up of the fungal spores. This felt may be of different colours, depending on the species of *Aspergillus* involved: yellowish-green in the case of *A. flavus*; grey-green in the case of *A. fumigatus*; and black in the case of *A. niger*. Once the larvae die, which occurs in the capped cells, their

bodies harden, appearing as small stones that are difficult to crush; hence, the name "stonebrood". The mummified larvae are difficult to remove from the brood cells, even with tweezers.

The adult bees remove the dead larvae from the cells, and green mummified larva can be found on the hive floor or at the entrance of the hive.

In rare cases, the infection may also affect adult bees, by ingestion of fungi-contaminated food. Infected bees will initially appear excited and restless, this state giving way to paralysis, inability to fly and death, usually occurring far away from the hive.

Although the death of entire colonies of bees affected by the fungus has been observed, the disease usually has a transitory character and tends to be overcome naturally.

11.3 TRANSMISSION

The disease is transmitted through food containing the fungal spores (honey and pollen) or through direct contact between bees.

The disease can spread from sick hives to healthy ones through the drifting of infected bees, the looting by healthy bees of infected hives, or swarming. The beekeeper can also transmit the disease by using infected tools or by moving combs from sick colonies to healthy ones.

11.4 DIAGNOSIS

One of the first signs of the disease is a spotted and irregular brood pattern (high quantity of empty cells, mixed with other cells containing eggs, larvae and nymphs of all ages).

The larvae first appear wrinkled, cream-coloured, and lose segmentation. They then change in colour, going from grey to greenish. Finally, the larvae mummify, adhering to the cell walls, making them difficult to remove (Figure 37). The mummy can be covered with a greyish-white felt or can be greenish-yellow, grey-green or black, depending on the fungal species of the disease.

With adult honeybees, paralyzed adult bees can be seen on the infected combs. After death, the body becomes hard. In the presence of high humidity, the body can be covered with a grey-white fungal felt, typical of the infection.

Positive identification of the fungus requires specialized laboratories to perform cultivation (*Aspergillus* spp. can be grown on potato dextrose or Sabouraud dextrose agars) or biomolecular identification.

Many species of *Aspergillus* can produce aflatoxins, which are carcinogenic for humans when ingested orally (e.g. via contaminated hive products, especially pollen). For this reason, in many countries, stonebrood is a disease that must be reported to the appropriate sanitary authorities when diagnosed. Measures should be taken to protect the health of beekeepers and consumers.

11.5 PREVENTION

Beekeepers should destroy the severely infected mouldy combs containing the affected brood, and should not use any of the hive products obtained from sick hives for human consumption or in healthy hives (e.g. honey, pollen, royal jelly, wax and propolis).

The adoption of GBPs is the most effective way to prevent the disease. Table 14 provides a list of good practices that can help to maintain healthy colonies and prevent the disease.

11.6 CONTROL

More research is needed to determine the proper control measures to adopt. The replacement of the queen can be very useful. To date, there are no registered treatments to control this infection in honeybees, although experimentally it has been observed that the essential oils of cinnamon (*Cinnamomum zeylanicum*), *Litsea cubeba* and geranium (*Pelargonium graveolens*), as well as their mixtures, are able to contain the growth of this fungus. Genetic selection for bee resistance to stonebrood could be an interesting sector to invest effort in, as it has been observed that a genetic predisposition may differ from colony to colony.

Figure 37: Larvae affected by stonebrood mummify, adhering to the cell walls, making them difficult to remove.

11.7 IMPACT OF STONEBROOD ON HUMAN HEALTH

The fungi causing stonebrood are found everywhere in the soil and are able to induce disease in insects, birds, mammals and also in humans, making it a zoonotic disease (a disease that can be transmitted from animals to people).

In humans, it can cause respiratory diseases, such as pulmonary infections (broncho-pulmonary aspergillosis, and pulmonary aspergillomas) or allergic bronchitis, if inhaled. It can also cause eye, pharynx, skin and open-wound infections in the event of direct contact with infected brood, bees or combs.

Moreover, the multiplication of fungi belonging to the *Aspergillus* genus may be responsible for the production of specific mycotoxins that may be dangerous when transmitted orally to animals and humans. In the case of stonebrood, the mycotoxins may be transmitted to humans through the consumption of pollen.

TABLE 13. Good beekeeping practices for prevention and control of stonebrood disease

Recommendation	Advantage or reason for practice
Choose a good location for the apiary, preferably with good exposure to the sun, avoiding wet areas.	Makes it difficult for fungi to grow.
Ensure proper management of the hive by: • favouring in-hive ventilation through the opening of a hole on the cover (also during the winter); • preventing water from entering the hive; • properly preparing for wintering by reducing the number of the combs; • removing unpopulated combs, and leaving only populated combs in the colony during winter.	Humidity is a favourable environment for fungi to grow.
Replace at least one-third of the brood combs every year.	Reduces the microbial population (fungi included) in the hive.
Ensure colonies have enough food during dearth periods. Provide additional feeding when necessary.	Avoiding nutritional stress reduces the possibility of the bees becoming infected.
Keep only strong colonies in the apiary. Weaker (healthy) colonies should be joined with another stronger colony.	Weak colonies may be more exposed to diseases than are healthy colonies.
Ensure a right balance between adult bees and brood, especially in spring.	In spring, there is an increase in brood to feed. If not enough adult bees are present, nutritional stress occurs.
Do not feed the bees mouldy pollen, and do not breed bees on mouldy combs. Frequently, these mouldy combs are the combs that remain unused inside the hives during winter in a lateral/external position.	Mouldy combs usually appear opaque-whitish or greenish in colour. They are strongly contaminated with the fungi.

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12 Viruses

Symptoms of the main viral honeybee diseases explained

Viral honeybee diseases are spread throughout the world and can cause serious economic losses if combined with other bee diseases.Their impact is usually underestimated by beekeepers.

12.1 INTRODUCTION

All viruses may be present in apiaries in latent or asymptomatic form (i.e. no symptoms are visible in the hive). Triggering events such as other hive diseases (e.g. varroosis or nosemosis) or stressful factors (e.g. starvation due to rainy weather or low temperatures) can lead to the development of the infection, the death of bees, and the need to destroy the affected combs and/or the entire colony. Seasonal factors and the region where the apiary is located strongly influence the onset of honeybee viruses.

Varroa destructor greatly contributes to increase in viral diseases. The varroa mite is a passive bee-virus carrier, with the virus being transmitted to the bees through the mite's saliva. In addition, varroa weakens the bees' immune systems, which can reactivate latent viral infections already present in the bees. Other bee diseases that set the conditions for the onset of viral diseases are nosemosis, EFB and amoebiasis.

The transmission of the viruses usually occurs through honeybee faeces, royal jelly, varroa, saliva or the beekeeper. However, the transmission of the main bee viruses is from the queen to the brood.

12.1.1 MAIN HONEYBEE VIRUSES

To date, many honeybee viruses have been identified and classified, but there is not enough information about their worldwide distribution:

- chronic bee paralysis virus (CBPV)
- sacbrood virus (SBV)
- acute bee paralysis virus (ABPV)
- deformed wing virus (DWV)
- black queen cell virus (BQCV)

- cloudy wing virus (CWV)
- slow paralysis virus (SPV)
- bee virus X (BVX)
- bee virus Y (BVY)
- filamentous virus (FV)
- Apis iridescent virus (AIV)
- Israeli acute paralysis virus (IAPV)
- Arkansas bee virus (ABV)
- Berkley bee picornavirus (BBPV)
- Kashmir bee virus (KBV)
- Egypt bee virus (EBV)
- tobacco ringspot virus
- Kakugo virus

12.2 SYMPTOMS OF THE MAIN VIRAL DISEASES OF THE HONEYBEES

12.2.1 CHRONIC BEE PARALYSIS VIRUS

Chronic bee paralysis virus (CBPV) causes an infectious and contagious disease in adult honeybees. The infection has no seasonal pattern, often remains latent, and is present in many countries. The virus is more frequently found in colonies infested with varroa.

Chronic bee paralysis virus is the only common viral adult bee disease that has well-described symptoms. For this, it has been given a variety of names, such as "hairless black syndrome" and "little blacks".

Affected bees become almost hairless, dark in appearance and suffer nibbling attacks from healthy bees of their colony. The beekeeper can observe wobbly and flightless bees in the upper part of the honeycomb, and bees crawling on the ground and on grass stems in front of the hive, where they die (Figure 38).

Sometimes, affected bees present enlarged abdomens (due to the accumulation of liquid in the honey sac) and wings spread out in a "K" form. Sick bees die within a few days of the onset of the symptoms.

Figure 38: Wobbly and flightless bees in the upper part of the honeycomb.

Figure 39: Bee with normal wings (left) and another with deformed ones (right)

Thousands of paralyzed bees from each colony die throughout the year, and severely affected colonies can collapse.

12.2.2 ACUTE BEE PARALYSIS VIRUS

Acute bee paralysis virus (ABPV) can normally be found in the bee's fatty tissue and does not show symptoms. Combined with varroa, the infection becomes particularly serious, causing mortality both in the brood and in adult bees. This virus is usually combined with CBPV. However, in the event of a massive varroa infestation, ABPV prevails over CBPV because of its rapid replication activity. It is possible to observe bees that die, larvae that are unable to emerge from capped cells, and adult bees with shivering wings, darkened hairless abdomens and thoraxes. This progresses into a state of paralysis and then death.

12.2.3 DEFORMED WING VIRUS

Deformed wing virus (DWV) is relatively widespread in apiaries, although often present in subclinical form (no symptoms are visible) if not associated with varroa. However, combined with varroa, DWV can cause the death of the brood and adult bees. This virus affects immature bees during their development in the cells. It is possible to observe wings and abdominal deformities such as: stubbiness; flightless wings; shortened, rounded, darkened abdomens; and leg and wing paralysis (Figure 39). Body and especially abdomen sizes frequently reduce, and the bees have a very short life expectancy.

12.2.4 SACBROOD VIRUS

Sacbrood virus (SBV) affects young honeybee larvae and sometimes adult bees. An uneven brood pattern with discoloured, sunken or perforated capping scattered throughout the brood is a typical symptom of an SBV infection. The affected larvae die shortly after capping, before they

transform into pupae. The larvae gradually change colour, from white to yellowish and brownish. Then, the internal organs become fluid while the outer skin remains intact, giving it the typical "saccular" appearance (a sack filled with liquid) (Figure 40). In adult honeybees, SBV infection is usually asymptomatic.

This virus is not very resistant to external agents (e.g. warm temperatures and direct sunlight). The virus in infected colonies remain infective if present in honey for up to six weeks. This is one reason why the disease can be transmitted to healthy colonies.

12.2.5. BLACK QUEEN CELL VIRUS

Black queen cell virus (BQCV) only affects the queen cells, and is one of the most frequent causes of mortality among the queen larvae. Affected queen bee pupae turn yellow, and the skin of the pupae becomes sac-like. The dead queen bee pupae may change to a brown-black colour. The walls of the queen bee cell also become brown-black in colour, hence the name of the virus (Figure 41). It is frequently associated with *Nosema*. Although worker bees and drone broods can be infected by BQCV, these do not generally develop any kind of symptoms.

12.3 TRANSMISSION

Every virus has different transmission routes into the colonies. They could be transmitted from queens to worker bees or drones, or from adult bees to other adult bees, of the same colony or of different colonies.

The prevalence of some viruses is also related to other diseases. For example, BQCV infection is more common when colonies are affected by nosemosis, as the lesions of the small intestine facilitate the passage of the virus in the haemolymph.

12.4 DIAGNOSIS

An exact diagnosis of the virus affecting the colony can be made using the PCR technique in a specialized analysis laboratory.

On-site diagnosis is possible for SBV, DWV and BQCV by observing the symptoms as described above.

12.5 PREVENTION AND CONTROL

Good beekeeping practices are essential to prevent diseases, and stress factors should be kept to a minimum level. Stress factors – such as chemical (e.g. drug treatments), physical (e.g. frequent visits in winter), metabolic and infectious factors – may serve as predisposing factors for viral

Figure 40: Sacbrood Virus.

Figure 41: Black queen cell virus.

disease outbreaks. It is fundamental to keep varroa and *Nosema* infestation under control. The occurrence of several viruses can be reduced by implementing GBPs and BMBs related to varroa and *Nosema*.

There are no specific and effective therapeutic remedies for bee viral diseases. In the case of particularly severe symptoms, the only remedy is destruction of the affected colonies. In other cases where the symptoms are less severe, you can try replacing the queen and the infected honeycombs, which should be destroyed.

The infected hives must be properly cleaned and disinfected before reuse. Disinfection can be carried out with bleach, and then by passing a blue flame over the hives.

Owing to the transovarian transmissibility of some viruses (an infected queen bee can produce infected eggs and brood), when introducing new queens into the apiary, the recommendation is to observe a quarantine period and monitor the health of the brood.

Annex 1

Good beekeeping practices

The good beekeeping practices (GBPs) listed below are the result of consultation and harmonization at the international level among experts (scientists and beekeepers). They are classified according to the general management operations in an apiary. They might be more or less applicable everywhere in the world (irrespective of climate, geographical location of the apiary, type of hive used, etc.). The GBPs are not listed in any particular order, and they are not listed according to their importance.

The adoption of GBPs can help the beekeeper maintain strong and healthy colonies, limit disease outbreak, and/or limit damage caused by disease.

LIST OF HARMONIZED GOOD BEEKEEPING PRACTICES

Table A1.1 General apiary management

Area of intervention	Appropriate measures
I. Transportation	1. Comply with legal obligations concerning restrictions on animal movement in cases of notifiable diseases. 2. Transport/move only healthy colonies. 3. Transport hives avoiding the warmer hours of the day, providing adequate openings for air ventilation in the hives.
II. Hygiene	1. Observe general hygiene rules, such as the periodic cleaning of suits, gloves and other beekeeping equipment. 2. Observe hygiene rules when dealing with dead colonies (combs, food stores, boxes, etc.). 3. Use disposable gloves when handling diseased hives. 4. Disinfect levers and other potentially contaminated equipment (e.g. gloves) after inspection of hives affected by transmittable diseases. 5. Do not place honey supers directly on the ground – to avoid honey contamination with *Clostridium botulinum*. 6. Avoid contact with dust during transport of the supers from the apiary to the honey house. 7. Do not place beehives directly on the ground.
III. Bee health	1. For nuclei, use only bees and brood combs from healthy colonies. 2. Balance colony strength among colonies by transferring frames only in the case of healthy hives. 3. Buy new bee colonies only after thorough inspection for honeybee diseases, preferably with a health certificate from a veterinarian. 4. Keep only healthy and strong colonies in the apiary. 5. Place apiaries in areas free from environmental pollutants (pesticides, heavy metals, etc.). 6. Do not imbalance the proportion between nurse bees and brood while equalizing the hives; use preferably combs with hatching bees to fortify weak colonies. 7. Perform genetic selection in order to have queens that are more resistant to disease and adapted to local climatic conditions. 8. Keep newly introduced colonies in a quarantine apiary, separate from the existing stock, for at least 1 month, in order to monitor them against diseases and prevent transmission of diseases. 9. Keep purchased or weak colonies/swarms in a quarantine apiary before introducing them into the final destination apiary. 10. Reduce bee stress (e.g. avoiding unnecessary winter inspections of the hives; limiting the use of the smoker; properly feeding the bees).
IV. Apiary management	1. Evaluate the melliferous and pollen capacity of the area and the availability of water resources. 2. Do not leave beekeeping material abandoned in the apiary. 3. Adjust the number of hives in a specific area to the amount of melliferous plants/pollen sources in the area. 4. Avoid placing apiaries in windy areas. 5. Place apiary in an area that is easy to access with a vehicle. 6. Adjust the number of hives in the apiary according to season, pollen, nectar, honeydew resources. 7. Place apiary in a dry area. 8. Prevent drifting: avoid keeping too many colonies in a single row. 9. Avoid having broken or not well-maintained hives with openings – to prevent robbing.

continued

Area of intervention	Appropriate measures
V. Wintering	1. Before winter, reduce the empty space in the hive. 2. Reduce the size of the hive entrance. 3. Perform beehive box maintenance: verify the integrity of hive boxes, replace damaged or broken parts or paint. 4. Verify there is enough food storage in the external frames. 5. Remove the unpopulated frames and adjust the hive volume to the size of the colony. 6. Insert a follower board frame to reduce the volume for the hive nest. 7. Wrap the hive in black tar paper, if needed.
VI. Human health	1. When required, ask the assistance of an expert (e.g. veterinarian, technician). 2. Use protective clothing and beekeeping tools when visiting honeybee colonies. 3. Avoid placing hives in areas of high presence of toxic plants (e.g. pyrrolizidine alkaloids in *Echium* spp., *Eupatorium* spp. and *Senecio* spp.). 4. During apiary inspections, always keep corticosteroids or other medicines within easy reach to guarantee health of operators (e.g. in case of anaphylaxis). 5. Limit the lifting of heavy weights (e.g. when harvesting supers or when moving hives) and, if needed, use back-protector devices. 6. Avoid areas where allergenic plants (e.g. *Ambrosia trifida* and *Artemisia vulgaris*) can be found in significant numbers.
VII. Colony management	1. Adopt hive management practices according to region, season, and strength of colony. 2. Replace the queens at least every 2–3 years, except those of high genetic value. 3. Comply with the planned schedule for beehive inspection. 4. Prevent swarming by splitting strong colonies in the spring. 5. Prevent swarming by insertion of new wax foundations. 6. Prevent swarming by placing of supers. 7. Prevent swarming by removing the entrance reducer. 8. Prevent swarming by adopting genetic selection of the queens. 9. Prevent swarming by insertion of drawn combs. 10. Use a queen excluder. 11. Reduce the opening of the hive entrance during robbing and cold periods, and increase the opening of the hive entrance during the hot season. 12. Mark the queen bee according to its year of birth. 13. Orient hive entrance in a way that the sun can reach it from the early morning hours. 14. Prevent drifting by painting numbers or different geometrical signs in different colours on the front and entrance of the hive. 15. Indicate the age of the combs on the top bar of the frame (e.g. the year of placing of the frame with foundation). 16. Prevent swarming by removing the beehive's bottom board. 17. Ensure adequate air circulation in the hive, if needed.

Table A1.2 Use of veterinary medicines

Appropriate measures
• Use only veterinary medicines for honeybees registered in your country or medicines legally imported. • Ensure that all treatments are carried out correctly as described in the instructions (respecting dosage and method of application). • Do not apply illegal treatments. • Use only pharmacological products registered for beekeeping use, following the instructions of use, and record the treatments. • Observe the withdrawal time of veterinary products, and ensure that products from treated hives are not used for human consumption until the withdrawal period has elapsed. • When using instruments for the application of a treatment (formic acid dispenser, sublimators for oxalic acid treatment) ensurethat they are appropriate and correctly calibrated for the administration. • Respect the required storage conditions for veterinary medicines and feeds. • Dispose of used instruments and devices in a biosecure manner.

Table A1.3 Disease management

Area of intervention	Appropriate measures
I. Precautionary measure	1. Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen in spring. 2. Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen at the end of the beekeeping season. 3. Replace queens from colonies with clinical history of American foulbrood. 4. Replace queens from colonies with clinical history of European foulbrood. 5. Quickly remove hives with dead colonies. 6. Take samples for laboratory analyses when sick or dead bees are found, if needed. 7. Clean equipment: scrape off wax and propolis, on a regular basis. 8. Remove and process wax of all combs from colonies that died as a result of an infectious disease. 9. Record the health status of the colonies: diseased/infected colonies (dates, diagnoses, ID of colonies affected, treatments and results). 10. Try to select and breed colonies that are more disease-tolerant/resistant. 11. Renew 30% of the hive combs every year.
II. Control and active measures	1. In cases of notifiable diseases, follow veterinary regulations from competent authorities. 2. In cases of infectious diseases, clean all beekeeping material after use (e.g. hive bodies, hive bottom boards, feeders, and hive tools). 3. Clean or disinfect (in cases of infectious diseases) the hive box before installing new colonies. 4. Verify promptly any symptom of disease, asking a veterinarian (or a specialist). 5. Do not move frames or any kind of biological material (e.g. to balance hives) from one hive to another if their health status is not well known. 6. Inspect diseased hives only after inspecting the healthy hives. 7. Select breeding queens and drones from the best-performing hives (good productivity, gentleness, resistance to diseases, etc.). 8. Record the origin and the use of all the disinfectants and other chemicals (e.g. repellent for bees, bites of other animals such as hornets, beetles, ants) used at the apiary level. Keep all the records relating to the cleaning and disinfection procedures used on equipment or honey house (including data sheets for each detergent or disinfectant used) as well as all the records showing that these procedures have been effectively implemented (task sheets, self-inspection checks on the effectiveness of the operations). 9. Disinfect equipment (for example, with NaOH, hypochlorite) on a regular basis. 10. Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen before supering the hives.

Table A1.4 Hygiene in cases of infectious diseases

Appropriate measures
• Disinfect iron and wooden beekeeping equipment through torching (blue flame). • Disinfect hives and beekeeping equipment (e.g. using bleach, or similar). • Burn affected colonies as necessary. • Disinfect hives and beekeeping tools (e.g. using high pressure or heat, unless legislation requires otherwise). • Use autoclaving as a method of disinfection of hives and beekeeping tools in cases of transmissible diseases. • Use gamma irradiation as a method of disinfection of beekeeping tools in cases of transmissible diseases.

Table A1.5 Bee feeding and watering

Area of intervention	Appropriate measures
I. Feeding	1. Do not feed the bees with honey, pollen or supplements, unless the absence of pathogens (spores of AFB, chalkbrood, *Nosema*, EFB, etc.) is certified. 2. Provide artificial feeding during times of shortage, or build up winter storage, when needed. 3. During winter: verify that there is a sufficient amount of stores in the hive. 4. Provide nuclei and swarms with adequate food supply when needed.
II. Watering	1. Ensure the bees have access to safe water sources. 2. Do not feed bees openly in the field – to prevent robbing and spread of diseases. 3. During transport, provide adequate watering if needed.

Table A1.6 Record-keeping

Level of documentation	Appropriate measures
I. Apiary level	1. Keep records of veterinary medicine treatments. 2. Registration of the beekeeper in the national beekeeping registry. 3. Record the exact position of each bee yard. 4. Identify with numbers/letters all the hives in each apiary. 5. Keep records of honeybee diseases and colony mortality or depopulation. 6. Keep records of movements of hives, swarms and queen bees. 7. Record period of collection of hive products from each apiary. 8. Keep detailed records of the origin and use of all medicines, including batch numbers, dates of administration, doses, treated hives and withdrawal times. Treated hives or apiaries should be clearly identified. 9. Keep all documents/certificates that indicate the raw materials used in feed manufactured by the beekeeper and given to the colonies. 10. Create a unique identification number for the apiary to easily trace the location of the hive (for stationary apiaries). 11. Keep records of breeding activities (e.g. all breeding stock, queens' birth date, the origin and arrival date of queens, the breeding date in case of instrumental insemination and outcomes, etc.).
II. Colony level	1. Set up a data-recording system that can be used to trace exactly which batches of commercial feed the colonies were fed with. 2. Keep all documents/certificates about the commercial feed used. 3. For each colony or group of colonies, require and keep all commercial and health documents enabling their exact itinerary to be traced from their farm or establishment of origin to their final destination. 4. Record all reared colonies. 5. Record origin and date of arrival of each new colony to ensure that movements of incoming colonies are traceable to their source. 6. Establish a data-recording system to ascertain the exact origin (batch) of bee products produced. 7. Keep all the documents regarding self-check and official controls on the proper management of the colonies and the sanitary and hygienic quality of the bee products. 8. Keep all documents proving that the bacteriological and physiochemical quality of the water used in the honey house, given to the colonies or used in feed preparation meets official national tap water standards. 9. Record the origin and use of all feeds used, keep all records of any feed manufacturing procedures and records for each batch of feed. 10. Keep a list of certified suppliers. 11. Record any other management changes that may occur. 12. Record any change in feeding. 13. Keep all laboratory reports, including bacteriological tests and sensitivity tests (resistance of bacteria to antibiotics). 14. Keep reference samples (at –20 °C) of all feeds administered to the bees.

TRAINING

Each beekeeper should undertake training on good beekeeping practices for a healthier and more successful apiary (Table A1).

Table A1.7 Training on good beekeeping practices

Subject matter	Appropriate measures
I. Beekeeping, apiary management and bee diseases	1. Follow a training programme in beekeeping and honeybee diseases to gain knowledge on honeybee diseases and symptoms. 2. Have adequate knowledge on honeybee diseases and symptoms. 3. Be able to display training certificates and/or qualifications obtained in beekeeping of all people working in the apiary.
II. Disease management	1. Keep the user instructions for the use of detergents/disinfectants to refer to in case of need. 2. Keep record of each detergent/disinfectant used and their method of use.

Annex 2

Biosecurity measures in beekeeping concerning the main diseases of the honeybee (*Apis mellifera)*

The biosecurity measures in beekeeping (BMBs) listed below are the result of consultation and harmonization at the international level between experts, mainly from countries of the European Union. They are classified according to the disease they aim to limit/control. They can differ between different regions due to local factors such as climatic conditions, beekeeping equipment used, or bee races, and the prevalence, virulence and importance of the honeybee diseases. They are general recommendations the beekeeper should implement and eventually adapt at the local level to reduce the incidence of disease, when needed.

LIST OF HARMONIZED BIOSECURITY MEASURES IN BEEKEEPING

Table A2.1 Varroosis

	Varroosis (causative agent: *Varroa destructor*)
I. Precautionary measures	1. Try to select and breed colonies that are varroa-tolerant/resistant. 2. Use hives with screened bottom boards. 3. Nuclei and swarms should originate from colonies with no clinical signs of varroa-related diseases (ABPV, DWV, IAPV, KBV, etc.). 4. Treat according to an integrated pest management concept, considering varroa thresholds. 5. Maintain the number of varroa mites below the harmful threshold in each colony. 6. Have good knowledge of the symptoms and of the transmission ways of varroosis and viroses.
II. Control and active/ immediate measures	1. Always treat varroosis according to the national legislation and regulations. 2. Adopt diagnostic tools for measuring varroa infestation levels (e.g. icing sugar method, CO_2 test, mite fall) after treatments and during the year (e.g. in spring at the beginning of beekeeping season or before harvesting). 3. Treat simultaneously all colonies of the apiary and in the same area. 4. Prepare colonies (e.g. absence of brood) before treatment to obtain the highest possible efficacy, depending on type of treatment and product used. 5. Monitor efficacy of acaricide treatments, e.g. verifying varroa fall after treatment. 6. Perform at least two treatments per year. 7. Monitor efficacy of acaricide treatments verifying the absence of varroosis symptoms in the colony (e.g. presence of varroa mites on adult honeybees) after treatment. 8. Rotate active principles of veterinary medicines to avoid varroa resistance. 9. Check the health status of drone-producing colonies, especially for viruses. 10. Use preferably medicines allowed in organic beekeeping to control varroa. 11. Provide sufficient number of healthy spare bee colonies to reinforce weaker colonies when the varroa infestation level is too high. 12. Treat nuclei and swarms (no brood) with oxalic or lactic acid.

Table A2.2 American foulbrood

American foulbrood (AFB) (causative agent: *Paenibacillus larvae*)	
I. Control and active measures in cases of infestation	1. Perform the ropiness test (toothpick test) to confirm clinical outbreak of AFB in the apiary. 2. Quick management of affected hives. 3. Check for *P. larvae* in **asymptomatic** *colonies* by submitting hive material for laboratory tests (e.g. stored honey in combs, hive debris). Take samples of colonies (hive debris, adult nurse bees, powder sugar, stores of honey in combs) **during the winter season**, to detect *P. larvae* (by PCR method or microbial isolation) to control the disease. 4. Submit colony samples (dead bees, hive debris, other) of **symptomatic (diseased) colonies** to a specialized laboratory for analysis (isolation and/or PCR) to confirm a clinical outbreak of AFB in the apiary. 5. Melt down brood and honeycombs of all colonies (with and without clinical symptoms) of the affected apiary, and process wax safely in order to control the disease. 6. Verify presence of the typical AFB scales (not removable, firmly adherent to the cell wall) to confirm clinical outbreak of AFB. 7. Promptly destroy or use shook swarm (according to national legislation) on hives that show clinical symptoms of AFB. 8. Disinfect/incinerate all beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of symptomatic hives. Disinfect all beekeeping equipment of asymptomatic hives located in apiaries with AFB outbreaks. 9. Increase the frequency of hive inspections in asymptomatic colonies (and in other apiaries of the same beekeeper) in cases of laboratory positivity to spores of *P. larvae* or in cases of symptoms of the disease in other hives of the same apiary. 10. Apply an AFB test (field kit) to confirm clinical outbreak of AFB in apiary.

Table A2.3 European foulbrood

European foulbrood (EFB) (causative agent: *Melissococcus plutonius*)	
I. Control and active measures in cases of infestation	1. Manage affected hives quickly to control the disease. 2. Search for the presence of removable scales, yellow and contorting larvae to diagnose a suspected EFB clinical outbreak. 3. Perform laboratory analysis (isolation and/or PCR) to confirm clinical suspicion of EFB. 4. Buy queens from breeders that can provide an EFB-free certificate. 5. Use shook swarm (according to national legislation) on hives that show EFB clinical symptoms. 6. Disinfect the infected beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of EFB-symptomatic colonies in cases of clinical outbreak. 7. Increase hive inspections in symptomless colonies in cases of laboratory positivity to *P. plutonius* or in cases of symptoms of the disease in other hives of the same apiary. 8. Take samples (hive debris, adult nurse bees, powder sugar, stores of honey in combs) from asymptomatic colonies for laboratory analysis in winter season or in cases of outbreaks, to confirm or not the presence of *P. plutonius* (by PCR method or microbial isolation). 9. Apply on-site EFB kit to confirm clinical outbreak of EFB on symptomatic hives. 10. Use a partial shook swarm (remove only brood combs, leaving store combs) on colonies that show EFB clinical symptoms. 11. Disinfect/incinerate all beekeeping equipment (beehives, nuc boxes, mating boxes, boards, frames, queen excluders, etc.) of EFB-asymptomatic colonies in cases of clinical outbreak in the apiary. 12. Be aware of the odour when opening the hive – typically, sour smell should raise suspicion of clinical form of EFB. 13. For a quick eradication of EFB in the apiary, all affected colonies should be destroyed. However, in the event of high rates (above about 20%) of cases of EFB in the apiary, follow point 5 above in order to avoid excessive economic losses.

Table A2.4 Nosemosis

Nosemosis (causative agent: *Nosema apis* or *N. ceranae*)	
I. Precautionary measures	1. Do not reuse combs (neither if empty or with stores of honey and/or pollen) originating from depopulated (few workers and the queen) or collapsed hives. 2. Prevent pollution of artificial water sources with faeces or drowned or dead bees. 3. Buy queens from breeders with stocks free of *Nosema* spp. 4. Select and breed honeybees resistant to *Nosema* spp., if possible. 5. Remove and destroy combs with signs of dysentery. 6. Take samples of forager honeybees (or powder sugar or hive debris) early in autumn or spring for laboratory analysis to diagnose nosemosis (PCR and microscopic methods). 7. Adopt a proper pathogen (e.g. *V. destructor*) control, to ensure a proper balance (nurse–forager bees) in the composition of the bee colony. 8. Strengthen and stimulate the colonies in autumn and spring with the administration of stimulant integrators or feed supplements.
II. Control and active measures in cases of infestation	1. Treat (if any registered/permitted products are available in your country) the colony against *Nosema* spp. when the percentages of infected bees are high (> 40%).

Table A2.5 Aethinosis (small hive beetle)

Aethinosis (small hive beetle [SHB]) (causative agent: *Aethina tumida*)	
I. Precautionary measures	1. Have good knowledge of the morphology of SHB eggs, larvae and adults. 2. Have good knowledge of hive inspection methods to detect SHB. 3. Do not leave frames, combs or other material outside of beehives that could be attractive and edible for *A. tumida*. 4. Have only healthy strong colonies in the apiary. 5. Have only young queens with hygienic behaviour. 6. Do not transport live material at risk (hives, queens, nucs, etc.) from areas where SHB is or could be present into your apiary. 7. Take care that the bees cover all frames in the hive (no empty spaces). 8. Use specific traps for quick visual detection of SHB. 9. Monitor periodically the presence of SHB by sampling debris or honey. 10. Do not transport material at risk (supers, wax, pollen, etc.) from areas where SHB is or could be present into your apiary. 11. Use queen-bee excluder in order to avoid the presence of brood in the supers.
II. Control and active measures in cases of infestation	1. Ensure that the bees cover all frames in the hive (no empty spaces must be left to SHB). Remove the empty frames. 2. Do not leave frames, combs or other material that could be attractive and edible for *A. tumida* outside the beehives. 3. Carry out hive inspections periodically to detect and eliminate the parasite (adults and larvae). 4. Trace meticulously the movement of hives (identify hives, dates of movements, exact position). 5. Control the transport conditions, adopting proper isolation of beekeeping equipment – to avoid spread of SHB during transport. 6. Stock combs in a cold chamber at a temperature below 10 °C or with a relative humidity below 34%, in order to prevent survival of SHB eggs and larval development. 7. Give artificial nutrition each time at low amounts so the bees can consume it in a short time (pollen, proteic feed, supplements are a good substrate for SHB reproduction). 8. Have only healthy strong colonies in the apiary. 9. Trace meticulously the movement of supers and wax. 10. Use traps to monitor and control SHB presence in the apiary. 11. Have only young queens with hygienic behaviour. 12. Use queen-bee excluder in order to avoid the presence of brood in the supers.

List of other beekeeping technologies and practices available on the TECA Platform

A large selection of technologies and practices related to different aspects of beekeeping can be consulted online on the FAO TECA Platform under the category "Beekeeping" (http://www.fao.org/teca/categories/beekeeping/en/). They include practices related to the construction of beekeeping equipment, beehive management, and extraction and processing of beehive products and cover different types of beekeeping (beekeeping with fixed comb hives, topbar hives and movable frame hives). Technologies and practices are available in English, French and Spanish. Following is an example of some beekeeping practices available on the TECA Platform.

Title	Available in English, French and Spanish (E, F and S)	Technology/practice provided by	URL
Practices related to honeybee health			
Method to dermine level of varroa infestation in the field	F, S	IRACH	F: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=9043 S: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=10003
Strategy for integrated varroa management: healthier colonies through brood removal	E	The Bee Institute in 'Kirchhain'	http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8401
How to make a varroa sampler for the alcohol wash method	S	Red Apícola Chile	http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=10003
Practices related to the production of beekeeping equipment			
How to construct a smoker for beekeeping	E, F, S	E: Swisscontact F: Beekeeping Network Nord-South S: Swisscontact	E: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=9114 F: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8871 S: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8295
The Kamara hive: an improved traditional hive	E, S	International Stingless Bee Centre	E: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=7289 S: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8667
How to build a topbar hive	E, F	E: National Bee Unit at The Food and Environment Research Agency (Fera) F: Beekeeping Network Nord-South	E: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=7274 F: http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8745, http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8744 and http://www.fao.org/teca/new-search-result/technology-detail/en/?uid=8707

This manual is a practical tool to help beekeepers, veterinarians and beekeeping advisory services to properly identify main honeybee diseases and to take the most appropriate actions in the apiary to control and/or prevent disease outbreaks. This publication is a sequel to the TECA publication "*Main bee diseases: good beekeeping practices*" published in 2018, which provided a more general overview on beekeeping practices.

This manual is a unique publication because, through its presentation of practical information, simple visuals, and understandable content, it helps beekeepers to correctly identify main honeybee diseases in a timely manner. More specifically, the manual creatively illustrates actions which facilitate the identification of disease symptoms. It also presents a comprehensive list of good beekeeping practices to adopt in the apiary as well as biosafety measures to reduce the risk of the introduction and the spread of main honeybee diseases. The manual's overall objective is ultimately to support a more sustainable beekeeping sector.

The information contained in this publication, together with a wealth of other beekeeping-related information, is available online: http://www.fao.org/teca/beekeeping.

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