BPRACTICES
(ERA-NET SusAn)
PROJECT

towards
a sustainable
European
beekeeping

BPRACTICES

Guidelines on sustainable management of honey bee diseases in Europe

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Edited by Giovanni Formato
BPRACTICES Project coordinator

This document was produced in the framework of BPRACTICES Project "New indicators and on-farm practices to improve honey bee health in the Aethina Tumida era in Europe". This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement n° 696231, ERA-Net SusAn – European Research Area on Sustainable Animal Production Systems. The main objective of BPRACTICES was to develop new management practices (Good Beekeeping Practices – GBPs) adopting new clinical methods, biomechanical and innovative biomolecular techniques respecting the natural behaviour of bees.

The BPRACTICES consortium

The BPRACTICES consortium, coordinated by the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (Italy), includes partners from five European countries:

- University of Namik Kemal (Turkey)
- Agricultural Institute of Slovenia (Slovenia)
- University of Maribor (Slovenia)
- Centro de Investigación Apícola y Agroambiental de Marchamalo (Spain)
- Austrian Agency for Health and Food Safety (Austria)
- Istituto Zooprofilattico Sperimentale delle Venezie (Italy)

and has the valuable collaboration of:

- University of Genova (Italy)
- Mississippi State University (USA)
- European Union Reference Laboratory for Bee Health (ANSES, France)
- International Federation of Beekeepers' Associations (Apimondia)
- FAO (TECA Beekeeping Exchange Group)
- European Professional Beekeepers' Association

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Foreword

The high value of beekeeping as a zootechnical sector and the even higher value given by the pollination service provided by the bees, must be worldwide preserved and improved with a sustainable approach.

Several stressors in Europe are affecting beekeeping, that is facing numerous challenges due to a variety of factors, mainly related to globalization, agrochemical pollution and environmental changes. Moreover, it is possible to record an increased emergence of pathogens like *Aethina tumida* and *Nosema ceranae*, that are introduced into a geographical area where they are not normally found, with serious negative consequences.

In this context, BPRACTICES project (European Union's Horizon 2020 research and innovation programme Grant Agreement n° 696231, ERA-Net SusAn – European Research Area on Sustainable Animal Production Systems) was originated. The Guidelines on Sustainable Management of Honey bee Diseases in Europe are the milestone of activities carried out during the 36 months of the project. Hereby you will find a definition and a list of the most relevant management practices (Good Beekeeping Practices - GBPs) and Biosecurity Measures in Beekeeping (BMBs), harmonized within Europe.

I would like to thank the partners who authored this work and all the BPRACTICES Consortium for the help in revising and improving the final version of the document.

Finally, the BPRACTICES Consortium and I would like to thank all the colleagues and individuals who were not involved in the project but equally contributed with their work to the implementation and success of the Project.

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Introduction

Good beekeeping practices (GBPs) are *"those integrative activities that beekeepers apply for on-apiary production to attain optimal health for humans, honey bees and environment". The implementation of the GBPs, therefore, would have a positive effect on colony health, on society and at the same time could favour high production standards.* GBPs are also meant to support beekeepers in decision making at the apiary level, leading them towards the most sustainable and resilient strategies.

GBPs should be clearly distinguished from BMBs that are "all those operational activities implemented by the beekeeper to reduce the risk of introduction and spread of specific honey bee disease agents". Biosecurity is the foundation of all disease control programmes and it is irrespective of the animal species. If biosecurity is well implemented, it is possible to reduce curative treatments at the apiary level to an absolute minimum.

GBPs at the international level may represent the universally accepted pre-requisites to guarantee a proper sustainability, competitiveness and resilience of the apiculture sector to face the current challenges of modern beekeeping. On the other hand, BMBs could vary between the geographical areas: due to local factors (e.g. climatic conditions, beekeeping technology, bee races or breeds) or the different prevalence, virulence and economic impact of the diseases. Moreover, BMBs are continuously evolving along the time, depending to changes in the prevalence of the different diseases and, more in general, to biotic and abiotic stressors. Finally, regulatory provisions may have a strong impact, especially on the control strategies of the diseases.

Concerning the relationship between the GBPs and BMBs, we can say that GBPs are the basis for a sustainable and resilient beekeeping and represent a pre-requisite for the implementation of BMBs in the day-to-day apiary management. Only if GBPs are systematically implemented by the beekeepers, BMBs can be properly tackled.

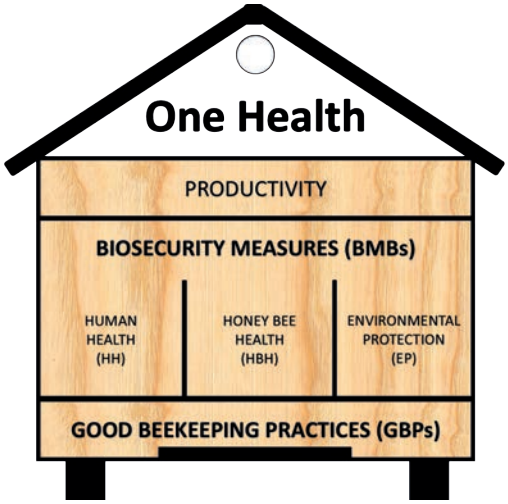

Figure 1. Visual representation of GBPs and BMBs (IZSLT)

GBPs, BMBs and the traceability system are just a starting point, a prompt, for all beekeeping stakeholders with the aim of creating a sector ready to face the new challenges of globalization. In fact, quality and safety of hive products is the logic consequence of a proper production at the apiary level as GBPs and BMBs permit to reach the top quality standards. The best way of making consumers aware of this is to set-up a cutting-edge traceability system using the QR-code/RFID technology that was implemented with the BPRACTICES project.

In this document it will follow the description of the main European Union honey bee diseases, their diagnosis, the related GBPs and BMBs and possible sustainable strategies to properly manage them. In conclusion, an example of application of the above mentioned traceability system will be provided.

The BPRACTICES consortium

1. Varroosis and virosis

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1.1. The disease in short

Varroosis is caused by the ectoparasitic mite *Varroa destructor* (Anderson & Trueman, 2000). The mite (Figure 1) is native to eastern Asia, originally the parasite of *Apis cerana*. Shift to new host *Apis mellifera* occurred in 1950s in Japan and has spread through Soviet Union to Europe and the rest of the world. Nowadays, it is present world-wide, the only exception is Australia and South Western Indian ocean islands (Muli et al., 2018). Two haplotypes are known; Japanese which is less virulent and Korean which is more aggressive.

Mites need bee brood in order to reproduce. A foundress mite invades the cell with larvae just prior the capping. After that, the mite starts laying eggs, in total up to six eggs. One of those eggs hatches to male and all others to female mites that feed on developing pupae and when they reach maturity they mate in the capped cell. After the mating male mites die and female mites hatch with the young adult bee. In the first days of life, adult female mites parasitize on adult bees, preferably nurse bees with still well-developed fat body. After this so called phoretic stage adult mites invade brood cells and the entire cycle is repeated.

Figure 1. Varroa mite (Shutterstock)

Mites feed primarily on fat body of developing larvae, pupae and adult bees (Ramsey et al, 2019). If affected larvae manage to survive the infestation (normally at lower infestation levels), the adult bees are affected having a smaller body size, weakened immune system and in some cases deformed wings. In the case of severe infestation the infested larvae/pupae dies. That could be observed as a spotty brood pattern. Mites are transmitted between colonies by robbing, drifting and transferring brood frames and adult bees.

Varroa is also a very important vector of many honey bee viruses. Most common are: acute (ABPV) and chronic bee paralysis virus (CBPV), sac brood virus (SBV) and deformed wing virus (DWV) which is also able to replicate in mites. Relationship between viruses and varroa mites is still full of mysteries that needs to be discovered but it is known for sure that with the occurrence of varroa incidence of clinically visible viral diseases increased throughout the world. Viruses are transmitted with feces, trophallaxis, eggs and semen. Viruses can survive in different materials that were in contact with bees and brood.

1.2. On-field diagnosis

There are many methods to assess varroa infestation levels in an apiary. We can determine percentage of

infested adult bees in colonies, percentage of infested pupae or assessment of mite population size by monitoring the natural death rate, so called natural mite fall.

Two on-field methods to assess mite infestation levels on at least 300 adult bees without sacrificing them are the so called "sugar shake method" and "CO_2 method". The bees are collected from the brood box (1g of bees are approximately 10 individual workers) and put in a jar with lid made of mesh (plastic, metal). For sugar shake method one tablespoon of powdered sugar is added and the jar is gently rolled to cover all the bees with sugar. Then the jar is shaken for about 1 minute above a white plate filled with water. At the end, the mites are counted on the water surface and the percentage is calculated according to the number of bees. Similar to sugar shake method, in the CO_2 test is used to anesthetize the bees and mites.

Alcohol or soapy wash and ether roll are also used to detect mites on adult bees. However, when using these methods, bees need to be sacrificed. Alcohol wash is done in a jar, then alcohol is added to the sample of bees and stirred to dislodge mites from the bees. Mixture of alcohol and bees needs to be poured over the sieve to separate parts of bees and mites. Mites are than counted in the alcohol. Instead of alcohol also soapy water can be used. For the ether roll method, a jar with a screen raised 2-3 cm above the bottom is sprayed for two seconds with automobile starter fluid to kill bees and mites. Then the jar is sprayed with ether and shaken for 1 min to dislodge the mites from the bees. After that the jar is put sideways and rolled three times along its vertical axis. The mites stuck to the sides of the jar and can be counted.

For the determination of percentage of infested pupae at least 200 cells of worker bees or drones must be uncapped and carefully examined for mites that need to be counted and their average number calculated. To get the number of naturally fallen mites, the hives must be equipped with a bottom board, on which debris is collected. The board must be protected by a mesh to prevent bees from discarding the dead mites. The mesh size should allow the mites to fall through. Investigating the hive debris in the summer in colonies with sealed brood is the most efficient method for detection of mites. Mites are also often found in hive debris at low infestation levels the first year of infestation without the use of chemicals. It is a method easy to apply but to some extent inexact due to mites removed by ants and other insects from the floorboard.

Viral diseases in bee colonies are mainly detected by clinical symptoms. Symptoms of deformed wing virus (DWV) can be recognized by smaller, darker bees with deformed wings and other extremities. Bees can also be more aggressive when the titer of DWV is high because it is replicating also in their brains. In case of chronic bee paralysis virus (CBPV) bees with trembling wings and body could be observed on the hive entrance. Bees also lose flight capability and their hairs, and are rejected by healthy members of the colony. Acute bee paralysis virus (ABPV) can normally be found in the fat body of bees. It can cause high mortality of adults and brood in combination with high varroa levels. Sac brood virus (SBV) affects larvae after the capping of the brood. Clinically affected cells have perforated cappings and contain bloated larvae which resemble a fluid filled sac. Later on these larvae became yellow-brownish and dry out to a typical brittle, brown-black coloured scale, which is easily removed from the cell.

1.3. Laboratory methods for diagnosis

For the laboratory diagnosis of varroosis, alcohol wash is normally used as described in Section 1.2. A sample of at least 300 bees from the brood box should be frozen and delivered to the laboratory on ice (frozen). Uncapping of the sealed brood as described above could also be used.

Viruses are most frequently detected by molecular methods where viral RNA is detected. RT-PCR is used to detect whether a sample is positive or negative and real-time PCR is used to quantify the number of virus particles. Beside that it is possible to detect viruses also by a serological method (ELISA, classic or sandwich ELISA could be used). Viruses can be detected from different matrixes: adult bees, brood and hive debris. Samples must be frozen as soon as possible after the collection and delivered frozen to the laboratory.

It is very important to mark the samples according to both the hive and the apiary. When sending samples to the laboratory, a short letter to accompany the sample should be written. The following information must be included: date of sampling, number of colonies in the apiary, number of infected colonies, last data about varroa infestation levels, the date of last varroa treatment and the veterinary medicine used.

1.4. Good beekeeping practices to prevent the disease

There are some good beekeeping practices that should be applied in every beekeeping operation to prevent varroosis and other diseases.

Figure 2. Frame placed in the trapping comb to perform brood interruption (AIS)

All live bees, brood and queens should originate from beekeeping operations and colonies that are free of diseases. All live bees should be examined by a veterinarian and a health certificate should be issued to guarantee the bees are free of diseases. Before introducing to the new apiary, bees should be kept for at least one month in a so-called quarantine apiary. It must be avoided, as far as possible, the introduction of swarms of unknown origin. When transferring frames of brood (Figure 2) or adult bees within colonies to balance the strength of the colonies the beekeeper must be sure that all those materials originate from the healthy colonies. All the tools that enter in contact with bees and hives should be periodically cleaned and disinfected, especially after handling diseased or dead colonies. Dead colonies should be removed quickly from the apiary and samples should be collected for further laboratory analysis. The risk for robbing must be minimized by reducing hive entrances, not feeding the colonies in the open, and having well maintained hives without any additional openings. The risk for drifting must also be minimized by painting and marking hives and not having too many colonies in a single row.

For treating varroa only authorized veterinary medicinal products (VMPs) can be used. All the treatments

must be carried out according to the label. The withdrawal period must be observed and the hive products from treated colonies are not to be used for human consumption until the withdrawal period elapsed. When using instruments for the application of VMPs (formic acid dispensers, oxalic acid sublimators), they must be appropriate and correctly calibrated.

The record of veterinary medicines used must always be kept. The apiaries must be registered in a national register of apiaries. All the colonies in the same apiary should be marked with unique identificatory. Beekeepers should frequently renew their knowledge of honey bee diseases.

1.5. Biosecurity measures to manage the disease

Before the treatment against varroa mites it is very important to use diagnostic methods to assess infestation levels. Mite levels should be always below the harmful threshold. To achieve that, at least two treatments should be done per year: one in summer and one in winter. All the colonies in the apiary must be prepared in advance for the treatment: honey supers removed, colonies well-fed and hive entrances narrowed to prevent robbing. All the colonies in one apiary must be treated simultaneously. It is also advised that beekeepers in a certain area organise and treat at the same time to prevent re-infestations.

Veterinary medicine products (VMPs) to treat varroosis must be always chosen from the list of authorized veterinary medicines for honey bees. VMPs, especially synthetic acaricides, must be rotated in order to avoid mite resistance. Preferably VMPs allowed in organic beekeeping should be used because they do not jeopardize hive products with residues and mites do not develop resistance to them. After the treatment verify the efficacy of the treatment with above described methods. To increase the efficacy of treatments and reduce the use of VMPs, different biotechnical measures should be used, like adoption of screened bottom boards (Figure 3), brood interruption or use of queens originating from varroa resistant/tolerant lines combined in so called integrated pest management (IPM).

Figure 3. Bottom board with mites as a tool to control efficacy of the treatment (AIS)

In order to prevent the spreading of mites and viruses, nuclei and swarms should originate from healthy colonies without clinical symptoms of any disease. Nuclei and swarms should also be treated with registered lactic or oxalic acid preparations when still broodless to obtain the maximum efficacy. Sufficient number of spare colonies should be provided to substitute lost colonies.

To manage viral diseases, an effective varroa management must be introduced and drone colonies should be checked and be free from clinical symptoms of diseases, especially those caused by viruses to prevent venereal transmission by mating, fertilization of eggs or oviposition.

1.6. Example of strategies for sustainable control

To manage varroa mite and viral disease effectively and sustainable, the IPM must be introduced. This a combination of beekeeping techniques and use of VMPs.

One of the possible yearly strategies is to treat varroa with formic acid in summer. Formic acid works also in brood but the beekeepers must pay attention on the external temperature, and it should not be too high (not above 30 degrees Celsius). Winter treatment could be done with oxalic acid in broodless conditions. Oxalic acid could be applied by dripping sucrose syrup with oxalic acid on the bees or by sublimating oxalic acid through the hive entrance. If brood interruption does not occur naturally, the queen can be caged.

To slow down the multiplication of the mites, all the queens should originate from lines with varroa sensitive hygienic behaviour or resistant lines and colonies should be housed in hives with screened bottom boards.

All the nuclei and swarms should be treated with registered oxalic or lactic acid preparations.

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2. American Foulbrood (AFB)

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2.1. The disease in short

American Foulbrood (AFB) is subject to the Regulation (EU) 2016/429 ('Animal Health Law') and a listed disease. Measures must be taken to prevent its spread associated with the import from third countries into the EU or by movements between EU-Member States. In case of an outbreak of the disease, restrictions on trade (quarantine zones) are put into force. AFB must be monitored and notified to the competent authorities.

AFB is distributed almost all over the world. It is caused by *Paenibacillus larvae*, a gram-positive spore-forming bacterium. It is highly infectious for young larvae while adult bees do not become infected. Honey bee larvae are most susceptible for the AFB infection 12-36 h after hatching from the egg. Only a few spores are necessary to initiate an infection in this early stage.

Four different *P. larvae* strains (ERIC I – IV) are known. They vary in their clinical symptoms, the virulence and in phenotypical characteristics like germination rate, temperature sensitivity, storage resistance, as well as sporulation rate.

The infection starts with the oral uptake of *P. larvae* spores by a bee larva. The spores germinate in the midgut, where the bacteria start massive proliferation. After breaking through the intestinal wall, they invade into the inner parts of the larva, causing its death and further decomposition to a ropy mass. The bacteria sporulate and the ropy mass dries down to a highly infective, hard scale, which is firmly attached to the cell wall.

P. larvae can survive in bee products for several years and up to 35 years in dry larval scales. The long survival rates and the contagiousness of AFB are serious problems in AFB control and need thorough and effective sanitation measures to eliminate the disease in an affected apiary. Some factors, like hygienic behaviour of bees, could reduce the AFB prevalence. However, without countermeasures AFB usually leads to a collapse of the infected hive.

The transmission of *P. larvae* can occur by honey bees through drifting, robbing and swarming as well as by the beekeepers themselves through the transfer of spore contaminated hive material like combs, honey, pollen or feeding honey or pollen from other apiaries, the exchange of beekeeping equipment between colonies and apiaries, or trade or migration of infested honey bee colonies.

To reduce the risk of transmission, comply with legal obligations for notification in case of suspicion or clinical outbreak of the disease. Strictly observe restrictions on colony movement and transportation of beekeeping equipment and hive products. Follow the instructions from the competent veterinary authorities. Generally, keep your data records and the exact position of the bee yards in the national beekeeping registry up to date.

2.2. On-field diagnosis

First signs of AFB are a spotty brood pattern, inward sunken and sometimes punctured cell caps with a moist

and dark discoloured appearance (Figure 1). Cells containing coffee-brown dead larvae or pupae without body segmentation are suspicious to be AFB-infected. Due to the decomposition of infested larvae to a ropy mass, there is a rotting smell when opening the colony in case of a heavy infestation. To confirm the suspicion in the field, perform a "matchstick" or "ropiness test" by touching the suspicious cell content with a stick and slowly pulling it back. In case of fresh infestation, a characteristic fine thread can be drawn from the cell (Figure 2). Alternatively, you may use an AFB-diagnostic test kit. At an advanced stage, the decomposed larvae have dried down to a black, hard scale, which is firmly attached to the lower cell wall.

Because of the serious consequences for the beekeeping operation concerned in case of a suspected AFB outbreak, the confirmation of the disease by proof of *P. larvae* as causative agent by an authorized laboratory is highly recommended. If the suspicion or outbreak of AFB is confirmed, comply with legal obligations in your country for notification to the competent authorities.

Figure 1. Brood comb with symptoms of AFB (spotty brood nest, discoloured, sunken cell cappings, scales)

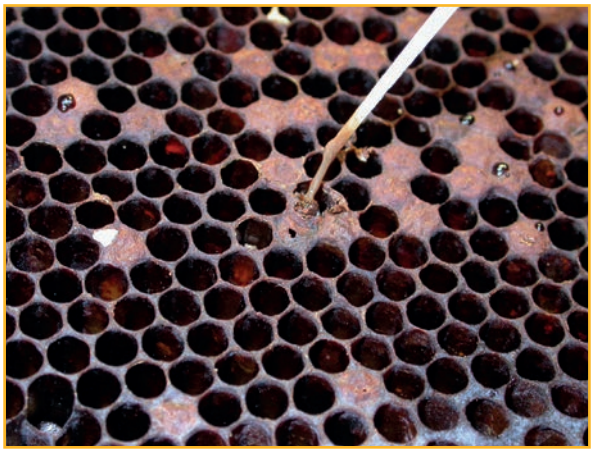

Figure 2. Ropiness test on suspicious brood cells

2.3. Laboratory methods for diagnosis

In colonies without clinical symptoms, for the purpose of prevention, adult bees, honey, wax, pollen or hive debris could be checked for *P. larvae* spores in the laboratory. From these matrices food store samples from brood combs have proven as a simple and effective way to collect authentic material from honey bee colonies to verify the presence of *P. larvae* as a preventive measure already in the preclinical stage.

In case of qualified suspicion for an AFB-outbreak (e.g. clinical symptoms, positive results of a ropiness test or from an AFB-diagnostic test kit), a piece of the tested brood comb should be sent to an authorized laboratory, preferably by the competent authorities.

Effective and established methods for the detection of viable *P. larvae* bacteria are incubation of suspected material on several media (e.g. MYPGP-agar, Columbia sheep blood agar, Columbia slant agar) to cultivate *P. larvae* to check for colony growth, catalase reaction and for giant whips by light microscopy. Biochemical profiling, antigen detection, conventional and real-time PCR as well as mass spectrometry are other methods to test for the presence of the pathogen.

2.4. Good beekeeping practices to prevent the disease

GBPs are in general highly recommended to prevent honey bee diseases. They are useful measures to avoid the introduction of pathogens, including *P. larvae*.

To have up-to-dates knowledge in beekeeping and honey bee diseases, take regular courses and training programmes.

Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen at least in spring after wintering, before supering for the first nectar flow and after the last honey harvest before preparing colonies for wintering. Check for *P. larvae* in asymptomatic colonies by laboratory tests (regularly once a year, e.g. stored honey from brood combs, hive debris) to detect the pathogen already in the subclinical stage to be able to apply promptly adequate countermeasures.

Record the health status of the colonies (healthy, diseased, infected, dead), dates of inspections, diagnoses, ID of colonies affected, treatments and results to have a current overview. Quickly inspect dead colonies for infectious diseases; remove all the material safely and melt down all combs from dead colonies. Scrape off wax and propolis and clean (for example with NAOH, hypochlorite) - as a measure of general hygiene. Do not have beekeeping material abandoned in the apiary.

Clean the equipment on a regular basis, before installing new colonies. Do not move frames or any kind of biological material (for example brood or food combs) from one hive to another in case their health status is not known. Do not feed your bees openly in the field to prevent robbing and spread of diseases.

Do not feed the bees with honey, pollen or supplements, unless the absence of pathogens (spores of AFB, chalkbrood, *Nosema*, EFB, etc.) is certified by a laboratory analysis.

Build your nuclei only with bees and brood combs from healthy colonies, i.e. negatively inspected for bee diseases. Only use material from healthy colonies for balancing colony strengths or food stores of different colonies. Buy colonies only after thorough inspection for bee diseases - preferably with a health certificate from a veterinarian. Avoid, as far as possible, the introduction of swarms from unknown origin and keep newly introduced colonies or swarms separate from the existing stock for an appropriate period (at least one month) in order to monitor them against diseases to prevent transmission. Respect hygiene rules (e.g. periodically cleaning of suits, gloves, veil, beekeeping equipment, etc.) and practice good hygiene when dealing with dead colonies (combs, food stores, boxes, etc.).

If *P. larvae* was already detected in your beekeeping operation, but clinical signs of the disease are absent (= subclinical state), apply the following measures in addition to the previously recommended.

Use disposable gloves when inspecting or handling possibly diseased hives. Inspect hives with pathogen negative status first, then those with known subclinical infection.

Check all your colonies in short intervals to spot early signs of the disease. Do not exchange any hive equipment, combs, etc. between colonies or apiaries. Install new colonies only by artificial swarms in new or disinfected hives on foundation in a separate apiary and feed them solely sugar syrup.

Quickly remove beehives with dead colonies and burn any equipment, which is not worth to be kept or not disinfectable. Clean and disinfect contaminated beekeeping equipment (beehives, nuc-boxes, mating boxes, boards, frames, queen excluders, etc.), the honey house, extraction tools/facilities (uncappers, centrifuge, sieves, pumps, spins, etc.) and bulk honey storing or packing materials (tanks, barrels) thoroughly in order to eliminate the pathogen. Clean and disinfect levers and other potentially contaminated equipment (e.g. gloves) after inspection of hives affected by transmissible diseases.

2.5. Biosecurity measures to manage the disease

Legal requirements are in place to monitor and manage the AFB-disease, for instance, the registration of the beekeeping operations in the national beekeeping registries, including regular updates and the notification of a suspicion or an outbreak of the disease.

The beekeeper has to promptly notify the suspicion or the outbreak of the AFB-disease to the competent authorities. To control the disease and to get rid of it, a quick and consequent management of affected hives is important. This includes to comply with legal obligations concerning restrictions of colony movement, transportation of beekeeping equipment or hive products, and to follow the instructions from the competent authorities concerning quarantine and sanitation measures.

In addition, several further actions and measures of GBPs are necessary in case of a clinical outbreak for a sustainable elimination of the disease and the pathogen from the apiary.

Most important is to perform the sanitation measures on all colonies of your apiary, regardless of AFB-symptoms. Perform the shook swarm procedure (see Section 2.6.), using new or disinfected hive material, foundation and sugar syrup for their reinstallation.

Melt down the combs of all colonies of the affected apiary, regardless of clinical symptoms, and get the wax safely processed by a certified producer of beeswax foundation.

Clean and disinfect all beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of the whole apiary, irrespective whether from AFB-symptomatic or asymptomatic colonies!

Burn all hive equipment, which is not worth to be kept or is not disinfectable with justifiable expense and effort.

Kill and incinerate affected colonies in case the disease appears in recently acquired colonies or swarms or affected colonies are too weak or if the season (late autumn or winter, early spring) does not allow a successful shook swarm sanitation procedure.

Disinfect heat insensitive hive equipment and beekeeping tools by torching (blue flame) in case of transmissible diseases. This is a practical method for most beekeepers. Alternatively, a treatment with bleach (soda, NaOH, etc.) is effective. Only use biocidal products that are registered for that purpose.

Require and keep all commercial and health documents for each colony or group of colonies, enabling their exact itinerary to be traced from their farm or establishment of origin to their final destination.

Remove queens from colonies with a clinical history of AFB disease and replace them with queens from a healthy stock. Perform genetic selection in order to have queens that are more resistant to diseases and adapted to local climatic conditions.

2.6. Example of strategies for sustainable control

The above mentioned GBPs and BMBs are an essential part of any strategy for sustainable control of AFB. As practice had shown, the shook swarm procedure is an effective method to eliminate *P. larvae* spores and to get rid of the disease in case of a clinical outbreak.

Because brood combs, pollen and honey stores, as well as the hive equipment are contaminated with *P. larvae* spores, bees have to be separated from these materials by the shook swarm procedure to achieve a successful and sustainable AFB control and elimination. As *P. larvae* spores could be in your colonies long before the occurrence of clinical symptoms it is necessary to submit all colonies of such an apiary to the shook swarm procedure – irrespective of AFB-symptoms.

Short outline of the shook swarm procedure for AFB-elimination and control

Preparations: Plan the procedure thoroughly and prepare the necessary equipment: swarm boxes, large funnel, water sprayer, hive brush, disposable gloves, a cool and dark room to keep the shook swarms in quarantine for two days - if available, new or disinfected hives and frames with foundation.

Check if infested brood combs and other material could a) be burned near the apiary or b) will need a transport to a waste incineration plant. In case a): prepare the incineration place and inform the local fire brigade about the activity. In case b): provide thick cardboard boxes for the brood combs of infested colonies and packing material for other equipment to be transported and burnt safely. If possible, ask for support in practical work when making the shook swarms and disinfection activities.

Figure 3. Making shook swarms in case of a clinical AFB-outbreak

Making the shook swarms: Ideally, the shook swarms should be made in the morning or evening when the bees' flight activity is low. Depending on the availability of a cool and dark quarantine room and the strength of the colonies, two different shook swarm procedures shall be applied: a) use of swarm boxes with wire mesh, and b) open shook swarm procedure in the apiary using new or disinfected hive boxes.

a. Put the swarm box onto the ground, put the funnel in, put disposable gloves on, open the hive, take out one comb after another and shake off the bees and the queen into the funnel and the swarm box (Figure 3). Take care not to lose the queen during shaking off the bees. To support this, you may put her into a queen cage if you discover her by chance and add her to the

swarm box later. Pass on the combs without bees to your helper to be sorted according to the following scheme: a) honey combs into a closed box with bottom and a lid – to be extracted later on in a bee-tight extraction room, b) brood combs of infested colonies into the cardboard boxes for incineration, c) empty combs into cardboard boxes – to be melted safely later at a wax processing company. Shake off all remaining bees from the hive cover, the hive boxes and the bottom board into the swarm box. Then close the swarm box tightly and put it on the ground in a shady place until all colonies of the apiary have been treated in the same way. If there are a lot of bees in the air you can leave a clean, empty hive box with bottom board and lid in the apiary to collect these homeless bees during the night. The next morning you can add them to one of the swarms in the quarantine room. Bring the swarm boxes into the quarantine room and leave them there for two days. During this quarantine phase the bees should consume the honey from their honey sac to eliminate *P. larvae* spores contained therein. If bees start falling from the swarm cluster during that time, give them a small amount of candy or sugar syrup. In the evening of the second day or the morning after, hive the swarms in new or disinfected hive boxes with foundation and feed them with at least five liters of sugar syrup.

b. **Attention**: if your colonies are very strong you must not put more than 2.5 kg of bees in one swarm box to prevent them from suffocation! In this case, or if there is no cool, dark place available to keep the swarms in quarantine, you have to do the whole shook swarm procedure at the apiary in the open. If possible, choose a period without or with only poor nectar or honeydew flow. Set the colony to be treated aside, put a new or disinfected hive box with foundation on the former place, install the funnel (if possible in the rear opening of the bottom board) and shake off the bees into the funnel comb by comb. They will run into the dark of the hive box and settle on the frames. Do not feed the bees for two days. Then check for comb building, presence of the queen and feed them with at least five liters of sugar syrup.

One week after application of either method, check for comb building and the presence of brood. Feed five liters of sugar syrup if there is no nectar or honeydew flow. As practice had shown, both methods are effective to eliminate *P. larvae* spores and get rid of the disease in case of a clinical outbreak.

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3. European Foulbrood (EFB)

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3.1. The disease in short

EFB is not restricted to Europe and can be found almost all over the world. Besides *Apis mellifera*, *M. plutonius* can also infect *Apis cerana* and *Apis laboriosa*. In Europe, occurrence of *M. plutonius* varies widely between different regions. In the last decade, increased numbers of EFB infections leading to massive outbreaks have been reported (e.g. in Switzerland and the UK). Also countries, which had been free of EFB outbreaks for several decades (e.g. Norway and the Czech Republic), suffered recent outbreaks.

EFB is a bacterial brood disease caused by the gram-positive bacterium *Melissococcus plutonius*. The causative agent *M. plutonius* mainly occurs as lanceolate coccus, is microaerophilic and needs carbon dioxide for growth. *M. plutonius* is a non-spore forming bacterium, which can persist and remain infectious in honey bee colonies without causing symptoms. It is separated into two subtypes: the typical and the atypical *M. plutonius* strains, which have different virulence and could lead to different disease development. *M. plutonius* infects unsealed brood, and the larvae become infected by feeding on contaminated food, with younger larvae being more affected than older ones. Larval mortality seems to be correlated with the quantity of the ingested pathogen. Infected larvae usually die when four to five days old, but may survive until the pupal stage, in which the faeces contain many viable cells of the pathogen. Dead larvae decompose and dry out to easily removable scales. *M. plutonius* infections are often associated with other bacteria (*Paenibacillus alvei*, *Lactobacillus kunkeei*) which have influence on the observed symptoms (e.g. the smell, or the consistence of decomposed larvae).

It seems that outbreaks occur more frequently under stress conditions (food shortage, small colonies, lack of queen, etc.). Genetic predisposition is another factor influencing the hygienic behaviour of the bees. Diseased colonies can recover spontaneously if moved to areas free of EFB.

Vectors of disease transmission may be the honey bee or the beekeeper. The disease can be transmitted between hives or apiaries by robbing, drifting or swarming, with the adult bee as carrier of *M. plutonius*. By this, food and pollen stores as well as all inner parts of the hive are contaminated. The pathogen is then transferred by nurse bees to the young larvae during feeding.

The beekeeper can act as a vector from hive to hive, if he uses contaminated beekeeping equipment or if he feeds contaminated honey to healthy colonies. Transferring contaminated combs (brood, pollen, honey) between colonies or apiaries to balance food stores or building nucs is another source of pathogen transmission. The migration or sale of sub-clinically infested colonies without any symptoms can also lead to a transmission and spread of the disease.

3.2. On-field diagnosis

Clinical signs of EFB are flaccid, yellowish and contorted or upwards curled larvae, which often lose their

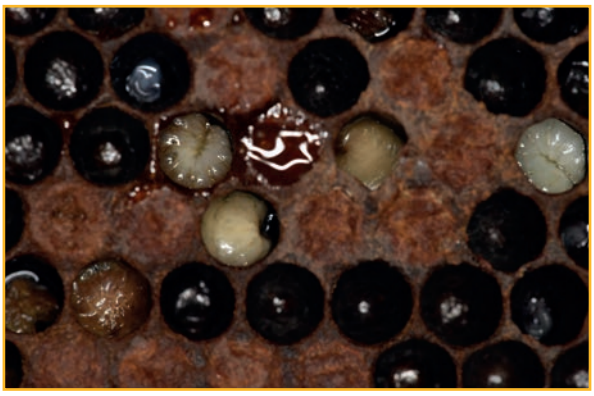

Figure 1. Brood comb with symptoms of EFB
Photo credits: Massimo Palazzetti

Figure 2. Brood comb with symptoms of EFB
Photo credits: Massimo Palazzetti

segmentation. Dead larvae appear twisted in the cell, slimy but not ropy as in case of AFB and later become dark but – in contrast to AFB – easily removable scales (Figures 1-2). Another clinical sign of EFB is a typical sour smell after opening the hive („Sauerbrut"). Other bacteria associated with the infection might have influence on the symptoms observed.

Please be aware that some symptoms of EFB resemble those of American Foulbrood (e.g. spotty brood pattern, dead and decomposed larvae, scales in cells). Therefore, take care not to confuse the two diseases in the field. In contrast to EFB, clinical symptoms of American Foulbrood are typically inward sunken and sometimes punctured cell caps with a moist and dark discoloured appearance and the coffee-brown ropy mass of decomposed larvae in the brood cells. Biosecurity measures and good beekeeping practice demand to verify promptly any suspected symptom of disease, e.g. with a diagnostic test kit for EFB. Furthermore, a laboratory analysis is recommended for clarification and identification of the causative pathogen. If the suspicion or outbreak of EFB is confirmed, comply with any related legal obligations in force in your country.

3.3. Laboratory methods for diagnosis

Beside the traditional methods such as cultivation of *M. plutonius* and microscopy, newer techniques such as immunology- or PCR-based methods are available for the unambiguous identification of *M. plutonius.*

3.4. Good beekeeping practices to prevent the disease

Complying with GBPs is generally highly recommended to prevent honey bee diseases. They are useful measures to avoid the introduction of pathogens, including *M. plutonius.* To have up-to-date knowledge in beekeeping and honey bee diseases, take regular courses and training programmes.

To reduce the risk of transmission of the disease and the pathogen, comply with legal obligations in your country, such as notification in case of suspicion or clinical outbreak of the disease. Observe restrictions on colony movement, and transportation of beekeeping equipment and hive products.

Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen at least in

spring after wintering, before supering for the first nectar flow, and after the last honey harvest before preparing colonies for wintering. Upon inspection, record the date, the ID of colonies, diagnoses and health status of the colonies (healthy, subclinically infected, diseased, dead), treatments and their effects to have a current overview. Quickly inspect dead colonies for infectious diseases, remove all the material safely, melt down all combs from dead colonies. Do not have beekeeping material abandoned in the apiary.

Scrape off wax and propolis, and clean the equipment (for example with soda, NAOH, hypochlorite) on a regular basis before installing new colonies, as a measure of general hygiene. Do not move frames or any kind of biological material (for example brood or food combs) from one hive to another in case their health status is not known. Do not feed your bees openly in the field to prevent robbing and spread of diseases. Do not provide the bees with honey, pollen or supplements, unless the absence of pathogens (spores of AFB, chalkbrood, *Nosema*, EFB, etc.) is certified by a laboratory analysis.

Build your nuclei only with bees and brood combs from healthy colonies, i.e. negatively inspected for bee diseases. Only use material from healthy colonies for balancing colony strengths or food stores of different colonies. Buy colonies only after thorough inspection for bee diseases - preferably with a health certificate from a veterinarian. Avoid, as far as possible, the introduction of swarms from unknown origin and keep newly introduced colonies or swarms separate from the existing stock for an appropriate period (at least one month) in order to monitor them against diseases to prevent transmission. Respect hygiene rules (e.g. periodically cleaning of suits, gloves, veil, beekeeping equipment, etc.) and practice good hygiene when dealing with dead colonies (combs, food stores, boxes, etc.).

If *M. plutonius* was already detected in your beekeeping operation but clinical signs of the disease are absent (= subclinical state) apply the following measures in addition to the previously recommended.

Use disposable gloves when inspecting or handling possibly diseased hives and inspect hives free of the pathogen first, then those with known subclinical infection. Check all your colonies in short intervals to spot early signs of the disease. Do not exchange any hive equipment, combs, etc. between colonies or apiaries. Install new colonies only by artificial swarms in new or disinfected hives on foundation in a separate apiary and feed them solely sugar syrup. Quickly remove beehives with dead colonies and burn any equipment, which is not worth to be kept or not disinfectable. Clean and disinfect spore-contaminated beekeeping equipment (beehives, nuc-boxes, mating boxes, boards, frames, queen excluders, etc.), the honey house, extraction tools/facilities (uncappers, centrifuge, sieves, pumps, spins, etc.) and bulk honey storing or packing materials (tanks, barrels) thoroughly in order to eliminate the pathogen. Clean and disinfect levers and other potentially contaminated equipment (e.g. gloves) after inspection of hives affected by transmissible diseases.

3.5. Biosecurity measures to manage the disease

Legal requirements may be in place to monitor and manage the EFB-disease in your country, for instance the registration of the beekeeping operations in the national beekeeping registries, including regular updates and the notification of a suspicion or an outbreak of the disease. If there are such requirements comply with them and

follow the instructions from the competent authorities concerning quarantine and sanitation measures. In case of a clinical outbreak, further actions and measures are necessary for a sustainable elimination of the disease from your apiary.

Most important is to perform the sanitation measures on all colonies of the apiary, regardless of EFB-symptoms. Perform the shook swarm procedure (see section "American Foulbrood", Figure 3), using new or disinfected hive material, foundation and sugar syrup for their reinstallation. Melt down the combs of all colonies of the affected apiary, regardless of clinical symptoms, and get the wax safely processed by a certified producer of beeswax foundation. Clean and disinfect all beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of the whole apiary, irrespective whether from EFB-symptomatic or asymptomatic colonies! Burn all hive equipment, which is not worth to be kept or is not disinfectable with justifiable expense and effort. Kill and incinerate affected colonies or swarms if they had been recently acquired. The same is recommended if they are too weak or the season (late autumn or winter, early spring) does not allow a successful shook swarm sanitation procedure.

Disinfect heat insensitive hive equipment and beekeeping tools by torching (blue flame) in case of transmissible diseases. This is a practical method for most beekeepers. Alternatively, a treatment with bleach (soda, NaOH, etc.) is effective. Only use biocidal products that are registered for that purpose.

Require and keep all commercial and health documents for each colony or group of colonies, enabling their exact itinerary to be traced from their farm or establishment of origin to their final destination.

3.6. Example of strategies for sustainable control

The above mentioned GBPs and BMBs are an essential part of any strategy for sustainable control of EFB. As practice has shown, the shook swarm procedure is an effective method to handle clinical outbreaks and to get rid of the disease in your apiary. If you keep your bees in an area where EFB is endemic or recurring outbreaks occur frequently, an effective strategy for sustainable control should include the regular survey of all hives in the apiary for subclinically diseased colonies. Thus, you will be able to apply appropriate preventive measures as described. The shook swarm procedure is described in detail in Chapter 2. "American Foulbrood" (Figure 3).

Figure 3. Shook swarm procedure in case of EFB. (Photo credits: Massimo Palazzetti)

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4. Nosemosis

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4.1. The disease in short

Nosemosis is a group of honey bee diseases caused by Microsporidia of the genus *Nosema*. These unicellular organisms are obligate intracellular parasites, which form spores as resistance structure to resist outside of the infected cell in order to reach other hosts. To date, three species of microsporidia have been described infecting *Apis mellifera*: *Nosema apis* (Zander, 1909) and *Nosema ceranae* (Fries et al., 1996), worldwide distributed, and *Nosema neumanni* (Chemurot et al., 2017), described in Uganda. Due to their more recent identification, little information is currently available about the effects of *N. neumanni* infection, while the other two species, *N. apis* and *N. ceranae*, produce nosemosis type A and type C, respectively (Higes et al., 2010). Each species presents a different clinical picture that unevenly affects the viability of bee colonies and, depending on their degree of pathogenicity and frequency, has different serious consequences for the viability of hives.

These two species infect the bee's ventriculi but they differ in the morphology of spores and in the size of the genome (Figure 1). *N. ceranae* seems to have a greater capacity of adaptation to temperature, also producing higher levels of infection (in terms of number of bees infected) than *N. apis*. Recent findings of *N. ceranae* in different species of the order Hymenoptera suggest a greater degree of distribution of infections by this microsporidium, which appears to be less host-specific and more capable of adapting to other species of different families and orders (reviewed in Martín-Hernández et al., 2018; Goblirsch, 2018). Therefore, the infection caused by *N. ceranae* is considered an emerging disease in different parts of the world.

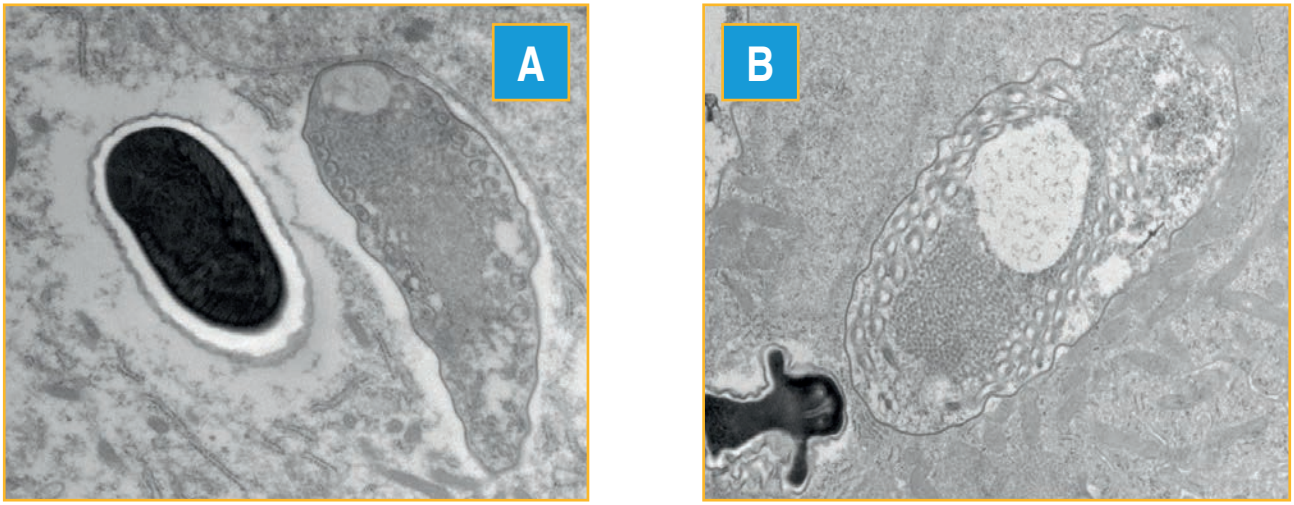

Figure 1. A. *Nosema ceranae* mature spore and immature stage in a ventricular cell of a honey bee. B. Sporoblast of *Nosema apis*. Notice the higher number of polar filament coils in *N. apis* than *N. ceranae*

4.2. On-field diagnosis

Nosemosis is a disease that does not produce easily identifiable signs as it does with other diseases affecting honey bees. As above mentioned, *N. apis* and *N. ceranae* infect the bee's ventricle (midgut) which is responsible for digestive functions, secreting substances that participate in the digestion of pollen and honey. Once the ventricle is infected by either species, the parasites multiply within the cells and the infection spreads throughout the organ. The cells of the ventricle (full of many parasitic forms), cease to perform their function adequately, which lead to the emergence of derived problems in the infected bees (reviewed in Martín-Hernández et al.2018). In this way, alterations are produced in the metabolism of carbohydrates that are translated into alterations in the flight and in a reduction in the capacity of the bees to return to their beehive. In addition, there is an alteration in bees' de-toxification capability and a modulation of their immune response, which makes them more vulnerable to certain toxins (e.g. some pesticides). There are also changes in some bee pheromones, which would explain why the infected bees begin duties normally undertaken by older bees (Bailey and Ball, 1991) as is the early onset of foraging activity. Finally, the life expectancy of bees infected with both *N. apis* and *N. ceranae* is noticeably reduced. They can infect all castes although workers and drones are more frequently found infected than queens.

Figure 2. Colony severely weak because of infection by *N. ceranae*

Consequently, all these effects on bees cause some clinical signs associated to the disease that could be detected in colonies after an inspection. These clinical signs are:

- progressive weakening of the colony, due to the constant loss of bees infected
- reduction in the efficiency of collecting pollen and nectar and therefore in pollination services and in colony productions. This is caused because the losing of bees infected, and because either the foragers infected or the younger bees recruited earlier for foraging have a reduced capability to collect nectar and pollen
- secondary brood diseases due to the reduction in interior bees that cannot take good care of the brood may occurr.

If the causes persist and these deficits (loss of bees and reduced food resources for the colony) are not compensated (supplementary feeding, population compensation, etc.), the colony weakens so much (Figure 2) that it ends up dying. The typical description of a colony depleted because of a Nosema infection, the queen can be observed surrounded by a few bees, attending to sealed brood (OIE 2018; Higes et al., 2008).

The main difference between the disease caused by *N. apis* and *N. ceranae* derives from the distinct epidemiology of both microsporidia. *N. apis* can be found in spring or autumn in temperate climates and it is rarely found in summer (Bailey and Ball., 1991) so the impact on the colony is limited. On the contrary, *N. ceranae* can be found infecting bees throughout the year (Higes et al., 2008), and the number of bees infected in a colony

is usually much higher than for *N. apis* (Martín-Hernández et al., 2012). Therefore, this constant presence of *N. ceranae* exerts a maintained chronic stress unbalancing the colony by exhaustion of the mechanisms of compensation of this one that has worse consequences.

There have been some attempts to design a field test based on Lateral-flow devices, but up to now, they are not commercially available. To check the suspicion of Nosema infection at the apiary, collect some foragers (as this population is the most infected in the colony) and dissect the ventriculus. To do this, hold the bee's abdomen and grip over the A7 abdominal dorsal and ventral segments using a forceps with the other hand. Pull it apart slowly so the posterior portion of the alimentary canal comes out up to the ventriculus. Infected bees' ventriculi usually have an appearance whitish and fragile, while it is usually more resistant and brown in healthy ones. However, this is not a pathognomonic sign as young bees or diseased by other causes could produce a similar look. In all cases, laboratory confirmation is required to evidence the presence of spores in bees. Finally, it should be noticed that although the infection by *N. apis* has been traditionally linked to dysentery in colonies, actually this is not a sign present in all colonies and there are some other diseases that can produce dysentery.

4.3. Laboratory methods for diagnosis

Due to the absence of specific clinical signs, a proper laboratory diagnosis should be made by determining the presence of spores and therefore confirming the infection.

One of the most used methods to confirm the presence of spores is by microscopy. This analysis should be done on the older bees in a colony, since this is the most infected population. So, collect forager bees at the hive entrance (or adult bees from a frame with no brood when foragers are not available), and at least analyze 60 bees (to detect 5% of sick bees with 95% confidence, Fries, 1993). Take whole abdomens or the digestive tract (see above for dissection) and macerate them in water. Examine the solution on a slide under a cover (x 400 magnifications) in a light field or preferably in a phase contrast microscope (Cantwell, 1970). Spores are refractory, with a well-defined dark edge. The spores of *N. ceranae* are smaller than those of *N. apis* which are oval. Fluorescence analysis has been also proposed to detect *Nosema* spp. spores (Snow 2016). However, mixed infections are frequent in colonies, and differentiating both species might be difficult. To confirm *Nosema* species use molecular tools as PCR, RT-PCR, or transmission electron microscopy.

4.4. Good beekeeping practices (GBPs) to prevent the disease

GBPs are designed to prevent honey bee diseases, so the full compliance with them would reduce the risk of the development of the disease and its spread among colonies and between apiaries. Any action that reduces the good general condition of colonies can favor the development of nosemosis, i.e. a productive stress, a non properly nutrition (scarce floral resources or honey stores, low-quality of supplementary feeding, etc.), a lack of renewal of wax and / or cleaning of the material, an inappropriate use of veterinary medicines, etc.

Some general recommendations to prevent nosemosis are:

- Selection of isolated apiaries as much apart from others as possible to reduce the risk of transmission from them. When available, select those location far away of pesticide treated crops, as those products have been demonstrated to produce a higher toxicity in the bees infected (mainly by *N. ceranae*)
- Avoid contact with sources of contamination:
  - » When bees have no access to running water and this is supplied by tanks, renew the water as much as possible and provide enough water supply points to avoid all bees drinking from the same place
- In *Nosema* positive colonies, bees crushed during handling can transmit the disease
- Decrease hives density: The higher the density, the greater the risk of diseases being transmitted. Apicultural intensification can also increase the risk of *Nosema* emergence, increasing the prevalence in the apiaries (Barlett et al., 2019)
- Eliminate / reduce stress factors: provide enough food and water. Reduce transport as in a colony closed during long time, the oldest bees (more probably infected and with higher parasitic load) get into direct contact with those younger so transmission is enabled
- Brood can be infected by Nosema spp., and honey and pollen can be contaminated with spores. Have it into account when equalizing colonies and use only these materials when you have tested them to be sure they are free of infection
- Test *Nosema* infection in drones for artificial insemination since they can be infected and transmit the infection to the queens
- Disinfection of beekeeping material (hives, tools, gloves, clothing, etc.) regularly and always after handling colonies and apiaries infected (Figure 3).

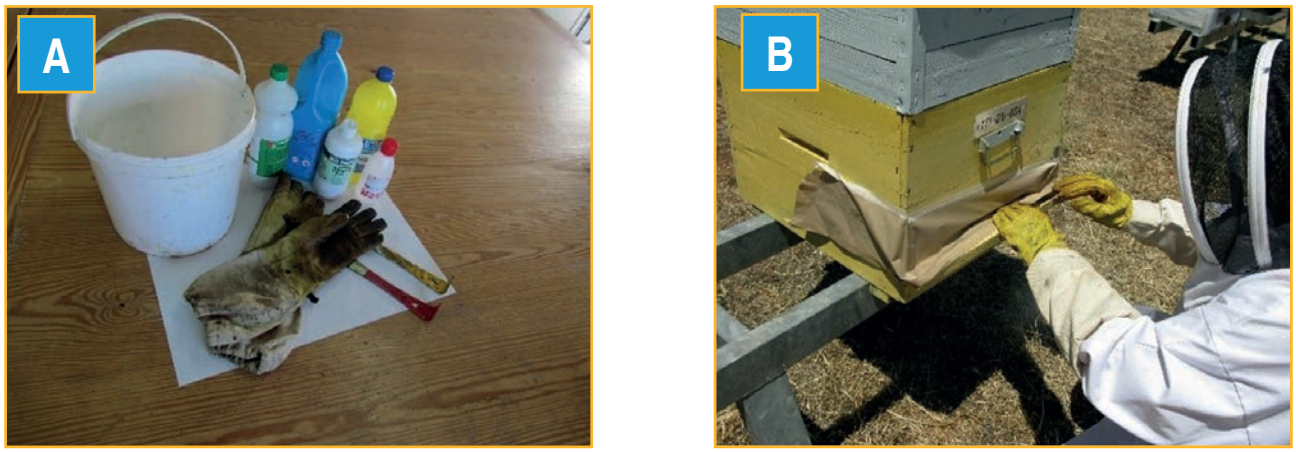

Figure 3. A. Disinfect beekeeping material after inspection of infected hives. B. Close the hive entrance to collect foragers as this is the most infected population.

4.5. Biosafety measures (BMBs) to manage the disease

Check colonies to determine if they are infected by using any laboratory method before to perform any control method. The best moment to get the colonies free or with low levels of Nosema infection is in autumn, as during winter confinement the infection will increase and it could involve a risk for the viability of the colonies.

It is also important to keep the size of positive colonies as much stronger as possible, as life expectancy greatly depends on this factor due to the early death of bees during winter or early spring. To this regard, the introduction of a new queen (spring, early summer) has been demonstrated to produce a dilution effect of parasitation, increasing production and reducing the risk of colony losses (Botías et al., 2012).

There is not any veterinary medicine product registered to control Nosemosis in colonies. Do not use any antibiotic to control *Nosema* infection (its use is forbidden). Some products registered throughout the EU mainly as feeding supplements for honey bee colonies (e.g. HiveAlive, Api-Herb, Nozevit, Figure 4) have been described to reduce the number of bees infected in colonies with a variable efficacy. Also oxalic acid (when applied trickled at the doses recommended for varroa control) have been shown to reduce the percentage of infection (Nanetti et al., 2015). Some other products have been tested at laboratory with promising results although they are not yet commercially available.

It is important to carry-out a careful disinfection of beekeeping material from those colonies dead after infection to avoid the transmission of the disease to future new colonies established in them. Spores of *N. apis* and *N. ceranae* can resist environmental conditions and high temperatures, being the latter more resistant (*N. ceranae* spores can survive up to 60°C). As well, acetic acid solution (60%) have been reported to inactivate spores of *N. apis* and it could be used to decontaminate sealed stack of boxes with combs (ventilated prior to their lately use). Ozone (Zanet et al., 2018) and gamma irradiation (Simone-Finstrom et al., 2019), have been also proposed to kill or reduce the viability of *N. ceranae* spores.

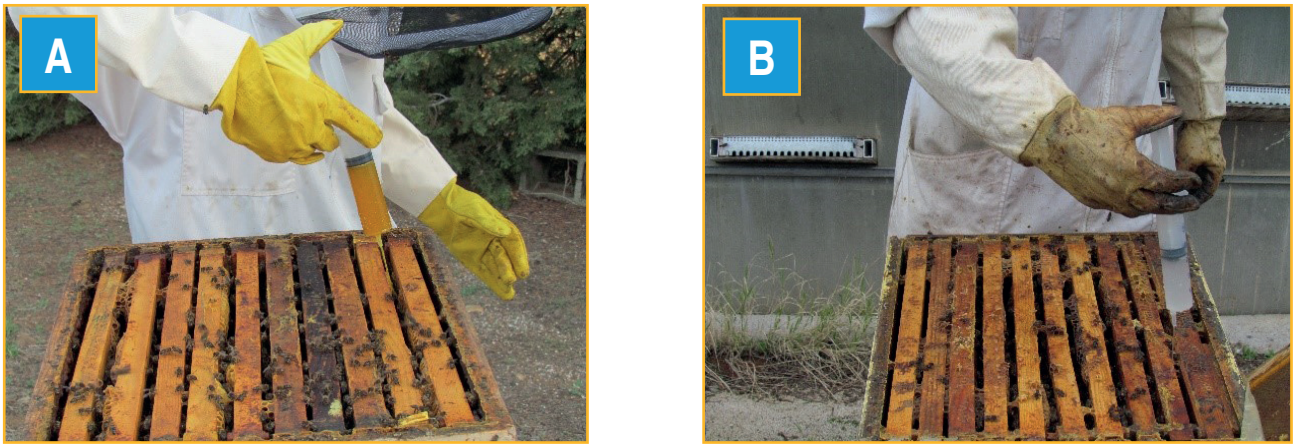

Figure 4. A. Beekeeper applying treatment with ApiHerb. B. Trickling oxalic acid for varroa and *Nosema* control

4.6. Example of strategies for sustainable control

After confirmation of nosemosis, the health status of the bee colony should then be evaluated (if there is a normal and correctly structured population and to check whether clinical signs are present). The prognosis is different according to the moment when the infection has been detected and for the same level of bees infected, the prognosis is worse when detected during autumn-winter, to one detected during spring or summer. In the wintering period, the colony has no capacity to raise new bees to compensate those bees lost because of the infection. On the contrary during the productive period, the colony is able to compensate for the premature death

of infected bees by raising new young bees that balance the colony (maintaining colony homeostasis).

For that reason, In the case of weak colonies in autumn and winter, it would be necessary to apply a product that prevents the percentages of parasitized bees continue to increase during the winter brood stop, which would cause their collapse during the winter or at the beginning of the following spring. However, when the parasite is detected in spring or summer, it would be more convenient to enhance the growth of the bee colony through appropriate beekeeping techniques, and then, after the end of the productive period, perform the application of any of the products available in the market to ensure the maintenance of low parasitic percentages (below 40%) during wintering.

Consequently, the application of a treatment, such as those described in section 3.5., is therefore essential before the winter stop or at the end of the winter. Spring treatments should only be applied if the colony shows obvious symptoms of depopulation and weakness.

Regarding the beekeeping practices that should be applied in the apiaries, we would highlight the annual or biannual renewal of queens to avoid the nutritional deficiencies of the colony (use of food of known composition and free of pathogens), annual renewal of wax from brood combs (if possible with pesticide-free wax), cleaning and disinfection of beekeeping material and beehives, as well as proper location of the hives.

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5. *Aethina tumida*

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5.1. The disease in short

Figure 1. *Aethina tumida*

The Small Hive Beetle (SHB), or *Aethina tumida* Murray, is a pest native to Southern Africa that affects the honey bee colonies and other pollinating insects of the Apoidea family such as the bumble bees (genus *Bombus*).

Beetles are attracted by the smell of live bees and combs containing pollen and/or larvae. Adult beetles can penetrate in hives through the entrance or cracks; and once inside the hives, females begin to lay hundreds of eggs, preferably on the brood combs by drilling the cap of the brood or in the hive cracks. Larvae cause enormous damage to the hives, digging tunnels among the cells of the honeycomb to feed on pollen, honey and bee brood. They defecate on honey and on the combs. The combs become slimy and acquire a characteristic smell of rotten oranges.

The SHB can spread very rapidly flying from apiary to apiary but also through the trade of bee packages, artificial swarms, queen bees, raw wax and beekeeping materials. Typically, SHB infestation leads to death of weak colonies already affected by other diseases (such as Varroosis). The presence of SHB in the hive can also cause swarming. In addition, as the SHB larvae are defecating on the honey, they compromise the quality of the honey. The SHB can also cause considerable damage on stored unextracted honeycombs in warehouses and honeyhouses.

Adults can survive several days without food so it can be easily introduced, even accidentally, in a SHB-free country through international trade. SHB represents a strong threat to the environment and to the economy of beekeeping.

5.2. On-field diagnosis

Adult SHBs are excellent flyers thus it is hard to see and collect them inside the hive. They are oval-shaped and with increasing age, adults are first yellow-reddish, then become gradually brown, dark brown and eventually black when they reach sexual maturity (Figure 1). Body is rather flattened, 0.5 - 0.7 cm long and 0.3 - 0.45 cm wide (about 1/3 of the adult bee size). Antennas are club-shaped and the rather long legs enable the SHB to move easily and quickly inside the hives.

Eggs of the SHB are white-pearly with a shape quite similar to those of bees but smaller (about 1/3). They are

1.4 mm long and 0.26 mm wide and are laid by the fecundated females of the SHB in the hive interstices and in the small gaps (difficult for bees to access and remove the eggs) or inside the capped brood cell (after perforating the cap). The incubation period of eggs varies from one to three days.

SHB's larvae are responsible for the greater damage inside the hive. They are cream-coloured and about 11 mm long at the end of their development stage. Larvae can be recognized by four rows of dorsal spikes along the back, three pairs of legs and two rear spines. These are three very clear characteristics that can allow the beekeeper to distinguish larvae of the SHB from larvae of the wax moth (*Galleria mellonella*).

Larvae penetrate the soil 5 to 60 cm deep for metamorphosis. The development of the SHB is strongly influenced by the type of soil in which the larvae will pupate: too hard or too muddy soils greatly reduce the birth rate of adults. Therefore, SHB prefers sandy soils for pupation. The period spent in the soil is usually 3-4 weeks (with variations from two to eight weeks, depending on the temperature and the soil properties). Pupation is a stage characterised by high mortality because the SHB is very vulnerable. Pupae, initially pearly-coloured, become light-brown and then brown-bluish (nymphs). Most of the adult beetles emerge after 3-4 weeks and promptly fly in the hives to feed.

Figure 2. The use of a divider to act as a refuge for SHB and improve the on-field diagnosis

On-field diagnosis of adults can be improved with the use of a divider (Figure 2) 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 divider should be installed at least 48 hours before the examination (Rivera-Gomis et al., 2017).

5.3. Laboratory methods for diagnosis

The best way to identify *Aethina tumida* is through morphological identification, when it is possible to have the complete insect. The method is fast and inexpensive and permit to discriminate between *A. tumida* from other nitidulid beetles and wax moth larvae. It requires a proper specimen collection and conservation until it reaches the official laboratory for confirmation. The specimens collected in or near honey bee hives should be killed before submission (by freezing or putting them in ethanol). Undamaged adults and larvae are the best samples to be collected. In order to ship the samples and preserve them in the best way, they should be put in 70% ethanol (not denatured ethanol). If the sample is incomplete and it is not possible to perform the morphological identification, confirmatory testing can be done in the laboratory by molecular methods (polymerase chain reaction [PCR]). These methods are particularly useful for larval identification or when specimens are damaged.

5.4. Good beekeeping practices to prevent the disease

Good beekeeping practices related to *Aethina tumida* are the best way to avoid the related-damage and to reduce the use of chemicals to control the pest. At the apiary level, avoid having broken hives with openings or not well-maintained hives is a good way to reduce the probability of entrance of the beetles into the colonies. Do not have beekeeping material abandoned in the apiary (especially built combs with honey and/or brood) and avoid feeding bees openly in the field is fundamental to prevent creating reproduction sites for the beetle. Good hygienic practice in dealing with dead colonies (combs, food stores, boxes, etc.) should be carried out by quickly removing beehives with dead colonies as soon as possible and melting/destroying all organic materials that could attract SHB.

In areas where the beetle is present, during the beekeeping active season (summer/spring), having only healthy strong colonies and, if necessary, balance colony strength among colonies transferring frames after verification of the absence of the adults, eggs and larvae are important measures that should be adopted. It is important to take care that the bees cover all frames in the hive (no empty space).

During autumn and before wintering it is important to verify the integrity of the hive boxes, reduce the number of frames in the hive box, reduce the size of the hive entrance, perform hive box maintenance (replace parts or painting, if needed) and insert a divider board to reduce the volume for the hive nest. During winter it is possible to perform a sampling of hive debris, in order to identify suspected hives/apiaries (preclinic winter diagnosis of AFB, EFB, SHB).

Technical support of an expert (for example, veterinarian, technician, etc.) to provide assistance in case of need and the following of a training programme in beekeeping and honey bee diseases is important to have knowledge on how to identify, prevent and control the disease.

Disease control should be carried out with the use of veterinary medicines for honey bees registered in the specific country or medicines legally imported and all treatments should be carried out correctly as described in the instructions (respecting dosage and method of application).

5.5. Biosecurity measures to manage the disease

Aethina tumida biosecurity measures are all those integrated measures implemented by the individual beekeeper to reduce the risk of introduction and spread of the specific honey bee disease agent. For definition, they can be adopted in areas where the beetle is endemic and in areas where it is not present.

Carrying out periodically hive inspections to detect and remove the parasite (adults and larvae); trace meticulously movement of hives (identify hives, dates of movements, exact position), supers and wax (also controlling the transport conditions adopting a proper isolation of beekeeping equipment); avoid the spread of SHB during transport are some biosecurity measures that can be adopted by beekeepers.

At the farm level, stocking combs in a cold chamber at a temperature below 10°C and/or less 34% relative humidity, prevent survival of SHB eggs and larval development (Figure 3).

Giving artificial nutrition should be done each time at low amounts so the bees can consume it in a short time, reducing probability to create a substrate for the reproduction of SHB.

A genetic selection of queens should be carried out in order to breed ones that are more resistant to the disease (hygienic behaviour) and adapted to local climatic conditions.

Figure 3. Stocking supers in a cold chamber at a temperature below 10°C and/or less 34% relative humidity prevent survival of SHB eggs and larval development

Concerning the practices that should be adopted at the honey house, it is fundamental to adopt pest control procedures, keep working rooms and equipment clean, tidy and in best order and apply general methods of hygiene (e.g. regular cleaning of equipment, etc.). More in detail, considering that SHB is attracted to unattended honeycombs and honey, supers or frames should be extracted as soon as possible (at least within 2 days) and extracted honey should be kept and stored without any access for bees or vermins in tight sealed packings (drums, hobbocks etc.).

In case of SHB is not being present in your area, it is important to have good knowledge of SHB morphology of eggs, larvae and adults and hive inspection methods. It is also mandatory not to transport unauthorized live material at risk (hives, queens, nucs, etc.) from areas where SHB is present and to periodically monitor the possible presence of SHB by sampling debris or honey.

5.6. Example of strategies for sustainable control

Control methods can be adopted at the apiary level and inside the honey house. The combination of different control strategies seems the best solution to apply. The first strategy should be to install mechanical traps or biological control methods and only subsequently chemical control methods (i.e. when the population of beetles threats the survival of the colony).

Visual inspections are of basic importance to regularly identify SHB and subsequently kill them. A divider, installed at least 48 hours before the examination, improves the success rate (Rivera-Gomis et al., 2017).

Mechanical traps (e.g. provided with glue or baits) are able to support the monitoring and controlling activities of the parasite inside the hives.

In the honey house a fluorescent light source positioned 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.

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6. The BPRACTICES traceability system: an innovative tool to connect beekeepers and consumers

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6.1. Traceability at the EU level

Food safety has been a growing concern among EU citizens over the last decades. Outbreaks of diseases in animals that could be transmitted to humans, or the presence of chemicals above acceptable limits in feed and food, can threaten both the quality and safety of products.

Traceability is a risk-management tool which allows food business operators or authorities to withdraw or recall products which have been identified as unsafe and it is very important for the protection of consumers, particularly when food and feed are found to be faulty. More in general, traceability:

- facilitates withdrawal of faulty food/feed from the market
- provides consumers with targeted and accurate information on specific products
- covers all food and feed, all food and feed business operators, without prejudice to existing legislation on specific sectors
- affects importers who are required to be able to identify from whom the product was exported in the country of origin
- obliges businesses to be able to identify at least the immediate supplier of the product in question and the immediate subsequent recipient, with the exemption of retailers to final consumers - one step back-one step forward (unless specific provisions for further traceability exist).

The **General Food Law Regulation (Regulation (EC) No 178/2002** of the European Parliament and of the Council of 28 January 2002) lay down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. For the purposes of the Regulation, "traceability" means the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution. Moreover, Article 18 of the same Regulation cites:

1. The traceability of food, feed, food-producing animals, and any other substance intended to be, or expected to be, incorporated into a food or feed shall be established at all stages of production, processing and distribution.

2. Food and feed business operators shall be able to identify any person from whom they have been supplied with a food, a feed, a food-producing animal, or any substance intended to be, or expected to be, incorporated into a food or feed. To this end, such operators shall have in place systems and procedures which allow for this information to be made available to the competent authorities on demand.

3. Food and feed business operators shall have in place systems and procedures to identify the other businesses to which their products have been supplied. This information shall be made available to the competent

authorities on demand.

4. Food or feed which is placed on the market or is likely to be placed on the market in the Community shall be adequately labelled or identified to facilitate its traceability, through relevant documentation or information in accordance with the relevant requirements of more specific provisions.

5. Provisions for the purpose of applying the requirements of this Article in respect of specific sectors may be adopted in accordance with the procedure laid down in Article 58(2).

More detailed traceability requirements in the context of the General Food Law Regulation are laid down for certain specific sectors and, in particular for foods of animal origin, there is the Commission Implementing Regulation (EU) No 931/2011. The Regulation apply to food defined as unprocessed and processed products in Article 2(1) of Regulation (EC) No 852/2004 and at Article 3 are defined the traceability requirements that food business operators shall ensure and made available to whom the food is supplied and, upon request, to the competent authority. Requirements are:

- an accurate description of the food
- the volume or quantity of the food
- the name and address of the food business operator from which the food has been dispatched
- the name and address of the consignor (owner) if different from the food business operator from which the food has been dispatched
- the name and address of the food business operator to whom the food is dispatched
- the name and address of the consignee (owner), if different from the food business operator to whom the food is dispatched
- a reference identifying the lot, batch or consignment, as appropriate
- the date of dispatch

Regulation (EU) No 1169/2011 of the European Parliament and of The Council of 25 October 2011 on the provision of food information to consumers at chapter IV “MANDATORY FOOD INFORMATION”, Article 9, lists mandatory particulars for labelling:

- the name of the food
- the list of ingredients
- any ingredient or processing aid listed in Annex II or derived from a substance or product listed in Annex II causing allergies or intolerances used in the manufacture or preparation of a food and still present in the finished product, even if in an altered form
- the quantity of certain ingredients or categories of ingredients
- the net quantity of the food
- the date of minimum durability or the ‘use by’ date
- any special storage conditions and/or conditions of use

- the name or business name and address of the food business operator referred to in Article 8(1)
- the country of origin or place of provenance where provided for in Article 26
- instructions for use where it would be difficult to make appropriate use of the food in the absence of such instructions
- with respect to beverages containing more than 1,2 % by volume of alcohol, the actual alcoholic strength by volume
- a nutrition declaration

Honey in EU Regulation is defined as a natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on plants. Bees collect it, transform it by combining with specific substances of their own, deposit it, dehydrate it, store it and leave it in honeycombs to ripen and mature. European Union defines specific **rules for honey** supplementing its laws on foodstuffs (**Council Directive 2001/110/EC of 20 December 2001 relating to honey**). The directive supplements the general EU rules on food labelling set down in Regulation (EU) No 1169/2011. Essential consumer information must be included on labels and, in particular, must include the country of origin of the honey and the product names, as set out in Annex I. **Directive 2014/63/EU** clarifies that pollen is a natural constituent rather than an ingredient of honey. The Directive also clarifies the labelling requirements where honey originates in more than one EU country or a non-EU country. In these cases, the indicator of the country of origin may be replaced by one of the following indications, as appropriate:

- 'blend of EU honeys'
- 'blend of non-EU honeys'
- 'blend of EU and non-EU honeys'

In certain cases, these names may be replaced by the simple product name 'honey' (except in the case of 'filtered honey', 'comb honey', 'chunk honey or cut comb in honey' or 'baker's honey'). Information on regional, territorial or topographical origin, on floral or vegetable origin or on specific quality criteria may supplement this labelling (except for 'filtered honey' and 'baker's honey').

Directive 2014/63/EU allows the European Commission to adopt further laws (delegated acts) laying down two parameters for the criterion of 'mainly' as regards the floral or vegetable origin of honey and the minimal content of pollen in filtered honey following removal of foreign inorganic or organic matter.

6.2. Good Beekeeping Practices related to traceability

BPRACTICES project identified Good Beekeeping Practices (GBPs) related to traceability and reported here below in Table 1.

APIARY MANAGEMENT
ENVIRONMENT AND INFRASTRUCTURE
Keep the medical certificates of persons working in contact with bees and any document certifying their qualifications and training
Keep all laboratory reports, including bacteriological tests and sensitivity tests
Keep all documents proving that the bacteriological and physico-chemical quality of the water used in the honey house, given to the colonies or used in feed preparation meets official tap water standards for your country
Keep all the documents relating to self-inspections and controls (by the authorities and other official bodies) on the proper management of the colonies and the sanitary and hygienic quality of the bee products
Keep all documents sent by the official inspection services (distributors or the quality control departments of food-processing firms) relating to anomalies detected
Keep all documents and records and place them at the disposal of the competent authority (Veterinary Services and Food Control Services) and ensure that all these documents are kept long enough to enable any subsequent investigations to be carried out to determine whether contamination of food products detected at the secondary production or distribution stage was due to a dysfunction at the primary production level
ANIMAL HANDLING
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
Identify with numbers/letters all the hives in each apiary
Create a unique identification number for the apiary to easily trace the location of the hive (for stationary apiaries)
Registration of the beekeeper in the National Beekeeping Registry
Record all reared colonies

Record the exact position of the beeyards
Record all colonies arrivals, origin and date of arrival, to ensure that movements of incoming colonies are traceable to their source
Keep records of movements of hives, swarms, queen bees
Keep records of breeding activities (e.g. all breeding stock, when queens were born, their origin and arrival, the breeding dates in case of instrumental insemination and outcomes, etc.)
Record any other management changes that may occur
Record period of collection of hive products from each apiary
Keep a list of certified suppliers
HONEY HOUSE MANAGEMENT
ENVIRONMENT AND INFRASTRUCTURE
Identify the supers in the honey house coming from different apiaries
HIVE PRODUCTS HANDLING
Establish a data-recording system to ascertain the exact origin (batch) of bee products produced
Establish a data-recording system to ascertain the destination of bee products produced
HONEY BEE HEALTH MANAGEMENT
VETERINARY MEDICINES
Keep records of veterinary medicine treatments
DISEASE MANAGEMENT
Record the health status of the colonies: diseased/infected colonies (dates, diagnoses, ID of colonies affected, treatments and results)
Record the health status of the colonies: mortality (dates, diagnoses, ID of colonies affected)
Record the origin and use of all disinfectants and consumable items used, 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 effectively been implemented (task sheets, self-inspection checks on the effectiveness of the operations)
Comply with legal obligations concerning restrictions on animal movements in case of notifiable diseases

Table 1 GBPs related to traceability

Good Beekeeping Practices (GBPs) related to traceability were evaluated by BPRACTICES partners. Each GBP depending on its importance through the adoption of a score ranging from 1 to 4 (1 = not important; 2 = slightly important; 3 = important; 4 = very important). A score of 4 was given to those beekeeping practices deemed of crucial relevance according to the legal requirements within individual countries and in accordance to the experience of the participants, based the magnitude of the impact they can have in the context of the "One Health" approach. The detailed results are reported in the article by Rivera-Gomis et al. 2019.

6.3. The innovative traceability system

With a multidisciplinary approach (economic, environmental and societal) granted by the multi-actor involvement of the BPRACTICES consortium, an innovative traceability system based on QR-Code/RFID technology has been developed. The system is a web tool addressed to both producers and consumers able to provide mandatory (see chapter above) and optional information on honey bee products origins and characteristics. Thanks to the collaboration in the project of the Danish Beekeepers Association, the traceability system is available at: www.hivelog.dk.

A logic scheme of the traceability system is reported in Figure 1.

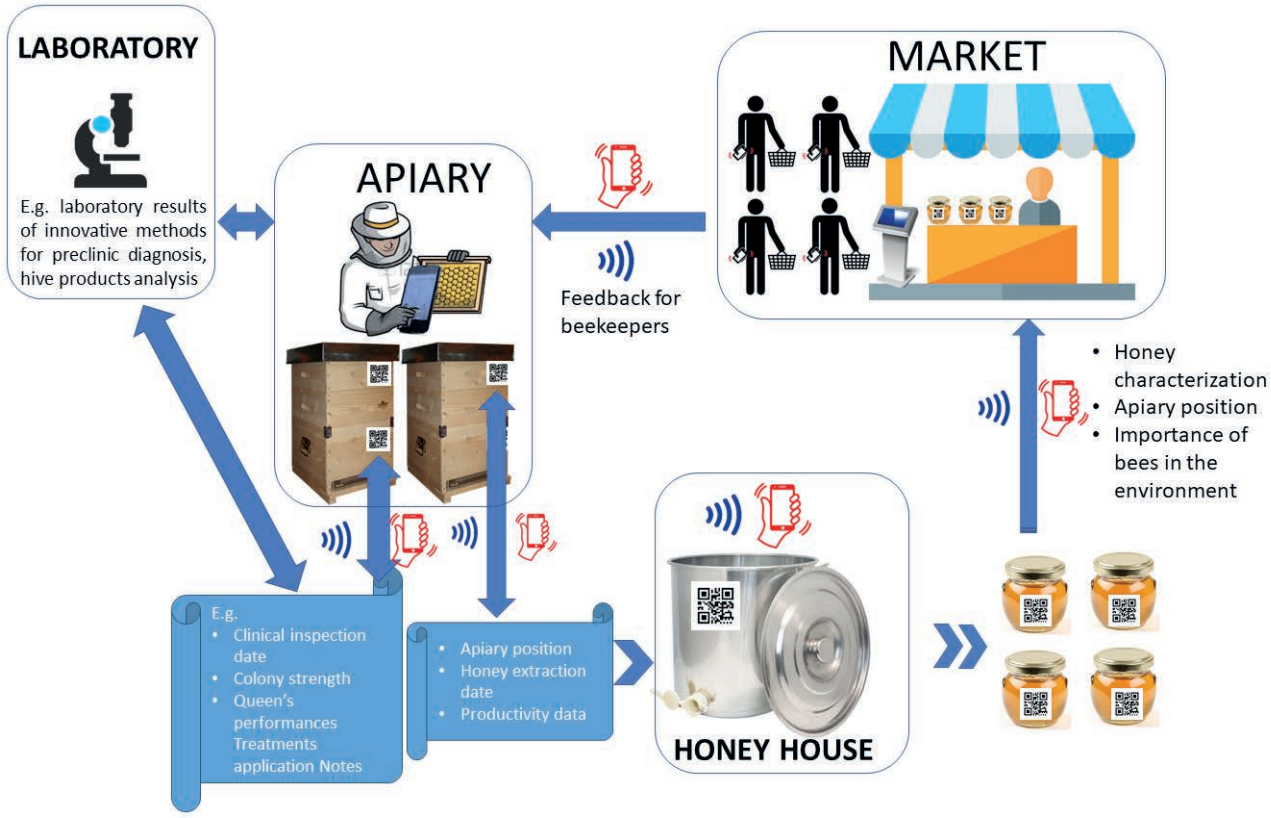

Figure 1. The traceability system implemented in the BPRACTICES project

Beekeepers, using the web application, can be informed on the innovations proposed with the new management system developed by the project. The application provides a long-lasting system able to record apiary management data (e.g. colony strength, queen's performances) and act as a surveillance system (recording diseases presence and showing distribution maps). The same web application has an interface to be used during the hive product processing to help beekeepers to maintain product traceability. Consumers, accessing the application directly from the jar, are educated to responsible consumption and made aware of the benefits of consuming a product deriving from an environment-friendly management, increasing the development of local productions. Moreover, the positive environmental impact of beekeeping and the ecosystem services provided by the bees are pointed out in the web application.

The minimum fields needed in order to implement bee-products traceability were defined and are reported in Table 2.

WHAT	**TYPE OF DATA**
User details (First name, Last name, Organization, Street, Zip code, City, Country)	Text
Beekeeper photo	File
Contact data (phone number, website, email)	Text
Treatments applied at the apiary level	Text
Apiary address/coordinates (to create a map)	Google maps coordinates
Apiary photo	File
Honey house address/coordinates	Google maps coordinates
Honey house photo	File
Bottling/Packaging address/coordinates	Google maps coordinates
Bottling/Packaging address photo	File
Final destiny of the product (If known)	Text
Certificates (Organic…)	File
Laboratory analyses	File
Type of harvest (frames, supers, propolis, pollen, royal jelly)	Text
Honey type (botanical origin)	Text
Date harvest start	Text
Date harvest end	Text
Date of extraction	Text
Date of bottling	Text
Lot number	Text
Date of minimum durability/Expiration date (royal jelly)	Text

Product quantity	Text
Lot visibility to public (private archive/public/delete)	Select
Select visible fields to public	Select

Table 2 Information required

The beekeeper layout of the traceability system is reported in Figure 2.

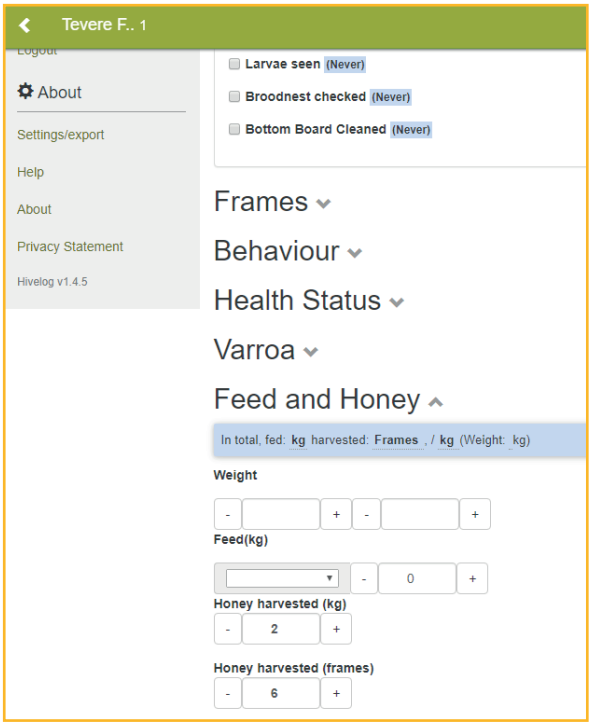

Figure 2. An example of the page where the beekeeper can add management data

In this page, the beekeeper can record all management data and the date of harvest. In another page (Figure 3) it is possible to upload pictures and add meta-data about the apiary and its surroundings.

In the store page, the beekeeper can attribute the production data (that is also linked to the management information, Figure 4) to a specific lot number (Figure 5) and more information among those reported in Table 2.

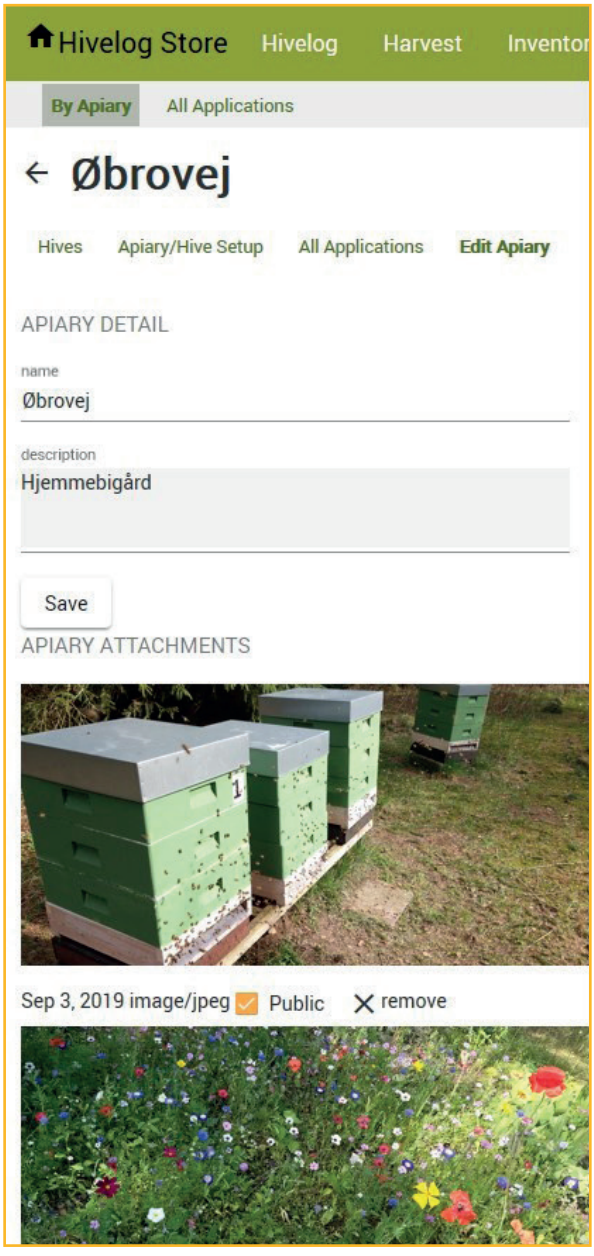

Figure 3. Image upload to add meta information about the apiary

Consumers can access all information concerning the product by reading a QR-Code provided on the label (Figure 6) or a RFID (NFC) tag added on the label or accessing a web site and entering the specific lot number of the product (Figure 7).

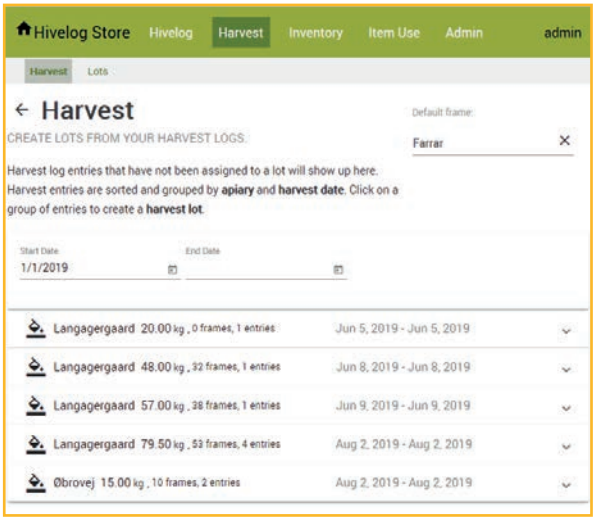

Figure 4. A screenshot of the store page with all harvests collected by the beekeeper

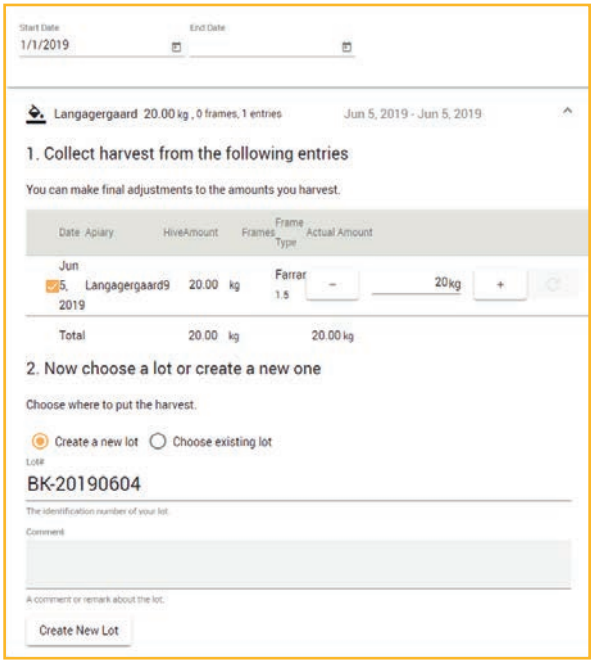

Figure 5. A screenshot of the page where the beekeeper can attribute a lot number to specific harvests

Figure 6. The reading of a QR-Code on a honey jar

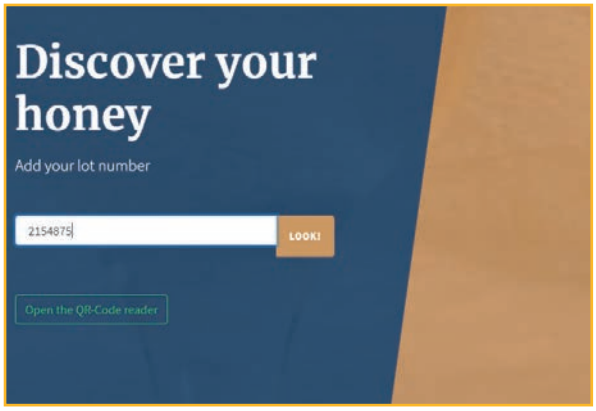

Figure 7. An example of the page where the consumer can add the lot number

After writing the lot number, consumers can access all data regarding the honey: products characteristics (Figure 8), producer's data (Figure 9), management information (Figure 10), and apiary position (Figure 11).

Figure 8. Honey characteristics accessible through the webpage

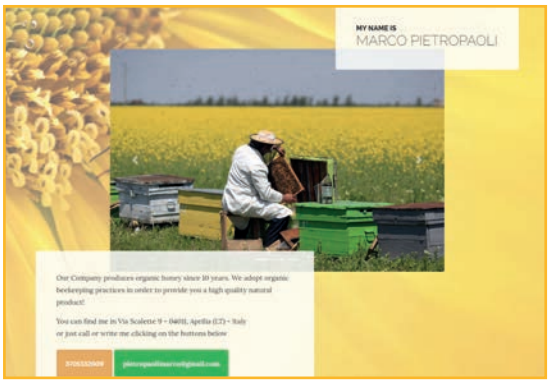

Figure 9. Pictures and a short story of the producer

Figure 10. Management information accessible from the webpage

Figure 11. A map of the surroundings of the apiary

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7. Traceability tips: consumers' insights to help beekeepers improving the traceability system

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7.1. Introduction

Traceability of food products has become a central issue in the European Union since an integrated approach aimed at guaranteeing food safety "from farm to table" through mandatory traceability requirements has been adopted (Charlier & Valceschini, 2008). The compulsory information reported on product labels fosters the recall of risky or unsafe food and facilitates frauds identification but it does not provide consumers with complete and clear information about the products they are purchasing.

Furthermore, consumers are increasingly interested in the origin of the food they eat and in production systems. In consumers' opinion, both quality and safety are related to traceability; indeed, food safety, health, quality and control guarantee are the main benefits associated to products traceability (Mora & Menozzi, 2008; Van Rijswijk et al., 2008).

Generally speaking, consumers consider honey a healthy and safe product (Pocol & Bolboaca, 2013). However, traceability is important because honey is a processed food and it is connected with environmental issues, that might contribute to generate concerns over its safety and origin (Menozzi et al. 2015).

The traceability systems, such as the one implemented in the BPRACTICES project (Chapter 6), promote food chain transparency, improving consumers' confidence in the food system and bringing them closer to the product.

The tips and the data presented in this Chapter are based on research activities realized by the Health Awareness and Communication Department of the Istituto Zooprofilattico Sperimentale delle Venezie. The social investigation aimed at evaluating the traceability system developed and implemented during the project and at identifying which kind of information on honey consumers require.

A semi-structured questionnaire was administered to a sample of 1,011 Italian honey buyers through the computer-assisted web interviewing (CAWI) method. Purchasing and consumption behaviours, knowledge and perceptions on honey and its production chain were investigated.

The web application and the traceability system proposed in the project were evaluated by means of two focus groups held in Bologna and Padova (Italy), involving a total of 25 honey buyers and a paper-and-pencil self-administered survey, carried out at FICO Eataly World (Bologna). Participants were asked to access the webpage containing the honey and beekeeper information via the QR Code applied on the honey jar. The design of the web application and the information available through the QR Code were evaluated to identify the weaknesses and the strengths of the traceability system.

7.2. Tips to improve the traceability system

The QR code technology to access information about honey

According to the results of the data collected in Italy:

- the QR Code technology to provide access to the information on honey and producers is considered 'suitable'. More than 60% of the respondents stated that they would use the QR Code to access information about the honey they buy
- almost 70% of the respondents declared they would be willing to pay a higher price for a package of honey that offered more information about the product

In the consumers' opinion, the QR Code should be integrated into the label, and not be used as a substitute of it. Moreover, the link to the website containing the information should be written on the label/jar.

Information consumers would like to receive through the traceability system

The webpages the consumers should access should guarantee good usability. In particular, the participants in the study highlighted:

- the importance of easy navigation and search for information
- the importance of interaction, e.g., inserting links to producer's social network accounts and/or webpage
- the importance of *adaptability* to different devices (visualization on pc, smartphone, tablet)

As regards contents on the honey jar to make available by means of QR Code, the basic information required were:

- Farm contact data: name of the producer, phone number, website, email
- Comments on the jar honey type: e.g. botanical origin, flavour description, water content
- Date of minimum durability. In addition, consumers are interested in the date of harvest, date of extraction, date of bottling
- Lot number
- Total product quantity of the lot
- Presence of certificates (e.g. local or organic production)

Moreover, participants positively evaluated the presence of further information, such as:

- Beekeepers' biography and apiary photo. Photos, as well as biography, need to be personal, coherent, realistic and authentic. Avoid the use of photo stock from the web
- Localization of the hives. The information on the precise positioning of the hives through a geolocalisation map is highly appreciated. However, if it is not possible or appropriate to show precisely where the hives are, it is preferable to omit the use of the localization tool. Information on the area where the hives are and the description

of the environment are suggested

- Laboratory analyses performed on the product. The access to these data is considered useful to stimulate curiosity, provide official guarantee on the product and to foster transparency. However, it is important to help consumers in understanding such information, explaining the meaning of the analysis and substances analysed, and improving the readability by outlining the main results

The consumers also suggested some other information they would like to receive about honey and beekeeping. This information may be added in the webpage or on the producer website and linked to the QR Code webpage. In particular, consumers are interested in honey experience, characteristics, production methods, bees and beekeeping practices. Having information on the bee world and their role for the environment is considered very important to sensitise consumers on beekeeping practices. They requested and would appreciate the authenticity of the information received as this transmits confidence.

Honey experience

- Tips on food pairings with the type of honey on the jar
- Tips on honey use/suggested recipes
- Comparison with similar products of the beekeeper

Honey characteristics

- Information on honey benefits for health
- Information on nutritional values
- Information on preservation methods, preservation temperature and time

Honey production

- Information on the honey production chain
- Methods of harvesting and extraction

Bees and beekeeping

- Information on bee health and welfare
- Information on beekeeping practices (e.g. migratory beekeeping)

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Conclusion

The Guidelines here presented are the conclusion of the BPRACTICES project that is aimed to develop a transnational European new system for Bee Healthcare focused on: preclinical disease approach, prevention, surveillance and control adopting a sustainable low-environmental impact approach respecting the product's quality and consumer's safety. Moreover, BPRACTICES includes an innovative traceability system (QR Code/ RFID based) applied for the first time throughout the entire hive production chain (from the hive to the jar) to the advantage of beekeepers and consumers.

Output of BPRACTICES aimed to impact on: the EU "economy pillar" improving the productivity, resilience and competitiveness of European production from the hives; the "environment pillar" promoting a sustainable and environment-friendly bee management; the "society and farmers' pillar" sharing and disseminating innovative on-farm practices and enhancing consumer acceptability and awareness of high-quality products coming from a sector able to improve biodiversity and to provide ecosystem services.

The sustainable beekeeping approach here presented will be disseminated at the global level. Future perspectives will be to interface and dialogue with other beekeeping systems in use worldwide, finding synergies and further implementations.

The BPRACTICES consortium

Annex 1 - Guidelines of harmonized Good Beekeeping Practices (GBPs)

1. GENERAL APIARY MANAGEMENT

Comply with legal obligations concerning restrictions on animal movements in case of notifiable diseases
For nuclei use only bees and brood combs from healthy colonies only (negatively inspected for bee diseases)
Respect hygiene rules (e.g. periodically cleaning of suits, gloves, etc.)
Transport/move only healthy colonies
Practice good hygienics when dealing with dead colonies (combs, food stores, boxes, etc.)
Disinfect levers and other potentially contaminated equipment (e.g. gloves) after inspection of hives affected by transmissible diseases
Balance colony strength among colonies transferring frames only in case of healthy hives
Transport hives avoiding the warmer hours of the day, providing adequate openings for air ventilation in the hives
Practice hive management according to region, season, strength of colony
Do not place honey supers directly on the ground (avoid contamination with *C. botulinum*)
Buy new bee colonies only after thorough inspection against bee diseases, preferably with a health certificate from a veterinarian
Replace the queens at least every two or three years except for those of high genetic value
Avoid the contact with dust during the transport of the supers from the apiary to the honey house
Keep only healthy strong colonies in the apiary
Do not place beehives directly on the ground
Evaluate the melliferous and pollen capacity of the area and the availability of water resources
Use disposable gloves when handling diseased hives
Have the support of an expert (e.g. veterinarian, technician, etc.) to provide assistance in case of need
Avoid areas with environmental pollutants (e.g. pesticides, heavy metals, etc.) to place apiaries
Do not have beekeeping material abandoned in the apiary
Do not imbalance the proportion between nurse bees and brood while equalising the hives; use preferably combs with hatching bees to fortify weak colonies
Keep a good proportion between the number of hives and the amount of melliferous plants/pollen sources in the area where the apiary is located
Prevent swarming by insertion of new wax foundations
Avoid windy areas to place apiaries

Perform genetic selection in order to have queens that are more resistant to diseases and adapted to local climatic conditions
Place apiary in an accessible area
Comply with the planned schedule for beehives inspection
Prevent swarming by colony splitting
Before winter, reduce the empty space in the hive
Adjust the number of hives in the apiary according to season, pollen, nectar, honeydew resources
Adjust the number of hives within a flight range according to season, pollen, nectar, honeydew resources
Wintering: reduce the size of the hive entrance
Keep newly introduced colonies separate from the existing stock for an appropriate period (at least 1 month) in order to monitor them against diseases to prevent transmission
Avoid, as far as possible, the introduction of swarms from unknown origin, colonies or queens from other apiaries
Place apiary in a firm area
Reduce the opening of the hive entrance during robbing and cold periods and increase the opening of the hive entrance during the hot season
Use personal protective clothing and equipment when visiting honey bee colonies
Keep purchased or weak colonies in a quarantine apiary
Prevent swarming by placing of supers
Prevent swarming by taking off the entrance reducer
Prevent swarming by adopting genetic selection of the queens
Use a queen excluder
Wintering: perform bee hive box maintenance (replace parts or painting, verify the integrity of hive boxes, if needed)
Place apiary in an area accessible to vehicles
Prevent drift occurrence: avoid keeping too many colonies in a single row
Mark the queen bee according to the date of birth
Avoid having broken hives with openings or not well maintained to prevent robbing
Avoid areas where toxic (e.g. with pyrrolizidine alkaloids) plants (e.g. *Echium spp.*, *Eupatorium spp.* and *Senecio spp.*) can be found in a significant quantity
Orientate hive entrance in a way that sun can reach them since the early morning hours.
Wintering: Verify the external position of the frames with stores in the hive.
Keep during apiary inspections corticosteroids or other proper medicines ready to use to guarantee health of operators (for example, in case of anaphylaxis)

Limit the weight lift (e.g. when harvesting supers or when moving hives) and, if needed, use back protector devices
Wintering: reduce the number of frames in the hive box.
Wintering: insert a divider board to reduce the volume for the hive nest.
Prevent drift occurrence: paint/draw numbers or identification signs on the front and entrance of the hive.
Prevent swarming by insertion of drawn combs
Avoid areas where allergenic plants (e.g. *Ambrosia trifida* and *Artemisia vulgaris*) can be found in a significant quantity.
Indicate the age of the combs on the top bar of the frame (e.g. the year of placing of the frame with foundation)
Reduce bee stress (e.g. avoiding unnecessary winter inspections of the hives; limiting the use of the smoker; properly feeding the bees etc.)
Wintering: wrap the hive in black tar paper, if needed.
Prevent swarming by removal of the beehive's bottom board
Provide adequate openings in the hive for air circulation, if needed

2. VETERINARY MEDICINES

Use only veterinary medicines for honey bees registered in your country or medicines legally imported
Ensure that all treatments or procedures are carried out correctly as described in the instructions (respecting dosage and method of application)
Do not carry out illegal treatments
Use only pharmacological products registered for beekeeping use, following the use instructions 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 periods have elapsed
In case of using instruments for the application (formic acid dispenser, sublimators for oxalic acid treatment) ensure that 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

3. DISEASE MANAGEMENT

In case of notifiable diseases follow the instructions from the veterinary regulations and competent authorities
In case of infectious diseases clean all beekeeping material between uses (e.g. hive bodies, hive bottom boards, feeders, hive tools)
Clean or disinfect (in case of infectious diseases) the hive box before installing new colonies
Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen in spring
Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen at the end of the beekeeping season
Quickly remove beehives with dead colonies
Take samples for laboratory analyses when sick or dead bees are found, if needed
Clean equipment, scrape off wax and propolis, on regular basis
Remove and process wax of all combs from dead, affected colonies
Record the health status of the colonies: diseased/infected colonies (dates, diagnoses, ID of colonies affected, treatments and results)
Renew 30% of the hive combs every year
Record the health status of the colonies: mortality (dates, diagnoses, ID of colonies affected)
Verify promptly any symptom of disease, asking a veterinarian (or a specialist)
Do not move frames or any kind of biological material (for example, to balance hives) from one hive to another in case their health status is not well known
Inspect diseased hives only after healthy hives inspections are ended
Select best performance stocks of honey bees
Burn dead colonies
Remove queens from colonies with clinical history of American Foulbrood disease
Remove queens from colonies with clinical history of European Foulbrood disease
Try to select and breed colonies that are more disease tolerant/resistant
Record the origin and use of all disinfectants and consumable items used, 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)
Disinfect equipment (for example, with NaOH, hypochlorite) on regular basis
Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen before supering the hives

4. HYGIENE

Torching (blue flame) as a disinfection method used in hives and beekeeping tools in case of transmissible diseases
Bleaching (soda, NaOH, etc.) as a disinfection method used in hives and beekeeping tools in case of transmissible diseases
Incineration of affected colony, if needed in case of transmissible diseases
Always incinerate affected colony in case of transmissible diseases
Water under high pressure and heated (90°C) as a disinfection method in hives and beekeeping tools in case of transmissible diseases.
Autoclaving as a method of disinfection of hives and beekeeping tools in case of transmissible diseases.
Gamma-irradiation as a method of disinfection of beekeeping tools in case of transmissible diseases.

5. ANIMAL FEEDING AND WATERING

Do not feed the bees with honey, pollen or supplements, unless the absence of pathogens (spores of AFB, chalkbrood, Nosema, EFB, etc.) is certified
Provide with artificial feeding during times of shortage or to build up winter stores, when needed
Wintering: verify that there is a sufficient amount of stores in the hive
Provide nucleus and swarms with adequate food supply when needed
Ensure the bees access to safe water sources
Do not feed your bees openly in the field to prevent robbing and spread of diseases
During transport provide adequate watering if needed

6. RECORD KEEPING

Keep records of veterinary medicine treatments
Registration of the beekeeper in the National Beekeeping Registry
Record the exact position of the bee yards
Identify with numbers/letters all the hives in each apiary
Keep records of honey bee diseases and colony mortality or depopulation
Set up a data-recording system that can be used to trace exactly which batches of commercial feed the colonies were fed with
Keep all documents/certificates about the commercial feed used
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
Record all reared colonies
Record all colonies arrivals, origin and date of arrival, to ensure that movements of incoming colonies are traceable to their source
Keep records of movements of hives, swarms, queen bees
Record period of collection of hive products from each apiary
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
Keep all documents/certificates that indicate the raw materials used in feed manufactured by the beekeeper and given to the colonies
Create a unique identification number for the apiary to easily trace the location of the hive (for stationary apiaries)
Keep records of breeding activities (e.g. all breeding stock, queens birth dates, their origin and arrival, the breeding dates in case of instrumental insemination and outcomes, etc.);
Establish a data-recording system to ascertain the exact origin (batch) of bee products produced
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
Keep all documents proving that the bacteriological and physico-chemical quality of the water used in the honey house, given to the colonies or used in feed preparation meets official tap water national standards
Record the origin and use of all feeds used, keep all records of any feed manufacturing procedures and records for each batch of feed

Keep a list of certified suppliers
Record any other management changes that may occur
Record any change in feeding
Keep all laboratory reports, including bacteriological tests and sensitivity tests
Keep reference samples (-20°C) of all feeds administered to the bees

7. TRAINING

Training/knowledge on honey bee diseases and symptoms
Follow a training programme in beekeeping and honey bee diseases
Attend a personal training on beekeeping
Record datasheets for each detergent/disinfectant used
Record disinfection procedures used
Record that disinfection procedures have been implemented
Keep the documents certifying qualification and training of persons working with bees

Annex 2 - Guidelines of harmonized Biosecurity Measures in Beekeeping (BMBs)

Introduction

VARROOSIS (*Varroa destructor*)

Treat the varroosis always according to the national legislation and registration
Adopt/provide hives with screened bottom boards
Nuclei and swarms should originate from colonies with no clinical signs of diseases Varroa-related (ABPV, DWV, IAPV, KBV, etc.)
Treat according to an integrated pest management concept taking Varroa thresholds into account
Maintain the number of Varroa below the harmful threshold in each colony
Adopt diagnostic tools for measuring Varroa infestation levels (for example, icing sugar method, CO_2 test, mite fall, etc.) after treatments and during the year (for example, in spring at the beginning of beekeeping season or before harvesting)
Treat simultaneously all colonies of the apiary and in the same area
Prepare your colonies (e.g. absence of brood) before treatment to get the highest possible efficacy, depending on type of treatment and product
Monitor efficacy of acaricide treatments: verifying Varroa fall after treatment
Have good knowledge of the symptoms and of the transmission ways of varroosis and virosis
Perform at least 2 treatments per year
Monitor efficacy of acaricide treatments: verifying the absence of varroosis symptoms in the colony (for example, presence of Varroa mites on adult honey bees) after treatment
Rotate veterinary medicines active principles to avoid Varroa resistance
Check the health status of drones producing colonies, especially for viruses
Use preferably medicines allowed in organic farming to control Varroa
Provide sufficient number of healthy spare bee colonies at the right time depending on climate and vegetation conditions
Try to select and breed colonies that are Varroa tolerant/resistant
Treat nuclei and swarms (no brood) with oxalic or lactic acid

AMERICAN FOULBROOD (*Paenibacillus larvae*; AFB)

Perform the ropiness test to confirm clinical outbreak of AFB in the apiary
Quick management of affected hives
Check for *P. larvae* in asymptomatic colonies by laboratory tests (e.g. stored honey in combs, hive debris) to control the disease. Take samples of colonies (hive debris/adult nurse bees/powder sugar/stores of honey in combs), in winter season, to detect *P. larvae* (by PCR method or microbial isolation) to control the disease
Perform laboratory analysis (isolation and/or PCR) to confirm a clinical outbreak of AFB in the apiary
Melt down the combs of all colonies (with and without clinical symptoms) of the affected apiary and process wax safely in order to control the disease
Verify presence of AFB typical scales (not removable, firmly adherent to the cell wall) to confirm clinical outbreak of AFB
Destroy only hives that show AFB clinical symptoms
Disinfection/incineration of all beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of symptomatic hives. Disinfect all beekeeping equipment of asymptomatic hives located in AFB outbreaks.
Disinfection/incineration of all beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of asymptomatic hives. Disinfect all beekeeping equipment of asymptomatic hives located in AFB outbreaks.
Make shook swarms of hives that show AFB clinical symptoms
Increase frequency of hive inspections in asymptomatic colonies (and in other apiaries of the same beekeeper) in case of lab positivity to spores of *P. larvae* or in case of symptoms of the disease in other hives of the same apiary
Apply an AFB-test (field kit) to confirm clinical outbreak of AFB in apiary
In case of AFB outbreak, make shook swarm of all colonies (with and without AFB symptoms)
Stamping out (destruction) of all colonies in the apiary (with and without AFB symptoms), only if you can already reach the eradication

EUROPEAN FOULBROOD (*Melissococcus plutonius*; EFB)

Manage quickly affected hives to control the disease
Search for the presence of removable scales, yellow and contorting larvae to diagnose a suspect of EFB clinical outbreak
Perform laboratory analysis (isolation and/or PCR) to confirm clinical suspect of EFB
Perform laboratory analysis (isolation and/or PCR) to confirm clinical suspect of EFB
Disinfect/incinerate the infected beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of EFB symptomatic colonies in case of clinical outbreak
Increase hive inspections in symptomless colonies in case of lab positivity to *M. plutonius* or in case of symptoms of the disease in other hives of the same apiary
Take samples (hive debris/adult nurse bees/powder sugar/stores of honey in combs) from asymptomatic colonies for the laboratory in winter season or in case of outbreaks, to detect presence of *M. plutonius* (by PCR method or microbial isolation)
Apply on-field EFB kit to confirm clinical outbreak of EFB on symptomatic hives
Make a partial (take off only brood combs, leaving store combs) shook swarm on colonies that show EFB clinical symptoms
Disinfect/incinerate all beekeeping equipment (beehives, nuc-boxes, mating boxes, boards, frames, queen excluders, etc.) of EFB asymptomatic colonies in case of clinical outbreak
Be aware of the odour opening the hive - typically sour smell to suspect clinical form of EFB
Make a shook swarm of all colonies of whole the apiary (with and without EFB symptoms) in case of EFB outbreak, if you want to reach eradication
Make a partial (take off only brood combs, leaving store combs) shook swarm of all colonies of the apiary (with and without EFB symptoms) in case you want to control the disease
Destroy affected colonies of the apiary if you want to reach eradication

NOSEMOSIS (*Nosema apis*, *N. ceranae*)

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
Prevent pollution of artificial water sources from faeces or drowned or dead bees
Select queen breeders with *Nosema spp.* free stocks
Select and breed *Nosema spp.* resistant honey bees, if possible
Remove combs with signs of dysentery
Take samples of forager honey bees (or powder sugar or debris) early in autumn or spring to diagnose nosemosis (PCR and microscopic methods)
Adopt a proper pathogen (e.g. *V. destructor*) control, to ensure a proper balance (nurse-forager bees) in the composition of the bee colony
Treat (if there are available any registered/permitted products in your country) against *Nosema spp.* the colony when percentages of infected bees are high (>40%)
Strengthen and stimulate the colonies in autumn and spring with the administration of stimulant integrators or feed supplements

AETHINOSIS (*Aethina tumida*; SHB)

Take care that the bees cover all frames in the hive (no empty space)
Do not leave outside of beehives frames, combs or other material that could be attractive and edible for *A. tumida*
Carry out periodically hive inspections to detect and eliminate the parasite (adults and larvae)
Trace meticulously movement of hives (identify hives, dates of movements, exact position)
Control the transport conditions adopting a proper isolation of beekeeping equipment avoiding spread of SHB during transport
Stock combs in order to prevent survival of SHB eggs and larval development in a cold chamber at a temperature below 10°C
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)
Have only healthy strong colonies in the apiary
Trace meticulously movement of supers and wax
Use traps to monitor and control SHB presence in the apiary
Stock combs in order to prevent survival of SHB eggs and larval development in a chamber at less than 34% relative humidity
Have only young queens with hygienic behaviour
Use queen bee excluder in order to avoid the presence of brood in the supers
In case of SHB is not being present in your area
Have good knowledge of SHB morphology of eggs, larvae and adults
Have good knowledge on hive inspection methods to detect SHB
Do not leave outside of beehives frames, combs or other material that could be attractive and edible for *Aethina tumida*
Have only healthy strong colonies in the apiary
Have only young queens with hygienic behaviour
Do not transport live material at risk (hives, queens, nucs, etc.) from areas where SHB is present into your apiary
Take care that the bees cover all frames in the hive (no empty space)
Do not transport material at risk (supers, wax, pollen, etc.) from areas where SHB is present into your apiary

Adopt specific traps for quick visual detection of SHB
Monitor periodically the presence of SHB by sampling debris or honey)
Do not transport live material at risk (hives, queens, nucs, etc.) from areas where SHB could be present into your apiary
Do not transport material at risk (supers, wax, pollen, etc.) from areas where SHB could be present into your apiary
Use queen bee excluder in order to avoid the presence of brood in the supers


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