New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* era in Europe
BPRACTICES

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FINAL REPORT

Work package 1 (WP 1) - "Varroosis and virosis". Leader: Partner n. 3

WP coordinator: Dr Maja Ivana Smodiš Škerl (Agricultural Institute of Slovenia)

Milestone M1.1: List of GBPs sent to WP5

Contributors:

Dr Maja Ivana Smodiš Škerl, Jernej Bubnič, Janez Prešern

Description:

The definitive list of Good Beekeeping Practices - GBPs (general) and Biosecurity Measures in Beekeeping-BMBs (disease specific) for *Varroa destructor*

Output:

The list of general Good Beekeeping Practices is reported in Table 1

Table 1

APIARY MANAGEMENT
ENVIRONMENT AND INFRASTRUCTURE
Buy new bee colonies only after thorough inspection for bee diseases, preferably with a health certificate from a veterinarian
For nuclei use bees and brood combs from healthy colonies only (negatively inspected for bee diseases)
Keep colonies of new introduction separate from the existing stock for an appropriate period (at least 1 month) to monitor them for diseases and infestations in order to prevent transmission of diseases
Orientate hive entrance in a way that sun can reach them since the early morning hours;
Avoid having broken hives with openings or not well maintained to prevent robbing
Prevent drift phenomenon: paint/draw numbers or identification signs on the front and entrance of the hive
Prevent drift phenomenon: avoid keeping too many colonies in a single row
Do not have beekeeping material abandoned in the apiary
Reduction of the hive entrance during robbing and cold periods and opening during the hot season
Place apiary in an area accessible to vehicles
Place apiary in a firm area
Place apiary in an accessible area
Keep a number of hives well-proportioned with the amount of melliferous plants/sources of the area where apiary is located
Evaluate the melliferous capacity of the area and the availability of water resources
Avoid areas where allergenic plants (e.g. *Ambrosia trifida* and *Artemisia vulgaris*) can be found in a significant quantity.
Avoid areas where toxic (e.g. with pyrrolizidine alkaloids) plants (e.g. *Echium* spp., *Eupatoriu*m and *Senecio* spp.) can be found in a significant quantity
Avoid areas pollutants (e.g. pesticides, heavy metals, etc.) in the environment where the apiary is placed

Avoid windy areas to place apiaries
Use personal protective clothing and equipment to visit honeybee colonies
Limit the weight lift (e.g. when harvesting supers or when moving hives) and, if needed, use back protector devices
Keep during apiary inspections corticosteroids or other proper medicines to guarantee health of operators (for example, in case of anaphylaxis)
Do not place beehives directly on the ground
Respect hygiene rules (e.g. periodically cleaning of suits, gloves, etc.)
Use disposable gloves to visit sick hives
Disinfect lever and other potentially contaminated equipment (e.g. gloves) after inspection of hives affected by transmissible diseases
Perform genetic selection in order to have queens that are more resistant to diseases and adapted to local climatic conditions
Respect the planned schedule for beehives inspection
ANIMAL FEEDING AND WATERING
Do not feed the bees with honey or pollen or supplements, unless the absence of pathogens (spores of AFB, chalkbrood, nosema, EFB, etc.) is certified
Ensure the bees access to safe water sources
Provide artificial feeding during times of shortage or to build up winter stores, when needed
Provide adequate food supply to nucleus and swarms, when needed
During transport provide adequate watering if needed
Do not feed your bees openly in the field, to prevent robbing and spread of diseases
ANIMAL HANDLING
Have only healthy strong colonies in the apiary
Keep purchased or weak colonies in a quarantine apiary
Indicate age of combs on the topbar of frame (= year of first placing a frame with foundation)
Replace the queens as maximum every two or three years except those of high genetic value
Balance colony strength among colonies transferring frames only in case of healthy hives
Do not imbalance the proportion between nurse bees and brood while equalising the hives; use preferably combs with hatching bees to fortify weak colonies
Prevention of swarming: insertion of new wax foundations
Prevention of swarming: colony splitting
Prevention of swarming: insertion of built combs
Prevention of swarming: removal of the beehive's bottom board
Prevention of swarming: placing of supers
Prevention of swarming: taking off the entrance reducer
Prevention of swarming: adopting genetic selection of the queens
Use of the queen excluder
Mark the queen bee according to the date of birth
Before winter, reduce the empty space in the hive

Transport hives avoiding the warmer hours of the day, providing adequate openings for air circulation in the hives
Transport/move only healthy colonies
Have the support of an expert (for example, veterinarian, technician, etc.) to provide assistance in case of need
Attend a personal training plan on beekeeping
Good beekeeping practices in general to prevent bee diseases: Which are important?
Hive management according to region, season, strength of colonies
Good hygienic practice in dealing with dead colonies (combs, food stores, boxes, etc.)
Number of hives in the apiary according to season, pollen, nectar, honeydew resources
Number of hives within a flight range according to season, pollen, nectar, honeydew resources
Wintering (in Autumn)
Verify the integrity of the hive boxes
Verify that a sufficient amount of stores is in the hive
Verify the external position of the frames with stores in the hive
Reduce the number of frames in the hive box
Insert a divider board to reduce the volume for the hive nest
Wrap the hive in black tar paper, if needed
Reduce the size of the hive entrance
Perform bee hive box maintenance (replace parts or painting, if needed)
HONEY HOUSE MANAGEMENT
ENVIRONMENT AND INFRASTRUCTURE
Adopt pest control procedures
Bee-tight room to extract the honey and store combs and equipment
Keep working rooms and equipment clean, tidy and in best order
Apply general methods of hygiene (e.g. regular cleaning of equipment, etc.)
Use a hygiene plan according to HACCP to control vermins and other pests
Avoid the contact with dust during the transportation of the supers from the apiary to the honey house
Don`t put honey supers directly on the ground (avoid contamination with *C. botulinum*)
HIVE PRODUCTS HANDLING
Super harvesting neither too early (avoid high water content) nor too late (risk of robbing behaviour)
Do not use repellents to get full honey boxes free of bees
Limit the use of the smoker during super harvesting to prevent the honey contamination
Wear clean clothing and hair protection when handling honey combs, extraction, straining and other manipulation of extracted honey
Extracted honey should be kept and stored without any access for bees or vermins in tight sealed packings (drums, hobbocks etc.)
Thoroughly skim and strain the honey before bottling

HONEY BEE HEALTH MANAGEMENT
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)
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
Dispose of used instruments and devices in a biosecure manner
Do not carry out antibiotic illegal treatments
Use only pharmacological products registered for beekeeping use following the use instructions and register the treatments
Register and identify the treated hives
Store veterinary products properly
DISEASE MANAGEMENT
Carry out a sampling from bottom hive debris or adult bees in the winter period, in order to identify suspected hives/apiaries (preclinic winter diagnosis of AFB, EFB, SHB)
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 before supering the hives
Carry out thorough inspections for clinical symptoms of bee diseases and presence of the queen at the end of the beekeeping season
Verify promptly any symptom of disease, asking a veterinarian (or a specialist) suggestions, even taking samples for laboratory investigations, if needed
In case of notifiable diseases follow the instructions of the veterinary law and authorities
Eliminate queens from colonies with clinical history of American foulbrood disease
Eliminate queens from colonies with clinical history of European foulbrood disease
Seek the support of an expert to provide assistance if you have concerns about a disease
In case of infectious diseases clean all beekeeping material between uses (e.g. hive bodies, hive bottom boards, feeders, hive tools)
Follow a training programme in beekeeping and honey bee diseases
Renew 30% of the hive combs every year
Do not move frames or any kind of biological material (for example, to balance hives) from one hive to another in case you are not sure of their health status
Inspect sick hives only after healthy hives inspections are ended
Clean or disinfect (in case of infectious diseases) the hive box before installing new colonies
Select best performance stocks of honeybees
Quickly remove beehives with dead colonies as soon as possible
Take samples for laboratory analyses when sick or dead bees are found, if needed
Burn dead colonies

Clean equipment, scrape off wax and propolis, on regular basis
Disinfect equipment (for example, with NaOH, hypochlorite) on regular basis
Remove and process wax of all combs from dead, affected colonies
Try to select and breed colonies that are more disease tolerant/resistant
Disinfection methods in case of contagious disease to be applied to hive and beekeeping tools
Torching (blue flame)
High pressure heated (90°C) water
Bleaching (soda, NaOH, etc.)
Autoclave method
Gamma-irradiation
Incineration of affected colony, always
Incineration of affected colony, if needed
Disinfection methods in case of contagious disease to be applied in the honey house equipment
Torching (blue flame)
High pressure heat (90°C) water
Bleaching (soda, NaOH, etc.)
Autoclave method
Gamma-irradiation
Cleaning with detergent

The list of Biosecurity Measures in Beekeeping - BMBs (disease specific) for *Varroa destructor* is reported in Table 2

Table 2

APIARY MANAGEMENT
ENVIRONMENT AND INFRASTRUCTURE
Adopt/provide hives with screened bottom boards
ANIMAL HANDLING
Nucleus and swarms should originate from colonies with no clinical signs of diseases (AFB, EFB, DWV, SBV, etc.)
Prepare your colonies before treatment to get the highest possible efficacy, depending on type of treatment and product
Provide sufficient number of healthy spare bee colonies at the right time depending on climate and vegetation conditions
HONEY BEE HEALTH MANAGEMENT
VETERINARY MEDICINES
Treat the varroosis always according to the national situation of legislation and registration
Treat nuclei and swarms (no brood) with oxalic or lactic acid
Treat according to an integrated pest management concept taking varroa thresholds into account

Use preferably biological methods like selection and breeding Varroa tolerant colonies, Varroa sensitive hygiene, etc.
Use preferably medicines allowed in organic farming to control Varroa
Treat simultaneously all colonies of the apiary and in the same area
Monitor efficacy of acaricide treatments: verifying varroa fall after treatment
Monitor efficacy of acaricide treatments: verifying varroa mite presence in the brood, after treatment
Monitor efficacy of acaricide treatments: verifying the absence of varroosis symptoms in the colony (for example, presence of varroa mite on adult honey bees) after treatment
Rotate veterinary medicines active principles to avoid varroa resistance
Perform at least 2 treatments per year
DISEASE MANAGEMENT
Try to select and breed colonies that are more varroa tolerant/resistant
Check the health status of drones producing colonies, especially for viruses
Maintain the number of varroa below the harmful threshold in each colony
Good knowledge of the symptoms of varroosis and viroosis
Good knowledge of the transmission ways of varroosis and viroosis
PRE-CLINIC INDICATORS
Adopt diagnostic tools for measuring varroa infestation levels (for example, ice 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)

Tables are published online in the project website at this link:

http://www.izslt.it/bpractices/wp-content/uploads/sites/11/2019/12/BPRACTCES-GBPs-BMBs.pdf

Milestone M1.2: GBPs to prevent Varroa and viruses

Contributors:

Dr Maja Ivana Smodiš Škerl, Jernej Bubnič, Janez Prešern

Description:

The definitive list of innovative GBPs to prevent Varroa and viruses

Output:

Preclinic indicators to prevent Varroa and viruses are published at this link:

http://www.izslt.it/bpractices/wp-content/uploads/sites/11/2019/12/BPRACTCES-GBPs-BMBs.pdf

Milestone M1.3: Laboratory methods for Varroa and viruses

Contributors:

Dr Maja Ivana Smodiš Škerl, Jernej Bubnič, Dr Antonella Cersini

Description:

The definitive list of harmonized laboratory methods for Varroa and viruses

Output:

For the laboratory diagnosis of varroosis, alcohol wash is normally used as described in chapter. A sample of at least 300 bees from the brood box should be frozen and delivered to the laboratory on ice (frozen). Alcohol wash is done in a jar: 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. Uncapping of the sealed brood could also be used 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 needs to be counted and average number calculated. 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 the hive and an 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.

Milestone M1.3.1: Varroa control methods review

Contributors:

Dr Maja Ivana Smodiš Škerl, Jernej Bubnič

Description:

A review of the best low environmental impact methods for Varroa control

Output:

A review of the best low environmental impact methods for Varroa control is under revision.

ANNEX 1.

Milestone M1.3.2: Varroa control methods - trials

1. Contributors:

Dr. Josef Mayr, Dr. Alexandra Ribarits, Dr. Rudolf Moosbeckhofer

Output:

Field test – Post harvest varroa control in summer - oxalic acid (OA) trickling after queen caging compared to formic acid (FA) evaporation

Several oxalic acid (OA) preparations are registered as veterinary medicinal products for varroa control. Because OA does not kill varroa mites in capped brood the efficacy is highest if applied to broodless winter colonies. In order to reduce the varroa infestation level and the risk of virus infections after the last honey harvest, some beekeeping management protocols use either the removal of the entire brood or a temporary caging of the queen to make the colonies artificially broodless. The field trials in Austria compared two OA treatment protocols (groups A, B) for post harvest varroa control with the frequently used application of FA (group C) in summer in the years 2017 and 2018. Parameters to evaluate the different groups were: percentages of queen losses, colony survival, and the colonies ready for the next year's spring nectar flow. According to the experimental design, queens should be caged for 24 days (A) or 19 days (B). Colonies were treated with OA („API-Bioxal"; trickling), either immediately after releasing the queen (A) or in the state of young unsealed brood (B). The control group (C) was treated twice with FA („AMO Varroxal"; Liebig dispenser) without caging of the queen. Additionally, all colonies were treated once by trickling „API-Bioxal" during their broodless winter period. The experiment was implemented in cooperation with beekeepers in a "Citizen Science" approach. Consequently, unlike in controlled conditions, the caging time of queens varied considerably within groups A and B. Queen losses increased with caging time and queen age, and were highest in group A, lower in B and less in C. Colony losses were lowest in group C, followed by A and B. The highest percentage of colonies ready for the next year's spring nectar flow was reported in group C (83.6%), followed by groups A (78.3%) and B (58.5%). In conclusion, the post harvest application of FA (group C) in summer was clearly superior to the two OA-variants (queen caging + oxalic acid treatment). This applies for the parameters queen and colony losses, percentage of colonies ready for the following spring nectar flow, as well as the additional time needed for searching and caging of the queens.

2. Contributors:

Dr Maja Ivana Smodiš Škerl, Jernej Bubnič, Dr Janez Prešern, Dr Marco Pietropaoli, Dr Giovanni Formato

Output:

Field test: Queen caging and trapping comb techniques in association with oxalic acid treatment to control *Varroa destructor*: efficacy and impact on honey bee and viruses population

The field trials were conducted in central Slovenian region and Latium region in central Italy. The aim of the experiments was to evaluate treatment efficacy, effects on population dynamics of honey bees and virus titre (ABPV, DWV) in adult bees using different brood interruption techniques (queen caging or trapping comb) and to determine the effect of selection pressure Varroa mites undergo while brood is interrupted, on virus titre in adult bees. These two beekeeping techniques were chosen due to low environmental impact of treatment and might be a good addition in combat against viral diseases in honey bees. Main hypothesis is that the viral load should be lower in trapping comb group than in queen caging group, because part of mite population is removed without forcing them to phoretic stage and thus reducing the chance for multiplying the viral particles. Results of trials in Slovenia and Italy showed that there were no differences in virus population between the groups. Brood interruption techniques were confirmed to be effective in decreasing varroa population level. Bee samples were sent to INRA (France) for further analysis (sequencing) and determination of viral strains.

Work package 2 (WP 2) - "American Foulbrood and European Foulbrood". Leader: Partner 5.

Dr Alexandra Ribarits (Austrian Agency for Health & Food Safety)

Milestone M2.1: List of GBPs sent to WP5

Contributors:

Rudolf Moosbeckhofer, Alexandra Ribarits, Oliver Alber, Hemma Köglberger, Irmgard Derakhshifar

Description:

The definitive list of Good Beekeeping Practices - GBPs (general) and Biosecurity Measures in Beekeeping- BMBs (disease specific) for AFB and EFB

Output:

The list of Good Beekeeping Practices has been published at this link (http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines/) and is reported in Table 1.

The lists of Biosecurity Measures in Beekeeping- BMBs (disease specific) for AFB and EFB are reported in Table 3 List of Biosecurity Measures in Beekeeping (BMBs) for AFB and Table 4.

Disease-specific lists of GBPs and BMBs were compiled for AFB and EFB, respectively, in cooperation with the BPRACTICES partners. To this end, GBPs and BMBs were identified, listed and ranked by relevance.

Table 3 List of Biosecurity Measures in Beekeeping (BMBs) for 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 swarms 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
ANIMAL HANDLING
Select queen breeders free of AFB
DISEASE MANAGEMENT
Which type of diagnostic method is important to confirm a clinical AFB-outbreak in an apiary?
Ropiness test
Search for AFB typical scales (not removable, firmly adherent to the cell wall)
AFB-test (field kit)
Laboratory analysis (isolation and/or PCR)
Which measures are the best to apply to control the disease?
Destroying only hives that show AFB clinical symptoms
Shook swarm only hives that show AFB clinical symptoms
Partial shook swarm (take off only brood combs, leaving store combs) only of hives that show AFB clinical symptoms
Stamping out (destruction) of all colonies of the apiary (with and without AFB symptoms)
Shook swarm of all colonies of the apiary (with and without AFB symptoms)
Partial shook swarm (take off only brood combs, leaving store combs) of all colonies of the apiary (with and without AFB symptoms)
Melting down the combs of all colonies (with and without clinical symptoms) of the affected apiary and safe wax processing
Increase hive inspections in symptomless colonies (and in other apiaries of the same beekeeper)
Check for P. larvae in asymptomatic colonies by laboratory tests (e.g. stored honey in combs, hive debris)
Quick management of affected hives
Disinfection measures in case of clinical outbreak: which measures are the best to control the disease?
Disinfection/incineration of the infected beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of AFB symptomatic colonies only
Disinfection/incineration of all beekeeping equipment (beehives, nucs, mating boxes, boards, frames, queen excluders, etc.) of the whole apiary (AFB symptomatic and asymptomatic)
Thoroughly cleaning with detergent of honey house extraction tools/facilities (uncappers, centrifuge, sieves, pumps, spins, etc.)
Thoroughly cleaning with detergent of hive product packaging materials (jars, tanks, barrels, etc.)
PRE-CLINIC INDICATORS
Sampling 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)
General GBPs for AFB: Do not feed the bees with honey or pollen or supplement, unless the absence of P. larvae is certified Move combs among hives only in case of healthy hives

Do not exchange honey or pollen combs between colonies in case of clinical or subclinical infection Select and breed AFB resistant honey bees Hygienic measures: - cleaning of equipment by scraping off wax and propolis - cleaning of equipment using registered alkaline cleaning agents (bleach: soda, NaOH, hypochlorite) after basic cleaning of equipment by scraping off wax and propolis - regular replacement of old, dark combs by beeswax foundation - wax processing of all combs from dead colonies Balancing the colonies or splitting colonies, avoiding reducing too much the amount of nurse bees respect the amount of brood Thorough hive clinical inspection in spring to search signs of AFB Thorough hive clinical inspection at the end of the productive season (end summer) to search signs of AFB Recognize the clinical symptoms of European foulbrood: spotty brood pattern, sunken cappings, holes in cappings, ropiness, scales tightly adherent to cell walls, rotting smell.

Table 4 List of Biosecurity Measures in Beekeeping (BMBs) for 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
Select queen breeders free of EFB
Make shook swarms on hives that show EFB clinical symptoms
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
Destroy hives that show EFB clinical symptoms
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 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

Milestone M2.2: GBPs to prevent AFB and EFB

Contributors:

Rudolf Moosbeckhofer, Alexandra Ribarits, Oliver Alber, Hemma Köglberger, Irmgard Derakhshifar

Description:

The definitive list of innovative GBPs to prevent AFB and EFB

Output:

The list of Good Beekeeping Practices has been published at this link (http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines/)

The lists of BMBs for AFB and EFB are reported in Table 3 List of Biosecurity Measures in Beekeeping (BMBs) for AFB and Table 4.

The lists of GBPs and BMBs created under M2.2 were evaluated, and ranked by their relevance. In addition to the evaluation by the BPRACTICES partners, beekeepers' associations contributed to the final list. The partners evaluated each GBP and BMB according to its importance through the adoption of a score. To rate each GBP, the mean result was calculated from the scores. All ratings were statistically processed to obtain a final list containing a reasonable number of GBPs. For the final ranking, only scores with means higher than the 75th percentile threshold were considered.

Milestone M2.3: Laboratory methods for AFB and EFB

Contributors:

Dr. Rudolf Moosbeckhofer, Dr. Alexandra Ribarits, Dr. Richard Gottsberger, Antonia Griesbacher, Hemma Köglberger, Dr. Irmgard Derakhshifar, Dr. Antonella Cersini, Dr. Mariano Higes, Dr. Laurianne Paris, Dr. Mustafa Necati Muz, Urška Zajc

Description:

The definitive list of harmonized laboratory methods for AFB and EFB

Output:

Guidelines were generated, in which the following procedures are described to prevent and check for the presence of *P. larvae*: 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 the mentioned matrices, food store samples from brood combs have proven as a simple and effective way to collect authentic material from honeybee 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. For *M. plutonius*, beside the traditional methods such as cultivation of the causative agent and microscopy, newer techniques such as immunology- or PCR-based methods are available for the unambiguous identification of *M. plutonius*.

Interlaboratory comparison (ILC)/Test performance study (TPS), “ring trial”

The ILC conducted in the frame of BPRACTICES was designed as TPS for the molecular detection of *P. larvae* and *M. plutonius*. The aim was to estimate the diagnostic sensitivity/specificity of different methods for detecting *P. larvae*/*M. plutonius* from debris, with the main focus on sensitivity. Six partner laboratories participated in the TPS: IZSLT, CIAPA, AIS, NKU, AGES, and EU-RL. The TPS aimed to assess the performance and the accuracy of the different selected PCR assays reported in the literature to be used for an early detection of *P. larvae* and *M. plutonius*, innovatively from beehive debris. Before performing the TPS, the lead partner of WP2, AGES, conducted a literature review, and selected the best performing PCR assays and qPCR (real time) assays (3 each) to be further used to monitor the two bacterial pathogens in a preclinical stage. Samples for the TPS were prepared according to the following general trial design: 20 blind samples (5 solely *P. larvae*, 5 solely *M. plutonius*, and 10 mixed samples, including “co-infected” samples), plus 1 positive and 1 negative control for *P. larvae* and *M. plutonius* to be tested with 3 assays per pathogen (2x PCR and 1x qPCR for *P. larvae*, 1x PCR and 2 qPCRs for *M. plutonius*) in double technical replicates (Table 6). For the positive samples, DNA was extracted from debris of AFB- and EFB-clinical samples. In addition, two samples containing a mix of other bacteria that are either closely related to the target organisms (different *Bacillus* spp., *Paenibacillus alvei*) or were detected in beehives before. The participating laboratories were provided with samples and controls. All participants received their samples numbered in a random order, along with primers and probes, mastermixes, an instruction protocol, and a form for sending the results.

Results of the TPS

Analysis was done in R (version 3.5.0; R Core Team 2018). Estimations of sensitivity (Table 4) and specificity (Table 5) were calculated for every method. The related confidence intervals were calculated with the method of Agresti-Coull (Brown et al. 2001).

Table 4. Estimation of sensitivity of the tested methods.

Method	**Estimation**	**Confidence interval**
Real-time PCR Dainat *M. plutonius*	64.1%	[53.0%, 73.9%]
Real-time PCR Dainat *P. larvae*	73.1%	[62.3%, 81.7%]
PCR Bakonyi	48.7%	[37.9%, 59.6%]
PCR Govan	44.9%	[34.3%, 55.9%]
PCR Kilwinski	48.7%	[37.9%, 59.6%]
Real-time PCR Roetschi	64.1%	[53.0%, 73.9%]

Generally, the real-time PCR assays showed, for both pathogens, a better sensitivity (ranging from 64.1% to 73.1%) than the conventional PCRs (44.9% to 48.7%, Table 4). The specificity was high for all tested assays (Table 5).

Table 5. Estimation of specificity of the tested methods.

Method	**Estimation**	**Confidence interval**
Real-time PCR Dainat *M. plutonius*	97.6%	[86.6%, 100.0%]
Real-time PCR Dainat *P. larvae*	92.9%	[80.3%, 98.2%]
PCR Bakonyi	92.9%	[80.3%, 98.2%]
PCR Govan	83.3%	[69.1%, 92.0%]

PCR Kilwinski	97.6%	[86.6%, 100.0%]
Real-time PCR Roetschi	97.6%	[86.6%, 100.0%]

Table 6 presents the results of the TPS. The columns show the type of the sample (negative, weak positive and strong positive). In the rows, the results submitted by the laboratories for these samples are summarised.

Table 6. Test performance study (TPS): General matrix per laboratory and pooled results for all laboratories per sample type.

General matrix		***M. plutonius***		
		negative	weak positive	strong positive
P. larvae	negative	2	2	3
	weak positive	2	3	2
	strong positive	3	2	1
		Sample type		
Real-time PCR Dainat – *M. plutonius*		negative	concentration	
			weak positive	strong positive
Pooled results	negative	41	28	0
	positive	1	14	36
Real-time PCR Dainat – *P. larvae*				
Pooled results	negative	39	18	3
	positive	3	24	33
PCR Bakonyi				
Pooled results	inconclusive	2	2	0
	negative	39	38	0
	positive	1	2	36
PCR Govan				
Pooled results	no result	7	7	6
	inconclusive	0	1	0
	negative	35	29	0
	positive	0	5	30
PCR Kilwinski				
Pooled results	inconclusive	1	0	0
	negative	41	40	0
	positive	0	2	36
Real-time PCR Roetschi				
Pooled results	negative	41	28	0
	positive	1	14	36

Not all participants in the TPS detected some of the very weak positive samples. This is reflected by generally low sensitivity values. Summarizing the comparative results from the pretesting and the ILC (TPS) for the detection of *M. plutonius* and *P. larvae* from beehive debris it can be concluded that real-time PCR approaches (here: Dainat et al. 2018; Roetschi et al. 2008) are more sensitive and should preferably be used.

Milestone M2.4: AFB and EFB control methods

Contributors:

Dr. Rudolf Moosbeckhofer, Dr. Alexandra Ribarits, Hemma Köglberger

Description:

A review of the best low environmental impact methods for AFB and EFB control

Output:

American Foulbrood (AFB) is subject to Regulation (EU) 2016/429 ('Animal Health Law') and a listed disease. AFB has to be monitored and notified to the competent authorities, and measures must be taken to prevent its spread. The use of antibiotics in honeybees is not permitted in the EU; moreover, their use would not be a method of low environmental impact. Alternative, low environmental impact methods are sacrificing and burning of clinically infested hives, and the so-called "shook swarm method", respectively. Because of the contagiousness of *P. larvae* and its ability to survive in bee products and hive equipment for several years, and up to 35 years in dry larval scales, thorough and effective sanitation measures are necessary to eliminate the disease in an apiary with clinical or subclinical infestation.

The disadvantage of burning only the clinically infested colonies is that this will not remove the pathogen from the subclinically infested hives, apiary and beekeeping operation. As *P. larvae* spores could be in colonies long before the occurrence of clinical symptoms, the only way to overcome this deficiency is to submit all colonies of such an apiary to the shook swarm procedure – irrespective of AFB-symptoms. To re-establish the sanitised colonies, only use new or disinfected hive material, foundation and sugar syrup.

Kill and incinerate affected colonies in case the disease appears in recently acquired colonies and 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.

Apart from carrying out the shook swarm procedure, the following steps are indispensable for control and elimination of AFB and EFB by low environmental impact methods:

- 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 cannot be disinfected with justifiable expense and effort.
- 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.

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. This also applies to EFB.

More details about the shook swarm procedure for AFB- and EFB-elimination and control are given in Chapter 2. – American Foulbrood (AFB) of the "Guidelines on sustainable management of honeybee diseases in Europe" compiled by the partners in the BPRACTICES project (please see M8.2, Honeybee diseases control in sustainable beekeeping).

AGES tested the efficiency of the shook swarm method in practice by applying it to subclinically infested honeybee colonies. To this end, food store samples were analysed in the laboratory of AGES (Department of Apiculture and Bee Protection) employing the in-house culture method before and after performing the shook swarm procedure. To evaluate the molecular methods defined within BPRACTICES, in addition to the culture method, debris samples were collected and analysed using the protocols that were defined as the most suitable based on the selections procedure and the TPS/ILC "ring trial".

Work package 3 (WP 3) - "Nosema". Leader: Partner 4

Dr Mariano Higes (Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA))

Milestone M3.1: List of GBPs sent to WP5

Contributors:

Dr Mariano Higes, Dr Raquel Martin Hernandez

Description:

The definitive list of Good Beekeeping Practices - GBPs (general) and Biosecurity Measures in Beekeeping-BMBs (disease specific) for Nosema

Output:

The list of Good Beekeeping Practices has been published at this link (http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines/) and is reported in Table 1.

The list of Biosecurity Measures in Beekeeping - BMBs (disease specific) for nosema is reported in Table 5.

Table 5

ANIMAL FEEDING AND WATERING
Prevent artificial water sources from faecal pollution or drowned or dead bees
HONEY BEE HEALTH MANAGEMENT
VETERINARY MEDICINES
Treat the colony if percentages of infected bees are higher than 40%, if there are any registered/permitted products in your country against Nosema
DISEASE MANAGEMENT
Remove combs with signs of dysentery
Strengthen and stimulate the colonies in autumn and spring with the administration of stimulant integrators or feed supplements
Adopt a proper pathogen (e.g. Varroa) control, to ensure a proper balance in the composition of the bee colony (equilibrium of nurse-forager bees)
PRE-CLINIC INDICATORS
Take samples of forager honey bees (or powder sugar or debris) early in autumn or spring to diagnose Nosemosis (PCR and microscopical methods)

General GBPs for Nosema:
Select queen breeders with Nosema-free stocks;
Verify the proper orientation (to South-East) and positioning of the hives: sunny and dry in the wintering places, avoiding humidity and wind and ground depressions
Destroy colony in case of heavy infection in weak colonies;
Strengthen and stimulate the colonies in Autumn and Spring with the administration of stimulant integrators composed by vegetal substances/molasses or vitamin integrators if they are registered/permitted products in your country
Disinfect beekeeping tools and equipment between uses: torching (Nosema ceranae spores are inactivated to over 60°C); gamma radiation; fumigation of combs with glacial acetic acid, sodium hydroxide 5% (caustic soda); sodium hypochlorite 0.5% (bleach). Prerequisite of any use of disinfectants is a legal status as a biocidal product in your country - check before any application
Do not feed extracted honey, combs with stores (honey or pollen) from Nosema infested to healthy colonies
Select and breed Nosema resistant honey bees

Milestone M3.2: GBPs to prevent Nosema

Contributors:

Dr Mariano Higes, Dr Raquel Martin Hernandez

Description:

The definitive list of innovative GBPs to prevent Nosema

Output:

The list of preclinic indicators for AFB and EFB is reported in Table 5.

Milestone M3.3: Laboratory methods for Nosema

Contributors:

Dr Mariano Higes, Dr Raquel Martin Hernandez, Dr. Antonella Cersini

Description:

The definitive list of harmonized laboratory methods for Nosema

Output:

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 analyse 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.

Milestone M3.4.1: Nosema control methods

Contributors:

Dr Mariano Higes, Dr Raquel Martin Hernandez

Description:

A review of the best low environmental impact methods for Nosema control

Output:

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 which clinical signs are present) and the period of the year in which the infection has been detected. 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 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 pro-duct 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, 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.

Milestone M3.4.2: Nosema control methods - trials

Contributors:

Dr Maja Ivana Smodiš Škerl, Jernej Bubnič, Janez Prešern

Output:

Two commercial food supplements for bees, BeeStrong and BV+, were tested in laboratory conditions in Slovenia. The supplement BeeStrong contains composition of aminoacids, similar to rojal jelly, and BV+ contains essential aminoacids, lipids, minerals, essential oils nad antioxidants. Bees were put in smal hoarding cages and fed sugar syrup with addition of food supplements or candy with pollen. Bees were individually inoculated (*per os*) with Nosema spores. Dead bees were counted daily and samples were taken to determine

the number of spores and the size of food glands. Results showed high level of Nosema spores in bee abdomen in all nosema treated groups (BeeStrong, BV+ and candy with pollen) but significantly higher mortality of bees in the groups with food supplements compared to the group with pollen. The pollen group had the best survival and gland development in treated bees in laboratory condition.

Three field trials were carried out in 3 Countries (Italy, Spain and Turkey) in order to verify the reduction in number of spores and any possible side effect after the administration of the same products.

The number of colonies involved into the trial is reported below:

PARTNER	BEESTRONG	BV+	CONTROL	Time of treatment
ITALY	3	2	3	16/4 – 24/5
SPAIN	5	5	5	26/2 – 2/4
TURKEY	0	25	0	
TOTAL	**8**	**32**	**8**	

The protocol adopted was:

Day -7: identification of honey bee colonies to include in the experiment and 1st nosema sampling

Selection of honey bee colonies was performed as described in Botías et al., 2013 and Higes et al., 2014. All bee colonies must have a similar population. Determine the number of combs occupied by bees and by brood, according to Botías et al., (2013).

Diagnostic/detection of *Nosema spp*. infected colonies: sample forager bees outside the entrance of the hive. Take a sample of 60 forager bees, to be analyzed with the OIE method, and 25 interior bees, from the external frames occupied by bees to avoid sampling nurse bees, to be analyzed by PCR (Martín-Hernández et al., 2012).

Create 3 statistically homogeneous groups respect the amount of infection and number of combs occupied by bees and by brood.

Colonies will be distributed in two different apiaries in order to reduce the risk of re-infecting the treated colonies with *N. ceranae* through contact with the untreated ones (Botías et al., 2013) (minimum distance of 500m and similar environmental and geographical conditions for the apiaries).

Day 0: administration of the products

Group BEESTRONG: add 50ml of BEESTRONG to 250ml of syrup. Mix with the help of a magnetic stirrer (the product can be prepared also the day before). Keep the solution at room temperature

and avoid direct light. Add this solution (300ml) into a feeder (suggested top feeders) and repeat the procedure for each colony.

Figure 1. BEESTRONG preparation and administration to the colonies

Group BV+: pour on top of frames containing bees, 30 grams of the dry BV+ product. In case of a different number of frames populated by bees, please refer to the table below.

Number of frames of bees	**Grams**
10	30
8	25
6	20
4	15

Please, be careful to distribute homogeneously on the entire area on top of frames.

Moreover, add one patty of medicated candy on the top of the frames.

>7 frames: administer 2Kg of patty/colony

<7 frames: administer 1Kg of patty for each colony

Open the plastic bag of the patty with a cutter, removing the plastic on the suface that will be placed directly in contact with the frames.

Figure 2. BV+ administration (powder and candy)

Group CONTROL: add the same syrup used to apply BEESTRONG.

Record environmental temperature and humidity in the area where the trials are carried out with a data-logger.

Day 5: consumption evaluation

Record the amount of BEESTRONG consumed with a graduated column.

Record the amount of BV+ patty consumed with a scale.

Day 5: BEESTRONG administration and consumption evaluation

Group 1: add 50ml of BEESTRONG to 250ml of syrup. Mix with the help of a magnetic stirrer (the product can be prepared also the day before). Keep the solution at room temperature and avoid direct light. Add this solution (300ml) into a feeder (suggested top feeders) and repeat the procedure for each colony.

Group 2: no action required. Record the amount of BV+ consumed with a scale.

Group 3: add the same syrup to apply BEESTRONG.

Day 15: consumption evaluation and 2nd nosema sampling (not mandatory)

Record the amount of BEESTRONG consumed with a graduated column.

Record the amount of BV+ consumed with a scale.

Day 20: consumption evaluation

Record the amount of BEESTRONG consumed with a graduated column.

Record the amount of BV+ consumed with a scale.

Day 30: last sampling and end of the trial

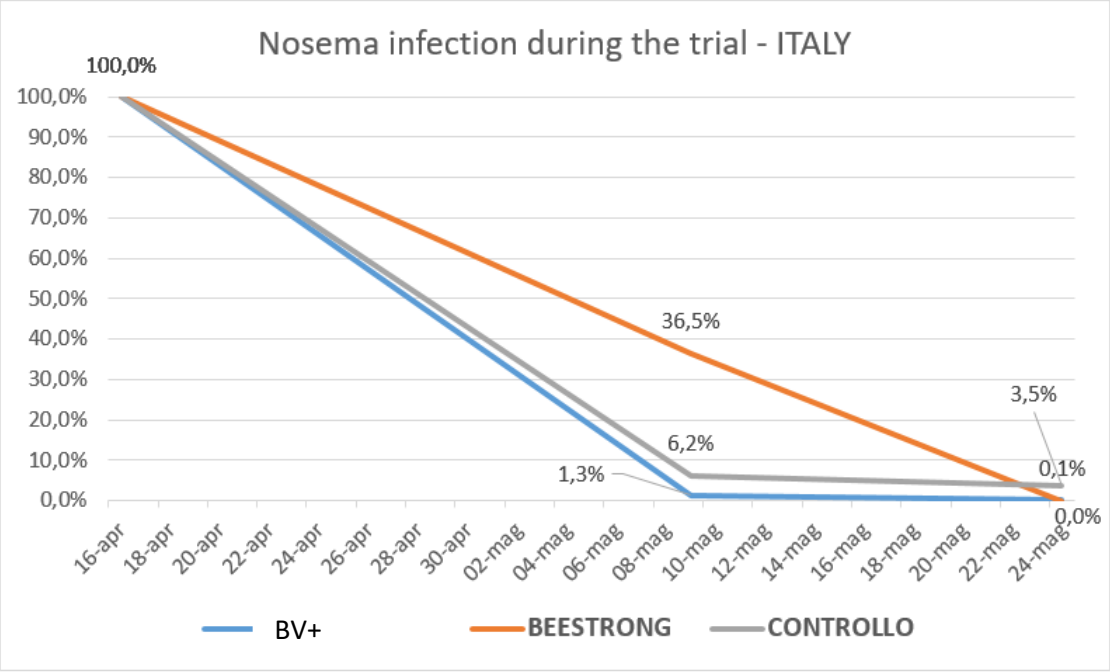
Diagnostic/detection of *Nosema spp*. infected colonies: sample forager bees outside the entrance of the hive. Take a sample of 60 forager bees, to be analyzed with the OIE method, and 25 interior bees, from the external frames occupied by bees to avoid sampling nurse bees, to be analyzed by PCR (Martín-Hernández et al., 2012).

Results

- **Reduction in *Nosema ceranae* infection**

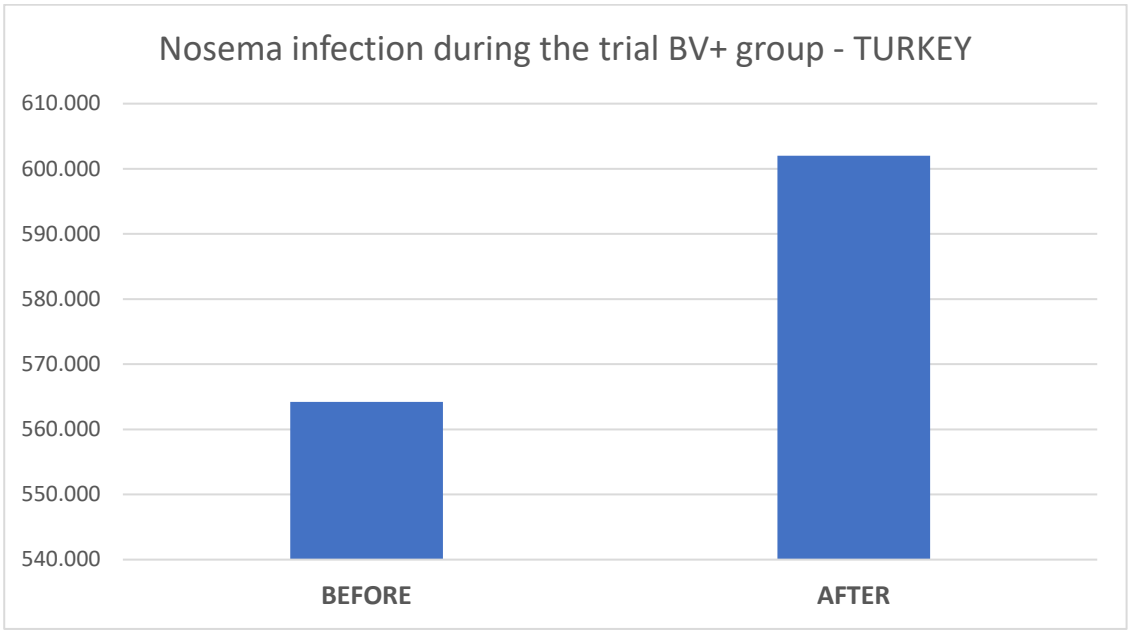
ITALY

The amount of nosema spores decreased after 23 days in all groups. At the end of the trial all groups except BEESTRONG reported some residual infection with Nosema.



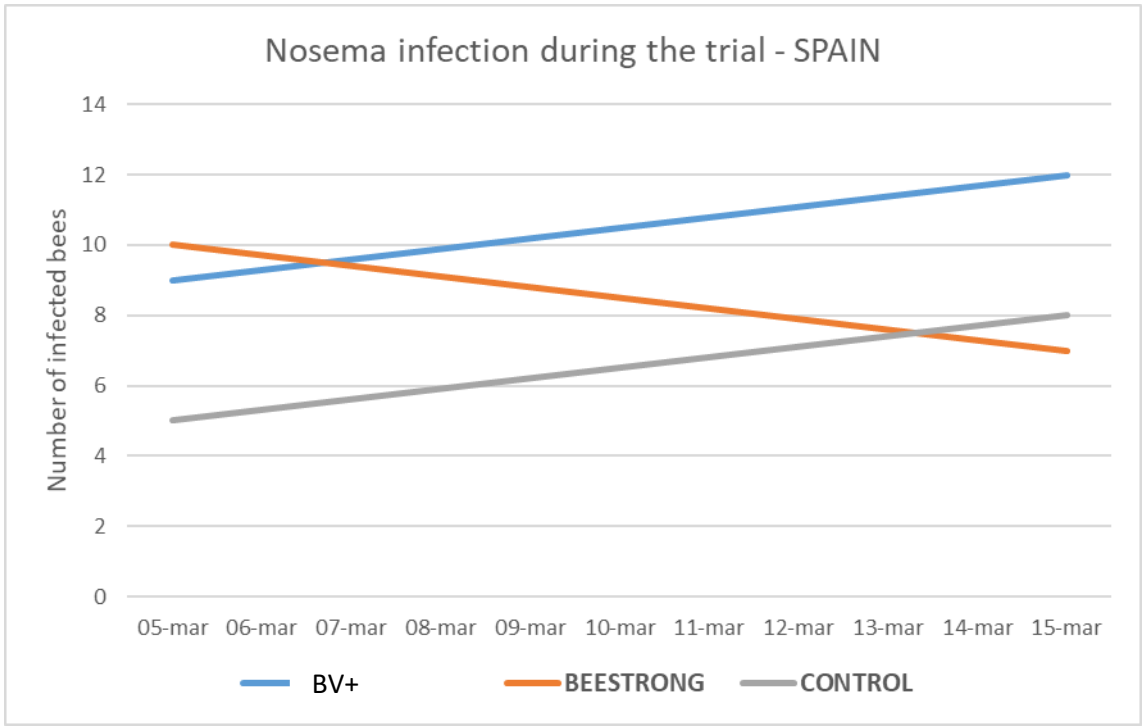
TURKEY

In Turkey the infection at the end of the trials increased respect the beginning of administration of BV+. No data are available for control group and BEESTRONG.



SPAIN

The percentage of infected bees into colonies after 10 days from the beginning of the administration increased in BV+ and CONTROL groups. BEESTRONG administration reduced the number of parasitized bees.



- **Consumption rates**

ITALY

All products (BEESTRONG and BV+) were completely consumed after administration in 5 days.

SPAIN

All BEESTRONG administrations were consumed. BV+ candy was consumed 71,18% after first administration and 83,40% after second one.

TURKEY

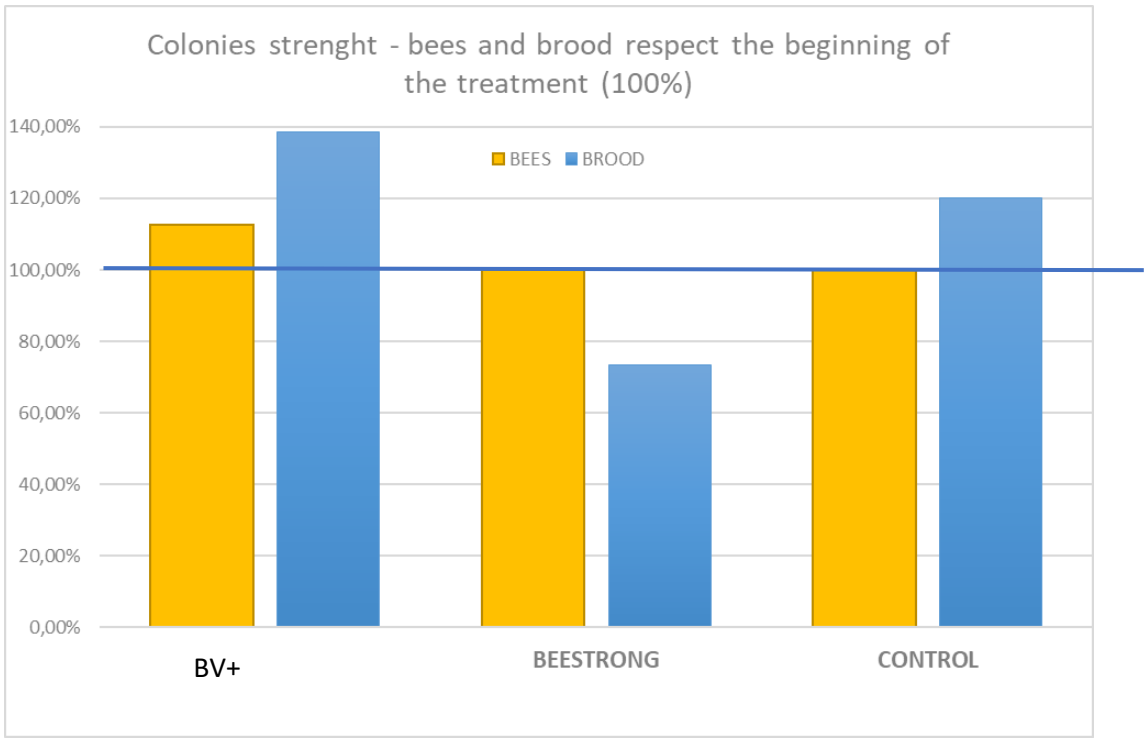
No data available.

- **Side effects**

ITALY

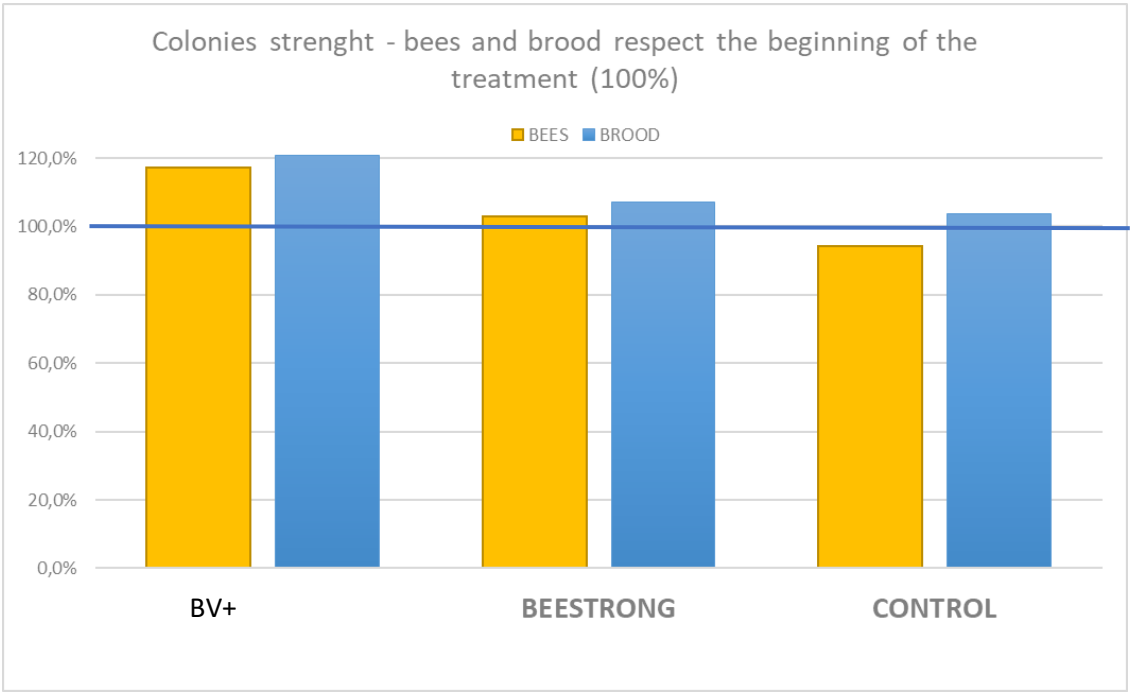
No reduction in colonies strength was observed after the administration of the product.

The reduction in brood coverage after BEESTRONG administration is due to a case of queen supersedure.



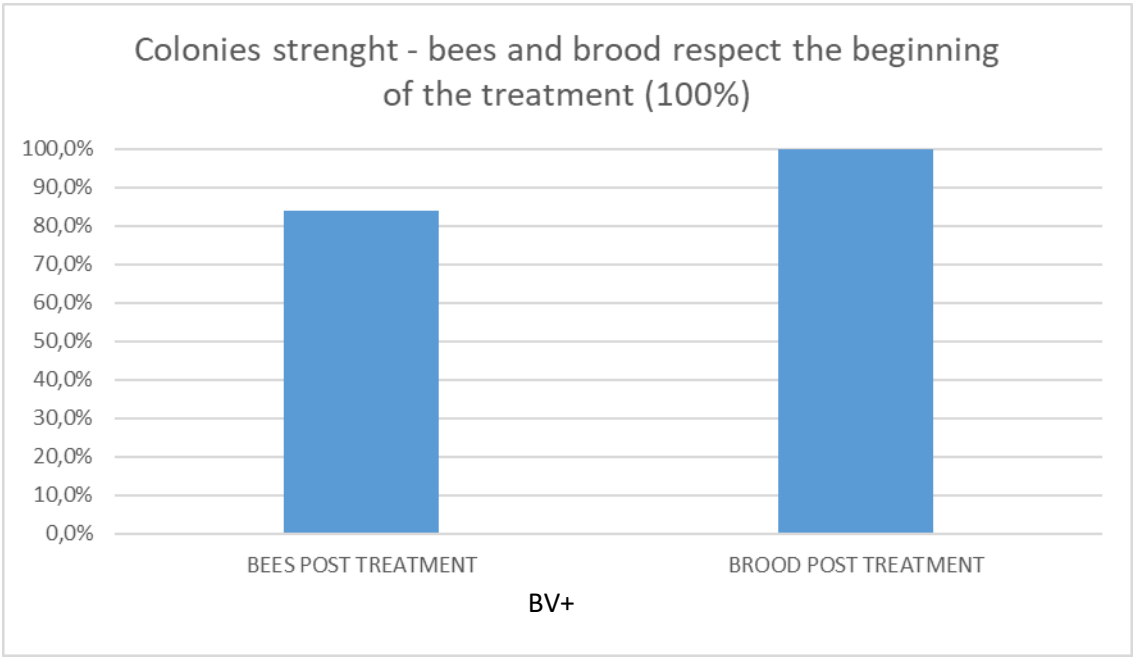
SPAIN

No reduction in colonies strength was observed after the administration of the products.



TURKEY

A reduction in the amount of frames covered by bees was observed. There are no available data for the control group.



CONCLUSIONS

BEESTRONG induced a reduction of nosema in Italy and Spain but this reduction was not statistically significant.

Consumption rates were very high for both products. A limited consumption of the candy is probably related to the lower environmental temperatures in Spain.

No reduction in colonies strength was observed after the administration of the product. BV+ induced an higher growth of the colonies.

Work package 4 (WP 4) - "*Aethina tumida*". Leaders: Partner 1, Partner 6

Dr Giovanni Formato (Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri")

Prof Ales Gregorc (Mississippi State University)

Milestone M4.1: List of GBPs sent to WP5

Contributors:

Dr Giovanni Formato, Dr Marco Pietropaoli, Dr Jorge Rivera Gomis

Description:

The definitive list of Good Beekeeping Practices - GBPs (general) and Biosecurity Measures in Beekeeping-BMBs (disease specific) for *Aethina tumida*

Output:

The list of Good Beekeeping Practices has been published at this link http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines/ and is reported in Table 1.

The list of Biosecurity Measures in Beekeeping - BMBs (disease specific) for *Aethina tumida* is reported in Table 6.

Table 6

APIARY MANAGEMENT in case of SHB being present in your area or your apiary has been in a SHB-zone (protection or surveillance zone) in the last two years
ENVIRONMENT AND INFRASTRUCTURE
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
Do not leave outside of beehives frames, combs or other material that could be attractive and edible for Aethina
Stock combs in order to prevent survival of SHB eggs and larval development in a cold chamber at a temperature below 10°C
Stock combs in order to prevent survival of SHB eggs and larval development in a chamber at less than 34% relative humidity
ANIMAL FEEDING AND WATERING
Administered artificial nutrition should be given each time at low amounts to be consumed by the bees within a short time. Pollen supplements (protein feed) could be a substrate for the reproduction of SHB
ANIMAL HANDLING
Have only healthy strong colonies in the apiary
Have only young queens with hygienic behaviour
Use queen bee excluder in order to avoid the presence of brood in the supers

Take care that the bees cover all frames in the hive (no empty space)
HONEY HOUSE MANAGEMENT in case of SHB being present in your area or your apiary has been in an SHB-zone (protection or surveillance zone) in the last two years
ENVIRONMENT AND INFRASTRUCTURE
Clean meticulously the honey house and warehouse
Use trap-lamps during the night in honey houses and warehouses with beekeeping material to diagnose SHB larvae presence
Trace meticulously movement of supers and wax
Use bleach (sodium hypochlorite) in the cleaning of honey houses and warehouses in order to prevent the development of SHB larvae and yeasts (*Kodamaea ohmeri*) if it is allowed as a cleaning agent in your country
Return the extracted supers to the hives in order to allow the bees to remove the remaining honey from the combs. (Prevent robbing!)
HIVE PRODUCTS HANDLING
Extract immediately the honey after the harvesting (at latest within two or three days)
HONEY BEE HEALTH MANAGEMENT in case of SHB being present in your area or your apiary has been in a SHB-zone (protection or surveillance zone) in the last two years
VETERINARY MEDICINES
DISEASE MANAGEMENT
Carry out periodical hive inspections to detect and eliminate the parasite (adults and larvae)
Use traps to monitor and control SHB presence in the apiary
APIARY MANAGEMENT in case of SHB not being present in your area and your apiary has not been in a SHB-zone in the last two years
ENVIRONMENT AND INFRASTRUCTURE
Do not leave outside of beehives frames, combs or other material that could be attractive and edible for Aethina
ANIMAL HANDLING
Have only healthy strong colonies in the apiary
Have only young queens with hygienic behaviour
Do not transport, into your apiary live material at risk (hives, queens, nucs, etc.) from areas where SHB is present
Do not transport, into your apiary live material at risk (hives, queens, nucs, etc.) from areas where SHB could be present
Use queen bee excluder in order to avoid the presence of brood in the supers
Take care that the bees cover all frames in the hive (no empty space)
HIVE PRODUCTS HANDLING

Do not transport into your apiary material at risk (supers, wax, pollen, etc.) from areas where SHB is present
Do not transport into your apiary material at risk (supers, wax, pollen, etc.) from areas where SHB could be present
DISEASE MANAGEMENT
Good knowledge of SHB morphology of eggs, larvae and adults
Good knowledge on hive inspection methods to detect SHB
PRE-CLINIC INDICATORS
Adopt specific traps for quick visual detection of SHB
Monitor periodically the presence of SHB sampling debris or honey

Preclinic indicators
"Monitor periodically the presence of SHB sampling debris or honey"

SHB-monitoring in Austria

As official entries in TRACES show, intra-Community movements of bees from Italy (non-restricted areas) to Austria have taken place in the last years. AGES (Department for Apiculture and Bee Protection) is the national reference laboratory (NRL) for bee diseases. Consequently, a monitoring system for the early detection of the presence of SHB was designed, with the following objectives: a) establishment of a molecular diagnostic method (PCR) for the detection of *A. tumida* at AGES, according to its tasks as NRL, b) identification of areas with increased risk of possible introduction of *A. tumida*, c) implementation of a PCR-supported SHB monitoring over a period of three years on debris samples provided by beekeepers from all Austrian provinces in a "Citizen Science"-approach. According to the project plan, about 60 apiaries are to be included each year. As a positive control, the EU Reference Laboratory (EURL) for Bee Health provided suspensions of homogenised SHB larvae and adult beetles as positive control material. SHB was successfully detected in the extracted DNA, which confirmed the successful establishment of the PCR detection method. Areas with an increased risk of *A. tumida* introduction were identified in a multi-step approach, which included TRACES entries reporting bee transports from Italy to Austria in 2015, 2016, 2017 and 2018, data on areas with the highest winter losses (Brodschneider and Krobath, 2019; bienenstand.at) , and areas with transit routes, transport hubs, and an increased offer of bee colony rental. Based on the thorough analysis of the available data, beekeepers were recruited to participate voluntarily in the SHB monitoring. The participants were provided with all materials necessary to take part in the study. Debris samples were obtained from 5 colonies from the apiaries of each participant. These samples were tested as a pooled sample for each apiary by PCR for the presence of DNA from the SHB. During the three-years monitoring, a total of 172 samples from 60 defined areas (political districts) were submitted for testing. As the results showed, none of the 172 samples contained *A. tumida* – neither as adult beetles, parts of them or larvae (as tested by visual screening) nor as DNA of the SHB (as tested by PCR).

Brodschneider R. and Krobath I. (2019) Zukunft Biene 2 – Grundlagenforschungsprojekt zur Förderung des Bienenschutzes und der Bienengesundheit, Modul U: Wintersterblichkeit (2. Zwischenbericht, S. 19 ff; Editors: K. Crailsheim, R. Brodschneider)

Milestone M4.2: GBPs to prevent *Aethina tumida*

Contributors:

Dr Giovanni Formato, Dr Marco Pietropaoli, Dr Jorge Rivera Gomis

Description:

The definitive list of innovative GBPs to prevent *Aethina tumida*. New hive inspection methods to improve the identification of the parasite within the hives. Indications on how to use sentinel nucleus to early detection of *Aethina tumida* in at risk areas for SHB.

Output:

The definitive list of innovative GBPs to prevent *Aethina tumida* is reported in Table 6.

Milestone M4.3: Sustainable protocols to SHB control

Contributors:

Dr Giovanni Formato, Dr Marco Pietropaoli, Dr Jorge Rivera Gomis

Description:

Best sustainable protocols to control SHB have been published with the collaboration of WG8.

Output:

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 sources positioned on the floor of the extraction room overnight, attract the SHB larvae. In this way they may be collected and destroyed by putting them in alcohol or detergent solution.

Milestone M4.4: Innovative biosensor method

Contributors:

Prof. Roberto Eggenhoffner

Description:

A new electrochemical biosensor laboratory methods for indirect diagnosis of SHB throw detection of Kodamaea ohmeri and OP has been developed.

Output:

ABSTRACT

The commitments of the research Unit in Medical Biophysics (MB) at the University of Genova in the BPRACTICE project are twofold: the development of biosensors for the detection of organophosphate

contaminations and the early detection of the *Aethina tumida* small beetle (SHB) that has contaminated in particular South Italy hives.

In connection with the first commitment, the MB Unit has developed primarily three biosensors of increasing complexity and accuracy: 1) a simple biosensor exploiting lateral flow principle capable of providing colorimetric response; 2) an electrochemical qualitative biosensor to investigate the electrochemical response from the interaction of specific enzymes with the organophosphates (OP) residues in honey and 3) quantitative electrochemical biosensors to measure the electrochemical amperometric response from the interaction of OP with the same specific enzymes deposited and entrapped on the biosensor surface.

The widespread findings that OPs are chemicals frequently used against the SHB affecting bees and environment as well well justify the reason for the twofold activity entering the project and also that the use of OP in agriculture has well-known consequences in depleting bees' immunity system. The second commitment of the MB Unit concerns the design and implementation of a biosensor based on a quartz crystal microbalance for the early detection of the Kodamaea ohmeri as a specific indicator of SHB's presence. Anti-yeast anti-peptide was immobilized on the gold surface of a quartz-crystal transducer to maximize yeast binding efficiency. The biosensor takes full advantage of proper transducer surface functionalization to give rise to an immunosensor in a quartz crystal microbalance. As discussed in detail in the present report, the MB Unit has fulfilled both the commitments stated in the project.

Finally, the Unit is committed to the final task duties that concern the publication of a comprehensive review of the results concerning GBPs approved by beekeepers, sustainable protocols for *Aethina tumida* control and the innovative use of biosensors.

1 General features of the strategy to adopt biosensors

The study of biosensors is still very up to date, although the first devices were introduced in the sixties of the last century, i.e., more than 50 years ago. Generally speaking, a biosensor exploits the signal of the biological component such as microorganisms, enzymes, antibodies implemented by a physiochemical transducer, and an electronic apparatus to amplify and comprehend the signal before it is transmitted to a computer or mobile phone.

The main reasons explaining such widespread interest involve the practical need to control suitable selected specific parameters representative of a general process to provide the required experimental evidence. Applications in environmental protection, clinical diagnosis in diverse areas of medicine, pharmacology, food, agriculture, safety, and defense are still increasing nowadays although it is well known that chemical investigations can be performed by bulky instrumentations such as HPLC, gas chromatography, mass spectrometry and biochemical investigations as, for instance, PCR and RTPCR analysis. However, these techniques are available in medium-large laboratory facilities, and they are expensive and difficult to be adapted to on-field applications. The introduction of a properly designed biosensor aims to overcome many of the disadvantages of analytical methods based on bulky instrumentations.

At present, however, technological troubles prevent the expected practical applications of biosensors that are used only rarely given their impracticability for real samples, whereas a biosensor developed in

laboratory concerning standards is not routinely applicable for actual examples. Hence, the challenge to achieve reliable results is to test and apply well accredited existing concepts for constructing biosensors suitable for real samples and usable in well-defined operating conditions, mostly throughout a specific protocol.

The main aim of the present section of the BPRACTICES project concerns the development of readily available instrumentation for applications to control environmental pollution due to pesticides and SHB. In the new management practices (Good Beekeeping Practices - GBPs), it emerges the need to adopt new clinical methods, biomechanical and innovative biomolecular techniques capable of providing reliable and quick answers concerning behaviour and health of bees.

Thus, the activities of the MB Unit focus on utilizing existing technologies for the detection of pesticides and developing new biosensors for honey to monitor and diagnose in advance honeybee diseases from SHB presence exploiting the results from RT-PCR.

2 – Involvement and Commitment of the Unit at the University of Genova in the framework of the project (WP4 section).

The central involvement in the project is summarized in Working Package 4 and, in particular, the subtasks 4.1 and 4.2 dedicated to organophosphate and *Aethina tumida* detection, respectively.

The Unit is developing an electrochemical biosensor to investigate the electrochemical response from the interaction of specific enzymes with the organophosphates (OP) residues in honey since OPs are chemicals frequently used against SHB affecting bees and environment as well. (http://www.izslt.it/bpractices/wp-content/uploads/sites/11/2017/04/BPRACTICES-WP4.pdf) Further, the Unit is studying the design and implementation of a biosensor based on a quartz crystal microbalance for the early detection of the Kodamaea ohmeri as a specific indicator of SHB's presence. Anti-yeast antibodies will be immobilized on the transducer surface to maximize yeast binding efficiency. The biosensor must take advantage of proper transducer surface functionalization to give rise to an immunosensor.

The Unit is committed to the final task duties that will concern the publication of a comprehensive review of the results with respect to GBPs approved by beekeepers, sustainable protocols for *Aethina tumida* control and the innovative use of biosensors.

The analyses will be performed on honey samples obtained from Partners to validate the biosensor method for Kodamaea ohmeri and OP residues. The evaluation of the environmental and biological impact of the increase in pollination services and the reduced use of chemicals to control diseases will be assessed by adopting the biosensor method of WP4.

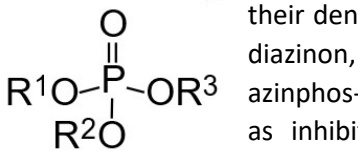
The use of dedicated biosensors can provide the quantitative basis for suggesting various degrees of attention to be translated into legislation at the European level (http://www.disc.unige.it/sites/www.disc.unige.it/files/pagine/fis-07-Biosensors%20of%20biomedical%20and%20environmental%20interest_Eggenhoffner.pdf).

The international collaboration will be useful for the preparation of a standard protocol for limiting the effects of the damage from the infection of *Aethina tumida* and hazardous chemical pollution on the health of bees and through the consumption of honey to humans (e.g., with pesticides and pathogenic yeast Kodamaea ohmeri). International collaboration is essential for the evaluation of the various and complex aspects that may arise locally following *Aethina tumida* infestation. Preventive measures and protocols accepted by all the involved subjects at the European level require international collaboration.

3 – Detection of OP

The need to control OP pollution in the current project arisen from the peculiar damage these chemicals cause to bee colonies and the possibility that farmers might use them to contrast *Aethina tumida* and other beetles diffusion.

Chemistry classifies organophosphates as phosphate esters of phosphoric acid, i.e., a class of compounds with the general formula $O=P(OR)_3$. OP occur in a diverse range of forms, including essential biomolecules such as DNA, RNA, ATP, but also many insecticides, herbicides, and nerve agents. Some of their denominations are parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, tetrachlorvinphos, azamethiphos, azinphos-methyl, and terbufos. Most organophosphates used as insecticides act as inhibitors of the enzyme acetylcholinesterase (AchE), breaking out a neurotoxic action determined by the accumulation of the neurotransmitter acetylcholine ring in the synaptic space with consequent overstimulation of the cholinergic transmission.



Acetylcholine **Choline** **Acetic Acid**

AchE is found in the synapses between nerve and muscle cells; after a signal is transmitted, it splits up the acetylcholine (Ach) in its two components, acetic acid, and choline. The two fragments are recycled to synthesize new neurotransmitters for the next contractions. This mechanism effectively stops the signal: acetylcholinesterase is one of the fastest enzymes since it degrades an acetylcholine molecule in about 80 microseconds. A recent variation was introduced in biosensor development: Thio-AcetylCholine (with the ensuing production of Thio-Choline) is used to exploit the highest efficiency of sulfur in electrochemical processes.

OP pesticides cause relatively minor adverse effects on adult humans for low dose exposures but can produce acute results in occupational exposure as farmers with convulsions, paralysis, neuropathies related to AChE inhibition more significant than 60% of control values. They degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water, but they contaminate drinking water by moving through the soil to the groundwater.

Thus, globally speaking, OP's effects on living species and the environment cannot be neglected.

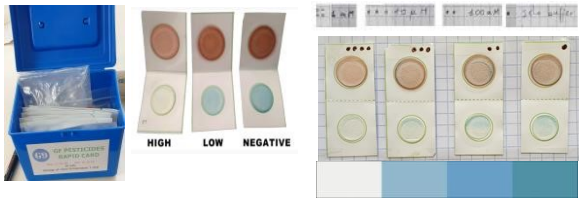
Many practical and economic biosensors widely used nowadays rely on optical or electrochemical transduction. Because of the different protocols commonly employed to treat honey, bees, and hive products, the scientific literature evaluates the reliability of a sensor or biosensor with respect to its behaviour in detecting a compound such as Syntostigmin (or neostigmine). The present work has fully adopted such a widely accepted criterion with respect to the development of all the biosensors, as discussed in the following.

3.1 - Experimental optical/colorimetric detection of OP

Commercially available pesticide "rapid cards" consist of a planar deposition on glossy/absorbing paper, the cholinesterase enzyme, and the chemical substrate with a chromogenic reagent to detect organophosphate in food tests. Both are placed in a card with two discs, respectively, that can be pressed together to allow the reaction to occur. A membrane coated with the enzyme and substrate solution is deposited on a PVC backing card. The diluted AChE enzyme (5mL) was immobilized onto the center of the membrane. A sample of honey or other hive products is placed in the middle of the discs after proper treatment. In the absence of OP analyte, the reaction at the enzyme side develops giving blue coloration; the presence of the OP analyte inhibits the enzyme, and the color remains at the beginning stage. The color pixels can be analyzed by color image software that sample pixel areas for a semi-quantitative concentration determination. The blue-green color change is induced by indoxyl acetate hydrolysis catalyzed by acetylcholinesterase (AChE) and the inhibition of AChE activity by pesticides. The time for the inhibition process and the color development were set to 15 and 10 min, respectively.

A suitable protocol was adopted for such optical biosensing card that involves the following steps:

a) Take about 5g sample into a bottle with a cover, add 10mL pure water or 10ml solution, then shake up about 50 times and stand it for at least 2 minutes.



b) Then mix/shake wet samples to extract pesticides
c) Remove the protector film from the sensor detection card.
d) Dip the white disc into the solution with the sample (from the last step) for about 2 seconds (or place a drop on the disc).
e) Stand the card for at least 10 minutes for pre-reaction
(place the card into thermostat device at 37°C if possible)
f) Fold the card in half and finger pinch the card for 3 minutes.
g) Let the white disc react with the red disc during this process at a reaction temperature around 37C (Body Temperature)
h) Run a control test by dropping the wash solution into the white disc of a new test card and let it stand for 10 minutes.
i) Fold the card in half and pinch the card for 3 minutes (www.renekabio.com/).
j) This will give the color for the negative result. Then compare the control color with the sample test color. An ideal result is reported below the cards in the above figure. Thus, the full concentration range can be split in four concentration ranges (Levels in the table below)

The LOD of these rapids cards ranges from 0.05 to 1.5 µg/mL, as detected for various pesticides. X Guo et al. [https://doi.org/10.1016/j.foodcont.2012.07.015] investigated the behaviour of rapid cards in comparisons of gas chromatographic-mass spectrometry analyses, with the following performance:

Table. The comparative results between GC–MS and test cards

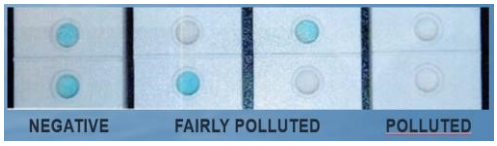
GC–MS results (µg/g)	0.05	1.43	5.24	9.73	16.71
Test card results (color levels)	Level 4 (0–1 µg/g)	Level 3 (1–5 µg/g)	Level 2 (5–10 µg/g)	Level 1 (>10 µg/g)	Level 1 (>10 µg/g)

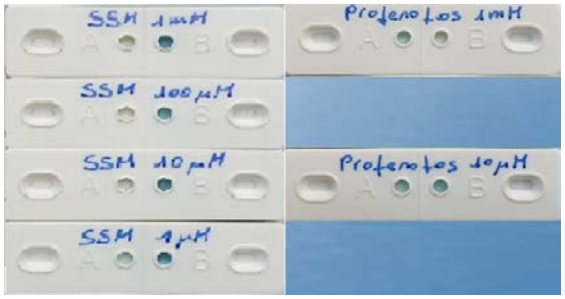
Detection results of pesticide residues showed that the influence of the sample natural color on the test card could almost be ignored, and thereby increasing sensitivity and reliability. The measurement results were entirely consistent with those of the official GC–MS method.

Thus, the rapid cards commercially available can be considered to be used for rapid class-specific screening of OP pesticides before official quantitative analysis in particular in reason of their simple, fast, and satisfactorily sensitive determinations.

Other simple devices commercially available employ a Lateral flow test [:DOI: 10.1042/EBC20150012].

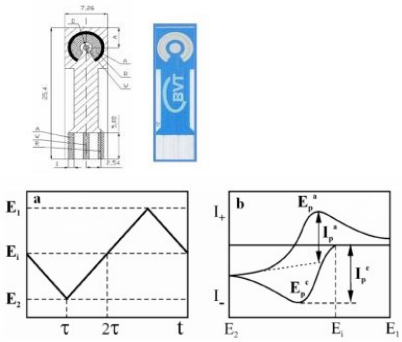
Mostly, these tests employ the reaction of a sample liquid into the surface of a pad with the reactive molecules that show an immediate visual positive or negative result. Typically, these tests are used for home testing and for medical diagnostics or even laboratory use. The home pregnancy test is a well-known and widely used application. These economic tests show results in around 5-30 minutes. The test adopts a straightforward protocol: samples are immersed in water to extract any possible contaminants like pesticides. It is left in the bottle at room temperature for 5-10 minutes, and then the sample is extracted. The external wells shown in the figure are filled on the support with five drops each, and the result is obtained in 5-8 minutes. The variation in coloring is observed: in the two smaller central wells. We report in the figure our results with the syntostigmin (SSM) drug that, as discussed above, is adopted as an international way to check the reliability of a biosensor based on the AchE mechanisms. Our results with a profenophos pesticide diluted in a buffer substrate are also reported for comparisons. It turns out a well-known result that such specific pesticide is very difficult to be traced with every analytical method, whereas we get for SSM comparable results with respect to rapid cards.





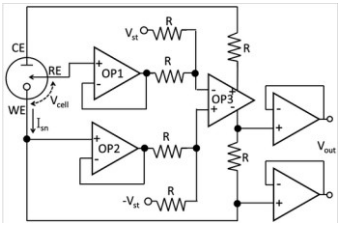
3.2 Cyclic Voltammetry and amperometric experiments

Voltammetry is an electroanalytical method used in analytical chemistry and industrial processes. The analyte is detected by measuring the current between the counter electrode (CE) and the working electrode (WE) at varying the potential applied between the reference electrode (RE) and the working electrode (WE); the resulting curves of current vs. potential are called voltammograms. The potential is varied step by step, and the actual current value is measured as the dependent variable. Voltammagram plots study the current produced by the analyte versus the potential of the working electrode, as shown in the sketch below.



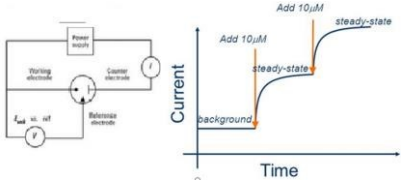
Typically, as shown in the figure, the above three electrodes are deposited on an insulating surface: the innermost is the WE, mostly gold functionalized with biological material in a biosensor, the outermost a metal - mostly platinum to form the CE and close the electrical circuit. The reference electrode (RE) is placed in the middle of such a planar configuration; it is mostly selected the Ag/AgCl reference electrode. In our case, we use gold electrodes by BVT, Inc., and

carbon WE electrodes by BIOPARD the latter chemically improved as discussed below. The codeposition of a binding/charge transfer agent is mandatory to assist the charge transfer of electrodes from the redox reactions. We also used a biofunctionalized gold WE by BVT with the deposition of two CFU enzyme units were also employed.



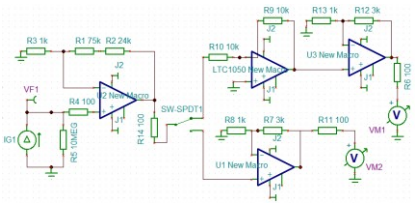
The experiments are performed by a potentiostat that effectively controls the voltage between the RE and WE, and measuring the current through the CE. A simple circuit with only five operational amplifiers provides the potentiostat behaviour at varying potential given by a control PC or microprocessor like ARDUINO as well as

the current measurement, as shown by the sketch in the Figure. The selection of an operational amplifier is critical: rail to rail precision amplifiers must be chosen to allow a potential scan of at least +/- 1V. Thus, the potential is applied and measured between RE and WE, whereas the current flow is measured between CE and WE in a closed electrical circuit. The WE is often connected to the ground to minimize electrical noise.



In conclusion, a standard CV experiment consists of measuring the current flowing through the CE and WE during a triangular potential perturbation applied to RE and WE.

Amperometry is intrinsically simpler since the potential is fixed at a chosen value, and the resulting current is measured as a function of time, as shown in the sketch above (for this reason, it is sometimes called Chronoamperometry). The enzyme must be deposited on the substrate in contact with the electrodes or, alternatively, the analyte must be exposed to the enzymatic action in a reaction chamber containing the electrodes. In both cases, it takes some time (ten minutes) to promote the enzyme reaction.



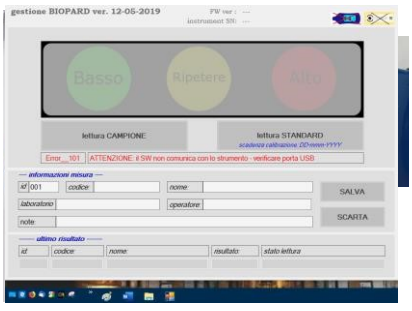
The circuit on the right represents instrumentation to measure electrical currents. It is written in TINA language (by Texas Inst) and realized with standard high-quality components. It allows measuring from a few pico to microampere currents, covering the most useful analytical ranges.

3.3 - Experimental QUALITATIVE amperometry

The second route selected for the present project was to build up a protocol employing a commercially available sensor for the OP detection in a still simple, although more sensitive method. We planned to perform conductometry (amperometry) to exploit the occurrence of the redox reactions outlined above and take full advantage of the dominant role of AChE/substrate in promoting ion migration resulting in the occurrence of electrical current. The measure of electrical current has many advantages in practical applications since:

- The redox reactions outlined in previous slides that take place at a suitable fixed potential
- The role of AChE is dominant in the redox currents as highly required for sensitivity
- The measure of the electrical current leads to qualitative/quantitative estimates of the concentration of redox species.
- One needs to deposit the enzyme on the substrate with the electrodes Ag/AgCl
- Alternatively, one has to expose the analyte to the enzyme action in a reaction chamber containing the electrodes
- In both cases, some time (ten minutes) is needed to promote the reaction of the enzyme.

An electrochemical detector for the rapid determination of organophosphorus pesticides in food (but not exclusively) can exploit the current measurement that is high in connection with the AChE action that depletes Ach (T-Ach) to Ch (TCh) increasing the ionic species in drop solution. The reagents necessary for the analysis are a buffer solution for the sample dilution and ph fixing and an enzyme solution. Thiol compounds are known as oxidable at the surface of solid electrodes, but the oxidation generally requires high potential values on a suitable electrode, i.e., 400-700 mV



[5,10]. These conditions can permit competitive reactions to happen, altering the reliability of the method: to overcome this drawback, chemically modified carbon electrodes are used.

We have selected the BIOPARD instrumentation developed by Ecobioservices & Researches s.r.l. based in Florence (Italy), see the homepage: https://www.ebsr.it/azienda/ also following the longstanding scientific collaboration between the groups of the Universities of Genoa and Florence that began in

1990. BioPARD is a sensor-based kit associated with an electrochemical detector for the rapid determination of organophosphorus and carbamic pesticides in food, water, and soil samples. The kit contains all the reagents necessary for the analysis, the selective disposable screen printing sensors for pesticides, and a portable WiFi detector for electrochemical remote control measurement. BioPARD provides in a few minutes an evaluation of the level of contamination due to the presence of organophosphoric and carbamic pesticides in the analyzed sample. It is designed for direct in-situ analysis; it does not require laboratory instrumentation and can also be used by non-expert personnel. The operation of BioPARD is based on the electrochemical measurement of the activity of a specific enzyme. This bioactivity is inhibited by the presence of organophosphoric and carbamic pesticides; therefore, by comparing the result obtained for a sample with a calibration curve obtained using specific standards, it is possible to obtain a correlation between the sample signal and its contamination level. Measurements are carried out using the detector with the appropriate selective sensors: the BioPARD detector is a remote-controlled portable electrochemical battery meter. It can be managed with all portable devices with WiFi connection and IOS, Android, or Windows systems (smartphone, tablet, or PC), by accessing the detector's web page directly and without the need to install any software. At the end of the measurement, it is not necessary to perform any data interpolation: the result will be immediately visible on the screen of the portable device. The BioPARD software provides all the step-by-step instructions for carrying out the measurement; it will be so easy to get the result of the analysis in less than 15 minutes.

The equipment for the detection of pesticides employs screen printing sensors with modified carbon electrodes by incorporating in the ink of the WE an optimized percentage of cobalt(II) phthalocyanine (CoPC). As reported in the literature, among the electrochemical mediators, CoPC was indicated as one of the most suitable for the detection of thiol-containing molecules, and the resulting oxidation signals occur at a lower voltage, i.e., around 100 mV, thus limiting the electrochemical interference of other oxidable compounds.

A calibration step instructs the apparatus to recognize high-level current (no OP) at a level of 2 10^{-9}M (some ppb). In a qualitative approach, the apparatus gives THREE possible qualitative answers for OP detection in the samples after an incubation time lasting 10 minutes, as shown above. The procedure to evaluate the pesticide inhibition effect begins by adding 10_L of Carbofuran (diluted solution) to 500_L of the buffer containing AChE in order to achieve the concentration range 10^{-11} to 10^{-6} M; afterward, the mixed solution was left to incubate for 5 min. Then, 200µL of this mixture was deposited onto the CoPC-modified sensor; a known volume of ATCh solution was also added in order to have a concentration of 1mM. After 10 min of incubation, a chronoamperometric measurement was performed (applied potential +0.1V versus pseudo-Ag/AgCl reference electrode). The current response at 30 s was evaluated. In the presence of AChE, ATCh is hydrolyzed in acetic acid and, thus, TCh concentration is strictly related to the enzymatic activity. Therefore, the incubation time between enzyme and substrate is a critical parameter. This value was evaluated experimentally by mixing 1mL of ATCh solution 1mM with 10 µL of enzyme solution (500 U/mL) in order to have 5 U/mL as a final activity. After mixing, 200 µL of it was deposited onto the modified electrode; the potential (+0.1 V) is then applied, and the current monitored for 30 min. The current increased very fast during the first 10 min, whereas after this time, a steady-state was reached (Fig. 3). Thus, 10 min was identified as the most suitable incubation time-value; the

$$I\% = 100 \times \frac{I_1 - I_2}{I_1}$$

inhibition effect of Carbofuran on enzyme activity was then checked by chronoamperometric measurements. An inhibition curve was calculated according to the following formula: where I_2 represents the oxidation current obtained for the solution of the sample (with any possible contaminants) and

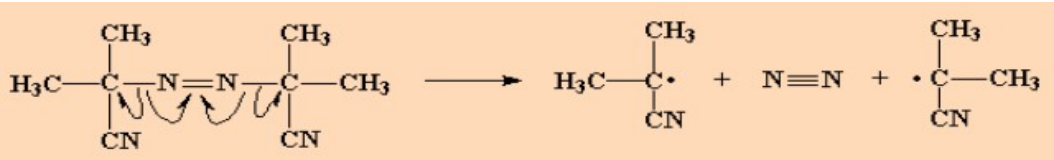
I_1 the oxidation current obtained for a control solution (prepared without pesticide for the whole incubation time).

3.4 - Experimental details and results from home-made development for QUANTITATIVE amperometry and cyclic voltammetry detection of pesticides.

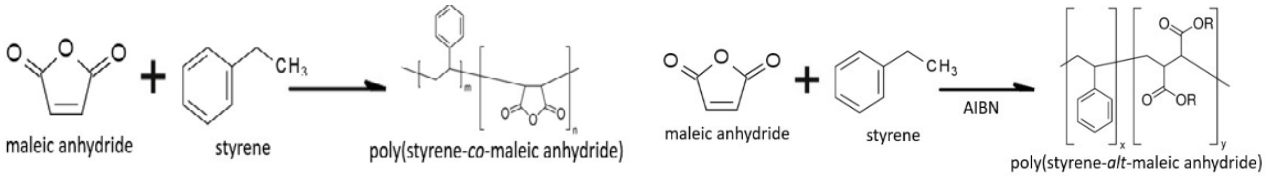
In the present section, the home-made implementations of planar sensors for electrochemical measurements are discussed, and their performances are reported both with scientific instrumentation commercially available and with devices developed in the MB laboratory in Genova. We prepared biofunctionalized electrodes based on BVT gold and carbon planar depositions by depositing a polymer suitable to capture the AchE enzyme (section 3.4.1-2) while maintaining its enzymatic function as proved below (section 3.4.3). In sections 3.4.4, electrochemical experiments and tests performed on these biofunctionalized electrodes are reported; the cyclic voltammetry measurements are discussed in section 3.4.5, also in comparisons with the biofunctionalized electrodes obtained by BVT. These electrodes are provided with two enzyme units deposited on the gold WE, as in the Figure above. Clearly, their treatment must respect the suggested recommendations, in particular concerning maintenance temperature thoroughly.

3.4.1 Experimental details on polymer deposition.

To achieve the optimal experimental conditions, one needs to deposit the enzyme on the surface of a metal electrode fabricated in a standard three-electrode configuration. Enzymes must be immobilized in a polymeric material capable of retaining their catalytic activity. Their immobilization is achieved typically through a site-specific reaction between reactive sites of the chosen material and an amino acid residue on the enzyme. These immobilized enzymes can catalyze degradation or neutralization reactions. These achievements must be tested to check the enzyme activity and to make calibrations before applying it to honey samples. Thus, various mandatory tests have been carried out following literature suggestions to identify the best solvent and the best concentration of polymer that provides a homogeneous molecular monolayer suitable for binding the enzyme. The first electrodes used (Crystal resonators) with Quartz Crystal Microbalance (QCM) were made suitable for this purpose by using a PSMA polymer on which the enzyme acetylcholinesterase (AchE) was deposited. The polymerization is fast and easy, catalyzed by the free radical polymerization through AIBN catalyst:



The polymer alternates maleic anhydride and styrene units to produce poly(styrene-alt-maleic anhydride) in one of the two following forms:



Both have a high molecular mass of the order of 350 000 Da and are soluble in the most common organic liquids. The P(St-*alt*-MA) fibers are known to be very brittle; the optimal formulation of mats containing P(St-*alt*-MA) and P(St-*co*-MA) must be investigated to match the optimal polymer depositions. In the optimized conditions, polymeric chains are envisioned to increase space separation allowing enzymes to be captured inside the resulting cavities.

Six electrodes were prepared for measurement with QCM after their functionalization. QCM measurements were performed on the electrodes selected before the coating with PSMA (A in the figure below), after the coating with PSMA (B) and after the deposition of the enzyme (C). Details on QCM will be discussed in connection with the biosensor for *Aethina tumida* detection, i.e., in section 4 below.

3.4.2 - Deposition of the enzyme as the sensitive element of the biosensor.

To achieve the optimal experimental conditions, one needs to deposit the enzyme on the surface of a metal electrode fabricated in a standard three-electrode configuration.

The freeze-dried AchE enzyme was purchased by Sigma Aldrich. It was employed in TRIS-HCl pH 7.5 and BSA 5mg / mL solutions. The enzyme was resuspended and aliquoted according to the supplier's instructions. Consequently, aliquots of 70µL in TRIS-HCl pH 7.5 at a concentration of 250U / mL were prepared. All subsequent dilutions were completed with a 5mg / mL BSA solution. Based on preliminary ^{10,000}experiments performed with the enzyme in solution, 100mU each in BSA 5mg / mL in a 1: 1 ratio was used on the electrodes.

We prepared a 1:1 enzyme and BSA 5mg/mL solution; afterward, 0,8µL of this solution (containing 100mU of AchE) was deposed on six quartz crystal resonators with gold electrodes. The enzyme was allowed to bind to the PSMA polymer coating, incubating it for 30 minutes at 22°C.

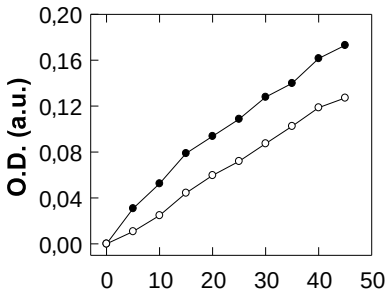
After evaporation of the water contained in the enzyme solution, the crystal resonators were washed using PBST (Tween 0,1%) one time for 5 minutes and the other two times with PBS for 5 minutes. After solution evaporation, we performed the final set of measurements with the QCM. As shown in the Figure, the frequency decreases very slightly from A to B. This result signals the deposition of a very thin polymeric film on the clean gold surface (A) and the persistence of the enzyme at the end of the procedures. As shown by the behaviour detected from B to C, the decrease is much more pronounced because of the higher enzyme mass. The figure reports frequency measurements on two different y-scale to account for the different base frequency of the two sets of quartz crystals used.

3.4.3 - Activity verification of the enzyme deposited on a solid substrate

The confirmation of the maintenance of the enzyme functionality fixed to solid support was performed by measurements to the spectrophotometer using the kit Acetylcholinesterase Assay Kit (Colorimetric) (ab138871 AbCam). Briefly, we prepared 0.1% BSA used for the preparation of the kit AEA and aliquoted all the components of the kit.

After having identified in the literature the possible concentrations useful for the purposes of the project, some verification measures were performed with the enzyme in solution.

Spectrophotometer measurements of the activity of the enzyme deposited on the electrodes were taken after the deposition of the polymer on the WE and the deposition of the enzyme at the identified suitable concentrations. Results verified that the enzyme activity was also maintained after its deposition as required. As shown in the Figure, such activity is maintained despite the fixing of the enzyme on the substrate, and it results proportionally to the units of the enzyme deposited.



To verify whether the enzyme activity was maintained fixing the AchE on the PSMA polymer also on planar electrodes, we experimented with the Acetylcholinesterase Assay Kit (Colorimetric) (Abcam, ab138871). The reaction solution was set up according to the supplier's instructions. Briefly, 450µL Assay buffer, 25µL DTNB Stock Solution, and 25µL Acetylcholine Stock Solution were placed into a cuvette and measured at 410nm by a spectrophotometer. To this purpose, we used one planar electrode having the Ache fixed on the PSMA layer. The planar electrode was introduced into a cuvette equipped with a magnetic stirrer to allow the substrate to interact better with the fixed enzyme. The activity of the enzyme fixed on the substrate was measured with OD results comparable to the behaviours reported above.

3.4.4 - Electrochemical experiments and tests

Starting from literature data to identify the concentrations of organophosphates (OP) detected in honey, bees, and pollen, the concentration of OP was established for use in experiments. The OP concentration

Al.Naggar et al., 2015	In honey	0,28	ng/g	0,00028mg/kg	Average 0,00626 mg/kg
Al.Naggar et al,2015	In pollen	11,6	ng/g	0,0116mg/kg	
Al.Naggar et al, 2015	In bees	6,9	ng/g	0,0069mg/kg	
Ghini et al., 2004	In bees from Granarolo	0,016	mg/kg		Average 0,13 mg/kg
Ghini et al., 2004	In bees from Ozzano	0,007	mg/kg		
Ghini et al., 2004	In bees from Bologna	0,017	mg/kg		

- Ghini S, Fernández M, Picó Y, Marín R, Fini F, Mañes J, Girotti S. Occurrence and distribution of pesticides in the province of Bologna, Italy, using honeybees as bioindicators. Arch Environ Contam.Toxicol. 2004 Nov;47(4):479-88.
- Al Naggar Y, Codling G, Vogt A, Naiem E, Mona M, Seif A, Giesy JP. Organophosphorus insecticides in honey, pollen, and bees (Apis mellifera L.) and their potential hazard to bee colonies in Egypt. Ecotoxicol Environ Saf. 2015 Apr;114:1-8. doi:10.1016/j.ecoenv.2014.12.039. Epub 2015 Jan 6.

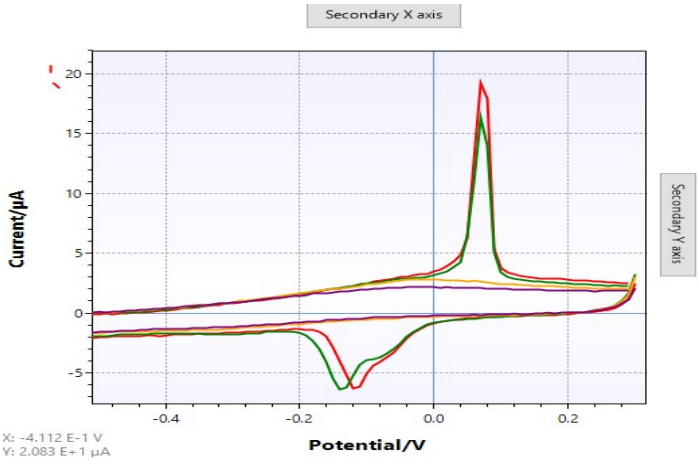
to be adopted in the present experiment was based on averaging the data provided by the following papers: the inspection of the data reported above suggested to adopt a concentration of 10^{-3}mg/L of OP. Concerning, for instance, Profenofos representative of OP with molecular weight 373,626 we used for testing operations the concentrations starting from 3 10^{-8} M approximately to three orders of magnitude higher. We used Profenofos packaging 45632-250MG from Sigma Aldrich.

A 10mM solution in milli-Q water and 1% DMSO was prepared and diluted to 1mM concentration as a mother solution. Preliminary experiments were performed using 20µL of Profenofos 10µM. Electrochemical experiments were performed using the functionalized electrodes in the presence of different OP concentrations. The results reported in the table experiments represent a guide to select the best conditions to evaluate the contaminant products from the enzyme inhibition.

3.4.5 – Measurements on AchE activity in cyclic voltammetry (CV)

We use a PalmSens EmStat Blue III potentiostat obtained by BVT to perform cyclic voltammetry measurements and detect the redox reaction of Ach, as reported above. A 5µL drop of an Ach solution was deposited on the planar electrode in the CV controller functionalized with the AchE enzyme.

A 15µL drop of an Ach solution was deposited on the planar electrode functionalized with the AchE enzyme in the above-reported polymer. The CV was measured by using the PalmSens controller. Further, 5µL of AgCl/KCl electrolytic solution and 5µL of a buffer phosphate solution at pH=7.4 were added to improve the conductivity of the solution. The voltammogram below turns out from these experimental setup conditions.



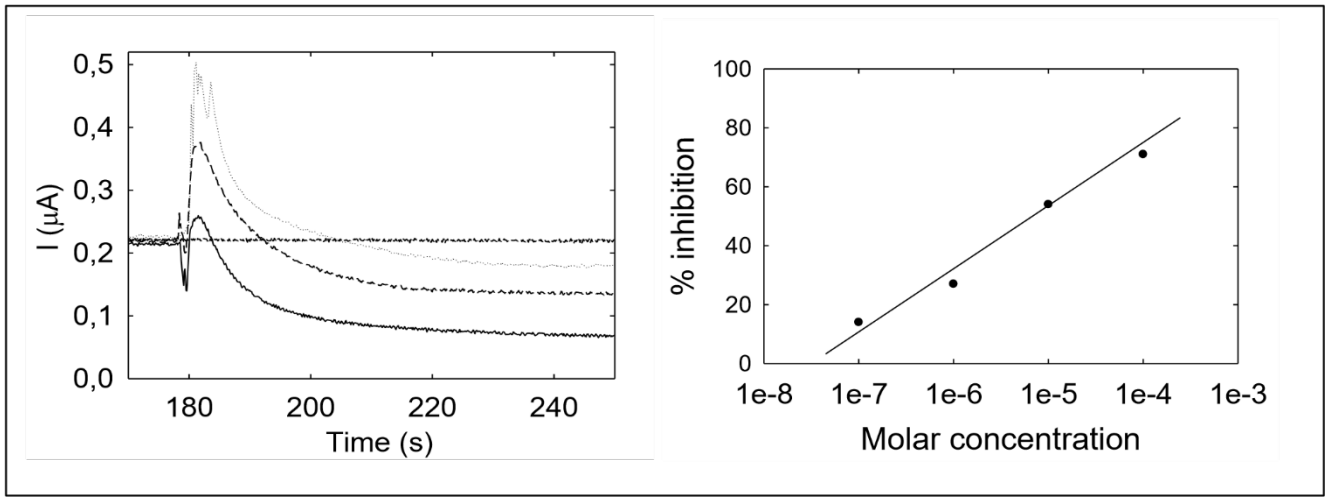
The inhibition of the entrapped enzyme from a contaminant agent was studied with the home-prepared electrodes as discussed above and also with commercial biofunctionalized

electrodes supplied by BVT. Both cyclic voltammetry (CV) and chronoamperometry were investigated employing the PalmSense potentiostat. We report below CV results with homemade electrodes and chronoamperometry with biofunctionalized BVT electrodes. Results for CV and amperometry measurements are entirely consistent.

In the Figure, the cyclic voltammograms collected for a solution with no OP contaminants are shown. The upper red curve is obtained at the very beginning, i.e., collected immediately after the contact of the drop on the electrode with the enzyme entrapped in the polymer in a fast mode CV of the potentiostat. The CV time behaviour of the Ach redox reaction (i.e., from the upper red to the green, yellow and the lowermost purple curves) shows the decreasing heights of the peaks at increasing time under the action of the enzyme that catalyzes the transformation of Ach in choline; curves are collected every 5 minutes. The peaks of the Ach dissolution reaction occur at 70 and -120 mV. The voltammograms reported are a clear indication of the enzyme activity in depleting the electroactive species in the drop solution and the possibility to detect currents starting from 200 mV.

The results of the chronoamperometry are reported for the four solutions of the standard Syntostigmin prepared in an Eppendorf safe-lock microcentrifuge tube with the following concentrations: 1 mM, 100

The results of the chronoamperometry are reported for the four solutions of the standard Syntostigmin



prepared in an Eppendorf safe-lock microcentrifuge tube with the following concentrations: 1 mM, 100 µM, 10 µM, and 1 µM. A 30 µM drop from the substrate solution containing Ach 1mM was deposited on the electrode plate, and the current measurement was started. As suggested by the cyclic voltammogram above, voltage is fixed at 200 mV by the PalmSense potentiostat that also collects current measurements between CE and WE covered by solution drop in the chronoamperometric operation mode. After 180 s, stable current measurements were observed, and a 3 µM small drop from one of the above four solutions was added to the substrate drop of the substrate covering the electrode plate. In these conditions, the effective concentration is one-tenth of the four preparation values. After initial instabilities, as shown in the chronoamperometry figure above, the inhibition activity of the enzyme takes place, resulting in a higher decrease of the current values in the plateau regions above

240 s at increasing the concentration of the agent, i.e., the Syntostigmin drug. As discussed above, this drug can is capable of simulating the same inhibition action on the AchE enzyme by any OP pesticide. The results for the four solutions are reported in the inhibition vs. concentration plot, suggesting a linear dependence on a semi-logarithmic scale.

3.5 Availability of samples

Honey and hive product were supplied by the Istituto Zooprofilattico IZSLT in Rome, directed by the project leader Dr.Giovanni Formato. These samples were analyzed for the presence of pesticides with

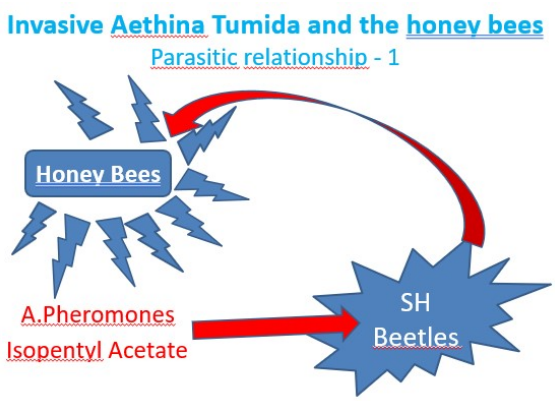
the methods described above. The samples turned out free of contaminations by OP pesticides. As reported by literature, residues of insecticide used in agriculture can hardly be found in honey. The concentration of OP in honey is up to a thousand times lower than that found in bees! This huge difference is due to a sort of filter effect of bees: it is not surprising! A bee can make up to 1000 microsamples per day; considering a hive with an average of 20,000 foragers, it translates that in a hive about 20 million micro-withdrawals per day. Since most pesticides are soluble in fat, they tend to settle in wax rather than honey. The samples were treated accordingly to literature suggestions as in https://doi.org/10.1016/j.talanta.2010.06.043. Ultimately the degree of concentration from pesticides in the various apiary products follows this order: bees > propolis > wax > pollen > honey

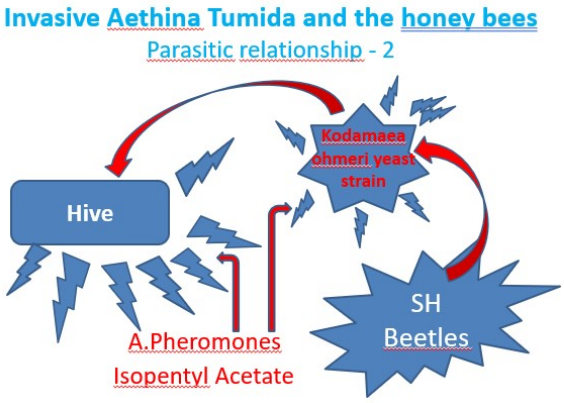
4 - Development of micro(nano)-gravimetric biosensor for *Aethina tumida* contamination

4.1 - Preliminary issues.

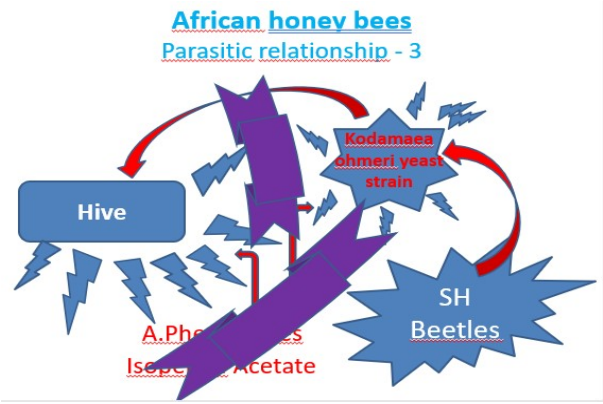
The beetle is not a pest of African honey bees because African bees have evolved effective methods to mitigate beetle infestation. Therefore, the environment of the European honey bee colony provides optimal conditions to promote the unique bee–beetle–yeast–pollen multitrophic interaction that facilitates effective infestation of hives at the expense of the European honey bee

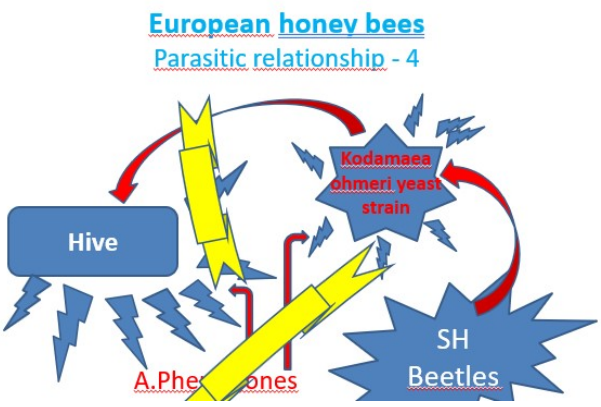
(https://doi.org/10.1073/pnas.0702813104). The hive is overrun with beetles and their larvae, causing the bees to abandon it.





Alarm pheromones are critically crucial to the survival of honey bee colonies. In the parasitic relationship between the European honey bee and the SHB, *Aethina tumida*, the honey bee's alarm pheromones serve a negative function because they are potent attractants for the beetle. Furthermore, the beetles from both Africa and the United States vector a strain of Kodamaea ohmeri yeast, which produces these same honey bee alarm pheromones when grown on pollen in hives as shown by Torto et al. in the reference above.





Interestingly, Kodamaea ohmeri yeast, which uses the beetles as a vector, also produces isopentyl acetate when grown on pollen in hives, mimicking of the bee alarm pheromones by yeast attracts even more beetles. In these conditions, the bees to abandon the hive. These findings contribute to suggest the detection of the Kodamaea ohmeri yeast, and, for the present reason. The development of a biosensor for such detection has been proposed since the early development of the current project on these bases.

Kodamaea ohmeri is an emerging pathogen that is studied by medical mycology. Kodamaea ohmeri is a rare yeast pathogen that has recently emerged as an essential cause of fungemia in immunocompromised patients. A case of catheter-related bloodstream infection caused by Kodamaea ohmeri in a 58-year-old patient was reported (https://doi.org/10.1016/j.diagmicrobio.2013.02.021). The patient improved after the removal of the venous catheter and micafungin treatment. Echinocandins are suggested as the first choice for therapy with respect to this pathogen.

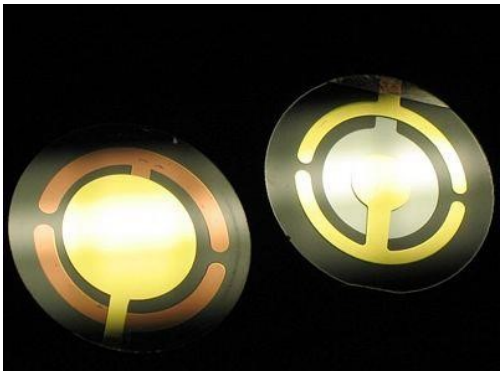
Earlier in the present project, we planned to extract cells of Kodamae Ohmeri yeast or their membrane proteins and to produce antibodies that must to be deposited and immobilized on the gold surface plated on a thin quartz crystal support. However, the existence of a peptide in honey was discovered by the Molecular Biology Group of the IZSLT (Dr.ssa Antonella Cersini at IZSLT) in conjunction with the presence of the yeast Kodamae Ohmeri. The specific antibodies for the peptide were subsequently produced, and these were immobilized on the gold surface of the quartz.

4.1 – Experimental details on microgravimetric biosensor

Nanogravimetry was selected since it offers the best detection limit for very low infestations corresponding to possible very early stages.

The technique employs a quartz crystal microbalance (QCM) instrumentation that has been for a long a standard tool to measure molecular adsorption under vacuum, i.e., to detect thin film thickness. A thin quartz crystal (generally AT-cut) sandwiched between two evaporated metal electrodes. It was introduced for monitoring the deposition on thin-film under vacuum.

It was adopted to determine the adsorption of biological systems (proteins, for instance) to d functionalized with bio-recognition sites (as antibodies). This technique provides frequency measurements with high precision; thus, inertial mass variations down to a level of below tens of picograms per square millimeter are experimentally accessible.



Photograph of typical quartz crystal resonators as used for QCM, metalised with gold electrodes (left: front electrode, right: back electrode) by vapor deposition (from Wikipedia).

QCM takes advantage of the so-called inverse piezoelectric effect accordingly to which if a voltage difference is applied across a quartz crystal cut, the crystal varies its reticular parameters contracting or expanding its bonds, hence its macroscopic size. The quartz crystal will oscillate in response to an applies an a.c. voltage: when the a.c. voltage is tuned to the resonance frequency of a particular crystal; the quartz frequency becomes extremely sensitive to the amount of mass adsorbed on it. The Sauerbrey equation describes the quantification of the correspondent frequency shift upon mass adsorption. The above scenario is exploited in biosensing for detecting tiny amounts of analytes that interact with a quartz surface that has been previously functionalized with suitable binding partners. Applications involve immobilized antibodies exposed to antigen solutions giving rise to sensitive immunosensors. Quartz crystal microbalance (QCM) has shown the pronounced ability for studying recognition behavior among biochemical molecules through changes of resonance frequencies of the quartz plate (doi:10.1016/j.bios.2007.03.003).

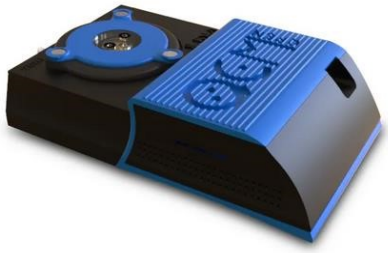
The Sauerbrey equation correlates without the need of calibrations the mass to measured frequency, which is independent mainly of electrode geometry and holds when the deposited mass is rigid, regularly distributed, and when the relative frequency change turns out below the critical value 0.02.

Sauerbrey's equation is defined as: where f_0 is the resonance (Hz), Δf the requency change (Hz), Δm the mass change (g), A the active crystal area (i.e., the area between the electrodes, cm^2), ρ_q = 2.648 g/cm^3 the quartz density, μ_q = 2.947x10^{11} g·cm^{-1}·s^{-2} (i.e., poise) the AT-cut quartz shear modulus (the ratio of shear stress to the shear strain). If a deposition varies, the frequency of the sensor at rest from 10 MHz up to 9,999,900 Hz, the detected mass that adheres to the sensor is varied by 850 10^{-9} grams (or 850 nanograms). For an organophosphate, the figure corresponds to approximately three nanomoles for a sequence peptide:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m.$$

GRHRGESRAARPPAPYKALSTSRVVWECSSWVVNSISIQARDRRTSTVMERKALK the calculated molecular weight is equal to 15300.58 Dalton; therefore the nanometer determines 56 10^{-12} moles, i.e., 56 picomoles.

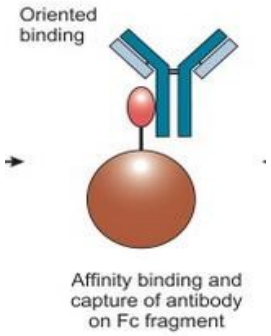
We have implemented an openQCM Wi2 QCM developed within an open-source project by openqcm.com and commercially available by Novaetech S.r.l.(Napoli, Italy), a Spin-off Company of the National Institute for Astrophysics (INAF). It is very compact and suitable for the measurements of the current project. The sensor module is designed to mount both 14mm and 25.4mm

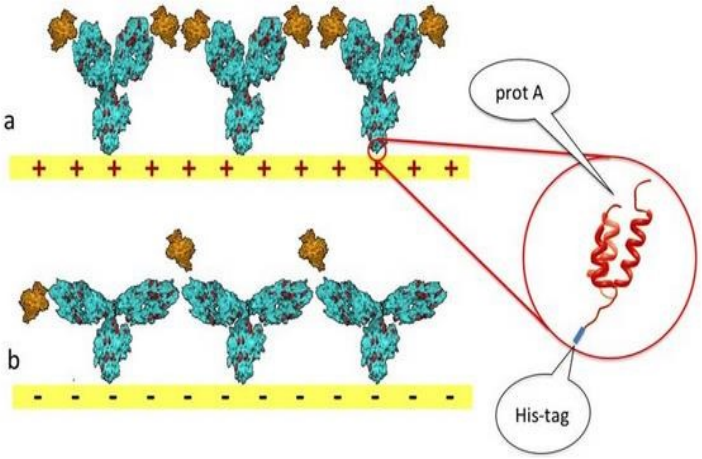


(1 inch) sensors and quartzes with different fundamental frequencies (5 and 10 MHz). It can mount both a microfluidic window with tubing interfaces and an open window for pipetting samples directly on the sensor surface. It adopts the new Teensy microcontroller capable of measuring frequencies up to 25 MHz, allowing the use of a wide range of quartz sensors with different sensitivities.

4.2 – His-tag Protein A deposition.

Protein A is often used for the purification and detection of antibodies such as IgG because of its quadrivalent domains that bind to the Fc region. (DOI:





10.1021/acs.analchem.5b00843). The first step was that of developing a surface immobilization chemistry for antibodies on a gold electrode surface that enabled their preferential orientation, hence that of their Fab fragments, towards the solution covering the electrode. (DOI: 10.1038/srep37779). We used protein A from Staphylococcus aureus, a molecule expressed in the outer membrane of the bacterium, with a high binding affinity for Fc fragments. The molecule we used was a mutant featuring 5 Fc-specific binding sites, deletions of non-specific adsorption sites a 6xHis tag at its N terminus. Given its high affinity for gold and other metals, the 6xHis tag was used for adsorbing a (sub)monolayer of protein A onto gold, providing unambiguous molecular orientation. The formed monolayer was then incubated with IgGs, giving rise to an IgG (sub)monolayer characterized by a preferential orientation, namely Fab fragments effectively exposed towards the solution. Such results were initially evaluated indirectly by measuring protein coverage and antigen-binding ability of IgG monolayers immobilized by the technique above vs. a more standard, non-orienting surface functionalization strategy; see Suppl.Fig. in DOI: 10.1038/srep37779.

4.3 - Microgravimetric biosensor for detecting *Aethina tumida* presence in honeybee hives

In order to develop a biosensing technology for the detection of honey contamination by *Aethina tumida*, we have focused on the detection of a particular yeast, Kodamaea ohmeri, which is brought about by *Aethina tumida* during its oocyte deposition in honey. Kodamaea ohmeri presence is revealed by the occurrence of a particular peptide found by ISZLT partner by RT-PCR analysis of ribosomal RNA sequence extracted from honey samples.

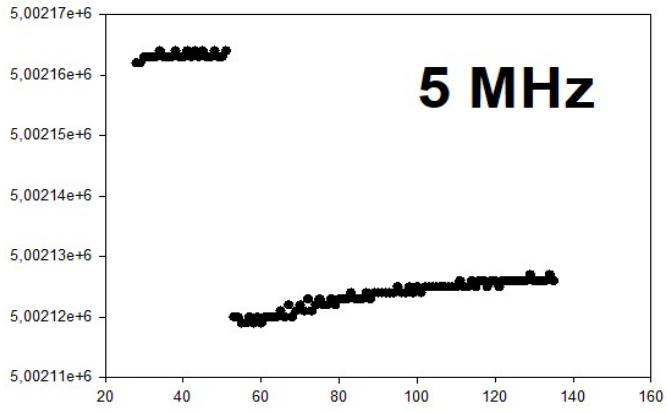
This peptide, whose sequence was conjectured *in silico* from that mentioned above, has been commercially synthesized and used for rising specific rabbit polyclonal antibodies.

The specific antibodies are then used to functionalize the quartz crystal surface, making it adhesive for our peptide. Its peptide sequence that has been used in our work is the following:

GRHRG*ESRAARPPAPYKALSTSRVVWECSSKWVVNSI* S*IQARDR*RTSTVMER*KAL*KES

The strategy to coat the surface of the quartz crystal cut, equipped with a gold electrode, exploits a specific method developed by us (ref. Ghisellini et al. Sci. Rep. 2016) that takes advantage of a recombinant His-tagged protein A originally isolated from the membrane of *Staphylococcus aureus*. His-tag shows a remarkable affinity for the gold surface and its position along with protein A sequence is such that it leaves exposed high-affinity binding sites of protein A for the Fc fragment of immunoglobulins. After forming a compact sub-monolayer of His-tagged protein A on the gold surface of our quartz transducer, further exposure to the polyclonal IGgs gives rise to a sub-monolayer of antibodies.

For the microgravimetric biosensor, it was decided to use an antibody of the peptide identified by the IZS of Rome instead of an aptamer. The latter was initially considered because new and attractive but discarded at the end because of the enormously high costs of these biosystems. Concerning the peptide, it was decided to use a specific portion as a target for the biosensor.



The quartz electrodes used in these experiments have a gold coating to which the antibody is bound by suitable chemistry. From the experimental point of view, similar tests were performed to those performed for the verification of the electrochemical biosensor.

The main result is reported in the Figure above. It shows the frequency decrease in time (due to increased mass deposited) before (a) and after (b) the following conditions:

a) The 5MHz quartz crystal was functionalized anchoring the Protein A to the gold surface as discussed above and also with the further step to capture the anti-peptide on the biosensor surface by the Protein A.

b) The functionalized biosensors prepared by the above steps are exposed to one solution containing the peptide from Kodamae Ohmeri and to a buffer solution (for control check)

As shown in the Figure, **the mass decrease with respect to the constant trend in the control experiment highlights the proof of the usefulness of the method developed to detect the signal from the peptide of Kodamae ohmeri infestation.**

The detail of the experimental procedure was documented by a series of films illustrating the steps described in Appendix 2. Their titles and lengths are as follows:

01-Preliminary descriptions.mp4 (1:25)

02-Cleaning the sensors.mp4 (2:38)

03-Reading the frequency.mp4 (0:21)

04-First deposition-Protein A.mp4 (0:54)

05-Washing after the first deposition.mp4 (0:52)

06-Measurement after Protein A deposition.mp4 (1:06)

07-Presentation 4 samples.mp4 (0:45)

08-Cleaning of the four samples.mp4 (0:54)

09-Sample with three complete depositions.mp4 (1.36)

10-Important-Demonstration of the capture of peptide.mp4 (1:21)

11-Mass calculations for first two depositions protein A and antipeptide.mp4 (0:37)

12-Measure on white-solvent only PBS.mp4 (1:30)

13-Second antipeptide deposition on white sample.mp4 (1:26)

14-Cleaning after second anti-peptide deposition on white.mp4 (0:30)

15-Details on white positioning.mp4 (0:47)

16-Measures on white peptide deposition.mp4 (0:32)

17-Addition of PBS to white with Protein A and antipeptide.mp4 (0:34)

18-Important-we show that with only PBS, the deposited mass does not change.mp4 (1:09).

APPENDIX 1 - Guidelines for organophosphate detection with an electrochemical device

A1.1 - Instrumentation

An electrochemical detector for the rapid determination of organophosphorus pesticides in honey and apiary products (but not exclusively) exploits the current measurement that is high in connection with the AChE action that depletes Ach to Ch increasing the ionic species in drop solution. Thus, the reagents necessary for the analysis are a measuring buffer for the sample dilution and pH fixing, an enzyme solution, and a substrate solution.

Screen printing sensors for pesticides allow establishing the proper electrochemical conditions on the drop above them through an electronic detector for control electrochemical measurement.

A calibration step instructs the apparatus to recognize highlevel current (no OP) at a level of 2.0×10−9M (some ppb). The biosensor is based on a sensitive element associated with an electrochemical detector for the rapid determination of organophosphorus pesticides and carbamates in honey or other apiary products. A kit has been designed for a qualitative assessment (presence/absence of pesticides), thus nonquantitative.

The system allows discrimination between defined values "High" - "Medium" – “Low”. The "Low" values indicate the absence or presence in minimal traces of organophosphorus and carbamate pesticide residues in the samples analyzed. The “High” answer indicates the presence of a large number of pesticides above the limit of 0.01 mg/kg or ppm.

The system includes a calibration step and contains a buffer for the extraction of residues from the sample matrix, enzymatic reagent, enzymatic substrate, selective sensor electrodes, and the electrochemical detector, as shown in the figure below.

A1.2 - Requirements

PC-notebook equipped with Windows 10, Micropipettes 20-200µl, Micropipettes 200-1000µl, mini vials (1.5 ml), and vials 15 ml.

A1.3 - Methods

Preparation of the interface computer: turn on the instrument and interface with a portable device. Install the BIOPARD software on the PC and proceed. It works with a USB-port for the control and data transfer.

A1.4 - Reagent preparation

Chemical reagents contained in solutions 1 and 2 must be stored at 4 ° C. Both can be prepared as follows:

- add 3 ml of measuring buffer to the powder in the reagent bottle 1, shake gently to avoid foaming.
- add 5 ml of measuring buffer to the powder in the reagent bottle 2 and shake gently.

In the middle-term delivery, the 1 and 2 solutions were prepared in the laboratory of the Biophysics Section.

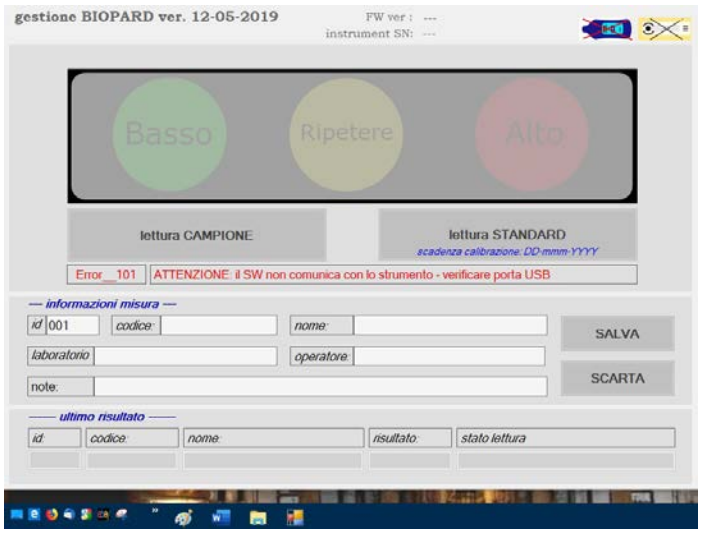
A1.5 - Calibration procedure

The instrument must be calibrated at the beginning of the session and whenever the instrument requires it, i.e., every 6 hours of use. The procedure suggested for the calibration follows these steps:

- Put 3 ml of measuring buffer (buffer) in the vial for analysis, add 30 µl of reagent 1, shake gently for a few seconds, add 90 µl of reagent 2, shake gently for a few seconds and wait 10 minutes.
- Take a strip with the three electrodes deposited. We suggest to cut the stripe reducing the global length (as shown in the figure) before inserting into the instrument, take 30 µl from the analysis solution, place them in the sensitive portion of the sensor and start the measurement after 20 seconds after selecting "calibration" item on the computer window.
- If the procedure is correct, the calibration screen will appear.

A1.6 - Sample analysis

- Take 1 ml of sample, put in a new tube and add 3 ml of measuring buffer; shake vortex 1 minute, centrifuge at a spin rate of 2000 rpm and take 3 ml of the supernatant.



- Add 30 µl solution 1 (enzyme acetylcholinesterase) to the supernatant extracted, shake, and wait 10 minutes. Add 90 µl of solution 2 (substrate, acetyl thiocholine chloride), shake gently, and wait 10 minutes. In the meantime, place a new electrode in the instrument, deposit 30 µl of the solution on the sensitive part of the sensor, wait 20 seconds and proceed with the measurement selecting the item "SAMPLE ACQUISITION" on the PC window of the software reported above.

If the measurement is carried out correctly, the sample contamination level will appear on the BIOPARD window of the PC monitor.

APPENDIX 2 - Nanogravimetric sensor user's protocol for *Aethina tumida*

A1.1 - Solutions

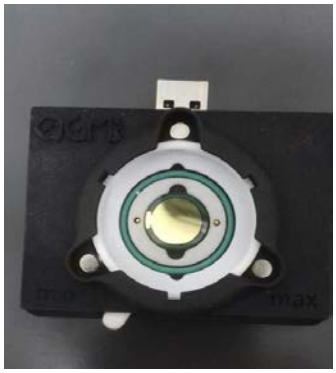
- Protein A 1 mg / ml in H_2O
- Anti-peptide 1.2 mg / ml in PBS Peptide 0.125 mg / ml in PBS

A1.2 - Nanogravimetric quartz functionalization protocol

- Dip the quartzes in ethanol and place them in a sonicator for 10 min
- Dry with oxygen flow
- Rinse in ethanol.
- Dry with oxygen flow

A1.3 - Measurement protocol on nanogravimetric sensor

- Place the quartz inside the sensor's measuring chamber, making sure to position the electrical contacts correctly



- Close the measuring chamber by placing the magnetic cover on the appropriate contacts
- Record the frequency measured by the sensor

A1.3 - Protein A deposition

- Take an aliquot (1 mg / ml) of Protein A in H_2O from the fridge. Deposit 200 µl of Protein A on the gold part of the quartz
- Are Incubate at room temperature for 2 hours Remove Protein A by sliding it off the quartz surface
- Rinse in H_2O limiting to the maximum the number of crossings of the air-water interface with the functionalized quartz
- Allow drying at room temperature

A1.4 - Protein A deposition

- Take an aliquot (1 mg / ml) of Protein A in H_2O from the fridge. Deposit 200 µl of Protein A on the gold part of the quartz
- Are Incubate at room temperature for 2 hours Remove Protein A by sliding it off the quartz surface
- Rinse in H_2O limiting to the maximum the number of crossings of the air-water interface with the functionalized quartz
- Allow drying at room temperature

A1.5 - Measurement on nanogravimetric sensor of the deposited Protein A

- Place the quartz inside the sensor's measuring chamber, being careful to position the electrical contacts correctly

- Close the measuring chamber by placing the magnetic cover on the appropriate contacts • Record the frequency measured by the QCM electronics

A1.6 - Anti-peptide deposition

- Take an aliquot of Anti-peptide 1.2 mg / ml in PBS from the -20 C freezer and defrost it in the fridge
- Deposit 200 µl of Anti-peptide on the gold surfaces of the quartz Incubate at room temperature for 2 hours
- Remove the Anti-peptide by sliding it off the quartz surface
- Rinse in H_2O limiting to the maximum the number of crossings of the air-water interface with the functionalized quartz
- Allow drying at room temperature

A1.7 - Measurement on nanogravimetric sensor of the deposited Anti-peptide

- Place the quartz inside the sensor's measuring chamber, being careful to position the electrical contacts correctly
- Close the measuring chamber by placing the magnetic cover on the appropriate contacts • Record the frequency measured by the QCM electronics

A1.8 - Detection of contamination (Peptide so far)

- Take an aliquot of Peptide 0.125 mg / ml in PBS from the fridge
- Deposit 200 µl of Peptide on the gold part of the quartz
- Incubate in the fridge at 4 C overnight
- Remove the uncaptured elements in the solution by sliding it off the quartz surface
- Rinse in H_2O limiting to the maximum the number of crossings of the air-water interface with the functionalized quartz
- Allow drying at room temperature

A1.9 - Measurement on nanogravimetric sensor of the detected Peptide

- Place the quartz inside the sensor's measuring chamber, being careful to position the electrical contacts correctly

Close the measuring chamber by placing the magnetic cover on the appropriate contacts • Record the frequency measured by the QCM electronics

Milestone M4.5: *Aethina* control methods

Contributors:

Dr Giovanni Formato, Dr Marco Pietropaoli, Dr Jorge Rivera Gomis

Description:

A review of best methods for *Aethina tumida* control has been published with the collaboration of WG8

Output:

Control methods that can be applied against SHB 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 sources positioned on the floor of the extraction room overnight, attract the SHB larvae. In this way they may be collected and destroyed by putting them in alcohol or detergent solution.

Work package 5 (WP 5) - "Validation". Leader: Partner 2

Prof Mustafa Necati Muz (University of Namik Kemal)

Milestone M 5.1: Compliance and feasibility study

Contributors:

Max Rünzel M.Sc. M.A., Dr Riccardo Jannoni-Sebastianini, Dr Joseph Cazier, Dr Edgard Hassler

Description:

A compliance and feasibility study of the new honey management and traceability system has been presented.

Output:

International surveys have been implemented in order to assess the compliance and feasibility of identified GBPs and BMBs for hobbyist and professional beekeepers. The links, available since 30th of April 2020, were published on TECA FAO website (http://www.fao.org/teca/forum/beekeeping/en/) and shared by project partners worldwide.

Surveys were available at those links:

Survey on Varroa management GBPs and BMBs

https://appstate.az1.qualtrics.com/jfe/form/SV_2tRRQOB02uZMFFz

Survey on antimicrobial resistance and related practices

https://appstate.az1.qualtrics.com/jfe/form/SV_79e4cEf0APggfGZ

Survey on honey bees infectious diseases and related practices

https://appstate.az1.qualtrics.com/jfe/form/SV_0rCAUp1fr9hCgXX

The above-mentioned surveys were compiled by 861 users (survey on Varroa management), 397 users (survey on antimicrobial resistance) and 388 users (survey on honey bees infectious diseases). Two third of users were from EU and UK, the rest from North America.

In annex 21 are available the surveys.

Moreove, another survey, that materialises as the cornerstone of the validation of the new management system, was undertaken during an official meeting on the topic of “Best Practices in Beekeeping” in Montefiascone on 30 November, 2019 under the lead of Massimo Palazzetti (ASL VT) and Giovanni Formato (IZSLT), where 24 beekeepers participated. The questionnaire can be found in the annex. Both WP 5 (Validation) and WP 6 (Economic Impact) draw on the results from this survey, focusing on the feasibility of organic beekeeping.

Notably, the part of the survey that addresses validation aimed to determine the feasibility of adhering to sustainable beekeeping practices, such as organic beekeeping. It was of importance to investigate what the benefits of promoting and keeping to these practices were and what obstacles beekeepers would face in their daily routines. As it can be seen in figure 6.1.1, sustainable beekeeping practices, I.e. organic beekeeping practices throughout this report are perceived to generate considerably higher amounts of costs compared

to conventional beekeeping practices. To be precise beekeepers perceive the cost of establishing and producing organic honey to be 53% and 43% respectively more expansive than keeping bees organically.

From a validation and feasibility point of view, these findings highlight the importance of providing beekeepers with tools to validate and proof effectively that sustainable beekeeping practices add value to keeping bees and beekeeping-related products. Beyond direct costs, beekeepers attest a higher perceived mortality of organic bees (27%) vis-à-vis conventionally kept bees (18%), which shows the importance of effective guidance with regards to beekeeping practices that promote colony strength and honey bee health, particularly while preparing the hives for overwintering.

As figure 6.2.1 illustrates, a majority of beekeepers (58%) agrees that adhering to sustainable beekeeping practices as organic beekeeping should be rewarded with price premiums superior to 20%. This further generates evidence that an innovative honey management and traceability could generate considerable support among beekeepers if it enables a pathway towards the generation of higher price premiums and increased sales.

Finally, this short assessment of the feasibility and validation of a new honey management and traceability system based on the resources needed for keeping bees organically gives reason to launch the profitably launch the system for initial trials. A Proof of concept in combination with further studies will help fortifying this notion.

Milestone M5.2: Laboratory analysis guidelines

Contributors:

All partners

Description:

Following are detailed the harmonized laboratory analysis guidelines.

The harmonized laboratory analysis guidelines as output of the Ring Tests results

1.Performance study for Hive debris (ring tests) to diagnosis Nosema spp., SHB, K. ohmeri

Antonella Cersini, Valeria Antognetti, Raffaella Conti, Gabriele Pietrella, Silvia Puccica.

Department of Virology, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri* Rome, Italy

Within the project BPRACTICES (approved within the transnational call of ERA-Net Net SusAn- European Research Area on Sustainable Animal Production Systems- in Horizon 2020 research and in the European Union innovation program) were set two objectives: a) selection of the best matrix for the research of major honeybee pathogens (*Paenibacillus larvae*-American Foulbrood, *Melisococcus plutonius*-European Foulbrood, *Nosema ceranae*, *Nosema apis* and *Aethina tumida*); b) selection and test of molecular protocols on the selected matrix, making a bank of reliable diagnostic methods and to share them with other research partners.

The partners that have collaborated and who are still working to achieve these objectives were reportet in the following table:

INSTITUTION	COUNTRY	ADDRESS	CONTACT

IZSLT – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri	ITALY	Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri", Via Appia Nuova 1411 – CAP 00178 Rome	Giovanni FORMATO giovanni.formato@izslt.it antonella.cersini@izslt.it
AGES – Austrian Agency for Health and Food Safety	AUSTRIA	Spargelfeldstr. 191, A-1220 Vienna	Richard GOTTSBERGER richard.gottsberger@ages.at Antonia GRIESBACHER antonia.griesbacher@ages.at
CIAPA – Centro de Investigación Apícola y Agroambientalal de Marchamalo	SPAIN	Centro Apícola de Marchamalo Camino de San Martín sn 19180 Marchamalo	Mariano HIGES mhiges@jccm.es rmhernandez@jccm.es
AIS – Agricultural Institute of Slovenia, Kmetijski Inštitut Slovenije	SLOVENIA	Inštitut za mikrobiologijo in parazitologijo, Veterinarska Fakulteta, Univerza v Ljubljani, Gerbičeva 60, 1000 Ljubljana	Urška ZAJC Urska.Zajc@vf.uni-lj.si Maja.Smodis.Skerl@kis.si
NKU – University of Namik Kemal	TURKEY	University of Namik Kemal. Faculty of Veterinary Medicine. Department of Parasitology 59030. Tekirdag	Mustafa Necati MUZ mustafamuz@gmail.com
EURL – European Union Reference Laboratory for Honeybee Health, **Anses Sophia Antipolis, Honeybee pathology Unit**	FRANCE	Les Templiers CS 20111, 105 route des Chappes, F. 06902 Sophia Antipolis cedex	Laurianne PARIS eurl.bee@anses.fr

The processing of the data obtained from the several Performance Study Test is carried out by AGES - Austrian Agency for Health and Food Safety and is currently still ongoing. The selected matrix on which the

tests have been effected the Performance Study Test is the honeybee hive debris. This matrix was selected because it is very useful under unfavourable conditions during the field inspection for example with adverse weather condition or bee aggressiveness) or in the case of apiaries with a significant number of hive, with the consequent reduction of monitoring plans costs. The DNA extraction protocol from the hive debris was proposed by the IZSLT partner (Italy), because it was developed for a specific accredited method (ACCREDIA Lab. N° 201) detect *Aethina tumida* on the same matrix. This DNA extraction protocol was experimentally evaluated by AGES partner (Austria), by comparing it with the DNA extraction methods used by AGES for hive matrices. This methods includes commercial kits and the classic DNA extraction protocols with phenol-chloroform method. In detail, the selected DNA extraction protocols from hive debris were performed using the commercial Nucleo Spin Mini kit (Macherey-Nagel), and requires the following steps:a) weigh 1 gram of hive debris; b) add 10ml of phosphate buffer (1X PBS) and incubate the sample in continuous stirring in thermostat set at 37°C for 2 hour; c) centrifugate for 10 minutes at room temperature at 20000 x g (14000 rpm); d) discard the supernatant; e) after collecting the pellet, proced according to the kit instruction. The extracted DNA yield is between 50 and 80 µg for 100 µl of eluted DNA.

Alternatively, the AGES partner (Austria) has proposed another DNA extraction kit, namely Dnesay Blood and Tissue kit (Qiagen), but it was not used for the preparation of the Performance Study Test samples.The End-Point and Real Time PCR qualitative protocols to test for the other honeybee pathogens (*Paenibacillus larvae*-American Foulbrood, *Melisococcus plutonius*-European Foulbrood, *Nosema ceranae*, *Nosema apis* and *Aethina tumida*) were proposed to other project partners and several methods on the basis of the experimental specificity and sensibility were selected.

Test Performance study for Nosema ceranae and *Nosema apis*

Regarding the Nosema ceranae and Nosema apis detection, the selected protocols are used at the moment from the IZSLT (Italy) partner, and consisting of qualitative Real time PCR methods. The Real Time PCR protocol for *Nosema ceranae* is only one: the PCR aimed to a 104 bp the internal transcribed spacer of the subunit ribosomal RNA target of N. ceranae (Genbank: DQ486027) (Bourgeois et al. 2010). In this case the N. ceranae CRA F/N. ceranae CRA R primer pair and a TaqMan probe labeled with JOE at 5' and BHQ-1 at 3 were used. We selected only one Real Time PCR protocol for *Nosema apis:*target was a 142 bp internal transcribed spacer of the small subunit ribosomal RNA sequence (Genbank: U97150)(Bourgeois et al. 2010), using N. apis CRA F/N. apis CRA R primer pair and a aqMan probe labeled with JOE at 5' and BHQ-1 at 3. For both Real Time PCR the Master Mix used was TaqMan® GTXpress 2X (Applied Biosystems).

The 2 selected molecular protocols for nosemiasis were tested on DNA extracted from hive debris, collected form hives without symptoms attributable to Nosema and located in areas at risk of contamination by *Aethina tumida*. In fact, the DNA positive samples for *N. ceranae* and *N. apis* were contaminated with both the TOP10-ITS-rDNA ceranae plasmid (containing the specific target Real Time PCR for *N. ceranae*) and the GeneStrand (containing the DNA fragment-ITS-rDNA apis representing the target Real Time PCR for *Nosema apis*), both at different concentration (high, medium, low of number target copies). The DNA negative samples were constituted by only negative hive debris for *N. ceranae* and *N. apis*.

The number of tested samples was established by AGES, considering: a) 95% confidence interval; b) expected sensitivity of 95%; c) expected specificity of 95%; d) number of participant partners. This participants were four in total:AGES (Austria); IZSLT (Italy), AIS (Slovenia) and NKU (Turkey).

For each protocol the sensibility and sensitivity estimation, on a total of 40 blind samples, of which 7 high positivity level samples (7,2 x 10^{11} target/µl of *N. ceranae* and 1x 10^{10} target/µl of *N. apis), 7* medium positivity level (7,2 x 10^{6} target/µl of *N. ceranae* and 1x 10^{4} target/µl of *N. apis*) and 7 low positivity level (72 target/µl of *N. ceranae* and 100 target/µl of *N. apis*) was carried out. A total of 16 negative samples was

tested. Therefore, for each unit the sensitivity of the applied molecular protocol has been calculated. All data about Performance Study Test for *N. ceranae* and *N. apis* were elaborated by AGES (Austria) :

a) for *N. ceranae*, in conclusion, a 65,5% general sensibility and a 71,1% general specificity was obtained;

b) for *N. apis*, in conclusion, a 73,8% general sensibility and a 92,1% general specifkicity was obtained.

The results obtained were commented both by AGES and by EURL. In fact, both have found a problem of specificity for most participants due to the misinterpretation of some negative samples.

The ANSES rechecked the Ct value for all participants in the Test Performance Study Nosema spp. Seasoning: a) Negative samples with a Ct value equal to 40,1 and b) Positive samples with Ct value below 40.

Consequently, the following results were considered valid and definitive:

a) *N. ceranae*, in conclusion, a 59.5% general sensitivity and a 84,2% general specificity.

b) *N. apis*, in conclusion, a 61,9% general sensitivity and a 94,6% general specificity

Follows the final report for *N. ceranae and N. apis* provided by AGES-Austrian Agency for Health and Food safety

########## RESULTS RING TRIAL NOSEMA SPP. ##########

****

N. ceranae

****

	Negative	**positive**
negative	**64**	**34**
positive	**12**	**50**

Sensitivity:

59.5% [48.8%, 69.4%]

Specificity:

84.2% [74.2%, 90.9%]

	negative	low	medium	high
negative	64	26	7	1
positive	12	2	21	27

Sensitivity high:

96.4% [80.8%, 100%]

Sensitivity medium:

75% [56.4%, 87.6%]

Sensitivity low:

7.1% [0.9%, 23.7%]

****

N. apis

****

	Negative	**positive**
negative	**53**	**24**
positive	**3**	**39**

Sensitivity:

61.9% [49.5%, 72.9%]

Specificity:

94.6% [84.8%, 98.7%]

	negative	low	medium	high
negative	53	18	6	0
positive	3	3	15	21

Sensitivity high:

100% [81.8%, 100%]

Sensitivity medium:

71.4% [49.8%, 86.4%]

Sensitivity low:

14.3% [4.1%, 35.5%]

Test Performance study for *Aethina tumida*

About the *Aethina tumida* detection, the protocol in use at the time in the IZSLT (Italy) partner was selected. This method is accredited (ACCREDIA Lab. N° 201) and consists of a Real time PCR protocol. This is aimed to a 109bp *cytochrome oxidase I* (*COI 1*) gene target (Ward et al., 2007), using SHB207/SHB315 primer pair and a TaqMan probe labeled with FAM. at 5' and TAMRA at 3'. The Master mix used is TaqMan® Universal PCR Master Mix II, with UNG (Applied Biosystems). This method to detect *A.tumida* was tested of DNA extracted from debris collected from hive located in areas at risk of contamination by *A. tumida* and subjected to specific contamination. In fact, the DNA positive samples for A. tumida were contaminated with the TOP10-COI plasmid (containing the specific target Real Time PCR for *A. tumida*) at different concentrations (high, medium, low of number target copies). The number of tested samples was established by AGES, considering: a) 95% confidence interval; b) expected sensitivity of 95%; c) expected specificity of 95%; d) number of participant partners. There were six participants in total:AGES (Austria); IZSLT (Italy), CIAPA (Spain); AIS (Slovenia), NKU (Turkey), EURL (France). For the molecular protocols the estimation of sensibility and sensitivity out of a total of 28 blind samples accredited. In particular, the positive sample were divided in: 5 high positive samples (4,4 x 10^8 target/µl of *A. tumida*); 5 medium positive samples (4,4 x 10^4 target/µl of *A. tumida*) and 5 low positive samples (44 target/µl of *A. tumida*). In total 13 negative samples were tested.

Therefore, for each unit the sensitivity of the applied molecular protocol has been calculated. All data about Performance Study Test for *A. tumida* were elaborated by AGES (Austria):

a) a 86,7% general sensibility and a 47,4% general specificity.

The results obtained were commented both by AGES and by EURL. In fact, both have found a problem of specificity for most partecipants due to the misinterpretation of some negative samples.

The ANSES rechecked the Ct value for all partecipants in the Test Performance Study Nosema spp. Seasoning: a) Negative samples with a Ct value equal to 40,1 and b) Positive samples with Ct value below 40.

Consequently, the following results were obtained:

a) a 87,6% general sensibility and a 70,7% general specificity.

However the AGES has advised to repeat the Test Performance study for *A. tumida* among a much smaller number of participants (AGES, EURL, IZSLT) in order to verify if it is possible to increase the sensitivity and the specificity with the criteria of interpretation of the above defined values for Ct.

For the molecular protocols is ongoing the estimation of sensibility and sensitivity out f a total of 60 blind samples. In particular, the positive sample were divided in: 15 high positive samples (4,01 x 10^7 target/µl of *A. tumida*); 15 medium positive samples (4,4 x 10^5 target/µl of *A. tumida*) and 15 low positive samples (40,1 target/µl of *A. tumida*). A total of 15 negative samples was tested.

Samples were sent by the IZSLT to AGES and EURL in the last week of August.

In this last Test Performance study for *A. tumida* they have been given precise rules for the interpretation of the values of Ct:

Samples negative: samples must be considered negative when they have average Ct values between 40 and 42, or, they have mean Ct values equal to 0. (**True negative**)

Samples low contamination: samples can be considere low contamination when they have average Ct values between 36 and 39 (**True positive- conclusive samples)**.

Samples medium contamination: samples can be considered as medium contamination when they have average Ct values between 27 and 30 (**True positive-conclusive samples**).

Samples high contamination: samples can be considered high contamination when they have average Ct values between 16 and 20 (**True positive – conclusive samples**).

The new results are

a) ***Aethina tumida*, in conclusion, a 97,0% general sensibility and a 84,4% general specificity**

Follows the final report for *A. tumida* provided by AGES-Austrian Agency for Health and Food safety

********** RESULTS RING TRIAL AETHINA TUMIDA 2 **********

	negative	positive	<NA>
negative	**38**	**4**	**0**
positive	**7**	**131**	**0**
<NA>	**0**	**0**	**0**

Sensitivity:

97% [92.4%, 99.1%]

Specificity:

84.4% [70.9%, 92.6%]

	Negative	low	medium	high
negative	38	0	2	2
positive	7	45	43	43
<NA>	0	0	0	0

Sensitivity high:

95.6% [84.4%, 99.6%]

Sensitivity medium:

95.6% [84.4%, 99.6%]

Sensitivity low:

100% [90.6%, 100%]

Test Performance study for *Kodamaea ohmeri*

About the *K. ohmeri* detection, was selected the protocol in use at the time in the IZSLT (Italy) partner.

For the End Point PCR for the detection K. Ohmeri a protocol targeting a region of 302bp internal at the 26S rRNA gene (I. Santino et al., 2013) was selected.

The pair of primers used consists of primers AW For/AWRev. The PCR End Point for K. Ohmeri was made using the kit Ampli Taq Twelve Paq Gold DNA Pol. Buffer II 12 x 250 (Applied Biosystems, ThermoFisher Scientific).

The number of tested samples was established by AGES, considering: a) 95% confidence interval; b) expected sensitivity of 95%; c) expected specificity of 95%; d) number of participant partners. This participants were five in total:AGES (Austria); IZSLT (Italy), AIS (Slovenia), NKU (Turkey) and CIAPA (Spain). For the moleculr protocols was carried out the estimation of sensibility and sensitivity out f a total of 31 blind samples. In particular, the positive sample were divided in: 4 high positive samples (50 CFU in total); 4 medium positive samples (30 CFU in total), 4 sub-medium positive samples (20 CFU in total) and 4 low positive samples (1 CFU in total). In total 15 negative samples were tested.

Therefore, for each unit the sensitivity of the applied molecular protocol has been calculated. All data about Performance Study Test for *A. tumida* were elaborated by AGES (Austria) and the data are considered valid and final:

a) ***K. ohmeri*, in conclusion, a 100% general sensibility and a 92% general specificity**

Follows the final report for *K. ohmeri* provided by AGES-Austrian Agency for Health and Food safety

########## RESULTS RING TRIAL KODAMAEA OHMERI ##########

	negative	**positive**
negative	**69**	**0**
positive	**6**	**80**

Sensitivity:
100% [94.5%, 100%]

Specificity:
92% [83.3%, 96.6%]

	negative	very low	low	medium	high
negative	69	0	0	0	0
positive	6	20	20	20	20

Sensitivity high:
100% [81%, 100%]

Sensitivity medium:
100% [81%, 100%]

Sensitivity low:
100% [81%, 100%]

Sensitivity very low:
100% [81%, 100%]

References

- Bourgeois A. Lelania, Rinder T. E, Lorraine D., Robert G. Danka
Genetic detection and quantification of Nosema apis and N.ceranae in the honey bee. (2010)
Journal of Invertebrate Pathology. Volume 103: pp.53-58.

- Ward L., Brown M., Neumann P., Wilkins S., Pettis J., Boonham N.
A DNA method for screening hive debris for the presence of small hive beetle (*Aethina tumida*). (2007)
Apidologie. Volume 38; pp.: 1-9.

-Kodamaea ohmeri isolate from two immunocompromised patients: first report in Italy. Santino I., Bono S., Borruso L., Bove M., Cialdi E., Martinelli D., Alari A. Mycoses, 2013, 56, 179-181.

Document sent to the operating units participating in the Test Performance study for *Aethina tumida*

RING TEST Molecular Detection of *Aethina tumida* from bee hive debris

1. Objective and principle

The current test is designed as a Ring Test, which aims at assessing the sensibility of selected PCR assay to detect *Aethina tumida* from bee hive debris.
DNA extracts from debris collected from hive located in areas at risk of contamination by *Aethina tumida* will be tested using 1 qPCR assay.

2. Participating laboratories

The participating laboratories will be assigned an anonymized lab code. The lab code should be provided along with the results to the Ring Test organizer only (giovanni.formato@izslt.it; antonella.cersini@izslt.it).

INSTITUTION	COUNTRY	ADDRESS	CONTACT

IZSLT – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri	ITALY	Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri", Via Appia Nuova 1411 – CAP 00178 Rome	Giovanni FORMATO giovanni.formato@izslt.it antonella.cersini@izslt.it
AGES – Austrian Agency for Health and Food Safety	AUSTRIA	Spargelfeldstr. 191, A-1220 Vienna	Richard GOTTSBERGER richard.gottsberger@ages.at
EURL – European Union Reference Laboratory for Honeybee Health, **Anses Sophia Antipolis, Honeybee pathology Unit**	FRANCE	Les Templiers CS 20111, 105 route des Chappes, F. 06902 Sophia Antipolis cedex	Laurianne PARIS eurl.bee@anses.fr

3. Time schedule

- Sample and reagents will be **shipped** in the **third week of August (expected August 27th)**. After receiving the sample, please check the integrity (60 blind samples, 1 negative control and 1 positive control and 3 tubes with primers and probe and 2 tube with Real Time mix), and confirm the date of receipt, integrity and good condition of the sending by mail (giovanni.formato@izslt.it; antonella.cersini@izslt.it).
- **The deadline for reporting** back the results is **October 14th**.
- The organizers will process the anonymized results. A report including statistical analysis of the Ring Test will be compiled and provided to the participants by November 2019.

4. Samples and reagents provided

The samples will be DNA extracts recovered using the NucleoSpin® Tissue (Macherey-Nagel) from hive debris.
The DNA positive samples will be contaminated with the TOP10-COI plasmid at different concentration (high, medium, low of number target copies).
The DNA negative samples will be constituted by only negative hive debris for *Aethina tumida*. The 1 negative control was constituted by $H_2O_{G.R.}$ and 1 positive control was constituted by plasmid TOP10-COI maintained in *Escherichia coli* strain.

Besides the samples, the organizer will supply the primers, probe and PCR mix:

- 1 bag with 2 primers and 1 probe (qPCR assays).
- 1 bag with 2 Real Time Mix.

IMPORTANT: Store the samples and reagents at ≤-20°C immediately upon receipt.

5. Material to be supplied by the participants

The participants have to use their own disposables, PCR water (molecular grade water) and equipment.

6. General Instructions

IMPORTANT: Please always spin down the reagents as well as the samples before use.

Molecular-grade water should preferably be used. Alternatively, prepared purified (deionized or distilled), sterile and nuclease-free PCR water.
All blind samples in all protocols should be tested in duplicate.

7. Results

Provide all results of the 2 replicates.
Fill in the results of the qReal Time test as cycle threshold values (Ct) in the result sheet (Excel format, provided by the organizer) with a conclusion concerning the test result (i.e. positive, negative or inconclusive).
Please also indicate if amplification curves are not exponential.
The results of the Real Time PCR should be provided as positive, negative or inconclusive.

Give additional information on the analysis including:

- Real Time cycler used (band + type),
- modifications done when required
- additional results with own in house methods, if relevant.

8. Interpretation of results

Samples tested in duplicate fall into 4 categories of contamination with *COI* target of *A. tumida* (negative, ie absence of contamination, low contamination, medium contamination and high contamination).

Samples negative: samples must be considered negative when they have average Ct values between 40 and 42, or, they have mean Ct values equal to 0. (**True negative**)

Samples low contamination: samples can be considere low contamination when they have average Ct values between 36 and 39 (**True positive- conclusive samples)**.

Samples medium contamination: samples can be considered as medium contamination when they have average Ct values between 27 and 30 (**True positive-conclusive samples**).

Samples high contamination: samples can be considered high contamination when they have average Ct values between 16 and 20 (**True positive – conclusive samples**).

9. qReal Time PCR protocol (L. Ward et al., 2007)

Target gene: cytochrome oxidase I of ***Aethina tumida*** (109bp)

Assay with the TaqMan probe (labeled with FAM) and primers. Whose sequences are shown below:

- SBH207 F: 5'-TCTAAATACTACTTTCTTCGACCCATCR-3'
- SBH315 R: 5'-TCCTGGTAGAATTAAAATATAAACTTCTGG-3'
- SBH245 T: 5'-FAM-ATCCAATCCTATACCAACACTTATTTTGATTCTTCGGAC-TAMRA-3'

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water	N.A.	6,66	N.A.
TaqMan® Universal PCR Master Mix II, with UNG	2X	12,5	1X
Forward primer (SBH207 F)	30µM	0,24	0,288µM
Reverse primer (SBH315 R)	30µM	0,24	0,288µM
Probe (SBH245 T)	10µM	0,36	0,144µM
Subtotal		20	
DNA samples (template)		5	
Total		25	

Amplification protocol:

Initial denaturation at 95°C for 10 min, followed by 45 cycles of (95°C for 15 s, 55°C for 30 s and 60°C for 30 s).
Fluorescence reading after every (60°C for 30 s) – step (**FAM channel**).

Reference

Ward L., Brown M., Neumann P., Wilkins S., Pettis J., Boonham N. (2007). A DNA method for screening hive debris for the presence of small hive beetle (*Aethina tumida*). *Apidologie. Volume 38; pp.: 1-9*

Document sent to the operating units participating in the Test Performance study for *Nosema ceranae* and *Nosema apis*

RING TEST Molecular Detection of *Nosema ceranae* and *Nosema apis* from bee hive debris

1. Objective and principle

The current test is designed as a Ring Test, which aims at assessing the sensibility of selected PCR assay to detect *Nosema ceranae* and *Nosema apis* from bee hive debris in the pre-clinical stage.

DNA extracts from debris, without symptoms attribuitable to nosemesis, collected from hive located in areas at risk of contamination by *Aethina tumida* will be tested after specific contamination using 2 qPCR assays.

2. Participating laboratories

The participating laboratories will be assigned an anonymized lab code. The lab code should be provided along with the results to the Ring Test organizer only (giovanni.formato@izslt.it; antonella.cersini@izslt.it).

INSTITUTION	COUNTRY	ADDRESS	CONTACT
IZSLT – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri	ITALY	Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri", Via Appia Nuova 1411 – CAP 00178 Rome	Giovanni FORMATO giovanni.formato@izslt.it antonella.cersini@izslt.it
AGES – Austrian Agency for Health and Food Safety	AUSTRIA	Spargelfeldstr. 191, A-1220 Vienna	Richard GOTTSBERGER richard.gottsberger@ages.at
AIS – Agricultural Institute of Slovenia,	SLOVENIA	Inštitut za mikrobiologijo in parazitologijo,	Urška ZAJC Urska.Zajc@vf.uni-lj.si

Kmetijski Inštitut Slovenije		Veterinarska Fakulteta, Univerza v Ljubljani, Gerbičeva 60, 1000 Ljubljana	Maja.Smodis.Skerl@kis.si
NKU – University of Namik Kemal	TURKEY	University of Namik Kemal. Faculty of Veterinary Medicine. Department of Parasitology 59030. Tekirdag	Mustafa Necati MUZ mustafamuz@gmail.com

3. Time schedule

- Sample and reagents will be **shipped** in the **fourth week of July (expected July 27th)**. After receiving the sample, please check the integrity (40 blind samples, 1 negative control, 1 positive control *Nosema ceranae* and 1 positive control *Nosema apis* and 6 tubes with primers and probe for *Nosema ceranae* and *Nosema apis*, 1 tube with Real Time mix for *Nosema ceranae* and 1 tube with Real Time mix for *Nosema apis*), and confirm the date of receipt, integrity and good condition of the sending by mail (giovanni.formato@izslt.it; antonella.cersini@izslt.it).
- **The deadline for reporting** back the results is **September 21th**.
- The organizers will process the anonymized results. A report including statistical analysis of the Ring Test will be compiled and provided to the participants by October 2018.

4. Samples and reagents provided

The samples will be DNA extracts recovered using the QIAamp® Blood Mini Kit (Qiagen) from hive debris.

The DNA positive samples will be contaminated with both the TOP10-ITS-rDNA ceranae plasmid (containing the specific target Real Time for *Nosema ceranae*) and the GeneStrand (containing the DNA fragment-ITS-rDNA apis representing the target Real Time for *Nosema apis*), both at different concentration (high, medium, low of number target copies).

The DNA negative samples will be constituted by only negative hive debris for *Nosema ceranae* and *Nosema apis*. The 1 negative control was constituted by $H_2O_{G.R.}$ and 1 positive control for *Nosema ceranae* was constituted by plasmid TOP10-ITS-rDNA ceranae maintained in *Escherichia coli* strain and 1 positive control for *Nosema apis* was constituted by DNA fragment ITS-rDNA apis synthesized by the Eurofins.

Besides the samples, the organizer will supply the primers, probe and PCR mix:

- 1 bag with 2 primers and 1 probe for Real Time *Nosema ceranae*, 2 primers and 1 probe for Real Time *Nosema apis* and 2 Real Time mix (a specific mix for *Nosema ceranae* and the other specific mix for *Nosema apis*).

IMPORTANT: Store the samples and reagents at ≤-20°C immediately upon receipt.

5. Material to be supplied by the participants

The participants have to use their own disposables, PCR water (molecular grade water) and equipment.

6. General Instructions

IMPORTANT: Please always spin down the reagents as well as the samples before use.

Molecular-grade water should preferably be used. Alternatively, prepared purified (deionized or distilled), sterile and nuclease-free PCR water.

All blind samples in all protocols should be tested in duplicate.

7. Results

Provide all results of the 2 replicates.

Fill in the results of the qReal Time test as cycle threshold values (Ct) in the result sheet (Excel format, provided by the organizer) with a conclusion concerning the test result (i.e. positive, negative or inconclusive).

Please also indicate if amplification curves are not exponential.

The results of the Real Time PCRs should be provided as positive, negative or inconclusive.

Give additional information on the analysis including:

- Real Time cycler used (band + type),
- modifications done when required
- additional results with own in house methods, if relevant.

8. FAST qReal Time PCR protocol

8a. FAST qReal Time PCR *Nosema ceranae* (Bourgeois et al. 2010)

Target gene: internal transcribed spacer of the small subunit ribosomal RNA (Genbank: DQ486027) of ***Nosema ceranae*** (104bp).

Assay with the TaqMan probe (labeled with JOE at 5' and BHQ-1 at 3'). The primers and probe sequences:

N. ceranae CRA F: 5'-AAGAGTGAGACCTATCAGCTAGTTG-3'

N. ceranae CRA R: 5'-CCGTCTCTCAGGCTCCTTCTC-3'

N. ceranae CRA Probe: 5'-JOE-ACCGTTACCCGTCACAGCCTTGTT-BHQ-1-3'

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water	N.A.	1,8	N.A.
TaqMan® GTXpress 2X	2X	6	1X
Forward primer (N.ceranae CRA F)	30µM	0,36	0,9µM
Reverse primer (N.ceranae CRA R)	30µM	0,36	0,9µM
Probe (N.ceranae CRA Probe)	10µM	0,48	0,4µM
Subtotal		9	
DNA sample (template)		3	
Total		12	

For the amplification used the TaqMan® GTXpress™ Master Mix; Cod.4401892. Applied Biosystems.

Amplification protocol for FAST Real Time PCR:

Initial denaturation at 95°C for 20 sec, followed by 50 cycles of (95°C for 1 s, 55°C for 10 s and 61°C for 20 s).

Fluorescence reading after every (61°C for 20 s) – step (**JOE channel**).

8b. FAST qReal Time PCR *Nosema apis* (Bourgeois et al. 2010)

Target gene: internal transcribed spacer of the small subunit ribosomal RNA (Genbank: U97150) of ***Nosema apis*** (142bp).

Assay with the TaqMan probe (labeled with FAM at 5' and BHQ-1 at 3'). The primers and probe sequences:

N. apis CRA F: 5'-GCCCTCCATAATAAGAGTGTCCAC-3'
N. apis CRA R: 5'-ATCTCTCATCCCAAGAGCATTGC-3'
N. apis CRA Probe: 5'-FAM-ACTTACCATGCCAGCAGCCAGAAGA-BHQ-1-3'

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water	N.A.	1,8	N.A.
TaqMan® GTXpress 2X	2X	6	1X
Forward primer (N.apis CRA F)	30µM	0,36	0,9µM
Reverse primer (N.apis CRA R)	30µM	0,36	0,9µM
Probe (N.apis CRA Probe)	10µM	0,48	0,4µM
Subtotal		9	
DNA sample (template)		3	
Total		12	

For the amplification used the TaqMan® GTXpress™ Master Mix; Cod.4401892. Applied Biosystems.

Amplification protocol for FAST Real Time PCR:

Initial denaturation at 95°C for 20 sec, followed by 50 cycles of (95°C for 1 s, 55°C for 10 s and 63°C for 20 s).

Fluorescence reading after every (63°C for 20 s) – step (**FAM channel**).

Reference

A. Lelania Bourgeois, Thomas E. Rinder, Lorraine D., Robert G. Danka (2010). Genetic detection and quantification of *Nosema apis* and *N.ceranae* in the honey bee. *Journal of Invertebrate Pathology. Volume 103: pp.53-58*.

Document sent to the operating units partecipating in the Test Performance study for *Kodamaea ohmeri*

RING TEST Molecular Detection of *Kodamaea ohmeri* from bee hive debris

1. Objective and principle

The current test is designed as a Ring Test, which aims at assessing the sensibility of selected PCR assay to detect *Kodamaea ohmeri* from bee hive debris.

DNA extracts from debris collected from hive located in areas at risk of contamination by *Kodamaea ohmeri* will be tested using 1 PCR End Point assay.

2. Participating laboratories

The participating laboratories will be assigned an anonymized lab code. The lab code should be provided along with the results to the Ring Test organizer only (giovanni.formato@izslt.it; antonella.cersini@izslt.it).

INSTITUTION	COUNTRY	ADDRESS	CONTACT
IZSLT – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri	ITALY	Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri", Via Appia Nuova 1411 – CAP 00178 Rome	Giovanni FORMATO giovanni.formato@izslt.it antonella.cersini@izslt.it
NKU – University of Namik Kemal	TURKEY	University of Namik Kemal. Faculty of Veterinary Medicine. Department of Parasitology 59030. Tekirdag	Mustafa Necati MUZ mustafamuz@gmail.com
AIS – Agricultural Institute of Slovenia, Kmetijski Inštitut Slovenije	SLOVENIA	Inštitut za mikrobiologijo in parazitologijo, Veterinarska Fakulteta, Univerza v Ljubljani, Gerbičeva 60, 1000 Ljubljana	Urška ZAJC Urska.Zajc@vf.uni-lj.si Maja.Smodis.Skerl@kis.si

AGES – Austrian Agency for Health and Food Safety	AUSTRIA	Spargelfeldstr. 191, A-1220 Vienna	Richard GOTTSBERGER richard.gottsberger@ages.at

3. Time schedule

- Sample and reagents will be **shipped** in the **fifth week of July (expected July 31th)**. After receiving the sample, please check the integrity (31 blind samples, 1 negative control and 1 positive control and 2 tubes with primers, 1 tube with Buffer 10X, 1 tube with $MgCl_2$ 25mM, 1 tube with dNTPs mix 10mM and 1 tube with AmpliTaqGold 5U/µl for conventional PCR), and confirm the date of receipt, integrity and good condition of the sending by mail (giovanni.formato@izslt.it; antonella.cersini@izslt.it).
- **The deadline for reporting** back the results is **September 21th**.
- The organizers will process the anonymized results. A report including statistical analysis of the Ring Test will be compiled and provided to the participants by October 2018.

4. Samples and reagents provided

The samples will be DNA extracts recovered using the NucleoSpin® Tissue (Macherey-Nagel) from hive debris.

The DNA positive samples will be contaminated with the field strain (*Kodamaea ohmeri* strain KBP: AP56; Sequence ID: MG367286.1) at different concentration (high, medium, low of CFU).

The DNA negative samples will be constituted by only negative hive debris for *Kodamaea ohmeri* diluted in water. The 1 negative control was constituted by $H_2O_{G.R.}$ and 1 positive control was constituted by *Kodamaea ohmeri* strain KBP: AP56.

Besides the samples, the organizer will supply the primers and PCR mix:

- 1 bag contenining also the 2 tubes with primers, 1 tube with Buffer 10X, 1 tube with $MgCl_2$ 25mM, 1 tube with AmpliTaqGold 5U/µl.

IMPORTANT: Store the samples and reagents at ≤-20°C immediately upon receipt.

5. Material to be supplied by the participants

The participants have to use their own disposables, PCR water (molecular grade water) and equipment.

6. General Instructions

IMPORTANT: Please always spin down the reagents as well as the samples before use.

Molecular-grade water should preferably be used. Alternatively, prepared purified (deionized or distilled), sterile and nuclease-free PCR water.

All blind samples in all protocols should be tested in duplicate.

7. Results

Provide all results of the 2 replicates.

The results of the conventional PCR should be provided as positive, negative or inconclusive. Give additional information on the analysis including:

- PCR cycler used (brand + type).
- Modifications done when required.
- Additional results with own in house methods, if relevant.

8. PCR based on the primers designed by Santino I. et al., 2013

Target gene: 26S rDNA gene ***Kodamaea ohmeri*** (302bp)

Primer sequences:

- K. ohmeri Fw: 5'-TAATTTGAAGATTGCGTCTTG-3'
- K. ohmeri Rv: 5'-TACCCACACTGACAATCTGAC-3'

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water	N.A.	15,15	N.A.
Pcr Buffer II	10X	2,5	1X
$MgCl_2$ Solution	25mM	3	3mM
dNTPs mix	10mM	1	0,4mM
Primer Forward	30µM	0,3	0,4µM
Primer Reverse	30µM	0,3	0,4µM
AmpliTaqGold	5U/µl	0,25	0,05U/µl
Subtotal		22,5	
DNA samples (template)		2,5	

Total		25	

For amplification used the AmpliTaqGold® DNA Polymerase with Buffer II & $MgCl_2$; cod. N8080245, Applied Biosystems.

Amplification protocol

Initial denaturation at 95°C for 10 min (Hotstart Polymerase), followed by 40 cycles of (94°C for 30s, 53°C for 30s and 72°C for 1min) and a final extension step of 72°C for 10 min.

Gel electrophoresis, visualization and result interpretation. Prepare an agarose gel of approx. 1,5%, load your samples, run and visualize the electrophoresis with your standard procedure. Use your own size marker (base pair ladder). The expected amplicon size is 302bp.

Reference

Santino I., Bono S., Borruso L., Bove M., Cialdi E., Martinelli D., Alari A. (2013). Kodamaea ohmeri isolate from two immunocompromised patients: first report in Italy. Mycoses*. Volume 56; pp.: 179-181; doi: 10.1111/j.1439-0507.2012.0232.*

Study and supply of protocols for the detection of Acute Bee Paralysis virus (ABPV) and Deformed Wing virus (DWV) to the units AIS – Agricultural Institute of Slovenia, Kmetijski Inštitut Slovenije and INRAE-Frenche Institut national de recherche en agriculture, alimentation et environment

qReal Time RT- PCR methods for DWV and ABPV

Scope:

This procedure describes the methods to detect the presence of Deforming Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV) using the RNA reverse transcription and amplification of target sequence in RNA extracted from bees.

Target sequence:

- DWV: sequence of DWVgp1 gene (NC_004830.2) coding a polyprotein.

- ABPV: non coding sequence of ABPVgp1 gene (NC_002548.1).

Reagents and solutions

Products	Notes	Storage

H_2O for molecular biology	Commercial	Stock: room temperature. Aliquotes : ≤ -18°C
Absolute ethanol	Commercial	Room temperature
QIAamp ® Viral RNA Mini Kit (250)	Qiagen Cat. No. 52906	≤ -20°C for carrier and troome temperature for the other components
High Capacity cDNA Reverse Transcription kit	Applied Biosystems No. 4368814	≤ -18°C
TaqMan ® Universal PCR Master Mix 2000 Reaction (10 X 5 ml)	Applied Biosystems No. 4318157	≤ -4°C
Primer DWV_brescia For	5'-ATGGGTTTGATTCRATATCTTGGAA-3' Commercial	≤ -18°C
Primer DWV_brescia Rev	5'-GATGTTCCRGGTGGCTTTAATGA-3' Commercial	≤ -18°C
Probe DWV	5'-FAM-ACTAGTGCTGGTTTTCCTTTGTC -MGBNFQ -3' (Applied Biosystems)	≤ -18°C
Primer Forward APV-1 F	5'-GCCCAGACAAGCGCAGTACT -3' Commercial	≤ -18°C

Primer Reverse APV-1 R	5'-AGCACGGAAAACGCGTCTT -3' Commercial	≤ -18°C
Probe ABPV-1	5'-FAM-TCCCCGATAGCRACCGA-MGBNFQ -3' (Applied Biosystems)	≤ -18°C

Procedure:

Bee or brood bee sample

Collect 30 bees (or 30 larvae) and put them in a sterile plastic packet. Add 30 ml of PBS 1X and omogenate the sample using pestle. Withdraw the supernatant using a 25ml pipettr and put them in a 15 ml test tube.

Collect 300 µl from the sample in a 1,5 ml test tube. Add 300 µl of DEPC H_2O or PBS 1X to obtain a diluition 1:2.

Sample treatment protocol

a) 30 honey bees + 30 ml PBS 1X in a sterile plastic bag

b) Ogomenate the sample with a glass bottle

c) withdraw the supernatant and put it in a 50 ml collection tube

d) withdraw 300 µl of homogenate and put it in a 2 ml collection tube

e) Add 300 µl of PBS 1X to obtatin the 1:2 diluition

f) withdraw 140 µl of sample and put it in a 1,5 ml collection tube

Start the RNA extraction according to the kit instruction

RNA extraction

The RNA extraction was carried out in according to kit instruction (QIAamp® Viral RNA Mini kit-Qiagen); section " Protocol: Purification of Viral RNA (Spin Protocol)"

Spectrophotometric quantification of nucleic acid

The quantification of the extracted RNA is carried out by means of a spectrophotometric reading at 260 nm, using the NanoDrop® ND-1000 or other spectrophotometer. If you use the NanoDrop® ND-1000, proceed as follows: 2 µl of sample, taken with micropipette and sterile tip with filter, is placed on the special plate reading of the instrument and analyzed. If the BioPhotometer Eppendorf spectrophotometer is used, proceed as follows: 5 µl of sample, taken with micropipette and sterile tip with filter, is placed in the appropriate cuvette (Uvette ® 220-1600nm type Eppendorf) and 95 µl of water / DEPC e is added to the sample analyzed. The special software on PC allows the automatic processing of the sample concentration, expressed in ng / µl, as well as the ratios Abs260 / Abs280 and Abs260 / Abs230, which represent an estimate of the degree of purity of RNA. [In particular, the 260/280 report allows to highlight the presence of one protein contamination (in pure RNA preparations this ratio is between 1.8 and 2.0). The report 260/230 allows to highlight the presence of contaminants such as phenol, aromatic compounds, peptides and carbohydrates (in preparations as well this ratio is around 2 -2.2)]. For the purpose of carrying out this test procedure, for which the use of a control is envisaged internal, the only data, among all those processed by the software, which is taken into account is that relating to sample concentration. At the end of the reading, the data are stored in a file and are taken care of print the quantification report. For each sample prepare a dilution to have a concentration of a total 1 µg. Of this 30µl will be used for cDNA synthesis.

Controls and reference materials

In the one step rRT-PCR were processed the following controls:

- Positive PCR amplification control;
- PCR reagents control (NTC)

.

cDNA synthesis

Each sample is precessed in single.

Reagents	Initial Concentration	Final concentration	Volume (µl) for 1 sample
DEPC H_2O	/	/	12,6
10x RT-Buffer	10X	1X	6

10x Random Primer	10X	1X	6
25 mM dNTP	25mM	1 mM	2,4
Multi Scribe Reverse Transcriptase	5U	0,25	3
RNA	/	/	30
Total volume			60

cDNA synhesis thermal profile

Cycle	temperature/time	Number of cycles
activation	25°C/ 1 min	1
Reverse trascription	37°C /45 min	1
cooling	4°C/10min	1

The samples are processed using the GeneAmp® PCR Systems.

DWV RT-PCR

Each sample is processed in double.

DWV Master Mix

Reagents	Initial Concentration	Final concentration	Volume (µl) for 1 sample
DEPC H_2O	/	/	5,38
Universatl TaqMan Master Mix 2X	2X	1X	12,5
DWV Fw	30 µM	0,9 µM	0,75
DWV Rv	30 µM	0,9 µM	0,75

DWV probe	10 µM	0,25 µM	0,625
cDNA	/	/	5
Total volume			25

ABPV RT-PCR

Each sample is processed in double.

ABPV Master Mix

Reagents	Initial Concentration	Final concentration	Volume (µl) for 1 sample
DEPC H_2O	/	/	5,25
Universatl TaqMan Master Mix 2X	2X	1X	12,5
APV 1F Fw	30 µM	0,9 µM	0,75
APV 1R Rv	30 µM	0,9 µM	0,75
APV1 Probe	10 µM	0,3 µM	0,75
cDNA	/	/	5
Total volume			25

DWV and ABPV real time thermal profile

- 50°C for 2 min;
- 95°C for 10 min;
- 50 cycles (95°C for 15 sec, 60°C for 1 min);
- 40°C for 30 sec.

The samples are processed using the ABI PRISM 7900HT software SDS 2.4

Results expression

The amplification results are accepted if:

- Negative process control: Negative
- Positive process control: Positive
- PCR reagents control: Negative

If the results are invalid repeat the test.

The sample is considered positive if the software detect a signal referable to target sequence.

The sample is considered negative if the software don't detect a signal referable to target sequence.

For the qReal time we use the standards set up by the targets Real Time RT PCR DWV and ABPV synthesized by Eurofins.

Standard DWV:

ATGTGGTGTGCCTGGTTTAGATGGGTTTGATTCGATATCTTGGAATACTAGTGCTGGTTTTCCTTTGTCTTCA TTAAAGCCACCTGGAACATCAGGYAAGCGATGGTTGTTTG

DWV STANDARD CURVE

Diluizione	Ct medio	N° molecole target/µl
tq	11,78	$3,9 \times 10^{8}$
Dil. 10^{-1}	14,15	$3,9 \times 10^{7}$
Dil. 10^{-2}	17,15	$3,9 \times 10^{6}$
Dil. 10^{-3}	20,70	$3,9 \times 10^{5}$
Dil. 10^{-4}	24,05	$3,9 \times 10^{4}$
Dil. 10^{-5}	28,10	$3,9 \times 10^{3}$
Dil. 10^{-6}	30,60	390
Dil. 10^{-7}	34,60	39
Dil. 10^{-8}	36,75	3,9

Efficiency = 1,027

R^2 = 0,9971

Standard ABPV:

TCTAAAGGAGCCGTTAGTCAGCCCAGACAAGCGCAGTACTTTAGAAGAGAGAAGTTCCCCGATAGCGACC
GAAAAGACGCGTTTTCCGTGCTAACTAATTTAAATGTGGGAA

ABPV STANDARD CURVE

Diluizione	Ct medio	N° molecole target/µl
tq	5,4	$5,8 \times 10^{23}$
Dil. 10^{-1}	7,35	$5,8 \times 10^{21}$
Dil. 10^{-2}	9,86	$5,8 \times 10^{20}$
Dil. 10^{-3}	12,87	$5,8 \times 10^{18}$
Dil. 10^{-4}	14,56	$5,8 \times 10^{15}$
Dil. 10^{-5}	16,32	$5,8 \times 10^{13}$
Dil. 10^{-6}	20,5	$5,8 \times 10^{11}$
Dil. 10^{-7}	22,56	$5,8 \times 10^{9}$
Dil. 10^{-8}	24,98	$5,8 \times 10^{7}$
Dil. 10^{-9}	26,28	$5,8 \times 10^{5}$
Dil. 10^{-10}	28,79	$5,8 \times 10^{4}$
Dil. 10^{-11}	30,91	$5,8 \times 10^{3}$
Dil. 10^{-12}	33,98	580

Dil. 10^{-13}	**35,78**	**58**
Dil. 10^{-14}	**38,75**	**5,8**

Efficiency = 1,089

R^2 = 0,9878

IPC (EUROGENTEC) for only control amplification

Universal Exogenous qPCR Positive Control (Yakima Yellow –TAMRA probe) 200 rx. EUROGENTEC

IPC Master Mix

Reagents	**Initial Concentration**	**Final concentration**	**Volume (µl) for 1 sample**
10X IPC mix	10X	1X	2,5
50X IPC y DNA	50X	1X	0,5
Universal Master mix 2X	2X	1X	12,5
DEPC H_2O	/	/	4,5
DNA template	/	/	5
Total			25

cDNA synhesis thermal profile

- 50°C for 2 min;
- 95°C for 10 min;
- 40 cycles (95°C for 15 sec, 60°C for 1 min);

Calculation of the number of target molecules/bee

We use this procedure to calcolate the number of target copies/bee and we actually detect for high Ct (es. Ct = 38 a low number of target copies/bee).
Example:
Suppose we obtain an Ct average (Ctm) = 30.91 for the Real Time ABPV which, according to our standard curve, is equivalent to 5.8 x 10^2 molecules target/microlitre.

The value of 5.8 x 10^2 molecules target/microlitre is multiplied for 140 microliters which is equivalent to the sample volume used for RNA extraction.
As a result, 580 x 140 microliters is obtained = 81.200 viral copies/ml.
Then I divide by 30 (number of bees that constitute the starting sample) = 2.706 viral copies ABPV/bee.

Examination of field samples for ABPV and DWV virus. The samples come from Ciampino apiary subjected to different *Varroa* treatmnent and control protocols

Below are the results obtained from samples supplied by Apiculture – IZSLT

Results for Real Time RT PCR ABPV

	ABPV Real Time 1° replicated Ct	ABPV Real Time 2° replicated Ct	ABPV Real Time average Ct	ABPV Real Time Molecules target/bee
PRE. 1 J	31,43	30,4	30,9	2.7 x 10^4
PRE. 7 J	11,47	11,93	11,68	2.7x10^{19}
PRE. 8 J	30,81	30,7	30,75	2.7x10^4
PRE. 9 J	33,7	34,23	33,96	2.7x10^3
PRE. 11 J	33,96	34,35	34.15	2.7x10^3
PRE. 14 J	33,8	33,02	33,41	2.7x10^3
PRE. 19 J	34,78	34,7	34,74	2.7x10^3
PRE. 20 J	33,7	31,79	32,74	2.70^3
PRE. 21 J	9,2	9,3	9,25	2.7x10^{21}
PRE. 23 J	29,74	31,95	30,84	2.7x10^4
PRE. 25 J	33,04	31,81	32,42	2.7x10^4
PRE. 29 J	24,56	24,5	24,53	2.7x10^5
PRE. 30 J	26,36	26,47	26,41	2.7x10^6
PRE. 31 J	27,83	28,26	28,04	2.7x10^6
PRE. 32 J	Neg	Neg	0	0
PRE. 33 J	30,2	30,76	30,48	2.7x10^4
PRE. 35 J	30,46	30,2	30,33	2.7x10^4
PRE. 36 J	33,38	34,3	33,84	2.7x10^3
PRE. 37 J	23,71	23,43	23,57	2.7x10^{10}
PRE. 38 J	31,88	31,01	31,44	2.7x10^4

PRE. 39 J	25,19	25,01	25,1	$2.7x10^{8}$
PRE. 40 J	28,67	28,54	28,6	$2.7x10^{6}$
PRE. 41 J	30,88	31,33	31,1	$2.7x10^{4}$
PRE. 43 J	30,71	30,77	30,74	$2.7x10^{4}$
PRE. 44 J	34,32	35,35	34,83	$2.7x10^{2}$
PRE. 47 J	35,45	34,83	35,14	$2.7x10^{3}$
POST. 1 V	28,25	28,55	28,4	$2.7x10^{6}$
POST. 7 V	17,07	17,14	17,1	$2.7x10^{14}$
POST. 8 V	30,32	29,99	30,15	$2.7x10^{4}$
POST. 9 V	32,36	32,28	32,32	$2.7x10^{3}$
POST. 11 V	32,38	33,74	33,06	$2.7x10^{3}$
POST. 14 V	32,87	17,78	25,32	$2.7x10^{8}$
POST. 19 V	28,94	28,64	28,79	$2.7x10^{5}$
POST. 20 V	31,75	31,46	31,6	$2.7x10^{4}$
POST. 23 V	14,35	13,99	14,17	$2.7x10^{16}$
POST. 25 V	33,8	33,37	33,22	$2.7x10^{3}$
POST. 29 V	17,95	17,83	17,89	$2.7x10^{14}$
POST. 30 V	29,83	29,93	29,88	$2.7x10^{4}$
POST. 31 V	30,52	30,33	30,42	$2.7x10^{4}$
POST. 32 V	30,09	29,61	29,85	$2.7x10^{4}$
POST. 33 V	28,42	28,31	28,36	$2.7x10^{6}$
POST. 35 V	28,27	27,8	28,03	$2.7x10^{3}$

POST. 36 V	29,28	29,26	29,27	$2.7x10^{5}$
POST. 37 V	17,24	20,88	19,06	$2.7x10^{12}$
POST. 38 V	29,24	28,93	29,08	$2.7x10^{5}$
POST. 39 V	32,01	30,91	31,46	$2.7x10^{4}$
POST. 40 V	28,26	28,54	28,4	$2.7x10^{5}$
POST. 41 V	29,43	29,17	29,3	$2.7x10^{5}$
POST. 43 V	27,98	27,91	27,94	$2.7x10^{5}$
POST. 44 V	30,77	31,36	31,06	$2.7x10^{4}$
POST. 47 V	31	30,96	30,98	$2.7x10^{4}$

Results for Real Time RT PCR DWV

	DWV Real Time 1° replicated	**DWV Real Time 2° replicated**	**DWV Real Time average Ct**	**DWV Real Time Molecules target/bee**
PRE. 1 J	13,87	14,15	14,01	$1.8x10^{8}$
PRE. 7 J	11,44	11,41	11,42	$1.8x10^{9}$
PRE. 8 J	13,18	12,96	13,07	$1.8x10^{9}$
PRE. 9 J	14,6	14,46	14,53	$1.8x10^{8}$
PRE. 11 J	24,48	25,21	24,84	$1.8x10^{5}$
PRE. 14 J	21,28	19,57	20,42	$1.8x10^{6}$
PRE. 19 J	12,8	11,81	12,3	$1.8x10^{9}$
PRE. 20 J	18,14	17,27	17,7	$1.8x10^{7}$
PRE. 21 J	9,2	6,71	7,95	$1.8x10^{9}$
PRE. 23 J	11,26	11,55	11,4	$1.8x10^{9}$
PRE. 25 J	13,6	14,9	14,25	$1.8x10^{8}$
PRE. 29 J	10,2	9,04	9,62	$1.8x10^{9}$
PRE. 30 J	9,57	10,13	9,85	$1.8x10^{9}$

PRE. 31 J	14,97	14,73	14,85	$1.8x10^{8}$
PRE. 32 J	31,49	31,49	31,49	$1.8x10^{3}$
PRE. 33 J	15,59	14,63	15,11	$1.8x10^{8}$
PRE. 35 J	11,58	12,15	11,86	$1.8x10^{9}$
PRE. 36 J	13,86	13,13	13,49	$1.8x10^{8}$
PRE. 37 J	12,47	11,38	11,92	$1.8x10^{9}$
PRE. 38 J	9,94	9,18	9,59	$1.8x10^{9}$
PRE. 39 J	13,63	13,62	13,62	$1.8x10^{8}$
PRE. 40 J	11,48	11,56	11,52	$1.8x10^{9}$
PRE. 41 J	9,48	9,79	9,63	$1.8x10^{9}$
PRE. 43 J	26,15	26,79	26,47	$1.8x10^{4}$
PRE. 44 J	15,32	14,62	14,97	$1.8x10^{8}$
PRE. 47 J	20,64	20,06	20,35	$1.8x10^{6}$
POST. 1 V	15,34	14,65	14,99	$1.8x10^{8}$
POST. 7 V	13,34	12,17	12,75	$1.8x10^{9}$
POST. 8 V	13,84	13,5	13,67	$1.8x10^{8}$
POST. 9 V	11,92	11,97	11,94	$1.8x10^{9}$
POST. 11 V	29,33	28,85	29,09	$1.8x10^{3}$
POST. 14 V	26,6	25,82	26,21	$1.8x10^{4}$
POST. 19 V	16,62	15,24	15,93	$1.8x10^{7}$
POST. 20 V	16,98	17,84	17,41	$1.8x10^{7}$
POST. 23 V	12,27	13,43	12,85	$1.8x10^{9}$
POST. 25 V	13,34	13,64	13,49	$1.8x10^{8}$
POST. 29 V	11,18	11,34	11,26	$1.8x10^{9}$
POST. 30 V	10,58	11,39	10,98	$1.8x10^{9}$
POST. 31 V	17,76	17,61	17,68	$1.8x10^{7}$
POST. 32 V	12,68	12,18	12,43	$1.8x10^{9}$
POST. 33 V	11,68	11,57	11,62	$1.8x10^{9}$
POST. 35 V	11,54	10,78	11,16	$1.8x10^{9}$
POST. 36 V	10,89	10,38	10,63	$1.8x10^{9}$
POST. 37 V	9,57	8,98	9,27	$1.8x10^{9}$
POST. 38 V	12,73	11,83	12,28	$1.8x10^{9}$
POST. 39 V	12,97	11,99	12,48	$1.8x10^{9}$
POST. 40 V	5,97	9,4	7,68	$1.8x10^{9}$
POST. 41 V	12,5	12,93	12,71	$1.8x10^{9}$
POST. 43 V	22,02	21,94	21,98	$1.8x10^{6}$
POST. 44 V	25,7	26,04	25,87	$1.8x10^{4}$
POST. 47 V	23,36	23,11	23,23	$1.8x10^{5}$

The cDNA related to the samples analysed in qReal Time RT-PCR for both ABPV and DWV were sent to INRAE-French Institut national de recherché en agricultural, alimentationet environment and to AIS – Agricultural Institute of Slovenia, Kmetijski Inštitut Slovenije.

The cDNA related to the samples analysed in qReal Time RT-PCR for both ABPV and DWV are currently undergoing the characterization of the DWV and ABPV strains, detected with the molecular analyses, by INRAE.

The IZSLT has supplied two packs of Top Taq DNA Polymerase (Qiagen) to INRAE for the molecular characterizations of the ABPV and DWV strains detected.

Selection of the *K. ohmeri* peptide to be used for the development of the micro(nano)-gravimetric biosensor for *Aethina tumida* contamination in honeybee hives

A highly conserved sequence within the DNA coding for ribosomal RNA gene (ITS2region)) was selected and wich was also chosen for the Test Performance study for *Kodamaea ohmeri*

The sequence ITS2 *K. ohmeri* is shown below:

GGACGTCACAGAGGGTGAGAATCCCGTGCGGCACGGCCCCCGGCTCCTTATAAGGCGC
TCTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAGTGGGTGGTAAATTCCATCTA
AAGCTAAATACAGGCGAGAGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAAA
AGCACTTTGAAAAGAGAGTGA

The sequencing of ITS2 PCR amplified was carried out and a 100% sequence identity and a 100% query-cover with sequence Accesion Number MG367286.1 (Kodamaea ohmeri strain KBP:AP56 internal transcribed spacer 2 and large subunit ribosomal RNA gene, partial sequence) was confirmed.

The sequence was converted into the corrisponding peptide by the program **http://in-silico.net/tools/biology/sequence_conversion):**

GRHRG*ESRAARPPAPYKALSTSRVVWECSSKWVVNSI*S*IQARDR*RTSTVMER*KA L*KES

The amino acid sequence of the peptide ITS2 *K. ohmeri* was provided to the University of Genova, the research Unit in Medical Biophysics.

Supply of the honey treatment protocol to be subjected to analysis with the micro(nano)-gravimetric biosensor for *Aethina tumida* at the University of Genova

The protocol provided to the University of Genoa has the purpose both of dissolving honey to make it suitable for analysis with the micro(nano)-gravimetric biosensor for *Aethina tumida* and of concentrating the peptides of *K. ohmeri* present in honey.

1) Transfer 10 grams of honey to a 50 ml falcon tube and add water-grade reagent up to a volume of 40 ml;
2) Incubate at 65°C mixing for 30 minutes;
3) Centrifuge at 3000xg (4000 rpm) for 30 minutes and at room temperature;
4) Remove the supernatant and let the pellet dry for 5 minutes at room temperature
   Proceed according to the requirements of the protocol required for the operation of the micro(nano)-gravimetric biosensor for *Aethina tumida*

Milestone M5.3: Honeybee diseases control guidelines

Contributors:

All partners

Description:

The guidelines for honeybee diseases control have been published online and on hardback format.

Output:

The Guidelines are available at this link (http://www.izslt.it/bpractices/2019/12/31/good-beekeeping-practices-gbp-the-bpractices-guidelines/)

Work package 6 (WP 6) - "Economic impact". Leader: Partner 2

Prof Mustafa Necati Muz (University of Namik Kemal)

Milestone M6.1: BPRACTICES economic impact

Contributors:

Max Rünzel M.Sc. M.A., Dr. Jannoni-Sebastianini, Dr Joseph Cazier, Dr Diego Pagani, Dr James Wilkes

Description:

A report on economic impact of BPRACTICES project.

Output:

The report analyses organic and conventional honey production in Italy focusing on the drivers and obstacles to organic beekeeping. Part 1 and 2 (WP 6.1) focus on the assessing the resources involved in organic honey production in Italy while Parts 3 and 4 (WP 6.2) assess the economics behind organic beekeeping as well as beekeepers' incentives to engage in organic beekeeping.

Both WP 6.1 and WP 6.2 use two common sources of data; a dataset and a survey. The dataset stems from CONAPI, one of Italy's principal honey producing cooperatives, including data on the annual variation of numbers of hives as well as the average production quantities per hive for organic and conventional beekeepers across Italy between 2014 and 2018. The CONAPI dataset is of particular interest as both beekeepers who keep bees organically (106 across 13 regions) and conventionally (101 across 14 regions) are adhering to the same standard beekeeping practices established by the cooperative. This creates a situation within which both organic and conventional beekeepers become very much comparable. In the dataset, 106 organic beekeepers across 13 regions within Italy have a total average of 29,098.4 producing hives per year, while 101 conventional beekeepers across 14 regions provide an average of 33,322.6 producing hives per year.

The survey, on the other hand, was undertaken during an official meeting on the topic of "Best Practices in Beekeeping" in Montefiascone on 30 November, 2019 under the lead of Massimo Palazzetti (ASL VT) and Giovanni Formato (IZSLT), where 24 beekeepers participated. The questionnaire can be found in the annex.

Part 1 – An assessment of the resources involved in organic honey production in Italy

Producing organic honey

Figure 6.1.1 The Perceived Costs of Establishing and Producing Organic Honey

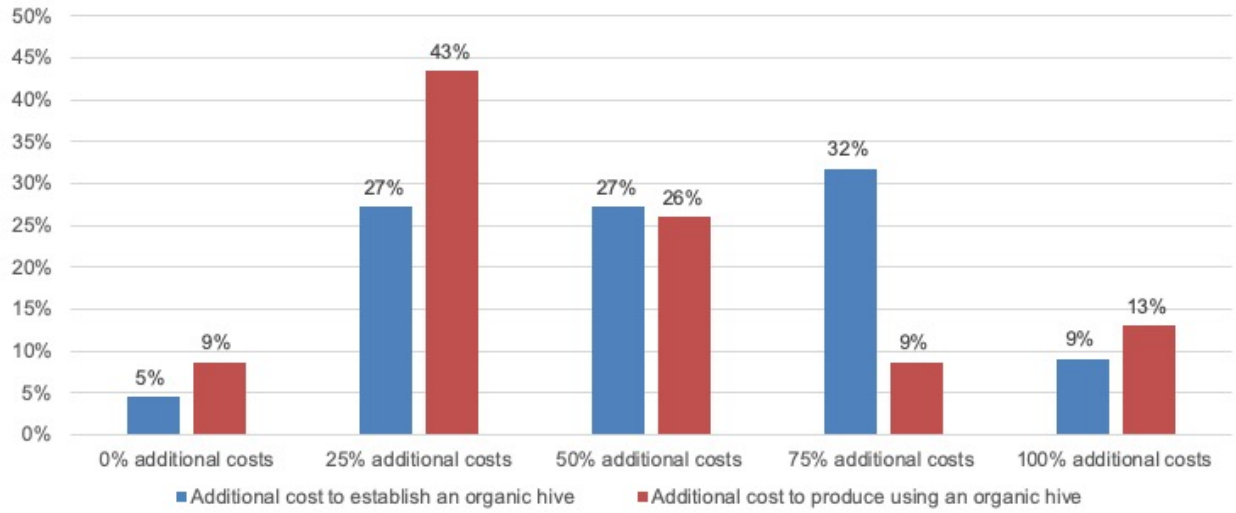


Figure 6.1.2 The Perceived Time it Takes to Manage Organic Hives

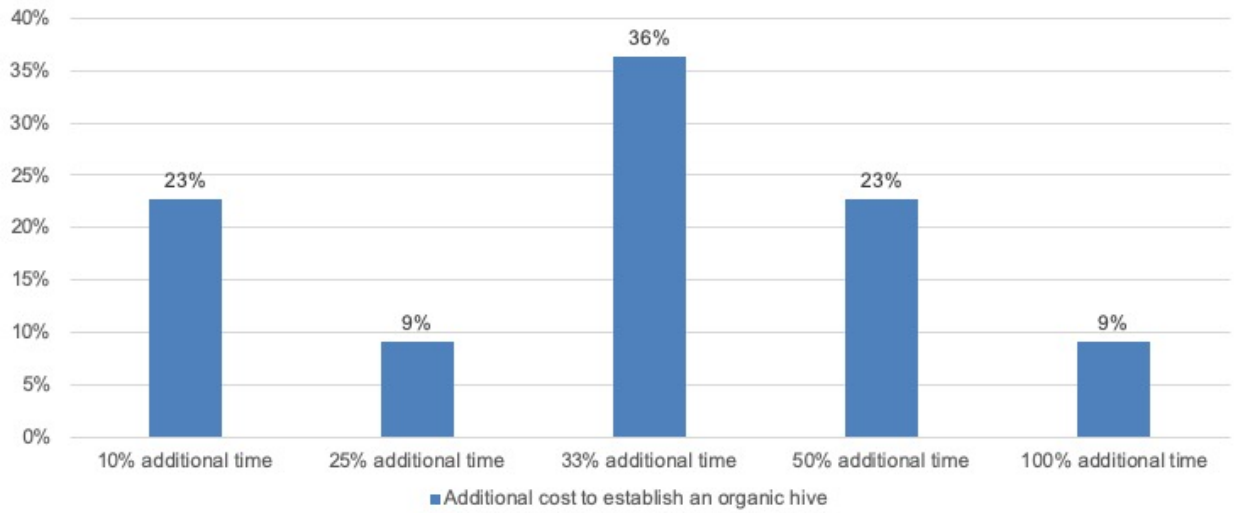
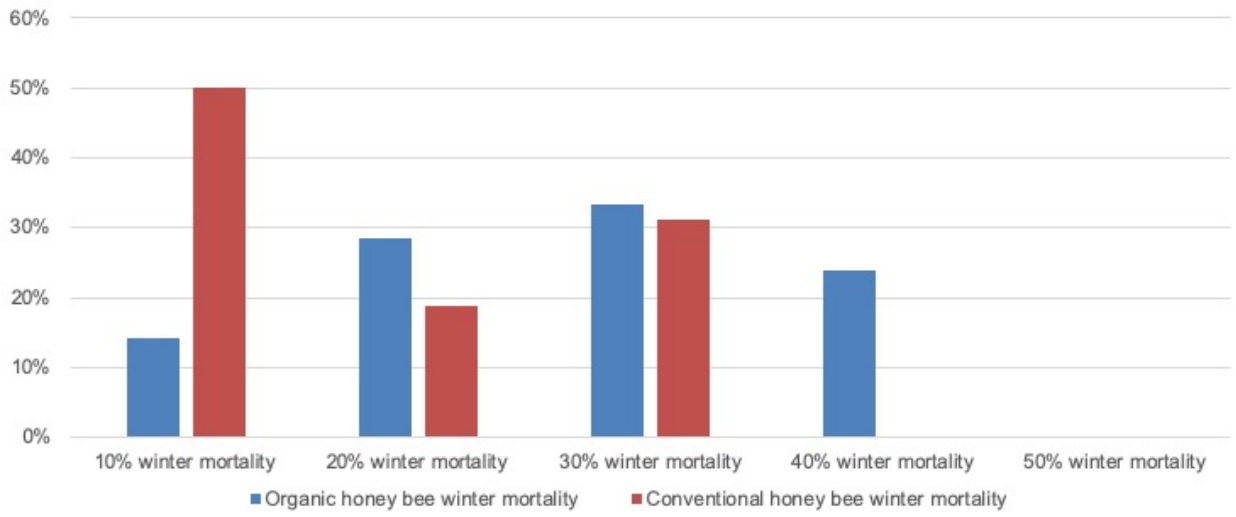


Figure 6.1.3 Perceived Winter Mortality of Organically and Conventionally Kept Bees



Summary of Part 1

Organic beekeepers perceive several disadvantages:

- 53% is the mean perceived additional average cost required to establish organic beekeeping operations vis-à-vis conventional beekeeping.
- 43% is the mean perceived additional average cost organic beekeeping production requires vis-à-vis conventional beekeeping.
- 37% is the mean perceived average additional time organic beekeeping requires vis-à-vis conventional beekeeping.
- 26.67% is the mean perceived average winter mortality of organically kept honey bees.
- 18.13% is the mean perceived average winter mortality of conventionally kept honey bees.

Part 2 – Comparison with CONAPI Data

In part 2 we compared the data observed through the survey with data from our base dataset. It is important to note that while the dataset contains data on the annual variation of bee hives, that is, the number of hives declared at the beginning of the year, we cannot derive the winter mortality from this figure as other factors may influence the initial annual declared number of hives. Notably, idiosyncratic or economically motivated decisions to increase or decrease the number of hives in any given year cannot be captured by the dataset.

Figure 6.1.4 Annual Variation of Bee Hives Using Declared Numbers to CONAPI

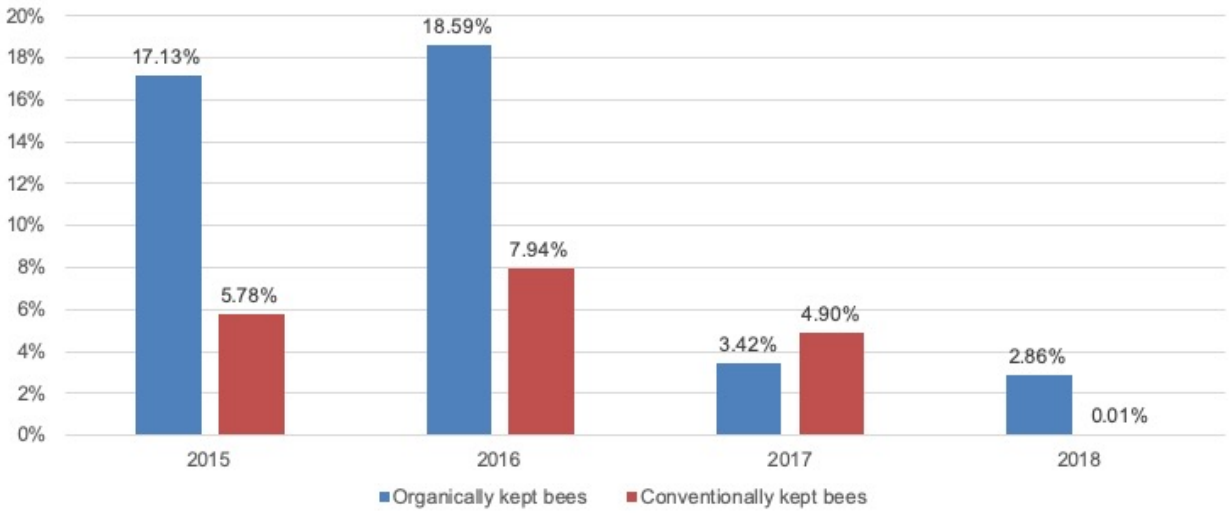


Figure 6.1.5 Annual Variation of Bee Hives Using Declared Numbers to CONAPI

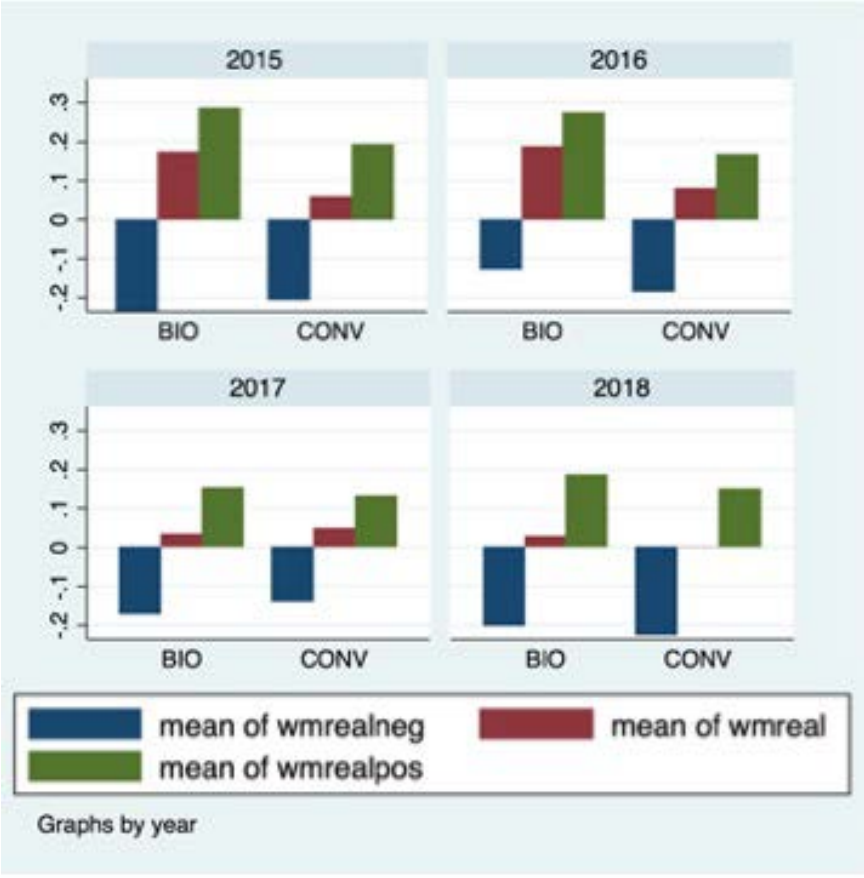


Figure 6.1.5 shows the annual variation with regards to the number of hives from 2015 to 2018. Please note that given the lack of 2013 data, the change in initially declared hives cannot be calculated for 2014. Notably, the graph locks at the annual variation from three different angles. First, the blue graphs show the variation among the beekeepers who have decreased the number of hives. Second, green shows the

variation in the number of hives for beekeepers who have increased the number of hives. Finally, red shows the average considering all beekeepers.

In all years but 2017, on average, the variation in the number of organically kept beehives is positive and higher and the variation in conventionally kept beehives.

Figures 6.1.6 and 6.1.7 show the same variation comparing 2014 with 2018 for conventional and organic beehives across all Italia's regions. Interestingly, the number of conventionally kept beehives as decreased or stayed stable across Italy. For organically kept beehives, the net number of hives as increased signifantly, particularly in the far north and south.

Figure 6.1.6 Net Change in the Number of Conventional Hives from 2014 to 2018

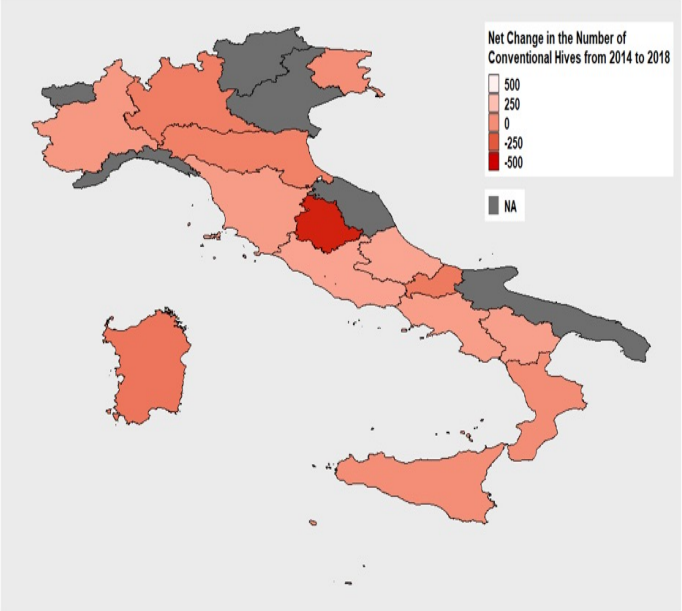


Figure 6.1.7 Net Change in the Number of Organic Hives from 2014 to 2018

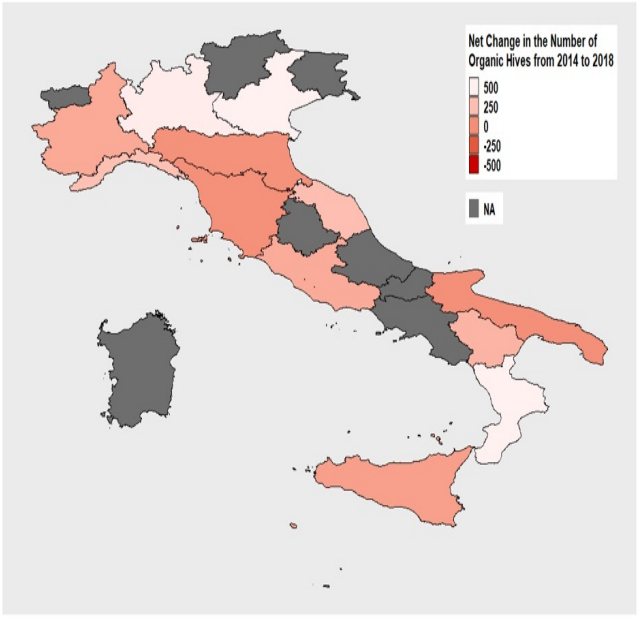


Figure 6.1.8 Comparing the Average Production of Organic and Conventional Honey Over the Years

Figure 6.1.9 Organic and Conventional Operations by Region

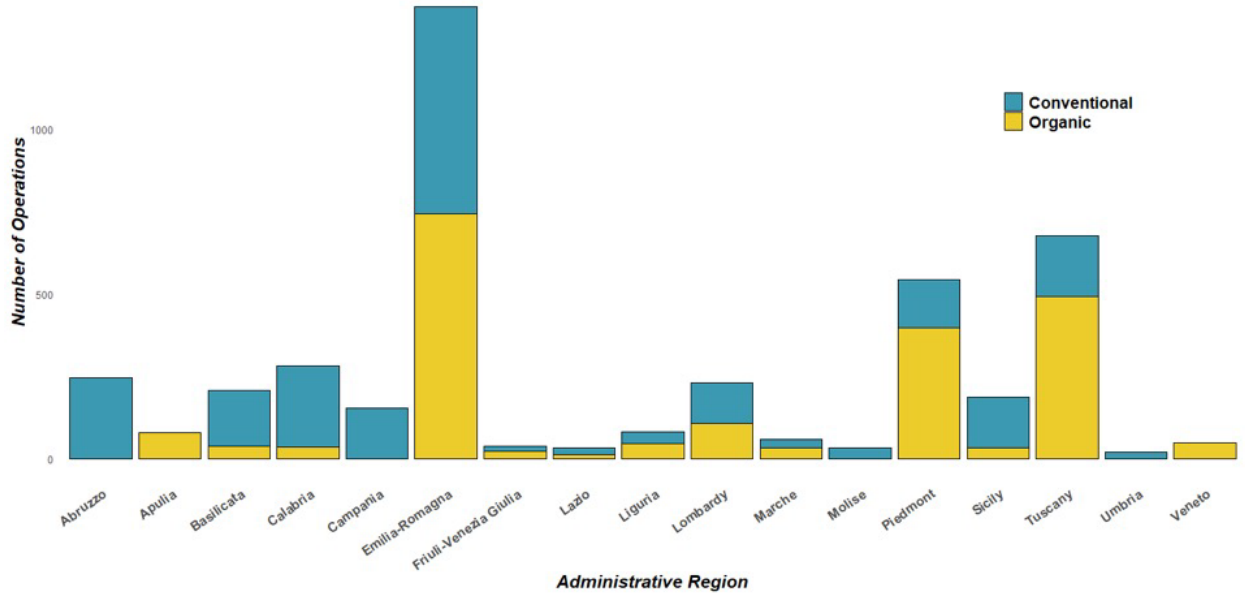


Figure 6.1.10 Organic and Conventional Actual Production from 2014 to 2018 for the Top 5 Producing Regions (kg)

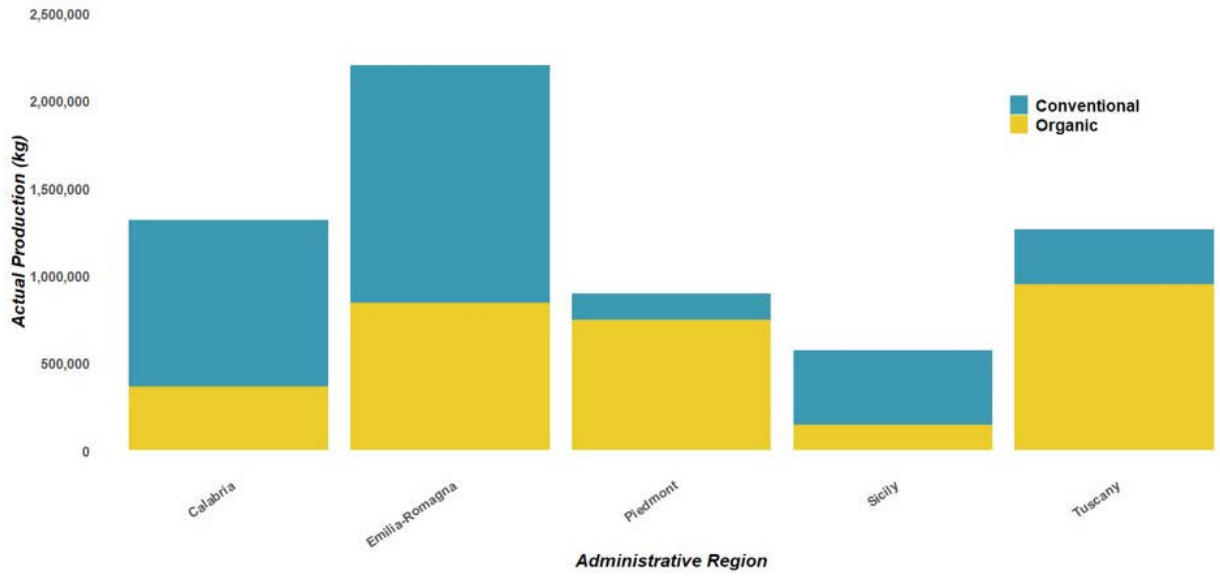
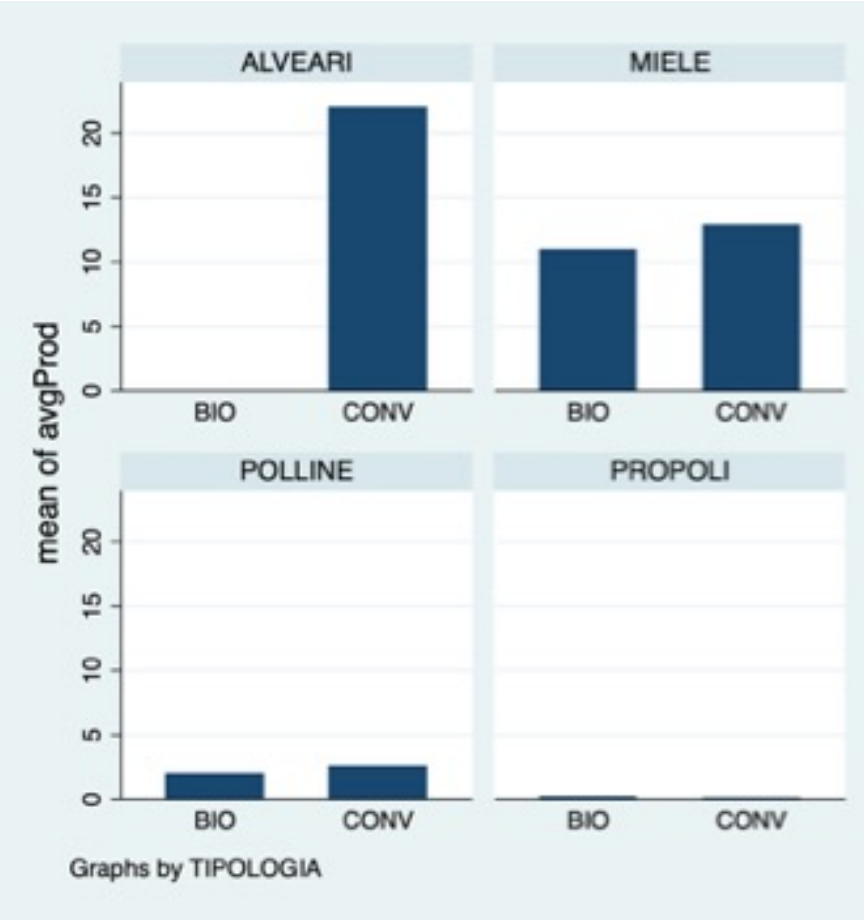


Figure 6.1.11 Type of Production



Milestone M6.2: EU beekeeping report

Contributors:

Max Rünzel M.Sc. M.A., Dr Joseph Cazier, Dr Riccardo Jannoni-Sebastianini, Dr Diego Pagani, Dr Norberto Garcia, Dr Giovanni Formato, Dr James Wilkes

Description:

A report on European beekeeping productivity, competitiveness and resilience

Output:

Part 3 – The economics behind producing organic honey

Figure 6.2.1 A Fair Price for Organic Honey

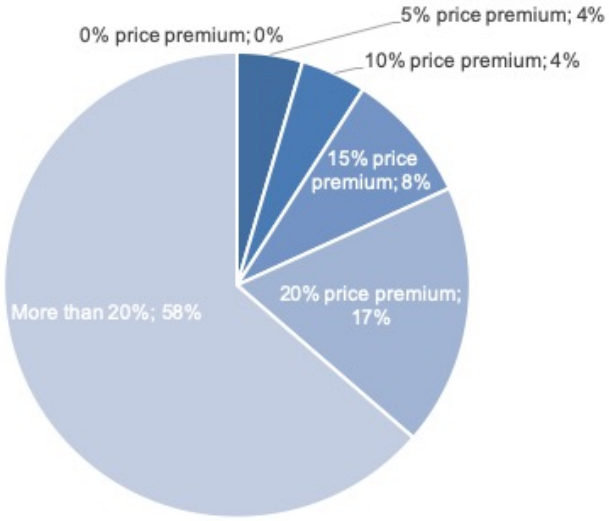


Figure 6.2.3 Price Differences for Mixed Flower and Specialty Honey

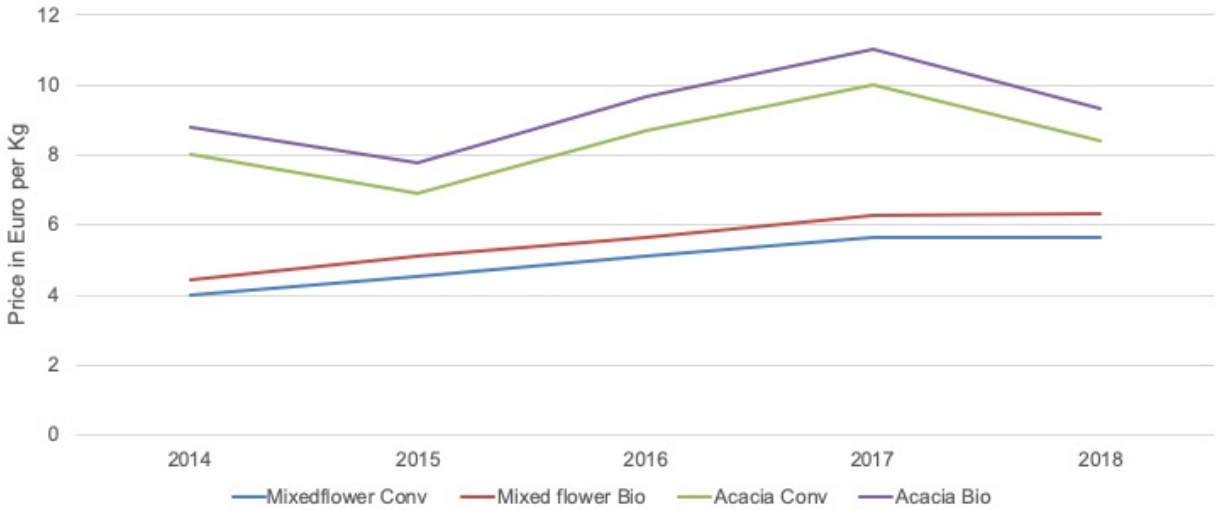
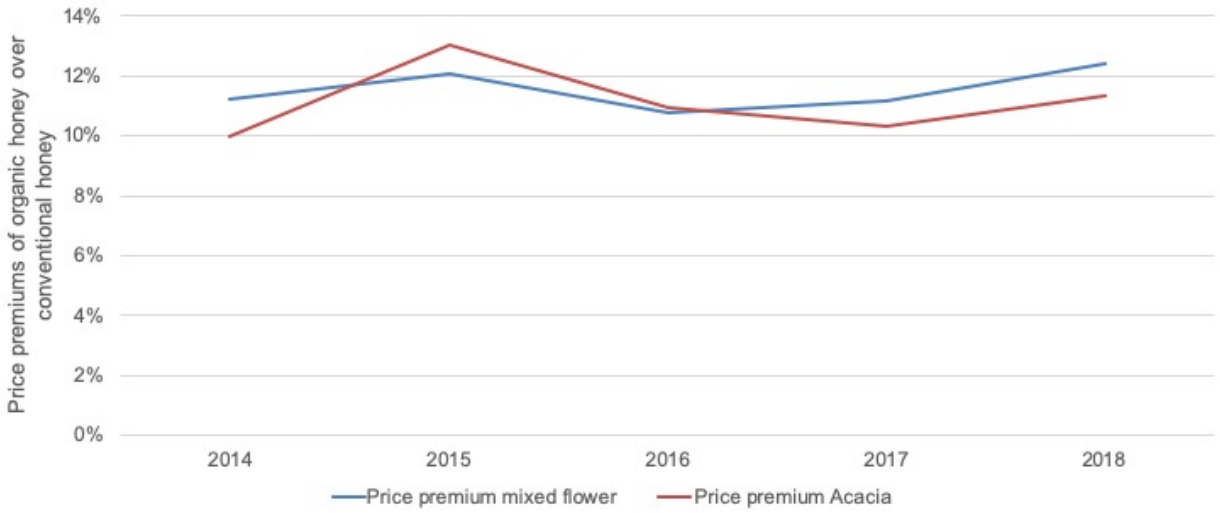


Figure 6.2.4 Price Premiums for Organic Mixed Flower and Acacia Honey over Organic Honey



Part 4 – What drives organic beekeepers?

Figure 6.2.5 Motivation for Carrying out Organic Beekeeping

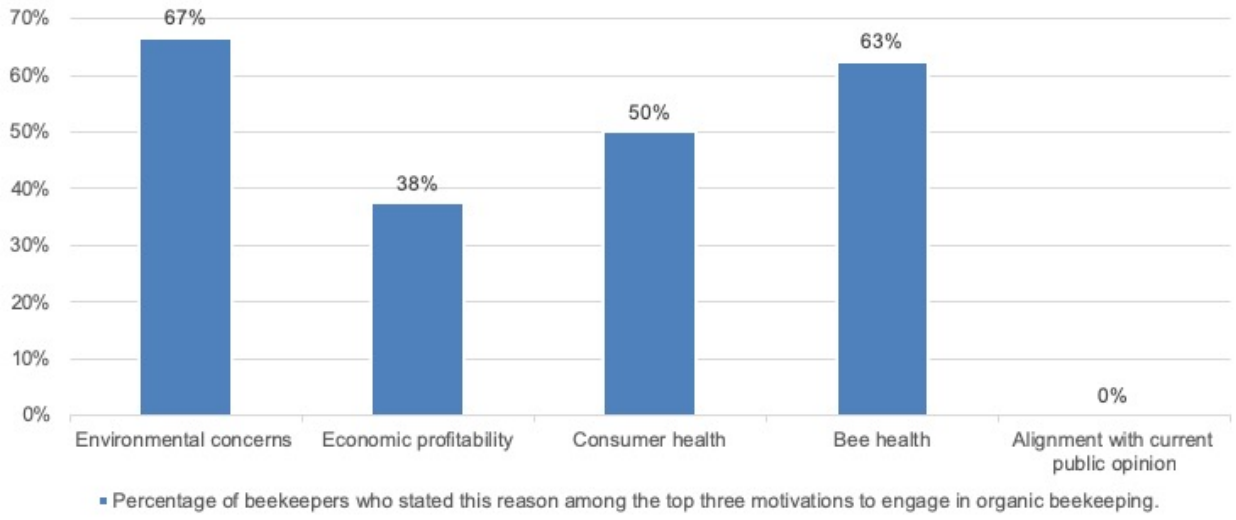


Figure 6.2.6 Obstacles for the Transformation to Organic Beekeeping

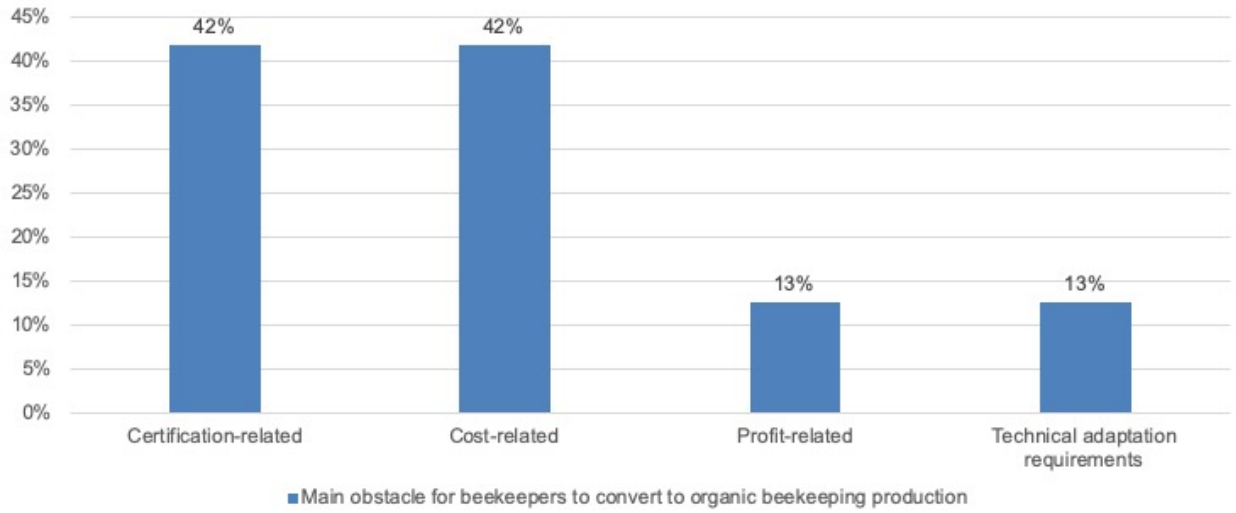
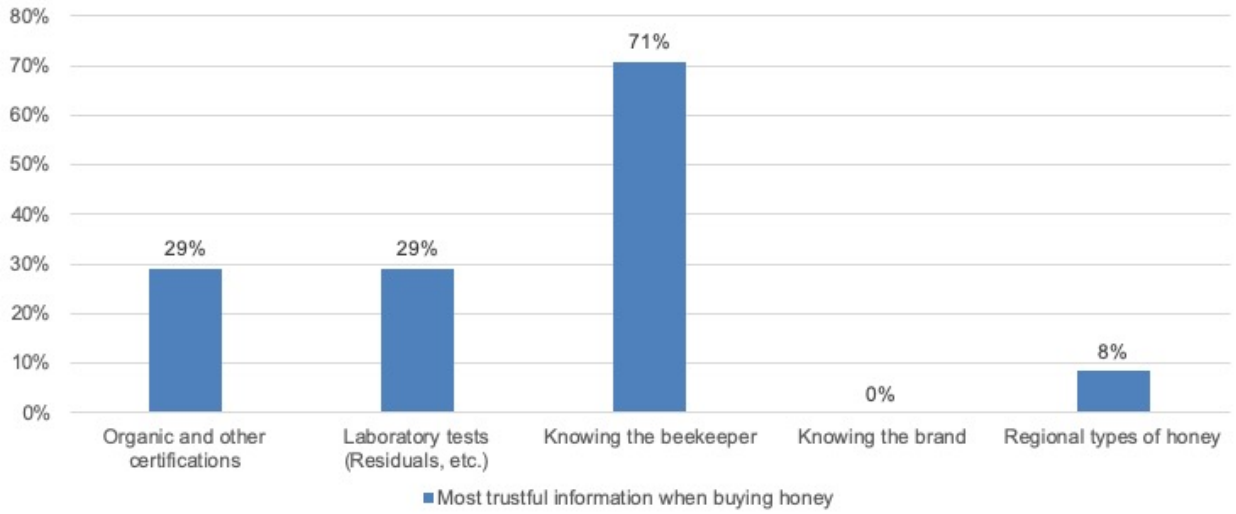


Figure 6.2.7 Honey and Trust



Conclusion

- Organic beekeeping does not seem to show to benefit beekeepers in terms of resources spent.
- Price premiums offered on the market seem not to cover additional resources spent.
- Economic incentives only show on position number four when it comes to justifying transforming conventional to organic beekeeping.
- Thus, the motivation for beekeepers to turn to organic is driven by environmental, veterinary and consumer health concerns.
- Finally, organic beekeepers' conviction is strong enough to support unprofitable business.

Limitations of the study presented in WP 6.1 and WP 6.2

- The survey does not cover the size, type or the location of beekeeping operations.
- Several biases may be present when beekeepers answer the survey questions.
- The CONAPI data available does not provide any information about the winter mortality but the annual variation in the number of hives.

Future Research

Future research should focus on

- The effects of price variations on the amount of hives declared in the beginning of a year.
- The influence of environmental factors on honey production and honey yields.
- Costs and efforts of keeping bees organically.

Work package 7 (WP 7) - "New traceability system". Leader: Partner 1

Dr Giovanni Formato (Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri")

Milestone M7.1: Traceability system online

Contributors:

Dr Marco Pietropaoli, Dr Flemming Vejsnæs

Description:

The traceability system and the web application are available online

Output:

The web application is available at this link: http://hivelog.dk/

More data are available into the Guidelines on project website.

Milestone M7.2: Consumers' point of view

Contributors:

Dr Licia Ravarotto, Dr Giulia Mascarello, Dr Anna Pinto, Dr Silvia Marcolin, Dr Valentina Rizzoli, Dr Stefania Crovato

Description:

Consumers' opinions, perceptions and behaviours related to the purchase and consumption of honey in Italy

A national survey was carried out with the aim of investigating the perception of risk and the purchasing and consumption preferences of honey consumers in Italy.

A semi-structured questionnaire was designed based on the existing literature. The questionnaire consisted of the following sections:

- Socio-demographic characteristics
- Purchasing behaviours
- Consumption behaviours
- Honey and production chain: Knowledge and perceptions

Before administration, the questionnaire was pre-tested on five honey buyers to identify and remove any unclear or dubious questions.

Between February 7th and 25th, 2019, a company specialized in opinion surveys administered the questionnaire through the computer-assisted web interviewing (CAWI) method to a sample of Italian honey buyers and consumers enrolled in the company's mailing list. The honey buyers were selected through a screening question placed at the beginning of the questionnaire: those who declared they had not bought honey in the last 12 months did not fill in the questionnaire.

A total of 1,011 honey buyers completed the questionnaire. Among them, the majority are female (51.1%) and aged between 50 and 62 years old (25.5%). They live in South Italy and the islands (Sicily and Sardinia) (36.3%), have an upper secondary school diploma (50.7%), have an occupation (49.1%), and meet their financial needs with some difficulties (41.9%).

The main results obtained in the Italian context are summarized below:

- Italian honey buyers prefer to purchase honey in hypermarkets/supermarkets/discount stores and directly from the producer
- In several parts of the questionnaire, it was revealed that the origin of the product plays a very important role in the respondents' purchasing and consumption behaviours. For example, 'That the honey is produced in Italy' is considered the most important aspect in the choice of which honey to buy. Moreover, even if the majority of respondents evaluated the information contained on the label as 'sufficient', the need to have more information about the exact origin of honey was observed. Again, the 'Place of origin' was considered the most important information on the label by those who declared that they usually read it
- More than 60% of the respondents stated that they would use the QR code to access further information about honey
- Most respondents stated that they were willing to pay a higher price for a package of honey if it offered them more information about the product
- 'It is good for health' is the main reason why respondents consume honey, while 'I don't like the taste' is the main reason reported by those who stated that they do not eat it
- Approximately 40% of the respondents thought that honey is not recommended for some categories of people, especially for persons affected by diabetes
- In general, the respondents defined honey quite traditional, tasty, healthy, usual, unspoiled, rural, sustainable, and very natural
- A lack of knowledge about honey and its production chain was observed among the interviewees
- In general, honey is not considered dangerous to health
- The label is considered a useful tool to obtain information about the product.

The collected data allowed to outline the purchasing and consumption behaviours adopted by Italian honey buyers and to deeply understand their opinions and perceptions towards honey in general and its production chain.

Consumers' opinions, perceptions and behaviours related to the purchase of honey in Austria and Slovenia.

The online survey was also carried out in Austria and Slovenia with the aim of investigating the opinions, perceptions and behaviours of honey consumers in these different contexts.

The survey is a pilot study developed from the results of the survey carried out at the Italian level.

The questionnaire was designed from a selection of the questions already used in the questionnaire administered in Italy. The questions chosen concerned the following aspects:

- Socio-demographic characteristics
- Purchasing behaviours

Project partners from Austria and Slovenia translated the English version of the questionnaire in German and Slovenian. The two surveys were created online by means of the IZSVe. Survey application (created from the LimeSurvey software) and disseminated between October and December2019, through all the communication channels of the project and of the project partners involved (web sites, social media, newsletters…).

The honey buyers were selected through a screening question placed at the beginning of the questionnaire: those who declared they had not bought honey in the last 12 months did not fill in the questionnaire.

736 respondents in Austria and 33 in Slovenia completed the survey, of which 636 honey buyers in Austria and 31 in Slovenia were considered in the sample. Generally speaking the sample both in Austria and in Slovenia was composed of females and the respondents have a rather high level of education.

The main results obtained in Austria and Slovenia are summarized below. The results are compared with those obtained in Italy. However, given the different sample sizes and the different data collection methods in the three countries, a comparison between the different countries would not give statistically reliable results. However, general trends can be outlined.

- The origin of the product plays a very important role in the respondents' purchasing and behaviours. 'That the honey is produced in the Country where the survey is conducted is considered the most important aspect in the choice of which honey to buy both in Austria and Slovenia. This result is consistent with the Italian one. Furthermore, it is interesting to notice that in Austria and Slovenia "that it is produced in the European Union" or "that it is produced Close to home" are the other most important aspects in the choice of the honey to buy. "That it is cheap", and "that it is from a popular brand/producer" are the aspects considered less important in both Austria and Slovenia and the same result emerged in Italy too.

- Austrian respondents who declare they always read the label on the honey they buy are the 78.5%, while the Slovenian who always read the label are 51.6%. In Italy, the percentage of label readers (56.5%) was closer to the Slovenian one.
- Both Austrian and Slovenian respondents think that the information contained on the honey label is sufficient (62.3% in Austria, 67.9% in Slovenia). It is interesting to notice that this percentage is higher in Italy (89.6%).
- Again, the "place of origin" is evaluated as the most important information to be found in the label, followed by the "presence of other ingredients" and the "information on the producer" in both Austria and Slovenia. Austrian respondents are less interested in "nutritional facts" while Slovenian in "brand".
- More than one third of the respondents declare they would use the QR code to access further information about honey (36.9% in Austria, 38.7% in Slovenia). In Italy those who stated they would use the QR code were more than 60%.
- Most respondents stated that they were willing to pay a higher price for a package of honey if it offered them more information about the product in all the Countries.

Output:

The overall report with the surveys results is available at Annex 19

Milestone M7.3: Consumer panel tests

Contributors:

Dr Licia Ravarotto, Dr Giulia Mascarello, Dr Anna Pinto, Dr Silvia Marcolin, Dr Valentina Rizzoli, Dr Stefania Crovato

Description:

Social research methods (focus groups and questionnaires) were applied to identify the weaknesses and strengths of a traceability system based on the QRCode/RFID technology. The traceability system allows consumers to access a web page with information on honey features suggested by beekeepers.

Participants were asked to access the web page via the QRCode applied on the honey jar.

www.smielatura.it/lotto/lotto.php?lotto=01072018

Two focus groups were held in Bologna and Padova (Italy) on May 20th and 28th.

- ✓ *First focus group*: May 20th, 2019, Bologna (IT)
  Participants: 11 honey buyers

- ✓ *Second focus group*: May 28th, 2019, Padova (IT)
  Participants: 14 honey buyers

Moreover, a paper-and-pencil self-administered survey was carried out between June 11 and 12, 2019, at FICO Eataly World (Bologna) with the support of the CONAPI (Italian National Consortium of Beekeepers) Association. Two experts belonging to the research team showed the interviewees the traceability system and provided support while they tested the QRCode/RFID technology. Then, the interviewees were invited to complete a questionnaire composed of 10 questions. A total of 59 honey consumers completed the questionnaire.

The obtained results were consistent between them: No differences were observed between what was detected through the focus groups and what was observed with the survey. A synthesis of the main findings is provided below.

- Participants seemed to positively welcome the proposal of the traceability system, even though most of them were unfamiliar with the QRCode technology
- In general, the information on honey provided on the webpage was considered by most to be 'complete', 'clear', 'original', and 'useful'
- Regarding the webpage content, most of the participants asked for more synthesis (e.g., on the chemical analyses) and interaction (e.g., social network)
- The possibility of having more information on the beekeeper is greatly appreciated, particularly if that information is authentic
- Tips on food pairings and honey usage were requested several times
- Participants evaluated the graphical aspects highly (in particular, the 'colours'), but they requested more adaptability to different devices

Output:

The results of the traceability system evaluation are summarized in the Report entitled "Consumers' opinions and perceptions related to the traceability system for accessing information on honey" available in Annex 20

Work package 8 (WP 8) – "Dissemination and sharing". Leader: Partner 7

Dr Licia Ravarotto (Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe))

Milestone M8.1: Website online

Contributors:

Dr Licia Ravarotto, Dr Barbara Tiozzo, Dr Mirko Ruzza, Dr Luca Lunardi, Dr Claudio Mantovani

Description:

The website of the project has been uploaded online on a public server

Output:

Website is available at: http://www.izslt.it/bpractices/

Milestone M8.2: Printed GBPs to prevent diseases

Contributors:

Dr Riccardo Jannoni Sebastianini, Dr Giovanni Formato, Dr Barbara Tiozzo

Description:

All the GBPs to prevent honeybee diseases have been printed as part of the activities of the Apimondia Regional and Scientific Commissions. Two documents have been produced

- A full version, that has been published on project's and partners' websites
- A shorter version, including only the list of those GBPs that project's experts rated as most important among those identified, that has been printed and will be distributed at major events to boost dissemination of BPRACTICES outputs even after the end of the project.

Output:

Guidelines (Annex) have been printed as part of the activities of the Apimondia Regional and Scientific Commissions. Moreover, one article have been published on OIE Journal:

J. Rivera-Gomis, J. Bubnic, A. Ribarits, R. Moosbeckhofer, O. Alber, P. Kozmus, R. Jannoni-Sebastianini, W. Haefeker, H. Köglberger, M.I. Smodis Skerl, B. Tiozzo, M. Pietropaoli, J. Lubroth, E. Raizman, C. Lietaer, R. Zilli, R. Eggenhoeffner, M. Higes, M.N. Muz, C. D'Ascenzi, M.P. Riviere, A. Gregorc, J. Cazier, E. Hassler, J. Wilkes & G. Formato (2019). Good farming practices in apiculture.

Available at: https://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/revue_plurithematique/2019/11122019-00160-EN_Rivera-Gomis-Formato_ANG.pdf

Milestone M8.3: Apimondia publications

Contributors:

Dr Riccardo Jannoni Sebastianini, Dr Giovanni Formato

Description:

Three publications have been published in Apimondia International Apicultural Congresses and Symposia. More publications are available on project website at this page: http://www.izslt.it/bpractices/dissemination/

Output:

- Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni-Sebastianini R., Lietaer C., McCabe P., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Pietropaoli M., Ravarotto L., Ribarits A., Riviere MP, Smodis Skerl M., Formato G. (2017). Preclinic Indicators at the Apiary Level to Prevent Honeybee Diseases. Proceedings of 45th APIMONDIA International Apicultural Congress September 29 - October 4, 2017. Istanbul - TURKEY [Abstract:0650]. Page 64.
- Rivera-Gomis J., Pietropaoli M., Cersini A., Necati Muz M., Muz D., Ozdemir N., Erat S., Smodis Skerl M.I., Higes M., Ribarits A., Moosbeckhofer R., Gregorc A., Ravarotto L., McCabe P., Haefeker W., Jannoni Sebastianini R., Eggenhoeffner R., Riviere M.P., Chabert M., Chauzat M.P., Lietaer C., Formato G. (2017). BPRACTICES project: New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* era in Europe. Proceedings of 45° APIMONDIA International Apicultural Congress, 29th September – 4th October 2017, Istanbul (Turkey): 116. (Abstract Reference N. 0624).
- Formato G., Pietropaoli M. (2016). Pre-clinic indicators as good beekeeping practices: sampling methods and new traceability systems. Proceedings of 6th Apimedica and 5th Apiquality International Simposium. Roma, 22-25 November 2016. Page 19

Milestone M8.4: Open-access paper

Contributors:

Dr Giovanni Formato

Description:

One open-access paper with the project aims and results has been sent to an open-access journal (Bee World)

Output:

The paper has been accepted and it will be available (open access) at this link: https://doi.org/10.1080/0005772X.2020.1757220

A copy of the manuscript follows:

BPRACTICES project: towards a sustainable European beekeeping

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[9]Centro de Investigacion Apicola y Agroambiental de Marchamalo, C/Camino San Matin s/n, 19180 Marchamalo, Spain

[10]International Federation of Beekeepers' Associations, Apimondia, Corso Vittorio Emanuele 101, I-00186 Roma, Italy

[11]Tecnologies and practices for small agricultural producers (TECA) platform of the Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy

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KEYWORDS

Good beekeeping practices; Biosecurity measures, BPRACTICES, Eranet SUSAN

INTRODUCTION

European beekeeping suffers significant regional differences in colony losses due to external impacts on beekeeping, including climate and prevalence of diseases (Potts et al., 2010; EPILOBEE, 2014; McMenamin and Genersch, 2015). The European EPILOBEE project (Laurent et al. 2015, Chauzat et al., 2014) underlined the lack of explanatory studies about risk factors affecting colony health like disease prevalence, environment condition and farming practices adopted by beekeepers to detect and control the major honeybee diseases: *Varroa destructor* and associated viruses, American Foulbrood (AFB), European Foulbrood (EFB) and *Nosema* spp.

Varroa destructor is the most widespread and hard to control disease (Rosenkranz et al., 2010). Quite all non-organic "hard treatments" produced resistant mites (Maggi et al., 2010; Kanga et al., 2010; Pettis, 2004) and reduced the quality and safety of hive products (Rosenkranz et al., 2010). American and European Foulbrood cause considerable economic losses (Forsgren, 2010; Genersch, 2010) and the use of antibiotics is still considered an illegal possible solution to pursue with the subsequent risk of residues in hive products and bacterial resistance. *Nosema* spp. (especially *N. ceranae*) is an emerging pathogen affecting adult honeybees and it is associated to a reduced lifespan and increase of winter mortality (Higes et al., 2010). Furthermore, with the spread of the exotic parasite *Aethina tumida* (Small Hive Beetle – SHB) from Italy (Neumann et al., 2016; Mutinelli et al., 2014) beekeeping trade in EU is facing a great risk of productivity reduction and exports halt.

Today, good beekeeping management at the apiary level is a crucial point to maintain a healthy bee population (ANSES, 2015). Cross-valuable methods or guidelines internationally adopted to prevent and control the above-mentioned honeybee diseases in a sustainable way, including harmonized methods and analytical techniques for laboratory diagnosis, at the EU level, have not been adopted so far (Laurent et al., 2015; Chauzat et al., 2014; Chauzat et al., 2013), leading to a very variable quality and quantity of EU hive productions.

The European Union funded project: "New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* era in Europe" (BPRACTICES), aims to support European beekeeping in the above

mentioned framework, in the context of the Horizon 2020 research and innovation program ERA-NET SusAn – European Research Area on Sustainable Animal Production Systems.

The BPRACTICES project tried to answer to the above-mentioned needs with an improvement of EU beekeeping production system towards the development of an innovative holistic approach (from apiary to jar) considering the good beekeeping practices (GBPs) and biosecurity measures in beekeeping (BMBs) application (Fig. 1).

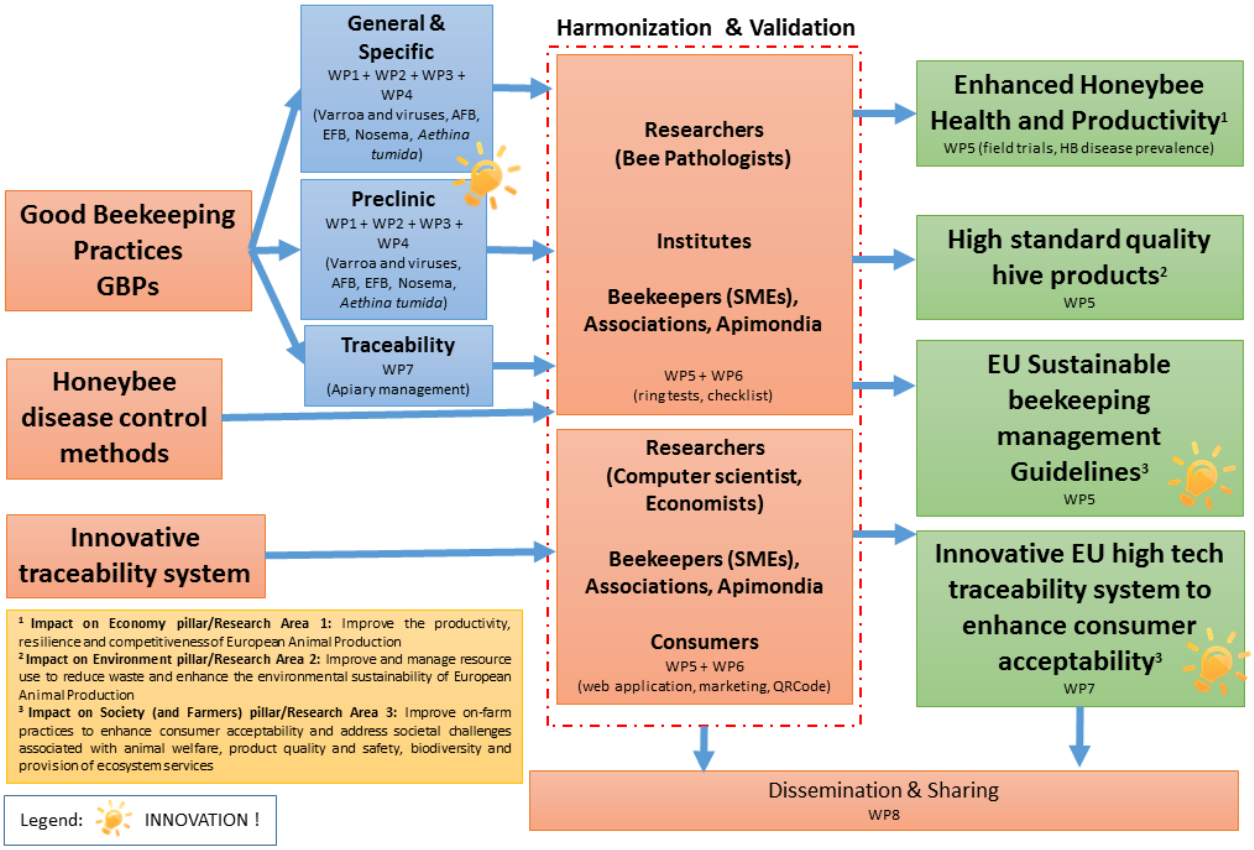

Figure 3 BPRACTICES project overall structure

GBPs and BMBs are considered as systematic tools to prevent honey bee diseases and to reduce the application of veterinary medicines at the apiary level, enhancing quality of hive products.

The outputs of the project have been:

- Definition and listing of GBPs and BMBs harmonized within partner countries involved into the project, providing a cross-EU stakeholders debate on it;

- A new approach on management of honey bee diseases based on prevention and on “preclinical” diagnosis. Adopting new clinical methods, biomechanical and innovative biomolecular techniques have been developed new biosensors from honey to monitor SHB presence and PCR techniques to diagnose in advance honeybee diseases (AFB, EFB, SHB) from hive debris.
- Guidelines on innovative laboratory diagnostic methods, harmonized among project partners, with the collaboration of the European Union Reference Laboratory for Bee Health (ANSES);
- Sustainable honeybee diseases control guidelines in respect of bee welfare and hive products quality (low-environmental impact approach);
- Economic study concerning the impact of the innovative GBPs system application;
- Dissemination of results and technical assistance/training, with the transnational participation of Apimondia (http://apimondia.com/) and FAO TECA platform (http://teca.fao.org/) and the release of a web-application as an innovative traceability system (QR Code/RFID based).

THE CONSORTIUM

All those objectives have been achieved by using multidisciplinary strategies: with the combination of scientific research, on-field experience for the validation of the methods, food safety control and economic, societal and commercial analysis. This wide approach has been possible thanks to the multi-actor involvement of the project that includes different specialities and abilities, with the practical and useful experience of the beekeepers.

Consortium partners: Research institutes (*), Food and Agriculture Organization of the United Nations (FAO), and the International Federation of Beekeepers’ Associations (Apimondia).

(*)*Research institutes*:

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) - Italy

Namik Kemal University - Turkey

Agricultural Institute of Slovenia - Slovenia

Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA) - Spain

Austrian Agency for Health and Food Safety (AGES) - Austria

Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) - Italy

University of Genova - Italy

European Union Reference Laboratory (EURL) for Bee Health, French Agency for Food, Environmental and Occupational Health Safety (Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail - ANSES) - France

FAO:

Beekeeping Exchange Group - TECA - FAO - Italy

Beekeepers' associations involved in the project:

International Federation of Beekeepers' Associations - APIMONDIA - Italy

European Professional Beekeepers Association - EPBA - Germany

Other collaborations:

Appalachian State University - USA

Danish Beekeepers' Association – Denmark

Box 1 Consortium composition

THE STRUCTURE OF THE PROJECT

The BPRACTICES project lasted 36 months and was structured in eight Work Packages (WPs). WP1 (varroosis and viruses), WP2 (AFB and EFB), WP3 (*Nosema*), WP4 (*Aethina tumida*) were finalized to identify the best cross-EU valid GBPs for the proper honeybee colonies management, develop innovative on-field methods to effectively prevent and control pathogens in a sustainable and holistic way, implement standardized laboratory methods for early disease diagnosis to guarantee a low environmental impact management and hive products quality and safety. WP5 (validation) performed the standardization of the GBPs, that was verified with a feasibility and compliance study by beekeepers; the validation of laboratory methods among partners through ring tests, the collaboration with the EU Reference Laboratory for Bee Health (ANSES) and the validation of best diseases control methods at apiary level with specific field trials. WP6 (economic impact) provided an economic evaluation of impacts of the new practice's application on the quality/safety/value of hive products and gave an overview of the added value of the innovations proposed within the project to the European beekeeping. WP7 (new traceability system) developed and applied to the entire honeybee food chain an innovative traceability system based on QRCode/RFID technology from hive to jar, previously tested by beekeepers and consumers in WP8. WP8 (dissemination and sharing) disseminated all the innovations developed by the project. Such achievement happened thanks to the active involvement of Apimondia (http://apimondia.com/), FAO TECA platform (http://teca.fao.org/) and all consortium participants.

RESULTS

Thanks to the combined work of the partners involved, a cross-EU list of GBPs and BMBs for the proper honeybee colonies management was identified. The list of GBPs has been published on an article (Rivera Gomis et al., 2019) and the list of BMBs is under review process (Pietropaoli et al., 2019). Both are available on project website (http://www.izslt.it/bpractices/).

Guidelines for sustainable honeybee diseases control and laboratory diagnostic methods, harmonized among project partners have been published on project website.

GBPs and BMBs applicability both for professional and hobbyist beekeepers were verified through the use of surveys available at TECA FAO website http://www.fao.org/teca/en/. The definitive compliance and feasibility study, the economic study concerning the impact of the new management system are available on project website.

Dissemination activities considered several papers (Pietropaoli et al. 2019; Della Marta et al. 2018 a b; FAO, 2018; Rivera-Gomis et al., 2017; Rivera-Gomis et al., 2018), proceedings (Rivera-Gomis et al. 2017 a, b; Formato & Pietropaoli, 2016; Rivera-Gomis et al. 2018b; Pietropaoli et al., 2018), publications on the FAO TECA platform (http://teca.fao.org/) and, on November 30th 2019, a popular dissemination event has been organized by Apimondia and IZSLT in Montefiascone (Italy).

The web-application with the innovative traceability system is available at this link: https://www.hivelog.dk/. Two reports have been published about consumers' opinions, perceptions and behaviours: one related to the purchase and consumption of honey in EU and the other one related to the traceability system for accessing information on honey. The two studies showed that the origin of the product plays a very important role in the respondents' purchasing and consumption behaviours. Moreover, even if most respondents evaluated the information contained on the label as 'sufficient', the need to have more information about the exact origin of honey was observed. Again, the 'Place of origin' was considered the most important information on the label by those who declared that they usually read it and more than 60% of the respondents stated that they would use the QR code to access further information about honey. Most respondents stated that they were willing to pay a higher price for a package of honey if it offered them more information about the product and a lack of knowledge about honey and its production chain was observed among the interviewees.

Weaknesses and strengths of the traceability system based on QRCode/RFID technology were identified by means of two different social research methods: focus groups and questionnaires. Participants seemed to positively welcome the proposal of the traceability system and in general, the information on honey provided

on the webpage was considered by most to be ‘complete’, ‘clear’, ‘original’, and ‘useful’. The possibility of having more information on the beekeeper was greatly appreciated, particularly if that information is authentic. Detailed reports are available on project website.

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Milestone M8.5: Publications on websites

Contributors:

All partners

Description:

Guidelines and the traceability system have been published on partners' institutional websites

Output:

- IZSLT: http://www.izslt.it/apicoltura/2019/01/01/il-sistema-di-tracciabilita-innovativo-del-progetto-bpractices/
- University of Namik Kemal: -
- Agricultural Institute of Slovenia: https://www.kis.si/Cebelarstvo_OZ/
- University of Maribor: -
- Centro de Investigación Apícola y Agroambiental de Marchamalo: -
- Austrian Agency for Health and Food Safety: https://www.ages.at/themen/umwelt/bienen/bienengesundheit/bpractices-neue-methoden-zur-voelkerfuehrung/ | https://www.facebook.com/agesnews/videos/665654433962168/
- IZSVe: https://www.izsvenezie.it/bpractices-buone-pratiche-allevamento-per-salute-api/ | https://www.izsvenezie.com/bpractices-new-management-practices-beekeeping/

Thanks to the collaboration with ERA-NET SusAn Communication team, BPRACTICES news have been shared also on ERA-NET SusAn communication channels (in particular through LinkedIn and e-newsletter), in order to promote dissemination and visibility of activities and results. Here below some examples are reported:

Newsletter

https://mailchi.mp/a8553a6c16a3/newsletter-susan-december-2019

https://mailchi.mp/3faeb34e4f60/susan-newsletter-june-2019

https://us16.campaign-archive.com/?u=515c601db26b9000ed1bfb35c&id=c6c5f85ae0

https://us16.campaign-archive.com/?u=515c601db26b9000ed1bfb35c&id=1ac73c13df

LinkedIn

https://www.linkedin.com/posts/era-net-susan-0534aa184_we-hope-you-enjoy-the-reading-of-our-latest-activity-6612333536437501953-tmNp

https://www.linkedin.com/posts/era-net-susan-0534aa184_researcher-giovanni-formato-pitches-the-bpractices-activity-6580758685612675072-haob

https://www.linkedin.com/posts/era-net-susan-0534aa184_bees-activity-6545222629714997248-J-hH

https://www.linkedin.com/posts/era-net-susan-0534aa184_beekeepers-lets-improve-bee-health-are-activity-6542680629056798720-x8sj

https://www.linkedin.com/posts/era-net-susan-0534aa184_bees-sustainability-activity-6534322534622605312-qspP

Milestone M8.6: FAO TECA publication

Contributors:

Dr Giovanni Formato, Dr Charlotte Lietaer

Description:

A page dealing with the project has been published in the FAO TECA platform (http://teca.fao.org/)

Output:

The page is available at this link: http://www.fao.org/teca/forum/beekeeping/en/

Milestone M8.7: Beekeepers event

Contributors:

All partners

Description:

Here below the list of beekeepers events organized by BRPACTICES partners.

- IZSLT: http://www.izslt.it/apicoltura/2019/10/31/le-buone-pratiche-in-apicoltura-dal-progetto-europeo-bpracties-alla-pratica-nella-tuscia/
- IZSVe: https://www.izsvenezie.it/convegno-salute-api-18-gennaio-2020-padova/…
- University of Namik Kemal: see atteched photos
- Agricultural Institute of Slovenia: Beekeepers meeting on December 2019, 17th (see attachment)
- University of Maribor: -
- Centro de Investigación Apícola y Agroambiental de Marchamalo: -
- Austrian Agency for Health and Food Safety: Project results have continuously been disseminated to beekeepers – as soon as they were available – at meetings of small, sideline and professional beekeepers, training courses for official beekeeping inspectors, and at diverse annual meetings. Below is a list of different events, including the venue (all in Austria), and the presenters. With such presentations, a large number of beekeepers (up to several hundred) were informed about the project. Unless otherwise indicated, general information about the BPRACTICES project and the related activities was provided.
    - 25.02.2017: Annual Meeting of Professional Beekeepers Association, Unterpremstätten; Rudolf Moosbeckhofer
    - 25.03.2017: Annual General Meeting of Beekeepers Association of Lower Austria, St. Pölten; Rudolf Moosbeckhofer
    - 28.04.2017: Annual Meeting of Health Advisors of the Austrian Beekeeping Association, Wien; Rudolf Moosbeckhofer
    - 25.04.2018: Annual Meeting of Health Advisors of the Austrian Beekeeping Association, Wien; Rudolf Moosbeckhofer (general information), Josef Mayr (activities within WP1)

- 16.2.2019: Annual General Meeting of Beekeepers Association of Styria, Gratkorn; Josef Mayr (activities within WP1)
- 23.2.2019: Annual Meeting of Professional Beekeepers Association, Premstätten; Josef Mayr (activities within WP1)
- 9.5.2019: Annual Meeting of Health Advisors of the Austrian Beekeeping Association, Wien; Alexandra Ribarits
- 9.5.2019: Annual Meeting of Health Advisors of the Austrian Beekeeping Association, Wien; Hemma Köglberger (activities within WP2)
- 19.10.2019: Annual Meeting of Beekeeping Instructors of the Austrian Beekeeping Association, Altlengbach; Josef Mayr (activities within WP1)
- 22.2.2020 Annual Meeting of Professional Beekeepers Association; Austria, Premstätten, Josef Mayr (agreed) (activities within WP1)

In autumn 2019, AGES organised a series of 4-hour-training courses for official bee inspectors from all Austrian Federal Provinces (Table 1). In total, 194 official bee experts were trained in six separate events by the lecturers Hemma Köglberger, Dr. Josef Mayr, and Dr. Linde Morawetz (all AGES, Department of Apiculture and Bee Protection). By these events, the whole Austrian territory was covered, reaching beekeepers in all Federal Provinces. The bee experts were informed according to the current state of knowledge on the small hive beetle, and sensitised to the importance of diagnosis. The trainings focused on the small hive beetle (*Aethina tumida*). The topics covered were biology, characteristics and procedure in case of suspicion, natural distribution and current dispersal, as well as possibilities for diagnosis, especially different inspection concepts and control of the small hive beetle. Each course commenced by presenting the BPRACTICES project, its major tasks and main results, ensuring that the project was widely disseminated.

Table 1. Beekeepers events in Austria in autumn 2019. Dates, venues, relevant Federal Provinces, and number of participants.

Date	Venue	Federal Province	Participants: Official bee experts
24.10.2019	Koppl	Salzburg	30
25.10.2019	Linz	Oberösterreich	26
30.10.2019	Wien	Burgenland, Niederösterreich, Wien	36
6.11.2019	Jenbach	Tirol	43
7.11.2019	Hohenems	Vorarlberg	9
22.11.2019	Frohnleiten	Steiermark	50
	Total number of participants		**194**

Kmetijski inštitut Slovenije
Agricultural Institute of Slovenia

SEZNAM UDELEŽENCEV NA PREDAVANJU 17.12.2019

IME IN PRIIMEK	ORGANIZACIJA	PODPIS
Nina Gunčič	UNI VF	
TINKARA REMIC	/	
TADEJ ŽETIC	/	
[illegible]	/	
Ivan Judkovič		
GORAZD TRUŠNOVEC	DRUŠTVO URBANI ČEBELAR	
SILVO ROSL	ČD Rogaška Slatina	
MARJAN MAROLT	MESTNO UR BANI ČEBELAR	
Vladimir Fajdiga	ČD Postojna	
Anastazija Jež Knebelj	ČD Postojna	
LEVIČAR MATJAŽ	SZČRP	
Boris Seražin	SZPC	
Andreja Kobentar	ČD ROGATEC	
Janez Dremelj	ČD LITIJA	
Simonič Ivan	ČD IVAN JOLAJEVEC	
MATTEO LUPI	ČD KRTINA DOB	
JERNEJ LONČAR	ČD LOGATEC	
Mitja Nakrst	ČEBELAR	
Tine Nusdorfer	ČEBELAR	
VESNA LOKAR	KII	
ALJOŠA KUŠEC	ČD URBANI ČEBELAR	

Kmetijski inštitut Slovenije
Agricultural Institute of Slovenia

SEZNAM UDELEŽENCEV NA PREDAVANJU 17.12.2019

IME IN PRIIMEK	ORGANIZACIJA	PODPIS
MIRA ŽENKO ZAGELJ	VF, NVI	
LUCIJA ŽVOKELJ	VF, NVI	
METKA FIŠCAK OCEPEK	VF NVI	
Mitja PALČIČ	ČD Loška dolina	
SUZANA SKERBIŠ	VF NVI	
Kris Pirih		
KATARINA GROS	KIS, CL	
IZTOK REŠKAR	URBANI ČEBELAR	
ROMAN NAVEC	KIS	
TRAJČE Vinološki	penzič	
NINA ILIČ	Zavod Emeja	
SIMONA GOSTINČAR	čebelarka	
DEJAN ILIČ	ČEBELAR	
SEBASTJAN KAPEL	ČD SLIVNICA	
MOČNIK TETE	ČD SLIVNICA	
ANDREJ JERNEJ	ČD BLAGOVNIT	
MARJAN PAPEŽ	ČD LOGATEC	
LJUBO STRUNJA	URBANI Č.	
MIHA BEZNIČAR	čd Sk. Loka	


ERA-NETSUSAN
BPRACTICES
BPRACTICES
ARIPRATİGİ
AVRUPA'DA Aethina tumida sürecinde BALARISI SAĞLIĞINI GELİŞTİRMEK İÇİN
YENİ GÖSTERGELER VE ARI ÇİFTLİĞİ UYGULAMALARI
29/11/2019 TEKIRDAG (TURKEY)
SURVEY and PRESENTATION MEETING
EUROPEAN RESEARCH AREA ON SUSTAINABLE ANIMAL PRODUCTION

Milestone M8.8: Popular dissemination event

Contributors:

Dr Riccardo Jannoni Sebastianini

Description:

A popular dissemination event has been organised by Apimondia

Output:

On November 30th 2019, it has been organized an event by Apimondia, IZSLT and ASL VT in Montefiascone (VT). Brochure and presentation of the event are available at: http://www.izslt.it/apicoltura/2019/10/31/le-buone-pratiche-in-apicoltura-dal-progetto-europeo-bpracties-alla-pratica-nella-tuscia/.

Milestone M8.9: Website section for consumers

Contributors:

Dr Licia Ravarotto, Dr Barbara Tiozzo, Dr Mirko Ruzza, Dr Luca Lunardi

Description:

A specific section of the project website for the consumers has been published.

Output:

A page dealing with the benefits for the consumers deriving from the output of the project has been published at: http://www.izslt.it/bpractices/the-traceability-system/

Articles published in the context of the project

2016

Rivera-Gomis, J., Gregorc, A., Ponti, A. M., Artese, F., Zowitsky, G., & Formato, G. (2016). Monitoring of Small Hive Beetle (*Aethina Tumida* Murray) in Calabria (Italy) from 2014 to 2016: Practical Identification Methods. Journal of Apicultural Science, 61(2), 257-262

2017

Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni Sebastianini R., Lietaer C., McCabe P., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Pietropaoli M., Ravarotto L., Ribarits A., Riviere MP, Smodis Skerl, M, Formato G. (2017) BPRACTICES (ERA-NET SusAn) PROJECT: towards a sustainable European beekeeping. Apimondia publication 2017.

2018

Rivera-Gomis j., Bubnic J., Cersini A., Chabert M., Chauzat MP., Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni-Sebastianini R., Lietaer C., McCabe P., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Pietropaoli M., Ravarotto L., Ribarits A., Riviere MP, Smodis Skerl M.I., Søgaard Jørgensen A., Formato G. (2018). Good Beekeeping Practices (GBPs) and disease prevention, in "Apimondia. Working for the benefit of bees and apiculture", released within the framework of the first World Bee Day (May, 20 2018)

Maja Ivana Smodis Skerl and Giovanni Formato (2018). The BPRACTICES project and its interaction with the COLOSS Varroa Control TF. Proceedings of the "Varroa Control TF 2018 Workshop" Zadar, Croatia 27th – 28th February 2018

Pietropaoli M., Vejsnæs F., Kilpinen O., McCabe P., Jannoni-Sebastianini R., Jørgensen A.S., Lietaer C., Formato G. (2018). BPRACTICES and Hivelog web application for honey bee products traceability. Proceedings of EURBEE Conference 2018. 18-20 September 2018, Ghent, Belgium.

Rivera-Gomis J., Bubnic J., Cersini A., Chabert M., Chauzat M.P., Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni-Sebastianini R., Lietaer C., Lubroth J., McCabe P., Moosbeckhofer R., Muz D., Muz M.N., Ozdemir N., Pietropaoli M., Ribarits A., Riviere M.P., Smodis Skerl M.I., Tiozzo B., Formato G. (2018). BPRACTICES: first attempt of definition of Good Beekeeping Practices (GBPs). Proceedings of EURBEE Conference 2018. 18-20 September 2018, Ghent, Belgium.

Della Marta U., Leto A., Pietropaoli M., Belardo V., Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Higes M., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Ribarits A., Riviere MP, Vejsnæs F., Kilpinen O., Bagni M., Ravarotto L., Tiozzo B., Ruzza M., Smodis Skerl M., Lietaer C., Mccabe P., Jannoni-Sebastianini R., Haefeker W., Formato G. (2018). « Nouveaux indicateurs et pratiques apicoles en Europe pour améliorer la santé des abeilles mellifères dans le domaine de la recherche européenne à l'ère d'*Aethina tumida*» [New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* ERA in Europe]. La Santè de l'Abeille. Maggio-Giugno n. 285 pag. 223-228.

Della Marta U., Leto A., Pietropaoli M., Belardo V., Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Higes M., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Ribarits A., Riviere MP, Vejsnæs F., Kilpinen O., Bagni M., Ravarotto L., Tiozzo B., Ruzza M., Smodis Skerl M., Lietaer C., Mccabe P., Jannoni-Sebastianini R., Haefeker W., Crovato S., Mascarello G., Mantovani C., Formato G. (2018). La proposta del progetto europeo B-PRACTICES. Argomenti. Numero 2/2018 pag. 66-69.

Rivera-Gomis J., Gregorc A., Maroni Ponti A., Artese F., Zowitsky G., Leto A., Della Marta U., Formato G. (2018). Monitoring of Small Hive Beetle (*Aethina tumida* Murray) in Calabria (Italy) from 2014 to 2016: practical identification methods. Proceedings of EURBEE 2018. 18-20 September. Ghent. P097

2019

G. Formato (2019). Talk: Pre-clinical indicators as innovative tools in beekeeping, in the context of the BPRACTICES project. Proceedings of Honey Bee Health Symposium 2019, Rome

J. Rivera-Gomis, M. Pietropaoli, F. Artese, G. Formato (2019). Talk: Comparison of two colony inspection methods for the detection of Small Hive Beetle (SHB) in Calabria region (Italy). Proceedings of Honey Bee Health Symposium 2019, Rome

J. Rivera Gomis, J. Bubnic, A. Ribarits, R. Moosbeckhofer O. Alber, P. Kozmus, R. Jannoni Sebastianini, W. Haefeker, H. Koeglberger, M. I. Smodis Skerl, B. Tiozzo, M. Pietropaoli, J. Lubroth, E. Raizman, C. Lietaer, R. Zilli, R. Eggenhoeffner, M. Higes, M. N. Muz, C. D'Ascenzi, M. P. Riviere, A. Gregorc, J. Cazier, E. Hassler, J. Wilkes, G. Formato (2019). Good Farming Practices in Apiculture (Good Beekeeping Practices GBPs). Proceedings of Honey Bee Health Symposium 2019, Rome, page 75 – 76

Rivera-Gomis, J., Bubnic, J., Ribarits, A., Moosbeckhofer, R., Kozmus, P., Jannoni-Sebastianini, R., Haefeker, W., Koeglberger, H., Smodis Skerl, M. I., Tiozzo, B., Pietropaoli, M., Lubroth, J., Zilli, R, Eggenhoeffner, R., Higes, M., Muz, M. N., D'Ascenzi, C., Riviere, M. P., Chauzat, M. P., Gregorc, A., Formato, G. (2019). Biosecurity Measures in Beekeeping. Proceedings of Honey Bee Health Symposium 2019, page 76 - 77

J. Rivera-Gomis, G. Formato, V. Antognetti, G. Pietrella, A. Cersini (2019). New *Aethina tumida* detection methods using Real Time PCR from hive debris and swab samples. Proceedings of Honey Bee Health Symposium 2019, page 80

Pietropaoli M., Jannoni Sebastianini R., Formato G. (2019). Apicoltura: sondaggi FAO. Partecipa! Apinsieme, December 2019, 8 – 11.

J. Rivera-Gomis, J. Bubnic, A. Ribarits, R. Moosbeckhofer, O. Alber, P. Kozmus, R. Jannoni-Sebastianini, W. Haefeker, H. Köglberger, M.I. Smodis Skerl, B. Tiozzo, M. Pietropaoli, J. Lubroth, E. Raizman, C. Lietaer, R. Zilli, R. Eggenhoeffner, M. Higes, M.N. Muz, C. D'Ascenzi, M.P. Riviere, A. Gregorc, J. Cazier, E. Hassler, J. Wilkes & G. Formato (2019). Good farming practices in apiculture. Revue scientifique et technique (International Office of Epizootics), December 2019, 38(3):1

2020

M. Pietropaoli, A. Ribarits, R. Moosbeckhofer, H. Köglberger, O. Alber, A. Gregorc, M.I. Smodis Skerl, J. Presern, J. Bubnic, M. Necati Muz, M. Higes, B. Tiozzo, R. Jannoni-Sebastianini, J. Lubroth, J. Cazier, C. Lietaer, M. Bagni, R. Zilli & G. Formato (2020). Biosecurity measures in European beekeeping. Revue scientifique et technique (International Office of Epizootics), January 2020. Under review.

M. Pietropaoli, M. Smodis Skerl, J. Cazier, MP Riviere, B. Tiozzo, R. Eggenhoeffner, A. Gregorc, W. Haefeker, M. Higes, R. Jannoni-Sebastianini, C. Lietaer, A. Ribarits, M. Necati Muz, F. Vejsnæs, G. Formato. (2020). BPRACTICES project: towards a sustainable European beekeeping. Bee World Online. DOI number 10.1080/0005772X.2020.1757220.

Annex 1 A review of the best low environmental impact methods for Varroa control

Abstract

Beekeeping sector is nowadays facing many challenges the biggest is defiantly how to keep healthy colonies that produce high quality products without any residues of veterinary medicines and with low environmental impact. The biggest obstacle to overcome is ectoparasitic mite *Varroa destructor*, the most damaging honey bee pests and a key factor in high colony losses all around the globe. To prevent the damage, beekeepers use different acaricides to treat varroosis whichi are unofficially divided into two groups: hard, synthetic acaricides and soft, organic acaricides, both having pros and cons. To overcome the dovnsides of both groups of acaricides they must be combined with different beekeeping techniques in so called integrated pest management.. In this review article we put together all the treatments and techniques that could be used in sustainible varroa management to guarantee high quality hive products and healthy colonies.

Key words: *Varroa destructor*, varroa treatment, control methods, low environmental impact, effectiveness

INTRODUCTION

Western honey bees *(*Apis *mellifera)* have a great economic importance due to the pollination of many agricultural crops and wild plant species, but also for the beekeeping industry. However, in recent years, big colony losses are reported world-wide (Neumann and Carreck, 2010). There are many different reasons for colony losses, some of them are the lack of forage diversity, intensive use of pesticides, and honey bee diseases, especially varroosis, which is caused by the mite *Varroa destructor* (Anderson & Trueman, 2000) and plays a crucial role in honeybee mortality.

Varroa mite is an obligate ectoparasite of different species in the genus *Apis*. Initially it was described as a parasite of *Apis cerana* but it in the middle of 20th century it shifted to *A. mellifera* (Oldroyd, 1999). Nowadays it is present world-wide except of Australia. At least six haplotypes of *V. destructor* are known (de Guzman and Rinderer, 1998), but only two shifted from *A. cerana* to *A. mellifera*: Korean haplotype which is distributed world-wide and is considered as more pathogenic and Japan haplotype which was reported in *A. mellifera* colonies only in North and South America, Japan and Thailand and is considered as less pathogenic (Anderson and Trueman, 2000; De Guzman et al., 1998; Muñoz et al., 2008).

In life cycle of varroa it is possible to observe two different stages: a phoretic and a reproductive stage. In the phoretic stage the adult mites feed on the fat body on the ventral side of adult bee's abdomen hidden under the sternits (Ramsey, 2019). Reproduction phase occurs in capped brood preferably in drone brood. Prior the capping of honey bee larvae (5th instar larva) adult (mother) mite invades the cell. Approximately 70h after cell capping mother mite lays the first egg which is normally an unfertilized male egg due to haplo-diploid sex determination. Eggs are laid in 30h intervals, up to 6 eggs are considered as normal. As reproduction occurs only in capped brood, males start to reproduce as soon as mature females arrive on mating site.

Beside its own negative effect on honey bees (negative impact on immune system, smaller bees, shorter life span), *Varroa* is also a vector for many viruses (Kashmir bee virus, Sacbrood virus, Acute bee paralysis virus, Israeli acute paralysis virus and Deformed wing virus) which also reduce the vitality of entire colony (Boecking, & Genersch, 2008). Considering honey bee colony as a super organism, *Varroa* can damage this super organism in two ways: drones which have been parasitized during development stages have reduced chances to mate and the infested colonies produce less swarms (Duay et al., 2002, Fries et al., 2003; Villa et al., 2008).

Many different approaches for managing *Varroa* mites have been described in literature and there are also many home-made approaches described on the internet. In this review article we selected the most effective approaches with low environmental impact to combat varroa in the honey bee colonies.

LEGAL REQUIREMENTS IN CASE OF VARROOSIS

The term "varroosis" is defined as disease of insects from genus *Apis* caused by mites from genus Varroa, primarily by *V. destructor* (OIE 2019). Appearance of the disease is very variable depending on infestation level and secondary diseases (Boecking et al., 2008). Typical clinical symptoms are spotty brood pattern, crippled bees and sudden weakening of the colony, which are frequently described as a parasitic mite syndrome (Shimanuki et al., 1994).

Based on Directive 92/65 EEC varroosis could be a notifiable disease in EU member states, based on decision made by member's veterinary authority (e. g. Austria: if a threshold of 30 % of hives is already dead or endangered to die).

METHODS TO CONTROL *VARROA DESTRUCTOR*

In general, varroa control methods could be categorized in different groups, according to the use of biotechnical control methods, chemicals, the use of attractants or repellents or using biological control agents.

Biotechnical control methods/ apitechnical measurements

Biotechnical methods for varroa control are applied to slow down the increase of mite numbers in colony, without using a chemical treatment or to increase efficacy of chemical treatments. Moreover, they also can be used in periods of nectar flow when medicaments are not allowed or not recommended to prevent residues in bee products.

Brood removal, drone brood removal, trapping comb and queen caging

All measurements are based to decrease mite population by removing mites in capped brood or »forcing« mites to phoretic stage where they are accessible for treatment.

For trapping of mites in worker brood with the trapping comb method, the queen must be confined on one comb by means of a queen excluder frame. Every week this comb has to be replaced by a new one and the queen relocated onto this new comb inside the queen excluder frame. This procedure must be repeated three to four timesin a row. The comb with eggs and young larvae must be left in the hive until the brood is capped and then removed from the hive and treated (formic acid, heat) or destroyed (freezing, melting). This method is quite attenuating for the colony. Trapping comb technique can reach efficacy of 95% if it is done correctly (enough worker cells available to queen) (Beetsma et al., 1999; Calis et al., 1999; Charrière et al., 2003; Engels et al., 1984)

Queen caging is a method of brood interruption, where the queen must be caged for 25 days, until all the brood is hatched. Queen caging alone kills up to 40.6% of the mites; in combination with other treatments with »soft« veterinary medicinal products the efficacy increases up to 97% (Giacomelli et al., 2016).

Quite similar works the method of drone brood removal. This method is based on the fact, that varroa mites prefer invading drone brood than worker brood - if both are present. After capping, the drone brood must

be removed from the colony before adult drones and varroa mites could hatch. This procedure could be carried out several times per season (Fuchs, 1990). .

Thermotherapy (Heat)

It has been experimentally verified that thermotherapy is highly effective in reducing *Varroa destructor* (Rosenkranz et al., 1987). If the temperature of the brood chamber is allowed to reach and is maintained at 40 – 47 °C over a period of 2.5 hours, mortality of the mites in the sealed brood is virtually absolute, whereas bee brood withstands this temperature unharmed. Different strategies for thermotherapy are in use (heat application on brood combs without bees in separate devices, heat application to the whole bee colony, heat application to bees only, etc.). In any case special devices for heat production and temperature control are necessary to get high efficacy and no damage to bees or brood. One of the possible ways of applying heat into the colony is also heated comb foundation. Huang (2001) tested this approach and reached efficacy up to 100% in preliminary trials.

Ultrasound

Anecdotally, ultrasound should disrupt mite life cycle. However Liebieg et al. (2017) showed, that ultrasound has no effect on mites or bees.

Rotation of brood combs

The Basic idea behind reducing mite numbers via rotating brood combs is that mites get confused when a brood cell is rotated. Mites normally orientate in sealed brood cells with the help of accumulation of feces, which is always on the top wall of the cell. When the cell is rotated the varroa mite is unable to find the feeding site on bee larva and therefore less varroa females are expected to hatch in theory (Aumeier et al., 2006). But in practice this method could not prevail.

Screen bottom boards

Screen bottom boards are normally used as a method for evaluating mite population in colonies by counting fallen mites. However, different studies showed that just using a screened bottom board can reduce mite population more than 20% (Pettis et al., 1999; Ostiguy et al., 2000; Ellis et al. 2001; Harbo et al., 2004; Sammataro et al., 2004).

Chemical varroa control methods

Chemical control methods are based on the use of chemicals inside beehives or on honey bees to get rid of varroa mites as effective as possible. Because they are used on food producing animals such substances must be approved and registered as veterinary medicinal products (VMPs) by the competent authorities. Also restrictions on use and precautions (e. g. withdrawal period after treatments) to keep residues below the maximum residue limits in bee products, e. g. honey, must be observed. Therefore, please keep in mind, that not all of the active substances, preparations and methods listed in this paper have been approved in the EU member states.

In practice beekeepers differentiate between “natural” chemical substances which are compounds of honey naturally (e. g. some organic acids like formic, oxalic, lactic acid or essential oils like thymol, menthol, camphor, eucalyptus oil, etc.) and other “synthetic” chemicals, not occurring naturally in honey (e. g.

pyrethroids: tau-fluvalinate, flumethrin, acrinathrin; amidins: amitraz; organophosphates: coumaphos; halocarbon compounds: chloro-, bromopropylate; etc.).

Beekeepers often call these different groups of chemical substances simply as “soft” (= environmentally friendly) and “hard” (= environmentally harmful) acaricides. Nevertheless, both type of acaricides are synthesized by pharmaceutical companies in industrial processes and not extracted from natural raw materials.

When applying any of these VMPs the instructions for use must be observed (e. g. time and maximum number of applications, withdrawal periods; mite resistance management, etc.) to ensure quality and safety of bee products. Besides that, statutory requirements are in force to record any use of veterinary medicinal products on honey bee colonies.

Monitoring the infestation levels in colonies and the application of less-persistent, low-residual acaricides could help reduce the amount of toxic active products applied each season and thus reducing residues in wax and other hive products and chances to induce resistance of mites. Attempt to demonstrate variety of diagnostic methods and control methods has been made in review publications (Rosenkranz et al., 2010; Zamene et al., 2015; Gregorc and Sampson, 2019)

Hard acaricides

Most frequently used synthetic acaricides are amitraz, coumaphos and the pyrethroids flumethrin and tau fluvalinate. Other compounds used in the past but nowadays not approved or registered in the EU had been bromopropylate and cymiazole. Amitraz and cymiazole are amidins that act on octopamin receptors, tau fluvalinate and flumethrin are pyrethroids that inhibit closure of sodium channels during repolarization period, coumaphos is an acetylcholinesterase inhibitor and mode of action of bromopropylat is unknown (Johnson et al., 2013; Van Leeuwen et al., 2015). Those so called synthetic acaricides are easy to use. Normally strips containing the active ingredient are placed between frames in the brood box or they are applied as vapour or trickled between frames. Products with these active ingredients are also cheap and beekeepers do not need special knowledge on varroa biology (Rosenkranz et al., 2010). However due to mostly lipophilic structure of those substance they can accumulate in bee’s wax and negatively affect honeybee’s larvae and hive products (Bogdanov et al., 1998; Wallner, 1999, 2000; Lodesani et al., 2008; Martel et al., 2007; Nasr and Wallner, 2003; Chauzat et al., 2009; Johnson et al., 2009; Wallner, 2005). Another major drawback associated with intensive use of synthetic acaricides is also the resistance of varroa mites. Resistance is reported for fluvalinate, amitraz and coumaphos (Milani, 1994; Milani, 1999; Trouiller, 1998)!

Soft acaricides

Formic acid

Many ways of applying formic acid are known. Definitely most commonly used is long term evaporation with different dispensers (Bracey & Fisher, 1989; Feldlaufer, Pettis, Kochansky, & Shimanuki, 1997; Fries, 1989; Hoppe, Ritter, & Steven, 1989; Lupo & Gerling, 1990). Efficacy against mites and effect on adult bees can vary greatly, depending on various conditions: commercial product and evaporator, position of the evaporator inside the hive, microclimatic conditions in hives, presence of brood and environmental temperature and humidity (Calderone, 1999; Calis et al., 1998; Eguaras et al, 2001; Rosenkranz et al., 2010).

High evaporation levels due to high environmental temperatures can cause mortality of queens and adult bees, or interruption of reared brood (Satta et al., 2005). On the other hand, some commercial product which contains formic acid in gel showed no negative effect on adult bees, queen and brood and also high acaricidal efficacy (Giusti et al., 2017). Formic acid works by passive evaporation in the hive, and it is the only active ingredient able to kill the varroa mites inside the capped brood cells and on adult bees (Fries, 1991, Amrine and Noel, 2006).

Oxalic acid

Oxalic acid is another so called organic acid used to combat Varroa mites. It is commonly used as a winter treatment in absence of brood when the efficacy is high (higher than 90%) (Bacandritsos et al., 2007; Marinelli et al., 2000; Nanetti & Stradi, 1997). The efficacy during the brood rearing periodin colonies with brood, ranged between 39 and 52%, but was 99% in the broodless period (Gregorc & Planinc, 2001, 2002) Usually is applied by trickling, dissolved in sucrose solution. It can also be applied by spraying or sublimation. Negative effects on bees and brood can occur if applied more than once per generation of adult bees (Higes et al., 1999). Toxicity is lower when it is applied by sublimation (Al Toufailia et al., 2015). Al Toufailia et al. (2015) also showed that colonies treated with sublimation had significantly more brood in spring that controls, and lower winter mortality, although this difference was not significant and this application method gives the greatest mite fall in comparison to spraying and trickling.

It was established that higher concentrations of OA mixed in sucrose solution will exhibit greater varroa efficacy than solutions with lower concentrations, with a similar toxicity response expressed in honey bees (Charrière and Imdorf 2002). In a study of Aliano et al. (2008), honey bees exposed to the recommended dose of 100 µg of OA per adult bee survived longer than 72 h, and mortality did not differ from the untreated control. Thus, it is thought that the dosage of OA dihydrate in sucrose solution has an impact on mortality rates in mites as well in bees (Milani 2001; Charrière et al. 2004; Al Toufailia et al. 2015). Oxalic acid is a natural constituent ofhoney with extensive research on its acaracidal efficacy both alone and in combination with a variety of biotechnical varroa control methods including queen caging or total brood removal. (Rademacher und Harz, 2006; Giacomelli et al. 2016; Gregorc et al. 2016). Oxalic acid has been found to be effective in controlling varroa in broodless colonies under a variety of climatic conditions, but less effective in colonies with capped brood (Brødsgaard, Jansen, Hansen, & Hansen, 1999; Nanetti, Massi, Mutinelli, & Cremasco, 1995)

Lactic acid

Lactic acid is normally used in broodless conditions (after brood interruption, in swarms, during broodless winter period). A 15 percent aqueous solution is applied via spraying (Koeniger et al., 1983). When 5ml of lactic acid per frame is used three times in broodless conditions efficacy was up to 96%, but strongly changing (Assmann-Werthmiiller et at., 1989) and when treated two times with 8ml per comb in broodless conditions efficacy up to 98% is reported (Kraus, 1992). In case of broodright colonies efficacy is up to 84% when treatment is repeated four times (Imdorf, 1989). There is no negative effect reported on eggs or larvae (Kraus, 1992a) but precise dosage is required in order to achieve high efficacy and no adult bee mortality (Kraus, 1991). Studies conducted with 5ml of lactic acid per frame side found frequently insufficient efficacy (Assmann-Werthmiiller et al., 1989). When 8ml of lactic acid was used, a more uniform efficacy was achieved (Kraus, 1991; Kraus, 1992 a; Kraus 1992 b). Lactic acid could also be used at low environmental temperatures

(4°C) and still achieve good efficacy and is well tolerated by adult bees (Weiss, 1987; Euteneuer, 1988; Weiss, 1991).

Thymol and other essential oils

Thymol is an essential oil of thyme (*Thymus vulgaris*) and is used by beekeepers for treatment of varoosis (Gregorc and Jelenc 1996; Imdorf et al. 1999; Lindberg et al. 2000; Fassbinder et al. 2002). However, despite its volatility it is possible to find its residues in honey ad wax that can affect the taste of the honey (Bogdanov et al. 1998; Bollhalder, 1998). Efficacy of thymol-based products depends on the evaporation rate of thymol which depends on environmental temperatures and colony conditions (El-Ghamdy 2002; Lodesani and Costa 2008). Great variation in efficacy is reported (Giacomelli et al., 2016).

Efficacy varies widely, from 50% to 97% (Mattila and Otis, 2000; Gregorc and Planinc, 2004; Fassbinder et al., 2002; Bollhalder, 1998) These large differences in the efficacy of using organic substances reflect different climatic and geographic conditions and hive management systems (Trouiller and Watkins, 2001).

Hop beta acids

Hop beta acids (HBA) are natural compounds of hops. They are applied on cardboard strips impregnated with HBA (DeGrandi-Hoffman et al 2012). The efficacy varies in different reports (Vandervalk et al., 2014; Rademacher et al., 2015; DeGrandi – Hoffman et al., 2012; DeGrandi – Hoffman et al., 2016). High toxicity, topically and *per os* on adult bees was observed (Moškrič et al., 2018) and also toxicity on adult bees of the commercial product Hopguard II was observed (http://scientificbeekeeping.com/a-test-of-hopguard-ii-as-a-late-summer-mite-treatment/ 2.10.2018). There is no product registered in the EU! https://www.hma.eu/fileadmin/dateien/Veterinary_medicines/CMDv_Website/Procedural_guidance/Miscellaneous/Bee_products_available_in_Europe2019.pdf [accessed 2020 01 08]

Possible biological varroa control methods

Use of bee-derived volatiles

Pernal et al. (2005) showed in a petry dish bioassay that varroa mites were attracted to live L5 larvae, just emerged young bees and freshly killed nurse bees. The main sources for orientation are two pheromone components produced by Nasonov glands: geraniol and nerolic acid. Pernal et al. (2005) also suggest that varroa mites in order to discriminate between adult bees and nourishing bees must detect relative concentration of these compounds. This study also proved that putative fatty acid esters that were previously identified as kairomones for varroa were inactive in this type of bioassay (Pernel et al., 2005).

Use of varroa derived volatiles

Ziegelmann et al (2014) tested the effect of varroa sex pheromone on mating behaviour of male varroa mites. In presence of sex pheromones male mites were not able to distinguish between receptive daughters, older females or immature females. In presence of sex pheromones also copulating time was shorter and males frequently fail to select receptive females. Pheromone components were also tested *in vivo*. Components were sprayed over empty comb prior to the egg laying activity of queen bee and after first brood cycle the number of spermatozoa of daughter mites was evaluated. 20% reduction was observed in the number of spermatozoa (Ziegelmann et al., 2014).

Use of pathogenic fungi

Many species of entomo-pathogenic fungi were tested on their pathogenicity against varroa. Most promising results were obtained with conidia (asexual spores) of the genera *Metarhizium*, *Beauveria* or *Verticillium* (Shaw et al., 2002). Some studies indicated that fungal control of varroa might be a good solution during broodless conditions (Garcia-Fernandez et al., 2008; Kanga et al., 2003; Meikle et al., 2008). Hamiduzzaman et al. (2012) reported efficacy up to 90% with certain strains of *B. bassiana.* (Shaw et al. 2002). Kanga et al. (2002) also reported that entomopathogenic Hyphomycetes showed significant virulence against *V. destructor*, but the application technology used was not efficient, economical and rapid enough. Another promising feature of this type of varroa control is that adult bees and drones can spread the fungus among colonies by drifting (Kanga et al., 2003).

Use of other pathogens

There are many entomopathogenic bacteria mainly belonging to *Bacillaceae* and *Micrococcaceae* but they are not varroa-specific (Rosenkranz et al., 2010). Efficacy varies greatly from 50% to 96.7% when only CRY and CYT proteins from *B. thuringiensis* were used (Tsagou et al., 2004; Alquisira-Ramírez et al., 2014). During *Varroa* research morphological pathological changes were described as black coloured changes on fat body. Up 8% of mite population showed the changes in capped brood and their longevity was shorter (Kleespies et al., 2000), studies revealed presence of spherical viral particles (Liu, 1996).

Further research and registration is needed before the findings of bee and varroa derived volatiles, pathogenic fungi and other pathogens could be used in practice for varroa control.

Predators or parasitoids

Pseudoscorpions are arthropods without stinging tail and not bigger than 8mm. More than 3000 species are present worldwide. They live in soil, plant litter and under the bark of fallen logs. Peudoscorpions are predators feeding mostly on small arthropods and their eggs. A few of those pseudoscorpion species live also in beehives and some are known to be phoretic. However, since the invention of movable frames pseudoscorpions are barley found inside the hives. In order to maintain sufficient numbers of pseudoscorpions in hives “save shelters” should be provided for pseudoscorpions to be able to hide from bees (Donovan et al., 2005). Methods for mass-rearing the *Nesochernes gracilis* were developed to provide specimens for research and introduction into beehives for biological control of Varroa (Read et al., 2014). mites.

Introduction of less virulent haplotypes of *Varroa destructor*

Varroa mite population that parasitize on *Apis mellifera* belongs to two haplotypes: Korean haplotype which is considered as more virulent and world-wide spread and Japan haplotype which is less virulent and present only in Japan, Thailand and North and South America (Anderson and Trueman, 2000; De Guzman et al., 1998; Garrido et al., 2003; Muñoz et al., 2008). Vetharaniam and Barlow (2006) suggested in their study, based on mathematical model that benign haplotype of varroa could replace the virulent haplotype due to competition for resources during the reproduction which resulted in increased offspring mortality (Fuchs and Langenbach, 1989; Martin, 1995). However, in the USA both haplotypes are present and this *in silico* model could be verified *in vivo* in the future.

New molecular technologies

RNA interference (RNAi) is an RNA-mediated sequence specific gene-silencing mechanism. The silencing pathway is initiated by the presence of endogenous or exogenous double stranded RNAs (dsRNAs) that is then cleaved by RNase III-like enzymes resulting in small (21–26 bp) interfering RNAs (siRNA). SiRNAs guide protein complexes to RNAs carrying homologous sequences and target the RNA for degradation, or RNA-directed DNA methylation or chromatin remodelling (Hannon, 2002, Zotti et al., 2015; Fire et al., 1998). Reciprocal horizontal transfer of dsRNA ingested by honey bees to Varroa mites and then on to Varroa-parasitized bees was demonstrated. Efficacy of this treatment was up to 61%. This technology is safe because dsRNA degrade in 6 days in hive conditions and selected sequences are not homologous to honey bee or human sequences (Garbian et al., 2012).

CONCLUSIONS

Substantial changes had to be made in the field of varroa control in the last decades. Driving forces of this process were resistance of mites to synthetic acaricides, problem of residues in honey and lack of efficient registered products without these limitations. With repetitive use of three main groups of synthetic acaricides selective pressure has been made on varroa mite population to a degree where resistant mite population causes serious winter losses. Besides that, selective pressure was also made on mite populations for a shorter phoretic period which reduced the time window when mites are directly exposed to acaricides. Another big problem in chemotherapy of varoosis are also residues of acaricides. Amitraz, coumaphos, pyrethroids and thymol are known to leave residues in wax and honey mostly due to their lipophilic structure. Regulation of European Commission 37/2010 laid down maximum residue levels for veterinary medicines which must be observed also when treating varoosis.

On the other hand, Nguyen et all. (2009) discovered residues of prohibited rotenone and bromopropylate in samples of honey. This discovery shows great lack of awareness and knowledge among beekeepers when it comes to varroa treatment. Jacques et al. (2017) also highlights the importance of beekeeping practices and beekeeper's background on winter losses due to Varroa and other diseases.

Residual concentrations of fluvalinate and coumaphos within a bee colony (mostly have negative side effects on worker bees and queens. Sanford (2001) reported about problems with maintaining productive queens in colonies after the use of acaricides increased. Haarmann et al., (2002) demonstrated that commercially available coumaphos and fluvalinate strips have negative effect on queen rearing process. Pettis et al. (2004) showed that coumaphos in wax that was used for queen cups caused lower expectancy rate in grafted larvae and lower body mass in queens that managed to develop. Residues of acaricides could be also toxic for worker bees when exposed to multiple residues stored in wax.

Synthetic acaricides could be also a risk for other animals and humans if they are not used correctly. For example, amitraz metabolite (2,4-dimethylaniline) could have teratogenic effect on frogs (Osano et al., 2002), coumaphos could also be toxic to vertebrates, including humans (Fang et al., 1995; Abou-Donia et al., 1982) and other arthropods (Sanchez-Fortun et al., 1995). Pyrethroids are toxic to other insects and marine invertebrates and fish (Gunning et al., 1999; Clark et al., 1989).

The development of acaricides on the basis of new active ingredients is not very likely (Dekeyser and Downer, 1994) and still not in sight. "Rotation" in the use of different acaricides within a "resistance management plan" may only be a short-term-solution, due to the mainly non-professional structure of the beekeepers´ community. Therefore, it is necessary to include alternative methods within the often chemical based Varroa control strategies (Lodesani, 2004; Milani, 2001b).

To address the problem of mite resistance and acaricides residues, so called integrated pest management (IPM) approach was introduced into beekeeping. IPM is defined as: *"The careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment" (FAO).* This approach is implemented in beekeeping as a combination and rotation of veterinary medicinal products and biotechnical measurements such as queen caging or use of screened bottom boards. This approach is gaining on recognition in scientific community and also among beekeepers (Lodesani et al., 2014).

Despite the promising IPM tools suggested by the experts and beekeepers in some countries, world-wide adoption of IPM has not been realized in many parts of the world yet. Few of the practices listed above can singly or indefinitely keep mites at non-damaging levels; computer modelling simulations indicate that non-chemical IPM practices delay damaging mite levels rather than prevent them (Hoopingarner, 2001; Wilkinson et al., 2001).

As a joined solution, taking into account one health approach, to all above mentioned problems in modern concepts of honey bee health management biosecurity measures in beekeeping (BMBs) and good beekeeping practices (GBPs) were identified. BMBs are defined as those integrated measures implemented to reduce the risk of introduction and spread of specific honey bee disease agent (Pietropaol et all., 2020, in press). Well implemented BMBs will reduce pathogen load which can result in decreased use of veterinary medicines thus ensuring improvements in production quantity, quality and safety (Dewulf et al., 2018). Prerequisite for the implementation of BMBs to beekeeping operations are GBPs that are defined as: *integrative activities that beekeepers apply for on-apiary production to attain optimal health for humans, honey bees and environment* (Rivera-Gomis et al., 2019).

Annex 2 Rivera-Gomis, J., Gregorc, A., Ponti, A. M., Artese, F., Zowitsky, G., & Formato, G. (2016). Monitoring of Small Hive Beetle (Aethina Tumida Murray) in Calabria (Italy) from 2014 to 2016: Practical Identification Methods. Journal of Apicultural Science, 61(2), 257-262

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Short Communication

MONITORING OF SMALL HIVE BEETLE (*AETHINA TUMIDA* MURRAY) IN CALABRIA (ITALY) FROM 2014 TO 2016: PRACTICAL IDENTIFICATION METHODS

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Abstract

The Small Hive Beetle (SHB), *Aethina tumida*, is an invasive pest of honey bee colonies that causes significant damage to the beekeeping sector. SHB was detected in southern Italy (EU) in 2014 and despite adopted eradication measures, is still present there. After three years of observations of SHB in Calabria (2014-2016), we provide here some practical tips for improving control measures. A new time-saving colony examination method, including the use of an internal divider reduced the time needed for hive inspections by 31.86 % on average. Prioritizating the inspection of pollen and honey combs rather than brood combs is advised. Sentinel apiaries with no more than five colonies without supers are suggested for each beekeeping location in order to attract and to monitor the early appearance of SHB. The use of these methods will enable early detection and prompt control measures application before this destructive pest can spread in the region.

Keywords: *Aethina tumida*, behaviour, monitoring, sentinel apiary, small hive beetle

MANUSCRIPT BODY

In September 2014, the presence of the Small Hive Beetle (SHB), *Aethina tumida* Murray, was officially confirmed in the Calabria and Sicily Regions (Southern Italy) (European Commission, 2014). Through December 2016, SHBs have been found in a total of 137 infested apiaries: 136 in Calabria and 1 in Sicily (INRC, 2017). Prevalence of the *Aethina tumida* infestation in the Calabria region was 3.59%, 1.89% and 2.80%, respectively, for the years 2014, 2015 and 2016. In Sicily, where eradication measures were effective, the prevalence was 0.04%, 0.00% and 0.00%, respectively, for the years 2014, 2015 and 2016[1].

Eradication measures have been applied since 2014, including the destruction of all colonies at apiary sites (8502 destructed colonies as of 08/10/2016) whenever a single infested colony was found. These measures, while resulting in SHB eradication only in the Sicily region (European Commission, 2017), maintained a low prevalence and slow spread of the pest in the Calabria region.

[1] Prevalence was calculated considering the number of outbreaks recorded (Italian National Reference Centre for Apiculture, 2017) in the populated apiaries registered in the Italian National Bee Registry (Italian Ministry of Health, 2010) on 31/12/2016

Fig. 1. Divider to be placed on the external side of the nest to act like a hiding place for the SHB (Photograph by Francesco Artese, FAI Calabria).

The official procedure to examine hives for SHB presence is effective but time consuming. As recommended by the Italian National Reference Centre for Apiculture, and adapted from Neumann & Hoffmann (2008), it includes detailed colony examination. More recent examination methods (Neumann et al., 2013; OIE, 2013) were not applicable in the field during routine colony inspections due to the high risk of robbing and honey bee aggressiveness (Mutinelli et al., 2014).

Due to its dark body colour and fast movements,SHB are not easily identified within a colony, particularly when at low population densities. Moreover, SHBs avoid light, hide in crevices or cavities of the hives or fly away from combs (Neumann, Pettis, & Schafer, 2016).

After three years of observing SHB colonization behaviour since its appearance in Calabria (2014–2016), we are now able to share our practical experience to neighbouring EU member states that are in danger of SHB introduction. In order to improve hive inspections, these practical tips should be followed:

1. A divider made of wood, felt, cardboard or a similar material should be placed laterally between the hive wall and the external comb (Fig. 1), to act as a refuge for SHB. This divider should be installed at least 48 hours before the examination, following recommendations for traps with a similar mechanism of action (Neumann et al., 2013). A similar trap is currently in use in Australia to detect the presence of SHB (Annand, 2008). In fact, we developed a new "time-saving protocol" (Tab. 1) recommending the inspection of the nest to be started on the opposite side from the divider, transferring combs one by one into an empty hive or nuc box. When three combs and the divider are

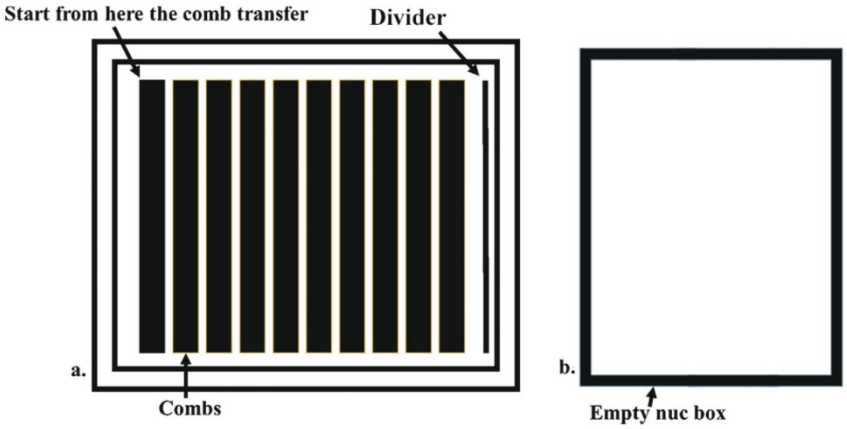

Fig. 2. Hive provided with divider placed at one extremity of the box to create a hiding place for SHB (a) and empty nuc box for transferring frames from the nest during the hive inspection (b).

Table 1

Comparison between the two hive inspection methods tested

Italian Ministry of Health (MoH) inspection protocol	Time-saving Protocol
1. Remove the outer cover. Examine the external surface of the inner cover, remove it and examine the internal surface. After that, put the outer cover on the hive supports or on the ground. 2. To inspect the nest, remove the first lateral comb and set it outside the hive. Then, inspect all the combs of the hive one by one observing both surfaces after removal of the first lateral comb. Alternatively, use an empty hive where inspected combs could be temporarily placed. 3. Once the comb inspection is finished, return the combs to their original position. 4. If a honey super is present, examine all combs one by one. After that, remove the super and set it on the outer cover. 5. Observe the content of the bottom board if present.	1. A divider made of wood, felt or cardboard (Fig. 1) should be placed between the last comb and the lateral wall of the hive, at least 48 hours before the inspection. 2. Remove the outer cover. Examine the external surface of the inner cover, remove it and examine the internal surface. After that, put the outer cover on the hive supports or on the ground. 3. Then, inspect the nest starting at the first lateral comb that is on the opposite side to the divider. The inspection of the combs with pollen and honey should be more diligent, while it can be much quicker for the other brood combs. Place the inspected combs one by one into an empty hive or into a nuc box. 4. In general, when combs are removed, always proceed with slow movements, in order to allow SHBs to move towards the remaining, not inspected, frames. 5. When three combs and the divider are left to be inspected, slowly move the combs to the opposite (empty) side of the hive. 6. After moving the last comb, carefully inspect the surface of the lateral divider and the space behind it, searching for the SHB. Carefully inspect also the corners, walls and bottom of the hive. 7. If the honey super is present, remove it and inspect the surface where it was placed carefully. Then, inspect the super combs quickly and, the lateral walls more carefully. 8. Observe the content of the bottom board if present.

left, the combs are moved to the opposite side of the hive. Removing the combs causes SHBs to move progressively towards the divider, where there will be a higher probability of finding them (Fig. 2).

2. Areas with higher probability of finding SHB's (e.g., corners and inner side of the walls of the hive, behind dividers, combs containing pollen and honey, etc.) should be inspected more thoroughly, while brood combs should only be quickly scanned due to the lower probability of finding SHBs there (Pietropaoli et al., 2015, Spiewok et al., 2007) (Tab. 1).
3. While inspecting combs, avoid removing the bees by shaking the comb as SHB could be dislodged with the bees; observe the frame at a distance further than normal, with arms fully extended, to guarantee a vision of the entire comb surface and facilitate identification of SHB movements across the comb; SHB is much easier to detect on the lighter wax of newly built combs, so more

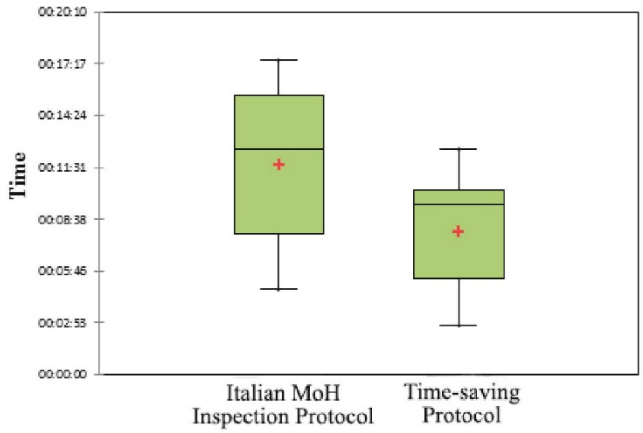

Fig. 3. Box plots of time needed for the hive inspection with the two different methods.

care should be used when inspecting older, darker combs.

4. Once SHB infestation is suspected, sampling is needed for ID confirmation. The body shield is small and hard, making them difficult to capture. While standard leather beekeeper's gloves are not useful for SHB sampling, tight fitting latex gloves are more convenient for examination, handling and sampling of beetles.

At the beginning of June 2017, a field trial was begun comparing this new protocol with the Italian MoH inspection protocol (Italian Ministry of Health, 2014) (Tab. 1), recording the time needed to inspect thirty potentially SHB-infested colonies in Calabria. Each inspection protocol was used on fifteen beehives and time needed for the inspection was recorded. The average time of application for the Italian MoH inspection protocol was 11 minutes and 43 seconds per hive, while our "time saving protocol" required only 7 minutes and 59 seconds per hive (standard deviation of 00:04:18 and 00:03:09 respectively). This was equivalent to a 3 minutes and 44 seconds (31.86 %) reduction in inspection time. Using the Mann-Whitney test (Mann & Whitney, 1947) with XLSTAT™ software (Addinsoft & S.A.R.L., 2010) we observed a statistically significant (P = 0.014) difference between the two methods (Fig. 3).

This time saving is indeed economically important, as the personnel expenses represent the greatest cost for SHB control measures. Moreover, this monitoring time reduction would favour beekeepers' compliance in collaborating with the authorities and could represent a key factor for the success of SHB management strategies. As the most efficient strategy, the person in charge of the apiary is supposed to place the divider at least 48 hours before the inspection is carried out (Neumann et al., 2013). As an alternative, the beekeeper is envisaged to keep the divider placed in the beehive as a good beekeeping practice to ease SHB detection, being always ready for the official controls and minimizing the workload of placing the dividers.

In order to improve monitoring activities, since 2014 Italian MoH has been using its own "sentinel apiaries" placed in areas potentially affected by SHB (Mutinelli et al., 2014; Italian Ministry of Health, 2015a; Italian Ministry of Health, 2015b; INRC, 2016). Due to the lower number and size of hives inspected per site, these sentinel apiaries have demonstrated advantages compared to the use of beekeeper's apiaries, including an easier and time-saving monitoring procedure. Moreover, sentinel apiaries can allow an easier and more accurate diagnosis compared with conventional apiaries, where a beekeeper may delay diagnosis and eradication procedures. In conclusion, sentinel apiaries ensure higher examination efficiency by revealing new infested areas more quickly.

According to our experience, these sentinel apiaries should be established using two to five colonies, to increase SHB attraction while limiting the time needed by inspectors for accurate inspection. Moreover, the colonies should be strong, healthy, queen right, as these are more attractive to the parasite (Annand & Spooner-Hart unpubl. data). Some final consid-

Fig. 4. Marks on the protein candy made by SHB feeding (Photograph by Francesco Artese, FAI Calabria).

erations are that, to ease inspections, sentinel colonies should never be provided with supers, and colonies should be placed in sunny and windy locations. In fact, we have observed SHBs to invade more often strong colonies placed in these conditions, as they facilitate propagation of attractive volatile compounds. Another useful tip for locating SHB in sentinel apiaries could be to insert protein candy or protein substrates into the hives to feed the bees, as both adult and immature stages of the SHB are attracted to protein substrates (Buchholz et al., 2008), and the presence of small holes in the candy are feeding signs of this parasite (Artese unpubl. data) (Fig. 4).

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BPRACTICES (ERA-NET SusAn) PROJECT: towards a sustainable European beekeeping

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Animal production is facing important problems worldwide, for example the global spread of diseases or the increasing demand of quality and quantity in food production. Beekeeping sector, even if it has peculiarities comparing to the other animal production systems, is not an exception, and it need to find its way into sustainability and resilience in order to adapt to the present and future challenges.

These demands include improvement in competitiveness, resilience and productivity, enhancement of environmental sustainability and consumer acceptability and address societal challenges associated with animal welfare, product quality and safety, biodiversity and provision of ecosystem services.

The BPRACTICES project (Fig. 1) was created in order to satisfy the requirements of the beekeeping sector in the given context. BPRACTICES is the acronym of “New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* era in Europe”.

Figure 1. *BPRACTICES logo*

This project is funded by the European Union’s Horizon 2020 research and innovation program ERA-NET SusAn – European Research Area on Sustainable Animal Production Systems (Fig. 2). The target of the project is the development of a sustainable bee breeding system by implementing innovative management practices in beekeeping (Good Beekeeping Practices - GBPs).

Figure 2. *Logo of the European Research Area on Sustainable Animal Production (ERA-NET SusAn)*

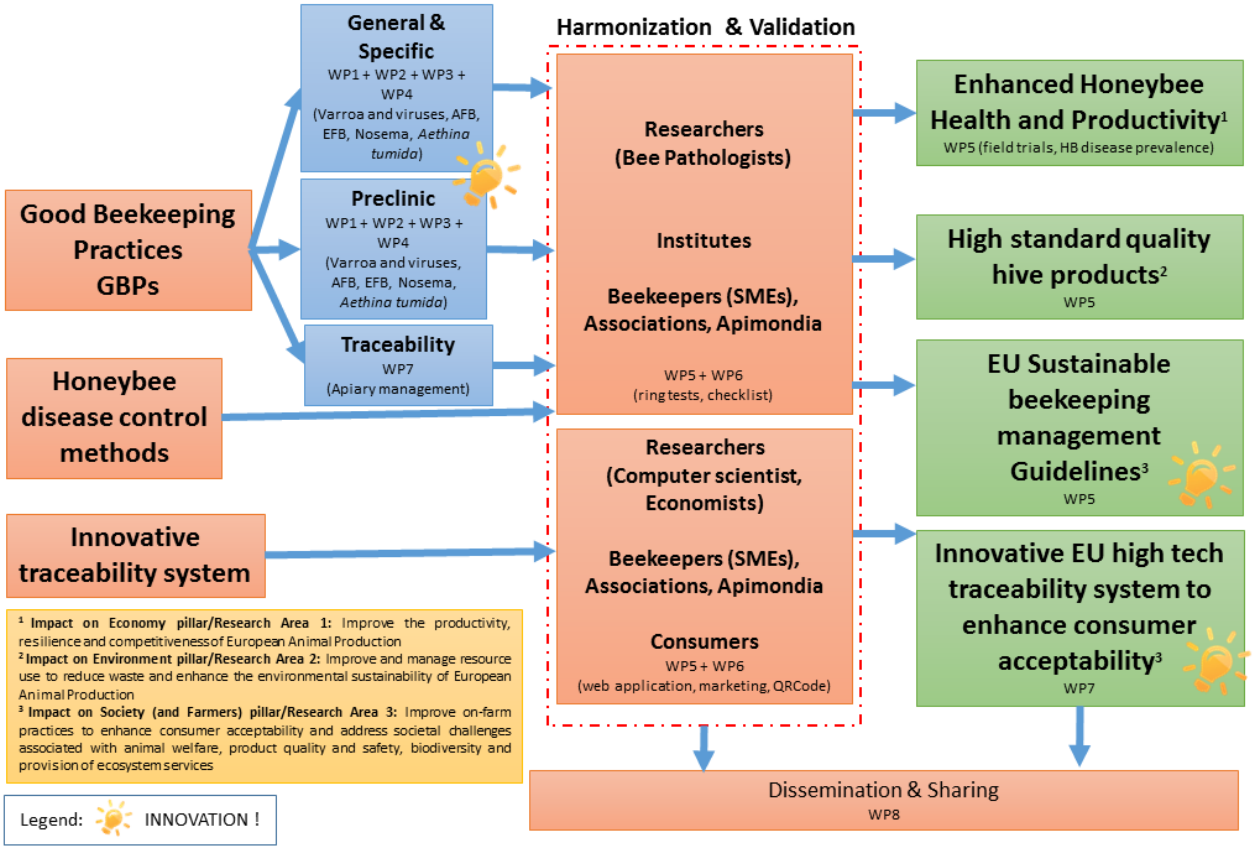
The project consortium, coordinated by the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana “M. Aleandri” (Italy), includes partners from five European countries: University of Namik Kemal (Turkey), Agricultural Institute of Slovenia (Slovenia), Centro de Investigación Apícola y Agroambiental de Marchamalo (Spain), Austrian Agency for Health and Food Safety (Austria), and Istituto Zooprofilattico Sperimentale delle Venezie (Italy). Moreover the project involves: the International Federation of Beekeepers Association (Apimondia), the University of Genova (Italy), and has the valuable collaboration of the European Union Reference Laboratory for Bee Health (ANSES, France), the Mississippi State University (USA) and of the Food and Agriculture Organization of the United Nations (FAO) Technologies and practices for small agricultural producers (TECA) platform.

The objectives of BPRACTICES include:

1. prevention and control of the main honeybee diseases adopting proper good beekeeping practices (GBP);
2. economic evaluation of competitiveness and resilience of European beekeeping;
3. development of an innovative traceability system that will benefit beekeepers and consumers giving information on the product's origin;
4. approval at the apiary level of all the innovations developed within the project and
5. dissemination of results by communication activities to ensure the visibility and sharing of the project results.

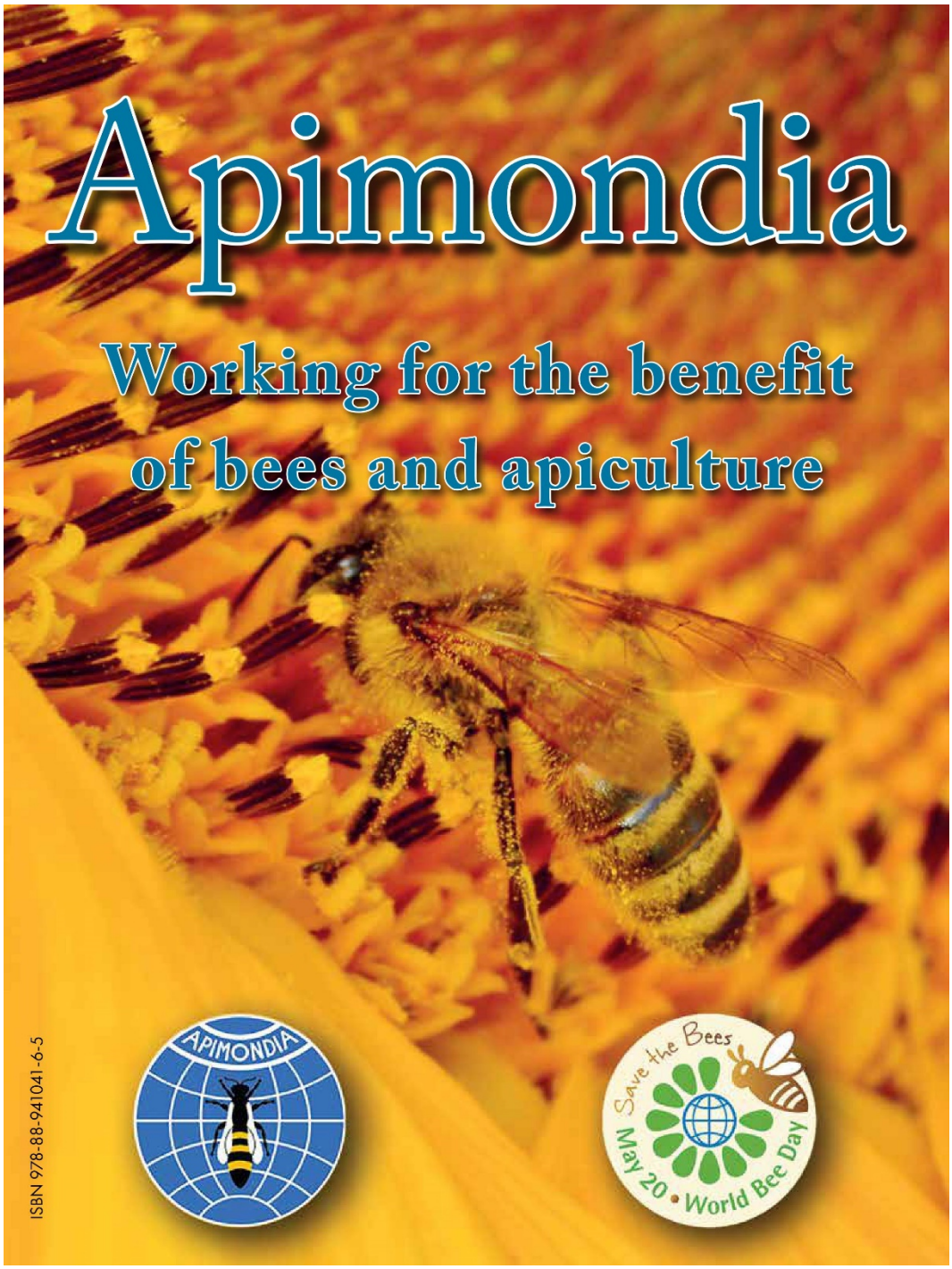
The avoidance of chemical treatments and the guarantee of quality and safety of hive products will be priority. This goal will be reached in collaboration with APIMONDIA.

Consumer acceptance and knowledge will be assessed by collecting data to identify weaknesses and strengths and optimize the system.



SCHEME OF THE B-PRACTICES PROJECT

Annex 4 Rivera-Gomis j., Bubnic J., Cersini A., Chabert M., Chauzat MP., Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni-Sebastianini R., Lietaer C., McCabe P., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Pietropaoli M., Ravarotto L., Ribarits A., Riviere MP, Smodis Skerl M.I., Søgaard Jørgensen A., Formato G. (2018). Good Beekeeping Practices (GBPs) and disease prevention, in "Apimondia. Working for the benefit of bees and apiculture", released within the framework of the first World Bee Day (May, 20 2018)



GOOD BEEKEEPING PRACTICES (GBPs)
AND DISEASE PREVENTION

Jorge Rivera-Gomis et al.

> *Improving beekeeping management, honeybee health and bee product quality will improve too, increasing the competitiveness and resilience of the apicultural sector at all levels.*

INTRODUCTION

Nowadays, beekeeping faces numerous challenges, and numerous disorders that affect honey bee colonies *(Vanengelsdorp & Meixner, 2010)*. An important menace to the development of the beekeeping sector, and thus bee product safety, is the potential introduction and spread of bee diseases, also affecting the trade of honeybee products and living honeybees. As an example, the presence of the honey bee pest *Aethina tumida* (small hive beetle, SHB) was recently detected in Southern Italy, leading to the subsequent reactions in Europe *(European Commission, 2014)*. Other factors to consider are also pesticides, climatic changes and high heterogeneity of the beekeeping industry *(Woodcock et al., 2017; Goulson et al., 2018; Chauzat et al., 2013)*.

In this context, the "BPRACTICES" project, funded from the European Union's Horizon 2020 research and innovation programme under Grant Agreement n° 696231, ERA-Net SusAn, aims to develop a system of sustainable apiculture by implementing innovative management practices (Good Beekeeping Practices - GBPs).

The project consortium, coordinated by the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (Italy), includes: University of Namik Kemal (Turkey), Agricultural Institute of Slovenia (Slovenia), Centro de Investigación Apícola y Agroambiental de Marchamalo (Spain), Austrian Agency for Health and Food Safety (Austria), Mississippi State University (USA) and Istituto Zooprofilattico Sperimentale delle Venezie (Italy). Moreover, the project involves: the International Federation of Beekeepers' Association (Apimondia), the European Professional Beekeepers' Association (EPBA), the University of Genova (Italy), European Union Reference Laboratory for Bee Health (ANSES, France) and the Food and

Apimondia

Agriculture Organization of the United Nations (FAO).

DEFINITION OF GOOD BEEKEEPING PRACTICES

GOOD BEEKEEPING PRACTICES (GBPs) are defined as those integrated and sustainable activities which beekeepers apply for the hive management to obtain an optimal health for honeybees, positive socioeconomic impacts (e.g. beekeepers and consumers health protection) and to ensure environmental protection.

The application of the GBPs results in a positive effect on the wellbeing of honeybee colonies, food safety and environmental protection, thus guaranteeing high production standards at all levels.

An essential part of the Good Beekeeping Practices are the preclinical indicators, which allow to diagnose an infection or infestation before symptoms appear, representing an essential tool for mitigation of the disease and prevention of the clinical symptoms.

Implementation of prevention practices leads to improvement of honeybee health and consequently increases the performance of honey bee colonies, the profitability of the beekeeping operation and the pollination service provided by honeybees and also reduces the amount of residues in honeybee products.

CLASSIFICATION OF GOOD BEEKEEPING PRACTICES

Starting from the OIE-FAO guidelines "Guide to Good Farming Practices for Animal Production Food Safety" *(OIE & FAO, 2009)*, we classified GBPs according to the following main headings: "general apiary management", "veterinary medicines", "disease management in general", "hygiene", "animal feeding and watering" and "GBPs specific to main honeybee diseases. For each category we created a list of GBPs which were then evaluated and given a score by the scientists involved in the "BPRACTICES" project.

(1) The GENERAL APIARY MANAGEMENT section proposes practices concerning movement of the colonies, positioning of the apiary, zootechnical measurements, winter preparations and general guidelines to maintain honeybee health.

(3) GBPs concerning the use of VETERINARY MEDICINES should be respected to ensure the efficacy of treatments, honeybee health and product safety.

(2) The section concerning the DISEASE MANAGEMENT IN GENERAL includes the practices concerning inspections of colonies for clinical signs of diseases,

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prevention, sampling in case of sick or dead bees, measures which should be adopted in case of dead colonies.

(4) The HYGIENE section is about different methods of disinfection and disease spread control.

(5) ANIMAL FEEDING AND WATERING GBPs are related to guarantee safety and hygiene of feeding and watering. It is also very important to have sufficient feed supplies all year round.

CONCLUSIONS

Resilience of the beekeeping sector, sustainability and the income of beekeepers increase when sanitary problems are prevented and costs (e.g. for treatments, colony losses, or caused by production decrease) are reduced. The on-farm practices firstly defined and identified in the "BPRACTICES" project provide a direct benefit to beekeepers. Improving beekeeping management, honeybee health and bee product quality will improve too, increasing the competitiveness and resilience of the apicultural sector at all levels.

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Preliminary researches regarding the effect of formic acid on varroa existed in bee brood artificially decapped

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The aim of the study was to establish the effect of formic acid on varroa found inside capped brood cells, which were artificially decapped, based on the scraping method, using a decapping fork. The experiments were carried out in the autumn 2017, on honeybee colonies highly infested with varroa. The treatments were done with formic acid impregnated in special cartons (150 mm X 170 mm X 4 mm). Each colony received between 25ml and 50 ml formic acid of 60-65% concentration, the exposing time being between 15h and 36 h. The nocturnal temperatures and the evaporated quantity of formic acid were also registered during the experiments. The researches were focused on establishing the mortality level of varroa in brood and the effect of formic acid on viability of capped bee brood artificially decapped. The results will be analyzed and commented.

The BPRACTICES project and its interaction with the COLOSS Varroa Control TF

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BPRACTICES is a project funded from the European Union's Horizon 2020 research and innovation program under Grant Agreement n° 696231, ERA-Net SusAn – European Research Area on Sustainable Animal Production Systems, that aims to develop a sustainable breeding system by implementing innovative management practices in beekeeping (Good Beekeeping Practices - GBPs). The project consortium, coordinated by the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (Italy), includes: University of Namik Kemal (Turkey), Agricultural Institute of Slovenia (Slovenia), Centro de Investigación Apícola y Agroambiental de Marchamalo (Spain), Austrian Agency for Health and Food Safety (Austria), Mississippi State University (USA) and Istituto Zooprofilattico Sperimentale delle Venezie (Italy). Moreover the project involves: the International Federation of Beekeepers Association (Apimondia), the University of Genova (Italy), and has the valuable collaboration of the European Union Reference Laboratory for Bee Health (ANSES, France) and of the Food and Agriculture Organization of the United Nations (FAO) Technologies and practices for small agricultural producers (TECA) platform. Eight work packages aim at the following specific accomplishments: prevention and control of the main honeybee diseases adopting proper good beekeeping practices (GBP), economic evaluation of competitiveness and resilience of European beekeeping, development of an innovative traceability system approval at the apiary level of all the innovations developed within the project and dissemination of results. Innovative biomolecular techniques will be used to detect preclinical signs of honeybee diseases (e.g. PCR analyses from innovative matrices), and will be validated and standardized at international level in collaboration with the EU reference laboratory. Methods to control honeybee diseases avoiding the

application of chemical treatments and guaranteeing quality and safety of hive products will be studied and tested at the apiary level, in collaboration with APIMONDIA and the other project partners. COLOSS Varroa Control TF finds an interesting sinergy with BPRACTICES collaborating in field trials and diffusion of results.

Visible symptoms of varroosis – what can they tell?

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In the surveillance study of the project "Future of honey bees" colonies in ca. 190 apiaries all over Austria were visually checked by bee inspectors for symptoms of varroosis and other bee diseases. The colonies were visited in summer and autumn 2015 and in spring 2016. Varroosis was diagnosed in 5 % (summer and autumn visit) and 1.5 % resp. (spring visit) of the colonies. The symptoms of varroosis reported by the bee inspectors were mostly "varroa mite on a bee" and "deformed wings" and less often "varroa mite embedded in a cell capping". The varroa infestation rate of the bees in summer and autumn-samples determined by the washing method was significantly higher in colonies with varroosis-symptoms than in colonies without symptoms ($p < 0.001$). Moreover the varroa infestation rate in colonies with two or three observed symptoms was significantly higher than in colonies with just one or no varroosis symptom ($p < 0.001$). Thus, it can be concluded that visible symptoms of varroosis give relevant clues on the varroa load of a colony. The project "Future of honey bees" (www.zukunft-biene.at) was funded by the Austrian Federal Ministry of Sustainability and Tourism (former: Federal Ministry of Agriculture, Forestry, Environment and Water Management), Biene Österreich, the Austrian federal provinces and own resources of the Austrian Agency for Health and Food Safety and the University of Graz (DaFNE Proj. 100972, www.dafne.at).

Citizen Scientist Initiative for Varroa economic damage thresholds: common efforts for data collection {CSI Varroa}

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SCI Varroa aims to collect as much data as possible, from as many colonies as possible, from treated and untreated colonies, with a uniform and standardized method, for at least 2 full years, from at least 2 apiaries per region and several regions per country. Participants could be professionals or amateur beekeepers and/or experts, therefore it is a CSI project. For each Country there will be a coordinator, therefore we will ask for volunteers. Country coordinators will have to promote this activity in any way they think it is relevant and best for their region, and at the same time they will

Meeting summary Varroa Control Task force
ACTIVITIES

Hereby we report the activities carried out in each working group and the future plans for each group.

WG 1. Varroa infestation assessments (Leader: Ole Kilpinen)

Aim of the working group is to compare currently used methods to assess the infestation levels of the colony (e.g. the soapy solution, powdered sugar, and natural mite-fall methods).

The WG 1 performed the field trials in 10 apiaries with 6 researchers in 2015/2016. The results, already discussed during the Varroa TF meeting in Bologna, will be collected in a manuscript and the WG will publish them on COLOSS website and/or on international journal. Into the same paper, as agreed during the Conference, a small review of available methods to evaluate Varroa infestation will be added in collaboration with the BPRACTICES project. Moreover, the same review will be added to the COLOSS Varroa Book (see WG 6).

During the Conference no new protocols were suggested. Considering that aspect, WG 1 will be closed as soon as the results of the 2015/2016 field trials will be published on the COLOSS website and/or on an international journal.

WG 2. Brood interruption (Leader: Ralph Büchler and Malgorzata Bienkowska)

Aim of the working group is to compare Varroa control methods using total removal of brood or temporary caging of queens combined with oxalic acid treatment.

Some trials of the WG 2 have been carried out during 2016 and the results have been collected in a shared file. At the spring workshop in Bologna in April 2017 the participants decided to repeat the trials in 2017/18. Both seasons will be evaluated together in spring 2018. The plan is to get the results published by the end of 2018. If further trials will be performed in 2017/2018 has to be decided during the taskforce spring meeting in 2018. All updates will be shared by email with participants and results will be available on COLOSS website.

An analitical core-group is consisted: Janez Prešern, Marin Kovačić, Aleksandar Uzunov. Aleksandar is the coordinator of the paper and he will contact all members of core-group.
The goal is to have all data prepared by 15th of March to write a paper by September 2018 for the COLOSS Congress in Eurbee 2018. The results are very important for other studies (e.g. BPRACTICES), to identify the best method to be suggested to the beekeepers. There is also a need for adaptation of the methods to local conditions.
Some ideas: to evaluate the impact of brood interruption on hives with viruses prevalence and population; mortality of the mites; to evaluate the duration of the queen being caged (18 or 25+ days).
In the future there will be collaborations with EurBeST-project and a Book on integrated Varroa control.

WG 3. Varroa Economic damage thresholds (Leaders: Fani Hatjina and Nikola Kezić, Janja Filipi)

Aim of the working group is to determine the range of economic damage threshold levels across Europe, and possibly link it to the environmental actors.

As suggested by WG 3 leaders, the new name of the working group will be: **CSI Varroa**, as the group will seek a help of beekeepers in building a data base.

Annex 6 Pietropaoli M., Vejsnæs F., Kilpinen O., McCabe P., Jannoni-Sebastianini R., Jørgensen A.S., Lietaer C., Formato G. (2018). BPRACTICES and Hivelog web application for honey bee products traceability. Proceedings of EURBEE Conference 2018. 18-20 September 2018, Ghent, Belgium.

distribution of bumblebees across Europe. Besides other European countries, Norway is also expected to be highly impacted by global warming. In this country, we have beenalready observing that the mountain bumblebees occur more than 100 meters further up than just a few decades ago. If these changes occur faster than the vegetation manages tofollow, the access to the resources must be a problem. The examples, the bumblebee species that would be affected by the global warming are: *B. alpinus*, *B. cingulatus*, *B. consobrinus*, *B. humilis* and *B. polaris*. In this communication, we will present the distribution and mode of living of the bumblebees recorded in Norway. Additionally, we will highlight the first results from the heat-shock(34°C) trial-experiments that have been conducted with the Norwegian bumblebees under controlled conditions. The bumblebees were collected from different parts of Norway in 50ml tubes (containing BioGluc solution and flowers), and were kept in the growth cabinet for 48h. Some bumblebees died within 24h of heat-shock, but many of them like *B. hypnorum*, *B. soroeensis*, and the *Bombus* sp. of the subgenus *Thoracobombus* survived 48h of heat-shock. This pilot study canprovideus the basic information about how the bumblebees will respond towards a heat-wave, which is becoming quite usual in a country like Norway, like in July this year, the day/night temperatures have been recorded >30°C/20°C.

P167 BPRACTICES and Hivelog web application for honey bee products traceability

Pietropaoli M.[1], Vejsnæs F.[2], Kilpinen O.[2], McCabe P.[3], Jannoni-Sebastianini R.[3], Jørgensen A.S.[2], Lietaer C.[4], Formato G.[1]
[1] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri", Rome, Italy; [2] Danish Beekeepers Association, Sorø, Denmark; [3] International Federation of Beekeepers' Associations, Rome, Italy; [4] Tecnologies and practices for small agricultural producers (TECA) platform of the Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

BPRACTICES (www.izslt.it/bpractices) is a project funded from the H2020 ERA-Net SusAn – European Research Area on Sustainable Animal Production Systems, that aims to develop a sustainable beekeeping breeding system by implementing innovative management practices (Good Beekeeping Practices). The Hivelog web application (www.hivelog.dk) is a free application for smartphones, tablets and personal computers, able to record the most important apiary management data like colony strength, queen's performances, feedings, honey harvest, varroa situation (treatments), colony behavior, sanitary status, developed by the Danish Beekeepers Association to improve the general data collection within danish beekeeping. The backbone of the program is to keep it simple and easy to use. The program is already translated into 8 languages. In the future the program will be open source, so that beekeepers groups are expected to continue the development. During the 36 months of BPRACTICES project, an innovative traceability system will be set up to inform beekeepers on the innovations proposed with the new management system . The traceability system will be integrated into the Hivelog program with an interface to be used during the hive products processing to help beekeepers to maintain product traceability thanks to QRCode/RFID technology (from flower to bee colony to extraction to filling to consumer). Users will be able to record harvest data (lot number, quantity), attach analytical results, and to know all details about the colonies that produced those products. Consumers, accessing the application directly from the jar, will be educated to responsible consumption and will be made aware of the benefits of consuming a product deriving from an environmentally-friendly management, increasing the development of local productions. The traceability system will be implemented thanks to a consumers' panel during the second and third year of the project through a social research technique.

P168 Possible side effects of sugar supplementary nutrition on honey bee health

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Food shortage, along with biotic stressors (e.g. parasites and pathogens) is a leading factor of winter honey bee colony losses. To support honey bee colonies, beekeepers normally supply homemade syrups which could contain compounds (e.g. hydroxymethylfurfural, HMF) with possible negative side effects. However, literature on this subject is unclear; in

Annex 7 Rivera-Gomis J., Bubnic J., Cersini A., Chabert M., Chauzat M.P., Eggenhoeffner R., Erat S., Gregorc A., Haefeker W., Higes M., Jannoni-Sebastianini R., Lietaer C., Lubroth J., McCabe P., Moosbeckhofer R., Muz D., Muz M.N., Ozdemir N., Pietropaoli M., Ribarits A., Riviere M.P., Smodis Skerl M.I., Tiozzo B., Formato G. (2018). BPRACTICES: first attempt of definition of Good Beekeeping Practices (GBPs). Proceedings of EURBEE Conference 2018. 18-20 September 2018, Ghent, Belgium.

9. To promote and organize training activities (e.g. conferences, workshops, seminars, courses of all types including undergraduate, master, round tables, etc.), both at national and international level;
10. To promote the activities of communication, dissemination and correct technical and scientific information of the beekeeping topics, also to public opinion.

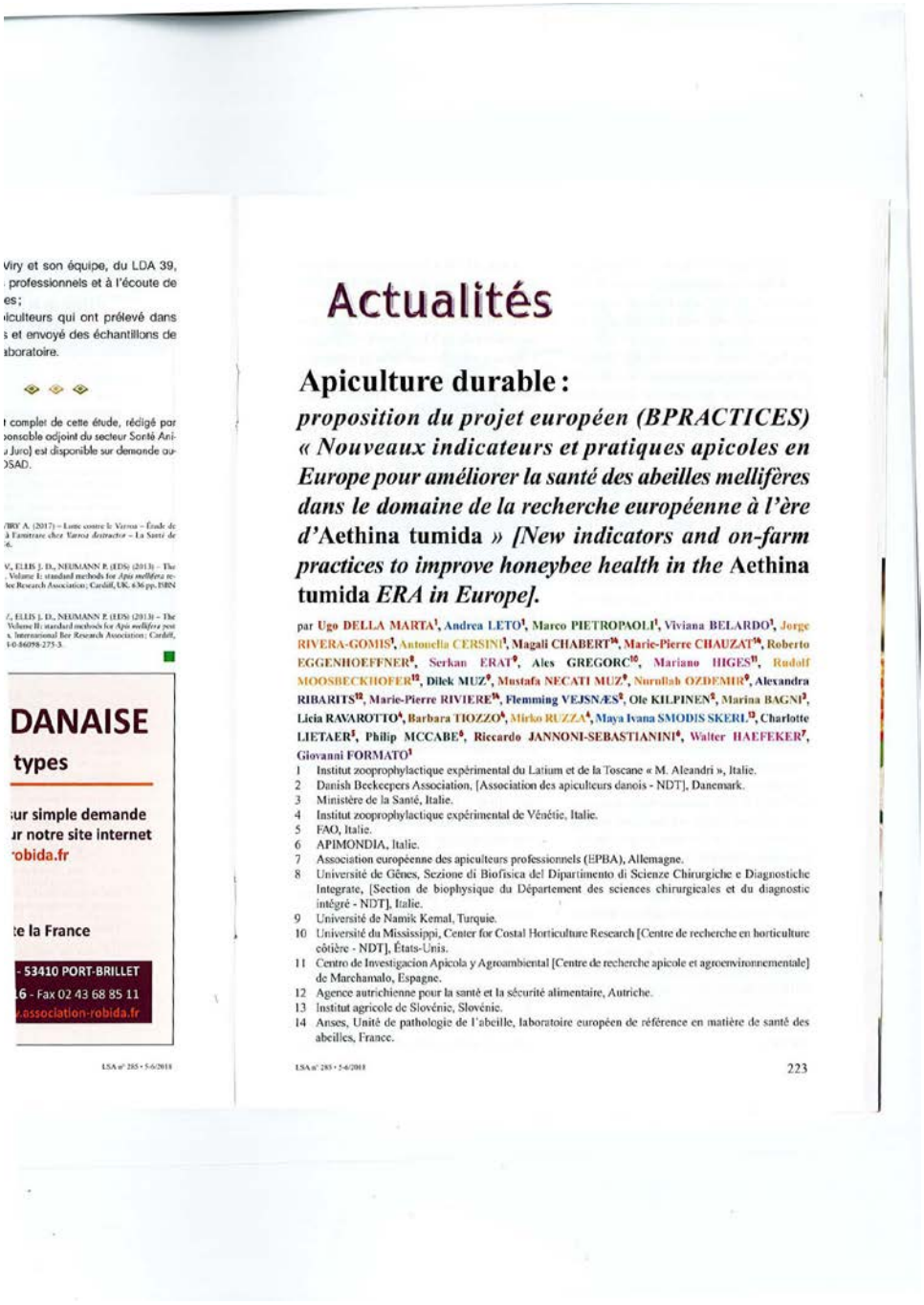
P179 **BPRACTICES: first attempt of definition of Good Beekeeping Practices (GBPS)**

Rivera-Gomis J.[1], Bubnic J.[1], Cersini A.[1], Chabert M.[2], Chauzat M.P.[2], Eggenhoeffner R.[3], Erat S.[4], Gregorc A.[5], Haefeker W.[6], Higes M.[7], Jannoni-Sebastianini R.[6], Lietaer C.[8], Lubroth J.[9], McCabe P.[6], Moosbeckhofer R.[10], Muz D.[4], Muz M.N.[4], Ozdemir N.[4], Pietropaoli M.[1], Ribarits A.[10], Riviere M.P.[2], Smodis Skerl M.I.[11], Tiozzo B.[12], Formato G.[1]
[1] Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Roma, Italy; [2] ANSES, Honeybee pathology unit, European Union Reference Laboratory for bee health, Sophia Antipolis, France; [3] University of Genova, Biophysic Section of Department of Surgery Sciences and Integrated Diagnostics (DISC), Genova, Italy; [4] University of Namik Kemal, Tekirdag, Turkey; [5] Mississippi State University, Center for Costal Horticulture Research, Poplarville, MS, USA; [6] International Federation of Beekeepers' Associations, Roma, Italy; [7] Centro de Investigacion Apicola y Agroambiental de Marchamalo, Marchamalo, Spain; [8] Tecnologies and practices for small agricultural producers (TECA) plattform of the Food and Agriculture Organization of the United Nations (FAO), Roma, Italy; [9] Animal Health Service, Animal Production and Health Division, FAO, Roma, Italy; [10] Austrian Agency for Health and Food Safety, Vienna, Austria; [11] Agricultural Institute of Slovenia, Ljubljana, Slovenia; [12] Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy

Nowadays, beekeeping faces numerous challenges, and numerous disorders that affect honeybee colonies, including the potential introduction and spread of bee diseases, the effects of pesticides and climatic change.
In this context, the "BPRACTICES" project, funded from the European Union's Horizon 2020 research and innovation programme aims to develop a system of sustainable apiculture by implementing innovative management practices (Good Beekeeping Practices - GBPs).
Good beekeeping practices(GBPs) are those integrated and sustainable activities which beekeepers apply for the hive management to obtain an optimal health for honeybees, positive socioeconomic impacts (e.g. beekeepers and consumers health protection) and to ensure environmental protection.
The application of GBPs results in a positive effect on the wellbeing of honeybee colonies, on food safety and environmental protection, thus guaranteeing high production standards.
An essential part of the Good Beekeeping Practices (GBP) are the preclinical indicators, which allow to diagnose infection or infestation before symptoms appear, representing an essential tool for prevention. These preclinical indicators will be identified and interpreted using innovative laboratory diagnostic methods and matrices from the hive. Examples are the preclinical diagnosis from powder sugar for American Foulbrood (*Paenibacillus larvae*, AFB) or European Foulbrood (*Melissococcus plutonius*, EFB), the preclinical detection of the SHB from bottom hive debris by Real-time PCR, or the yeast *Kodomaea ohmeri* as an indicator for the presence of SHB.
The risk of residues in honeybee products due to chemical treatments is reduced through the application of GBPs, thus guaranteeing quality and safety. GBPs also avoid productivity losses.
Preventive GBPs represent an opportunity to ensure the improvement of honeybee health and consequently increase the performance of honeybee colonies, the profitability of the beekeeping operation and the pollination service provided by honeybees.
Resilience of the beekeeping sector, its sustainability and the income of beekeepers increase when sanitary problems are prevented and costs (e.g. for treatments, colony losses, production decrease) are reduced. The implementation of GBPs provides a direct benefit to beekeepers, supporting the sector.
In conclusion, by improving beekeeping management through GBPs, honeybee health, bee products safety, and the competitiveness and resilience of the apicultural sector are improved at all levels.

POSTERS

Annex 8 Della Marta U., Leto A., Pietropaoli M., Belardo V., Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Higes M., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Ribarits A., Riviere MP, Vejsnæs F., Kilpinen O., Bagni M., Ravarotto L., Tiozzo B., Ruzza M., Smodis Skerl M., Lietaer C., Mccabe P., Jannoni-Sebastianini R., Haefeker W., Formato G. (2018). « Nouveaux indicateurs et pratiques apicoles en Europe pour améliorer la santé des abeilles mellifères dans le domaine de la recherche européenne à l'ère d'Aethina tumida» [New indicators and on-farm practices to improve honeybee health in the Aethina tumida ERA in Europe]. La Santè de l'Abeille. Maggio-Giugno n. 285 pag. 223-228.



Actualités

Apiculture durable :

proposition du projet européen (BPRACTICES) « Nouveaux indicateurs et pratiques apicoles en Europe pour améliorer la santé des abeilles mellifères dans le domaine de la recherche européenne à l'ère d'Aethina tumida » [New indicators and on-farm practices to improve honeybee health in the Aethina tumida ERA in Europe].

par Ugo DELLA MARTA[1], Andrea LETO[1], Marco PIETROPAOLI[1], Viviana BELARDO[1], Jorge RIVERA-GOMIS[2], Antonella CERSINI[3], Magali CHABERT[3a], Marie-Pierre CHAUZAT[3a], Roberto EGGENHOEFFNER[4], Serkan ERAT[9], Ales GREGORC[10], Mariano HIGES[11], Rudolf MOOSBECKHOFER[12], Dilek MUZ[9], Mustafa NECATI MUZ[9], Nurullah OZDEMIR[9], Alexandra RIBARITS[12], Marie-Pierre RIVIERE[3a], Flemming VEJSNÆS[8], Ole KILPINEN[5], Marina BAGNI[3], Licia RAVAROTTO[4], Barbara TIOZZO[4], Mirko RUZZA[4], Maya Ivana SMODIS SKERL[13], Charlotte LIETAER[3], Philip MCCABE[6], Riccardo JANNONI-SEBASTIANINI[6], Walter HAEFEKER[7], Giovanni FORMATO[1]

1 Institut zooprophylactique expérimental du Latium et de la Toscane « M. Aleandri », Italie.
2 Danish Beekeepers Association, [Association des apiculteurs danois - NDT], Danemark.
3 Ministère de la Santé, Italie.
4 Institut zooprophylactique expérimental de Vénétie, Italie.
5 FAO, Italie.
6 APIMONDIA, Italie.
7 Association européenne des apiculteurs professionnels (EPBA), Allemagne.
8 Université de Gênes, Sezione di Biofisica del Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate, [Section de biophysique du Département des sciences chirurgicales et du diagnostic intégré - NDT], Italie.
9 Université de Namik Kemal, Turquie.
10 Université du Mississippi, Center for Costal Horticulture Research [Centre de recherche en horticulture côtière - NDT], États-Unis.
11 Centro de Investigacion Apicola y Agroambiental [Centre de recherche apicole et agroenvironnementale] de Marchamalo, Espagne.
12 Agence autrichienne pour la santé et la sécurité alimentaire, Autriche.
13 Institut agricole de Slovénie, Slovénie.
14 Anses, Unité de pathologie de l'abeille, laboratoire européen de référence en matière de santé des abeilles, France.

La santé des abeilles est menacée par de nombreux facteurs (Laurent *et al.* 2015), parmi lesquels la pollution de l'environnement (en particulier les produits phytopharmaceutiques utilisés par l'agriculture intensive), les changements climatiques, la progression de l'urbanisation (qui entraîne une diminution des aires de butinage) et enfin les agents pathogènes spécifiques aux abeilles (en premier lieu *Varroa destructor*). Parmi ces derniers, il faut tenir compte de la propagation en Europe du coléoptère parasite des abeilles, *Aethina tumida* (petit coléoptère des ruches, en anglais « Small Hive Beetle » ou « SHB ») qui, à partir d'un seul foyer découvert en Italie en 2014, semble être en mesure de se disséminer dans le pays (EFSA 2015, Neumann *et al.* 2016) ce qui a des conséquences économiques négatives aussi bien pour l'apiculture que pour le secteur agrozootechnique, en raison de la potentielle baisse du service de pollinisation.

Le projet européen « New indicators and on-farm practices to improve honeybee health in the *Aethina tumida* era in Europe » (désigné par son acronyme BPRACTICES), lancé au mois de février 2017, est une étude sur trois ans dont le coordinateur est l'Institut zooprophylactique expérimental du Latium et de la Toscane (IZSLT), financée dans le cadre de l'avis 2016 ERA-NET SUSAN - (http://www.izslt.it/bpractices/home/). Ce projet a pour objectif d'améliorer la santé des abeilles grâce à la mise en place de bonnes pratiques d'élevage (BPE) et de stratégies ayant un faible impact sur l'environnement en vue de lutter contre les principales maladies des abeilles.

Outre l'IZSLT, les partenaires du projet sont l'Université turque de Namik Kemal, l'Institut agricole de Slovénie, le Centre de recherche apicole et agroenvironnementale de Marchamalo (Espagne), l'Agence autrichienne pour la santé et la sécurité alimentaire (AGES) et l'Institut zooprophylactique expérimental de Vénétie. La Fédération internationale des associations d'apiculteurs (APIMONDIA), l'Université de Gênes, la FAO avec sa plateforme TECA (Beekeeping Exchange Group – http://teca.fao.org/group/beekeeping-exchange-group), l'Association européenne des apiculteurs professionnels (EPBA), le laboratoire de référence de l'Union européenne (LRUE) pour la santé des abeilles (Laboratoire Anses – Agence nationale sanitaire de l'alimentation, de l'environnement et du travail – de Sophia Antipolis, France) ainsi que le professeur Ales Gregorc rattaché à l'Université du Mississippi (États-Unis) y participent également.

Dans ce projet, la nouveauté réside dans l'identification des bonnes pratiques d'élevage en apiculture qui permettent un échange entre chercheurs et apiculteurs, le but étant de définir des pratiques à la fois efficaces d'un point de vue scientifique et applicables à l'élevage des abeilles au quotidien. Les BPE intégreront pour la première fois des indicateurs dits « précliniques », qui sont des techniques de diagnostic innovantes (par exemple la PCR ou « Polymerase Chain Reaction ») utilisées sur des matrices apicoles jusqu'à présent peu prises en considération (par exemple les débris de fond de ruche, le sucre glace, etc.) afin de détecter de façon précoce la présence d'agents pathogènes dans la ruche

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sité de Gênes, la FAO
me TECA (Beekeeping
) – http://teca.fao.org/gr
exchange-group), l'As-
enne des apiculteurs pro-
BA), le laboratoire de
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phia Antipolis, France)
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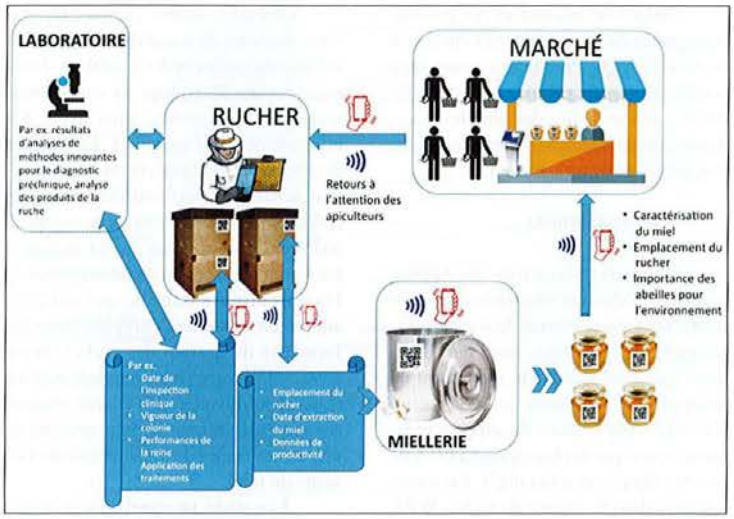

Fig. 1 : Système innovant de traçabilité développé dans le cadre du projet BPRACTICES.

et d'intervenir avant que la maladie ne soit cliniquement visible. Outre l'amélioration de l'état de santé des abeilles, l'objectif final est également de réduire l'impact des traitements chimiques et ainsi d'obtenir des produits de la ruche plus sûrs pour le consommateur.

En plus de garantir un élevage durable des abeilles, le projet soumet l'idée ambitieuse d'une sorte de certification du miel produit grâce à un système innovant de traçabilité basé sur les technologies QRCode/RFID. En effet, grâce à ces technologies, le consommateur disposera de nombreuses informations sur l'étiquette du pot de miel, y compris en ce qui concerne l'élevage des abeilles et les analyses effectuées en laboratoire (voir Fig. 1).

Les objectifs indiqués ci-dessus seront poursuivis au moyen d'un ensemble de tâches (« Work Packages » ou WP), avec une approche multidisciplinaire issue de la coopération du monde de la recherche scientifique, de celui des éleveurs et de leur expérience quotidienne au rucher et de celui des économistes ou autres experts du secteur.

Pour plus de détails sur les différentes tâches entreprises (WP), vous trouverez ci-après la liste des activités menées dans le cadre du projet :

- **WP1 (varroose et viroses), WP2 (loques américaine et européenne), WP3 (nosémose) et WP4 (infestation par *Aethina tumida*) :**

Objectifs : identifier les bonnes pratiques d'élevage au niveau européen et développer des méthodes innovantes pour le diagnostic précoce et le contrôle durable des maladies des abeilles. Évaluation de protocoles thérapeutiques au moyen d'essais sur le terrain.

- **WP5 (validation)** :

Objectifs : normaliser les bonnes pratiques d'élevage identifiées dans les différents pays et vérifier leur applicabilité pour les apiculteurs aussi bien amateurs que professionnels (par exemple méthode d'échantillonnage de la ruche) y compris par le biais du support technique fourni par la plate-forme TECA de la FAO (http://teca.fao.org/). En outre, toujours dans le groupe de tâches WP5, il conviendra de normaliser et de valider au moyen d'essais inter-laboratoires les méthodes de laboratoire pour le diagnostic précoce des maladies évoquées précédemment entre les partenaires du projet, en collaboration avec le LRUE pour la santé des abeilles.

- **WP6 (impact économique)** :

Objectifs : évaluer l'impact économique de l'application des bonnes pratiques d'élevage sur la qualité et la quantité de miel produit et vendu par les apiculteurs adhérant au projet, prévoir un logo spécial qui indique au consommateur que le miel est issu de la bonne gestion des ruches grâce au respect des bonnes pratiques d'élevage mises en place au rucher.

- **WP 7 (système de traçabilité innovant)** :

Objectifs : mettre en place un système innovant de traçabilité du miel dès la sortie du rucher et des modalités d'élevage des abeilles jusqu'au consommateur final, qui pourra ainsi avoir des informations sur l'apiculteur, les secteurs de production, les aspects relatifs au produit acheté ou aux contrôles effectués (par exemple propriétés/caractéristiques, analyses de laboratoire, etc.). Ces activités seront réalisées en collaboration avec les apiculteurs danois, qui ont déjà adopté un système d'enregistrement informatisé de gestion des ruches au niveau de l'élevage (http://english.stadekort.dk/about-hivelog-dk/) et sont intéressés par l'idée d'intégrer leur système au niveau des étapes de transformation et de vente du miel.

À ce stade, un panel de tests visant aussi bien les apiculteurs que les consommateurs est prévu afin d'évaluer la plate-forme Internet mise en place.

- **WP8 (communication et diffusion)** :

Objectifs : informer les acteurs du secteur apicole et les consommateurs sur le projet et les résultats qui en découlent, grâce à un support informatique (par exemple pour la construction du site Web, la diffusion des articles en accès libre, ou encore pour contacter les apiculteurs du monde entier par le biais de la plateforme TECA de la FAO, etc.) et au soutien des autres entités, à commencer par Apimondia (http://apimondia.com/) (Fig. 2).

Les résultats obtenus à ce jour sont reportés sur la page Internet du projet (www.izslt.it/bpractices).(Fig. 3).

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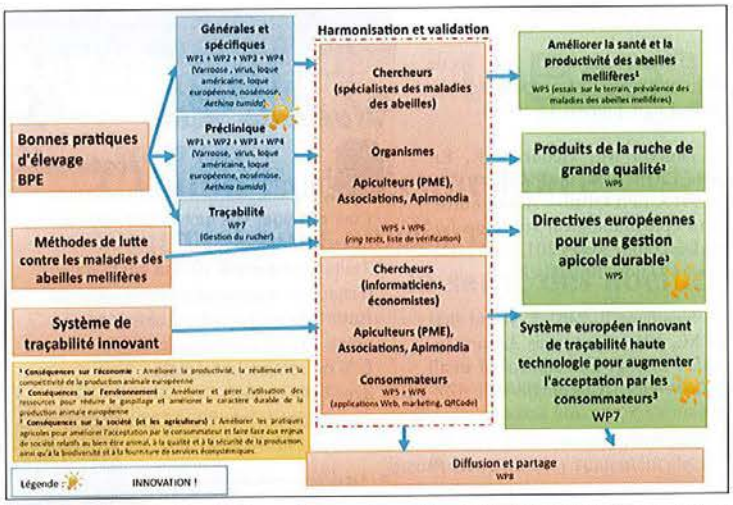

Fig. 2 : Schéma récapitulatif du projet européen « New indicators and on-farm practices to improve honeybee health in the Aethina tumida ERA in Europe » (BPRACTICES).

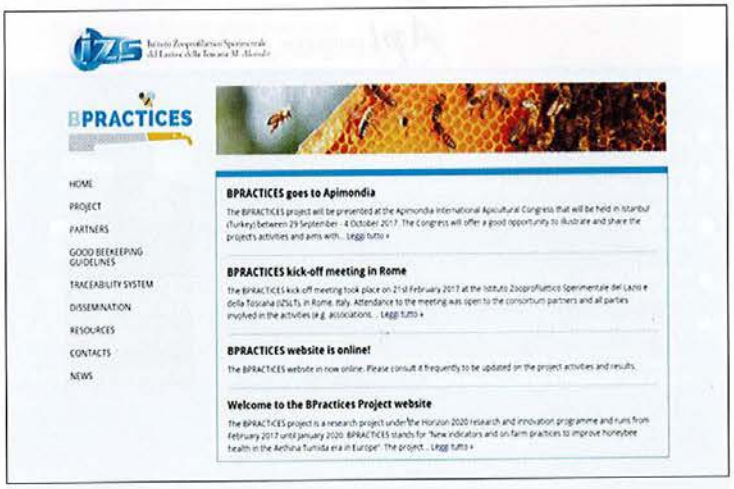

Fig. 3 : Page d'accueil du projet BPRACTICES (www.izslt.it/bpractices).

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Laurent M., Hendrikx P., Ribière-Chabert M. and Chauzat M.-P., 2015. EPILOBEE – A pan-European epidemiological study on honeybee colony losses 2012-2014. European Union, Brussels.

Neumann P., Pettis J. S. and Shäfer M., 2016. Quo vadis *Aethina tumida*? Biology and control of small hive beetles, Apidologie 47: 427-466.

Actua

L'Unio
un proje

Contact : **Andrés SAL**

Alors que les pertes d'abeilles continue ter les apiculteurs, les gro gistes, les législateurs et les nouveau projet vise à abo blème grâce à la technologie mation. Le projet Intern (IoBee) est une initiative fin Commission européenne do est de réaliser des progrès dent pour améliorer la santé Grâce à l'application de l'I Choses (IoT en anglais), les pourront observer leurs ruch des informations utiles et En raison des nouveaux ments, IoBee ouvrira la d'établir le premier réseau pour la gestion complète de abeilles. Le réseau permettra teurs, aux chercheurs et aux de prendre les mesures néc opportunes pour protéger les améliorer la qualité de l'envi

IoBee déterminera autom la santé des abeilles et l'état la colonie. Chaque ruche

La Santé de l'Abeille

Bimestriel Prix 4,50 € - ISSN 0036-4568

N° 285
Mai-Juin 2018

Annex 9 Della Marta U., Leto A., Pietropaoli M., Belardo V., Rivera-Gomis J., Cersini A., Chabert M., Chauzat MP, Eggenhoeffner R., Erat S., Gregorc A., Higes M., Moosbeckhofer R., Muz D., Necati Muz M., Ozdemir N., Ribarits A., Riviere MP, Vejsnæs F., Kilpinen O., Bagni M., Ravarotto L., Tiozzo B., Ruzza M., Smodis Skerl M., Lietaer C., Mccabe P., Jannoni-Sebastianini R., Haefeker W., Crovato S., Mascarello G., Mantovani C., Formato G. (2018). La proposta del progetto europeo B-PRACTICES. Argomenti. Numero 2/2018 pag. 66-69.

SIMeVeP

APICOLTURA SOSTENIBILE

La proposta del progetto europeo *B-PRACTICES*

UGO DELLA MARTA[1], ANDREA LETO[1], MARCO PIETROPAOLI[1], VIVIANA BELARDO[1], JORGE RIVERA-GOMIS[1], ANTONELLA CERSINI[1], MAGALI CHABERT[14], MARIE-PIERRE CHAUZAT[14], ROBERTO EGGENHOEFFNER[8], SERKAN ERAT[9], ALES GREGORC[10], MARIANO HIGES[11], RUDOLF MOOSBECKHOFER[12], DILEK MUZ[9], MUSTAFA NECATI MUZ[9], NURULLAH OZDEMIR[9], ALEXANDRA RIBARITS[12], MARIE-PIERRE RIVIERE[14], FLEMMING VEJSNÆS[2], OLE KILPINEN[2], MARINA BAGNI[3] , LICIA RAVAROTTO[4], BARBARA TIOZZO[4], MIRKO RUZZA[4] , MAJA IVANA SMODIS SKERL[13], CHARLOTTE LIETAER[5], PHILIP MCCABE[6], RICCARDO JANNONI-SEBASTIANINI[6], WALTER HAEFEKER[7], STEFANIA CROVATO[4], GIULIA MASCARELLO[4], CLAUDIO MANTOVANI[4], GIOVANNI FORMATO[1]

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[2] *Danish Beekeepers Association, Danimarca.*
[3] *Ministero della Salute, Italia.*
[4] *Istituto Zooprofilattico Sperimentale delle Venezie, Italia.*
[5] *FAO, Italia.*
[6] *APIMONDIA, Italia.*
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[9] *Univerità di Namik Kemal, Turchia.*
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[11] *Centro de Investigation Apicola y Agroambiental de Marchamalo, Spagna.*
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[14] *ANSES, Honeybee pathology unit, European Union Reference Laboratory for bee health, Francia.*

La salute delle api è minacciata da una molteplicità di fattori [1] tra i quali è possibile annoverare: l'inquinamento ambientale (soprattutto da agrofarmaci, impiegati in agricoltura intensiva), i cambiamenti climatici, la progressiva urbanizzazione (che comporta una riduzione delle aree di pascolo) e, non di minor importanza, gli agenti patogeni specifici delle api (*Varroa destructor in primis*). Tra quest'ultimi, va anche tenuta in considerazione la diffusione in Europa

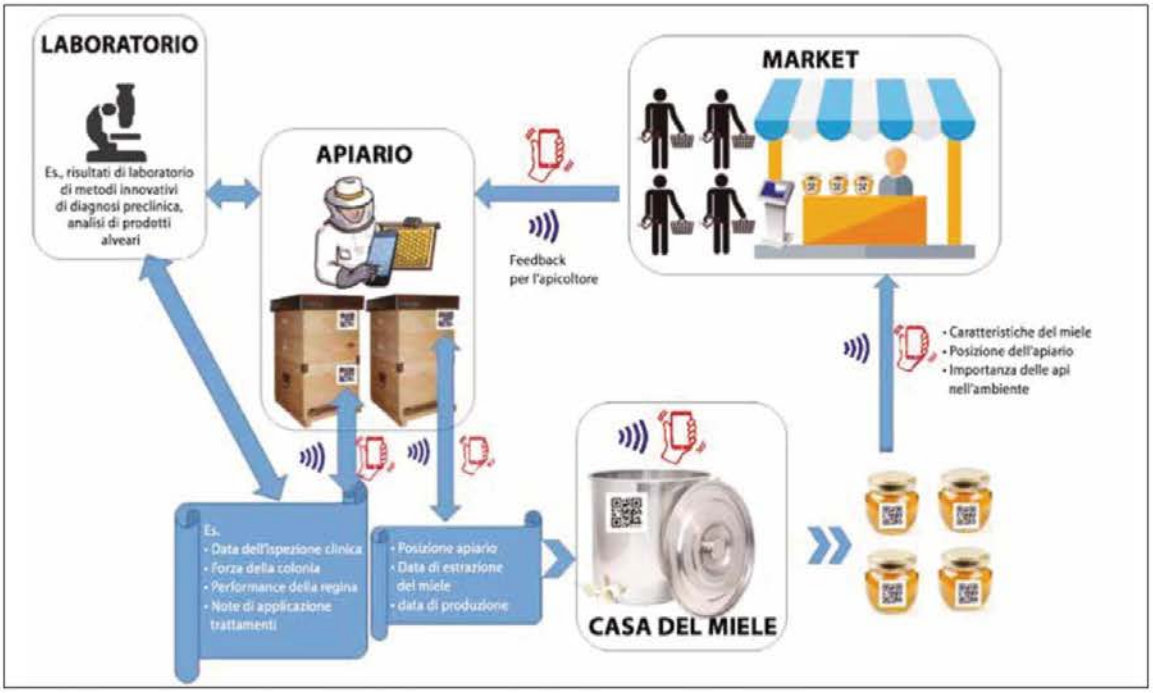

Figura 1. L'innovativo sistema di tracciabilità sviluppato nell'ambito del progetto B-PRACTICES

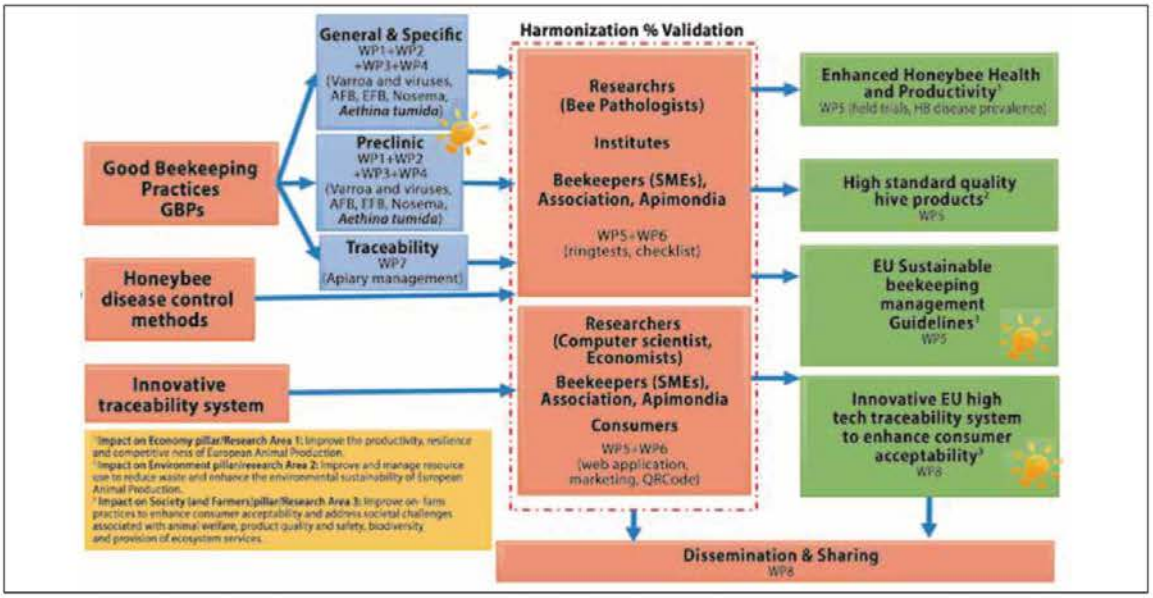

Figura 2. Schema riassuntivo del progetto europeo B-PRACTICES "*New indicators and on-farm practices to improve honeybee health in the Aethina tumida ERA in Europe*".

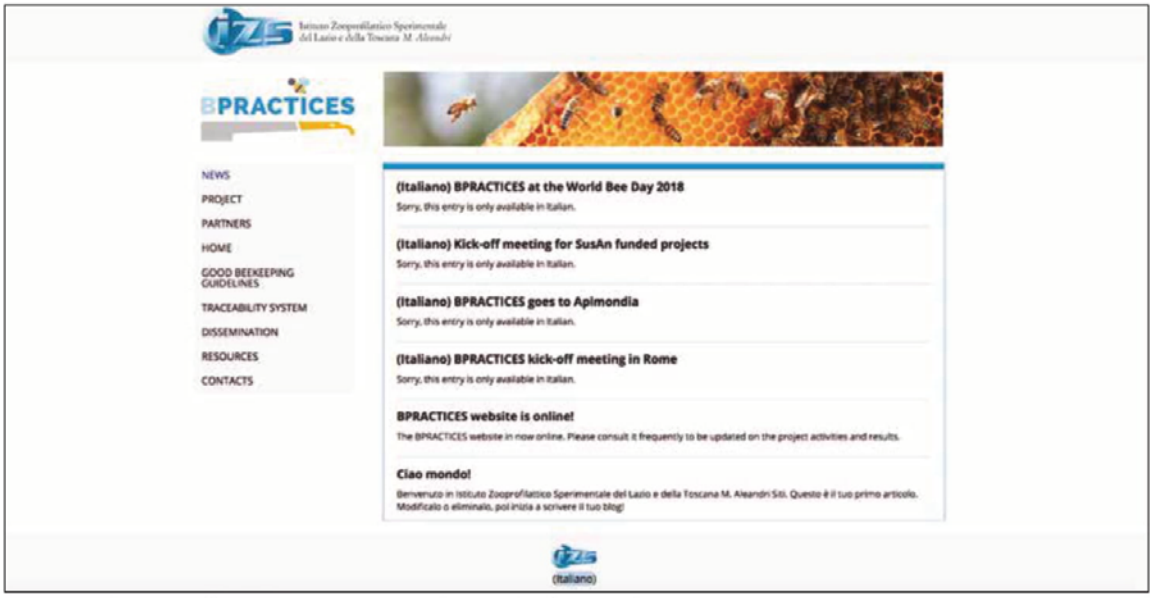

Figura 3. La homepage del progetto B-PRACTICES (www.izslt.it/bpractices).

del coleottero parassita delle api *Aethina tumida* (Small Hive Beetle - SHB) che, a partire dal suo primo focolaio registrato in Italia nel 2014, sembrerebbe essere destinato, più o meno lentamente, a diffondersi nel resto del Paese [2] con ripercussioni negative sia per l'economia del settore apistico, sia per quella del settore agro-zootecnico, in conseguenza della riduzione della biodiversità e del servizio di impollinazione.

Il progetto europeo

Il progetto europeo, "*New indicators and on-farm practices to improve honeybee health in the Aethina tumida ERA in Europe*" (acronimo: *BPRACTICES)*, iniziato nel mese di febbraio 2017, è uno studio di durata triennale che vede come capofila l'Istituto zooprofilattico sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT), finanziato nell'ambito del bando 2016 ERA-NET SUSAN - (*http://www.izslt.it/bpractices/home/*). Obiettivo del suddetto progetto è migliorare lo stato di salute delle api mediante l'applicazione di buone pratiche di allevamento (BPA o *Good beekeeping practices* - GBP) e di strategie di lotta a basso impatto ambientale nei confronti delle principali malattie delle api.
Partner del progetto, oltre all'IZSLT, sono: l'Università turca di Namik Kemal, l'Istituto sloveno di Agraria, il Centro spagnolo per la Ricerca Apistica e Agroambientale di Marchamalo, l'Agenzia per la Salute e la Sicurezza Alimentare Austriaca (AGES, Laboratorio nazionale di riferimento per l'apicoltura) e l'Istituto zooprofilattico sperimentale delle Venezie. Collaborano inoltre al progetto: la Federazione internazionale delle associazioni di apicoltori (APIMONDIA), l'Università di Genova, la FAO con la propria piattaforma TECA (*Beekeeping exchange group - http://teca.fao.org/group/beekeeping-exchange-group*), l'Associazione europea di apicoltori professionisti (EPBA), il Laboratorio di riferimento europeo (*European union reference laboratory* - EURL) per la Salute delle Api (*Sophia Antipolis honey bee health laboratory* in Francia) e il professor Ales Gregorc presso l'Università del Mississippi (USA).

Ricercatori e apicoltori lavorano insieme

Novità di questo progetto è quella di individuare le buone pratiche di allevamento in apicoltura in un'ottica di condivisione tra ricercatori e apicoltori. Tale condivisione ha il fine di individuare pratiche efficaci dal punto di vista scientifico e, al tempo stesso, fattibili nella pratica quotidiana di allevamento delle api. Nell'ambito delle BPA saranno comunque inclusi, in maniera inedita, i così detti "indicatori preclinici", rappresentati da tecniche diagnostiche innovative (es. *Polymerase chain reaction* - PCR) impiegate su matrici dell'alveare fino ad oggi poco considerate (es. detriti del fondo dell'alveare, zucchero a velo etc.), al fine di svelare precocemente la presenza di patogeni nell'alveare e intervenire opportunamente su questi prima che la malattia divenga clinicamente evidente. Il risultato finale, oltre al miglioramento dello stato di salute degli alveari, sarà anche quello di ridurre l'impiego di trattamenti chimici, ottenendo così prodotti dell'alveare più sicuri per i consumatori.

*shutterstock_753916444

Oltre a garantire un allevamento sostenibile delle api, il progetto si propone l'ambizioso obiettivo di prevedere una sorta di certificazione del miele prodotto grazie a un sistema innovativo di tracciabilità basato sulle tecnologie QRCode/RFID. Grazie a quest'ultimo infatti, i consumatori potranno conoscere molti dettagli dalla etichettatura del barattolo di miele che acquisteranno, inclusi aspetti relativi all'allevamento delle api e alle analisi di laboratorio effettuate (figura 1).

I Work packages

I sopra indicati obiettivi saranno perseguiti mediante specifici *Work packages* (WPs), con un approccio multidisciplinare dato dal confronto tra il mondo della ricerca scientifica, quello degli allevatori con la loro quotidiana esperienza in apiario e quello degli economisti e altri esperti di settore.
Entrando maggiormente in dettaglio sui WPs del progetto, possiamo elencare le attività di seguito riportate:

- **WP1** (varroosi e virosi), **WP2** (peste americana ed europea), **WP3** (nosemiasi) e **WP4** (Aethinosi), finalizzati a identificare le buone pratiche di allevamento a livello europeo e a sviluppare metodi innovativi per la diagnosi precoce e il controllo sostenibile delle malattie delle api. Valutazione di protocolli terapeutici mediante prove di campo;
- **WP5** (validazione): con il quale si provvederà a standardizzare le buone pratiche di allevamento individuate tra i diversi Paesi e verificare la loro fattibilità per gli apicoltori hobbisti e professionisti (es. metodi di campionamento in apiario), anche ricorrendo al supporto tecnico fornito dalla Piattaforma TECA della FAO (http://teca.fao.org/). Inoltre, sempre nello stesso WP5, si avrà cura di standardizzare e validare mediante ring-test i metodi di laboratorio per la diagnosi precoce delle suddette patologie tra i partner del progetto, in collaborazione con il Laboratorio di riferimento europeo per la sanità delle api (Honey bee health EURL);
- **WP6** (impatto economico): con il quale si valuterà l'impatto economico dell'applicazione delle buone pratiche di allevamento sulla qualità e sulla quantità del miele prodotto e venduto dagli apicoltori aderenti al progetto. Prevedere un logo specifico che indica al consumatore che è stata applicata una gestione degli alveari nel rispetto delle buone pratiche di allevamento in apiario;
- **WP 7** (sistema di tracciabilità innovativo): con il quale si realizzerà un sistema innovativo per la tracciabilità del miele partendo dall'apiario e dalle modalità di allevamento delle api, fino ad arrivare al consumatore finale. Quest'ultimo potrà ricevere informazioni sull'apicoltore, sulle zone di produzione, su aspetti relativi al prodotto acquistato o ai controlli ricevuti (es. proprietà/caratteristiche, analisi di laboratorio etc.). Tale attività si realizzerà in collaborazione con gli apicoltori danesi, che già adottano un sistema di registrazione informatizzato della gestione degli alveari a livello di allevamento (http://english.stadekort.dk/about-hivelog-dk/) e sono interessati a integrare il loro sistema nelle fasi di lavorazione del miele e di vendita. In tale fase è previsto un panel-test sia per gli apicoltori sia per i consumatori al fine di testare la piattaforma web realizzata;
- **WP8** (comunicazione e disseminazione): con il quale si provvederà a informare gli operatori del settore apistico e i consumatori in merito al progetto e ai risultati che ne conseguiranno, anche ricorrendo a un supporto informatico (ad esempio, per la costruzione del sito Web, per la divulgazione di articoli *open-access*, per contattare gli apicoltori di tutto il mondo mediante la piattaforma FAO TECA, etc.) e al supporto di altri Enti, tra cui Apimondia (http://apimondia.com/) (figura 2).

Il progetto riporta nella sua pagina Web (www.izslt.it/bpractices) i risultati ad oggi ottenuti (figura 3).

Annex 10 Rivera-Gomis J., Gregorc A., Maroni Ponti A., Artese F., Zowitsky G., Leto A., Della Marta U., Formato G. (2018). Monitoring of Small Hive Beetle (Aethina tumida Murray) in Calabria (Italy) from 2014 to 2016: practical identification methods. Proceedings of EURBEE 2018. 18-20 September. Ghent. P097

P097

Monitoring of Small Hive Beetle (*Aethina tumida Murray*) in Calabria (Italy) from 2014 to 2016: practical identification methods

Rivera-Gomis J.[1], Gregorc A.[2], Maroni Ponti A.[3], Artese F.[4], Zowitsky G.[1], Leto A.[1], Della Marta U.[1], Formato G.[1]
[1] Apiculture Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy; [2] Mississippi State University, Poplarville, Mississippi, USA; Agricultural Institute of Slovenia, Ljubljana, Slovenia; [3] Ministero della Salute, Rome, Italy; [4] Italian Beekeeping Federation (FAI) Calabria, Gioia Tauro, Italy

The Small Hive Beetle (SHB), *Aethina tumida*, is an invasive pest of honey bee colonies that causes significant damage to the beekeeping sector. SHB was detected in southern Italy (EU) in 2014 and despite the adopted eradication measures, it is still present there. After three years of observations of SHB in Calabria (2014-2016), we provide here some practical tips for improving control measures based on clinical inspection:
-use of a lateral divider as SHB trap;
-focus the inspection on areas with higher probability of finding SHB's;
-use of tight fitting latex gloves for examination, handling and sampling of beetles.
A new time-saving colony examination method, including the use of a lateral divider to be placed in the hive reduced the time needed for hive inspections by 31.86 % on average. Prioritizating the inspection of pollen and honey combs rather than brood combs is advised.
Moreover, concerning the sentinel apiaries used to monitor SHB's arrival in free areas, no more than five colonies without supers are suggested for each location in order to attract and to monitor the early appearance of SHB. The colonies should be strong, healthy, queen right, as these are more attractive to the parasite. Inserting protein candy or protein substrates into the hives to feed the bees could ease SHB detection, as both adult and immature stages of the SHB are attracted to protein substrates.
Integrative diagnose measures are essential to detect SHB, implementing sentinel apiaries in at risk areas and performing inspections and other diagnosis methods as detection of SHB DNA from hive matrices. An early detection and eradication is essential in free areas, as once it is stablished, this pest is extremely difficult to eliminate from the territory. The use of these methods will enable early detection and prompt eradication measures activation before this destructive pest can spread in a region where it is not present.

POSTERS

Annex 11 G. Formato (2019). Talk: Pre-clinical indicators as innovative tools in beekeeping, in the context of the BPRACTICES project. Proceedings of Honey Bee Health Symposium 2019, Rome

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2. Session: Good Beekeeping Practices - GBPs

Pre-clinical indicators as innovative tools in beekeeping, in the context of the BPRACTICES project

Giovanni Formato
IZSLT
Laboratory of "Apiculture, Honey Bee Productions and Diseases", Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT), Rome, Italy

"BPRACTICES" is the acronym of the EU co-funded project named: "New indicators and on-farm practices to improve honey bee health in the *Aethina tumida* era in Europe". This project is part of the European research area on sustainable animal production (EU Horizon 2020 Research and Innovation Programme - Grant Agreement n° 696231, ERA-Net SusAn).
The project consortium is coordinated by the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (Italy), and includes as partners: University of Namik Kemal (Turkey), Agricultural Institute of Slovenia (Slovenia), Centro de Investigación Apícola y Agroambiental de Marchamalo (Spain), Austrian Agency for Health and Food Safety (Austria), Mississippi State University (USA) and Istituto Zooprofilattico Sperimentale delle Venezie (Italy). Moreover, the project involves the collaboration of: the International Federation of Beekeepers' Association (Apimondia), the European Professional Beekeepers' Association (EPBA), the University of Genova (Italy), the European Union Reference Laboratory for Bee Health (ANSES, France) and the Food and Agriculture Organization of the United Nations (FAO).
Aim of the project is to develop a system of sustainable apiculture by implementing innovative management practices (Good Beekeeping Practices - GBPs).
Good Beekeeping Practices (GBPs) can be defined as those activities that beekeepers apply on-apiary production to attain optimal health for humans, honeybees and environment. The application of the GBPs, therefore, has a positive health effect on colonies, on society in general and at the same time, leads to high production standards. Resilience of the beekeeping sector, sustainability and the income of beekeepers increase when sanitary problems are prevented and costs (e.g. for treatments, colony losses, or caused by production decrease) are reduced. The risk of residues in honeybee products due to chemical treatments is reduced when applying GBPs, thus preventing the use of antibiotics or acaricides, guaranteeing quality and safety of hive products. GBPs also avoid productivity losses.
Starting from the OIE-FAO guidelines "Guide to Good Farming Practices for Animal Production Food Safety" (OIE & FAO, 2009), BPRACTICES partners and collaborators classified GBPs according to the following main headings: General apiary management, Veterinary medicines, Disease management (general), Hygiene, Animal feeding and watering, Record keeping and Training.
Moreover, considering the main honey bee diseases, we identified the following biosecurity measures:

VARROOSIS (*Varroa destructor*)

To prevent the clinical outbreak of varroosis, treatments (biotechnical, veterinary medicines) have to be applied timely to allow the honeybee colony to produce healthy brood and bees, according to natural changes of the bee population throughout the seasons.
Treat against varroosis always according to the national situation of legislation and registration;

Adopt/provide hives with screened bottom boards;

Treat according to an integrated pest management concept taking varroa thresholds into account;

Rotate veterinary medicines active principles to avoid varroa resistance;

Nuclei and swarms should originate from healthy colonies with no clinical signs of diseases (Varroosis, AFB, EFB, DWV, SBV, etc.);

Adopt diagnostic tools for estimation varroa infestation levels (for example, ice sugar method, CO2 test, natural mite fall, etc.) before and after treatments and during the year (for example, in spring at the beginning of the beekeeping season or before harvesting);

HONEY BEE HEALTH SYMPOSIUM 2019

Treat simultaneously all colonies of the apiary and in the same geographic area;

Perform at least two control treatments (biotechnical, veterinary medicines) per year;

Treat nuclei and swarms (no brood) with oxalic or lactic acid;

Have a good knowledge of the symptoms and transmission ways of varrosis and virosis;

Try to select and breed colonies that are more varroa tolerant/resistant.

AETHINOSIS (*Aethina tumida*)

Take care that the bees cover all comb surfaces in the hive (no empty space);

Clean meticulously the honey house and warehouse;

Good knowledge of SHB morphology (eggs, larvae and adults);

Good knowledge on hive inspection methods to detect SHB;

Extract the honey immediately after the harvesting (at latest within two or three days);

Carry out periodical hive inspections to detect and eliminate the parasite (adults and larvae);

Adopt specific traps for quick visual detection of SHB;

Monitor periodically the presence of SHB by sampling debris or honey;

Do not leave outside of beehives frames, combs or other material that could be attractive and digestible for *Aethina tumida;*

Stock combs in order to prevent survival of SHB eggs and larvae in a cold chamber at temperature below 10°C;

Give the artificial nutrition each time at low amounts so the bees can consume it in a short time because protein feed (pollen supplements) could be a substrate for the reproduction of SHB;

Use traps to monitor and control SHB presence in the apiary;

Have only healthy, strong colonies in the apiary;

Have only young queens with hygienic behaviour;

Do not transport live materials (hives, queens, nucs, etc.) and other materials at risk (supers, wax, pollen, etc.) from areas where SHB is present to your apiary.

AMERICAN FOULBROOD (*Paenibacillus larvae*, AFB)

Do not feed the bees with honey or pollen or supplement, unless the absence of *P. larvae* is certified;

Move combs among hives only in case of healthy hives;

Do not exchange honey or pollen combs between colonies in case of clinical or subclinical infection;

Select and breed AFB resistant honey bees;

Balance or split the colonies to avoid reducing the number of nurse bees below a critical point with respect to the amount of brood;

Inspect thoroughly the colonies for clinical symptoms of AFB on a regular basis (at least in spring, end of summer, before wintering);

Recognize the clinical symptoms of AFB: spotty brood pattern, sunken cappings, holes in cappings, ropiness, scales tightly adherent to cell walls, rotting smell;

Take samples of colonies (hive debris, adult/nurse bees, powder sugar, stores of honey in combs) in the winter season to detect *P. larvae* (by PCR or microbial isolation) to control the disease;

Replace regularly old, dark combs.

EUROPEAN FOULBROOD (*Melissococcus plutonius*, EFB)

Do not feed the bees with honey or pollen or supplement, unless the absence of *M. plutonius* is certified;

Move combs among hives only in case of healthy hives;

Do not exchange honey or pollen combs between colonies in case of clinical or subclinical infection;

Select and breed EFB resistant honey bees;

Balance or split colonies, avoiding reducing too much the amount of nurse bees with respect to the amount of brood;

Inspect thoroughly the colonies for clinical symptoms of EFB in spring;

Inspect thoroughly the hive for clinical symptoms of EFB at the end of the productive season (end summer);

Take samples of colonies (hive debris, adult/nurse bees, powder sugar, stores of honey in combs) in the winter season, to detect *P.larvae* (by PCR or microbial isolation) to control the disease;

Replace regularly old, dark combs.

NOSEMA (*Nosema apis, N. ceranae*)

For rearing queens, only use breeder queens and starter or finisher colonies from Nosema-free stocks;

Verify the proper orientation (towards South-East) and positioning of the hives: sunny and dry in the wintering places, avoiding humidity, wind and ground depressions;

Destroy weak colonies heavily infected;

Strengthen and stimulate the colonies in autumn and spring – in cases of insufficient natural resuources - with the administration of scientifically tested and certified (e.g. stimulant integrators composed by vegetal substances/molasses or vitamin integrators if there are registered/permitted products in your country);

Disinfect beekeeping tools and equipment between uses: torching (*Nosema ceranae* spores are inactivated by over 60 °C); gamma irradiation; fumigation of combs with glacial acetic acid, sodium hydroxide 5% (caustic soda); sodium hypochlorite 0.5% (bleach). Prerequisite of any use of disinfectants is a legal status as a biocidal product in your country - check before any application;

Do not feed extracted honey, combs with stores (honey or pollen) from Nosema infested to healthy colonies;

Select and breed Nosema resistant honey bee stocks;
Replace combs every three years;

Take samples of forager honey bees (or powder sugar or debris) early in autumn or spring to diagnose Nosemosis (PCR and microscopical methods).

Implementation of prevention practices leads to reduce the honey bee mortality, the improvement of honeybee health and consequently increases the performance of honey bee colonies, the profitability of the beekeeping operation and the pollination service provided by honeybees. Moreover, reducing the amount of the honey bee diseases, it reduces the use of veterinary medicines and the risk of residues in honeybee products.

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As a new approach, the project includes in the management practices, together with the set up of innovative diagnostic techniques, the monitoring of the so-called "pre-clinical indicators". These represent an essential part of the Good Beekeeping Practices and a crucial basis for an up to dated beekeeping.
Preclinical indicators allow to diagnose an infection or infestation before symptoms appear, representing an essential tool for mitigation of the disease and prevention of the clinical symptoms.

MONITORING OF PRECLINIC INDICATORS
· Take samples for laboratory analyses when sick or dead bees are found, if needed.
· Adopt diagnostic tools for measuring varroa infestation levels (for example, icing sugar method, CO2 test, mite fall, etc.) after treatments and during the year (for example, in spring at the beginning of beekeeping season or before harvesting).
· ake 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.
· Take samples from the colonies (hive debris/adult nurse bees/powder sugar/stores of honey in combs), in winter season, to detect *M. plutonius* (by PCR method or microbial isolation) in case of clinical outbreak to control the disease.
· Take samples of forager honey bees (or powder sugar or debris) early in autumn or spring to diagnose Nosemosis (PCR and microscopic methods).
· Adopt specific traps for quick visual detection of SHB.
· Monitor periodically the presence of SHB by sampling debris or honey.

Table - Practices that could be adopted to monitor preclinical indicators

The monitoring of preclinical indicators can be in some cases performed adopting modern laboratory diagnostic methods (for example, using PCR methods) on new matrices (for example, powder sugar or hive debris), taken from the inspected hives. Examples are the preclinical diagnosis from powder sugar for American Foulbrood (*Paenibacillus larvae*, AFB) or European Foulbrood (*Melissococcus plutonius*, EFB), the preclinical detection of the SHB from bottom hive debris by Real-time PCR, or;the yeast *Kodomaea ohmeri* as a potential indicator for the presence of SHB.
Monitoring preclinical indicators is a good preventive practice able to ensure the improvement of honeybee health and consequently increase the performance of the beehives, the profitability of the beekeeping operation and the pollination service provided by honeybees.
Improving beekeeping management, direct positive effects will follow on honeybee health and bee product quality, as the competitiveness and resilience of the apicultural sector at all levels. Even the application of chemicals at the apiary level will be reduced, increasing quality and quantity of bee products.

HIVELOG and the Internet of Things – IoT

Annex 12 J. Rivera-Gomis, M. Pietropaoli, F. Artese, G. Formato (2019). Talk: Comparison of two colony inspection methods for the detection of Small Hive Beetle (SHB) in Calabria region (Italy). Proceedings of Honey Bee Health Symposium 2019, Rome

Comparison of two colony inspection methods for the detection of Small Hive Beetle (SHB) in Calabria region (Italy)

Jorge Rivera-Gomis[1], Marco Pietropaoli[1], Francesco Artese[2], Giovanni Formato[1]
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The Small Hive Beetle (SHB), *Aethina tumida*, is an invasive pest of honey bee colonies that causes significant damage to the beekeeping sector. SHB was detected in southern Italy (EU) in 2014. It was eradicated in Sicily, but despite the adopted eradication measures, it is still present in the Calabria region. The gold standard diagnostic method is the direct visual detection of the beetle by inspecting the honeybee colony. In Italy, the Ministry of Health indicated the specific procedure to follow in the ministerial note 0020069-01/10/2014-DGSAF-COD_UO-P. This is a method not easy to apply in the field due to the high working load. We compared time needed and efficacy of the official inspection method with a time-saving protocol in Calabria Region in 2017 and 2018. The official inspection method consists in a systematic inspection of the beehive, giving attention to all parts of the hive. The new time-saving protocol can be adopted even during ordinary hive inspections performed by beekeepers, and a specific training is not needed. The time-saving protocol includes the inspection of a lateral divider placed between the last comb and the hive wall, acting as a trap for SHB. The comparison of time needed for the two inspection methods was carried out on thirty colonies. Each protocol was used on fifteen colonies and the time needed for the inspection was recorded. The comparison in efficacy of both methods was performed on

2 colonies divided in two groups recording the number of SHBs found. The colonies were housed in 10 ame Dadant-Blatt hives and homogeneously distributed in two apiaries in a SHB infested area in the alabria region. All groups were homogeneous in terms of strength and amount of brood. The average me needed to apply the official inspection protocol was 11 minutes and 43 seconds per hive, while the ime saving protocol" required only 7 minutes and 59 seconds per hive (standard deviation of 00:04:18 nd 00:03:09 respectively). This was equivalent to a 3 minutes and 44 seconds (31.86 %) reduction of the ıspection time. The difference between the methods was statistically significant (p=0.014). Adopting the fficial inspection protocol, 2.05±3.00 SHBs were found, while with the time-saving protocol 2.86±3.77 HBs were found. There was not a statistically significant difference between the two methods (p=0.151). he time-saving method reduces the inspection time by 31.86% while the efficacy remains the same of ıe official inspection method. Using an automatic instrument to capture the beetles could reduce the ıspection time. The time-saving inspection method represents a useful detection tool, easing the appli- ation of SHB control measures.

Annex 13 J. Rivera Gomis, J. Bubnic, A. Ribarits, R. Moosbeckhofer O. Alber, P. Kozmus, R. Jannoni Sebastianini, W. Haefeker, H. Koeglberger, M. I. Smodis Skerl, B. Tiozzo, M. Pietropaoli, J. Lubroth, E. Raizman, C. Lietaer, R. Zilli, R. Eggenhoeffner, M. Higes, M. N. Muz, C. D'Ascenzi, M. P. Riviere, A. Gregorc, J. Cazier, E. Hassler, J. Wilkes, G. Formato (2019). Good Farming Practices in Apiculture (Good Beekeeping Practices GBPs). Proceedings of Honey Bee Health Symposium 2019, Rome, page 75 – 76

Good Farming Practices in Apiculture (Good Beekeeping Practices – GBPs)

J. Rivera-Gomis[1], J. Bubnic[2], A. Ribarits[3], R. Moosbeckhofer[3], O. Alber[3], P. Kozmus[4], R. Jannoni-Sebastianini[4], W. Haefeker[5], H. Koeglberger[3], M. I. Smodis Skerl[2], B. Tiozzo[6], M. Pietropaoli[1], J. Lubroth[7], E. Raizman[7], R. Zilli[1], R. Eggenhoeffner[8], M. Higes[9], M. N. Muz[10], C. D'Ascenzi[11], M. P. Riviere[12], A. Gregorc[13], J. Cazier[14], E. Hassler[14], J. Wilkes[14] & G. Formato[1*]

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Beekeeping faces numerous challenges due to a variety of factors, mainly related to globalization, agrochemical pollution and environmental changes. As a consequence, new pathogens threaten the health of European honey bees. In this context, a proper honeybee colony management should consider a wider vision, where productivity aspects are linked to a "one health" approach in order to protect honeybees, humans and the environment. In order to achieve these objectives, with this paper we describe the novel tool of Good Beekeeping Practices (GBPs) to be applied in beekeeping operations. GBPs are defined as "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, will have a positive effect on colony health, on society and at the same time could favour high production standards. According to the OIE-FAO classification of Good Farming Practices (GFPs) we classified GBPs considering the following headings: General apiary management, Veterinary medicines, Disease management (general), Hygiene, Animal feeding and watering, Record keeping and Training. An international team, including researchers, animal health national authorities and international beekeepers' associations validated a list of GBPs that the BPRACTICES team had scored depending on their importance.. An overall list of 234 GBPs was identified, of which 140 were selected and validated. All the activities were carried out in the project "BPRACTICES" approved within the transnational call of ERA-Net SusAn (European Research Area on Sustainable Animal Production Systems) in the Horizon 2020 research and innovation programme of the European Union. The study aims at presenting an innovative and implementable approach for similar applications also in other livestock productions.

Annex 14 Rivera-Gomis, J., Bubnic, J., Ribarits, A., Moosbeckhofer, R., Kozmus, P., Jannoni-Sebastianini, R., Haefeker, W., Koeglberger, H., Smodis Skerl, M. I., Tiozzo, B., Pietropaoli, M., Lubroth, J., Zilli, R, Eggenhoeffner, R., Higes, M., Muz, M. N., D'Ascenzi, C., Riviere, M. P., Chauzat, M. P., Gregorc, A., Formato, G. (2019). Biosecurity Measures in Beekeeping. Proceedings of Honey Bee Health Symposium 2019, page 76 - 77

Biosecurity Measures in Beekeeping

Rivera-Gomis, J.[1], Bubnic, J.[2], Ribarits, A.[3], Moosbeckhofer, R.[3], Kozmus, P.[4], Jannoni-Sebastianini, R.[4], Haefeker, W.[5], Koeglberger, H.[3], Smodis Skerl, M. I.[2], Tiozzo, B.[6], Pietropaoli, M.[1], Lubroth, J.[7], Zilli, R[1], Eggenhoeffner, R.[8], Higes, M.[9], Muz, M. N.[10], D'Ascenzi, C.[11], Riviere, M. P.[12], Chauzat, M. P.[12], Gregorc, A.[13], Formato, G.[1]
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[6]Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy
[7]Food and Agriculture Organization of the United Nations (FAO), Rome, Italy
[8]University of Genova, Biophysic Section of Department of Surgery Sciences and Integrated Diagnostics (DISC), Italy
[9]Centro de Investigacion Apicola y Agroambiental de Marchamalo, Marchamalo, Spain
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The emergence of honeybee new pathogens represents an important threat to the development of the beekeeping sector in Europe, along with the increased drug resistance and contamination of hive products. The implementation of Good Beekeeping Practices (GBPs) and Biosecurity Measures in Beekeeping (BMBs) plays an essential role in supporting honeybee health. GBPs are a pre-requisite to the use of BMBs in the day-to-day apiary management. A group of experts, within the BPRACTICES project identified, defined and classified BMBs within the European context. BMBs are those preventive measures aimed at analysing and managing risks related to specific hazards relevant to honeybee, human and environmental health, with a focus on honeybee diseases, through a strategic and integrated approach. We distributed the BMBs in "headings" in relation to the five main honey bee diseases: *Varroa destructor*, American foulbrood (AFB), European foulbrood (EFB), *Nosema spp.* and *Aethina tumida* (Small Hive Beetle or SHB). BMBs were classified in "categories" properly adapted to consider productivity and the "One Health" approach: human health, honey bee health and hive products safety. A total of 94 BMBs were identified. We ranked the BMBs according to the average priority score attributed by the different experts considering the variability of the beekeeping sector between regions. The implementation of those concrete GBPs represents an essential step forward to increase the resilience and sustainability of European beekeeping.

NEW APPROACHES TO HONEY BEE HEALTH
Rome 13th - 15th Feb 2019

Annex 15 J. Rivera-Gomis, G. Formato, V. Antognetti, G. Pietrella, A. Cersini (2019). New Aethina tumida detection methods using Real Time PCR from hive debris and swab samples. Proceedings of Honey Bee Health Symposium 2019, page 80

New *Aethina tumida* detection methods using Real Time PCR from hive debris and swab samples

Jorge Rivera-Gomis[1], Giovanni Formato[1], Valeria Antognetti[1], Gabriele Pietrella[1], Antonella Cersini[1]*
[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy

In 2014, *Aethina tumida* or Small Hive Beetle (SHB), a parasite of honey bee colonies, was detected in Southern Italy. Currently, SHB is endemic to the Calabria region. As part of the surveillance activities in Italy, diagnosis is carried out by clinical inspection of the apiaries, which is expensive in terms of resources (personnel costs, above all). We developed two new DNA extraction methods to detect SHB using Real Time PCR from hive debris and swabs that could fasten the monitoring activities and reduce their cost. The matrices considered for biomolecular analysis were hive debris taken from the bottom board of the hive and swabs taken from the inner surface of the hive with more probability of finding SHB. Between 2016 and 2017 we tested 291 hive debris samples and 68 swabs from the inner surface of the hive. All samples were collected from 31 apiaries of the Reggio Calabria and Vibo Valentia provinces of the Calabria region, in Southern Italy. To extract the SHB DNA, 1g of hive debris was collected per hive. The samples were diluted in 10ml of PBS 1X and incubated two hours at 37°C in agitation. Subsequently, 2ml of treated hive debris were used for DNA extraction with the NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) according to manufacturer instructions. The swabs taken from the inner surface of the hive were kept in 1ml of PBS 1X at 4°C. The samples were vortexed and 200µl of buffer solution were taken for the DNA extraction with the NucleoSpin Tissue (Macherey-Nagel). The DNA concentration and purity were estimated by spectrophotometry (Bio Photometer, Eppendorf, Hamburg, Germany). The DNA was used immediately or stored at –20°C until the analysis was carried out. Specific primers and probes from literature that amplify a 109 bp fragment of *COI I* A. tumida gene were chosen for the Real Time PCR (Ward et al., 2007). The Real Time PCR was developed on the basis of specific primers and probes from literature, amplifying a 109 bp fragment of COI I A. tumida gene (Ward et al., 2007). An internal standard for the PCR was built by coning the Real Time PCR *A. tumida* product of 109bp in pCRII-TOPO vector. The Limit of Detection was set in the exponential phase of the reactions and Ct-values greater than 41 were regarded as negative. A standard curve for the quantification of the COI I *A. tumida* copies obtained in the Real Time PCR was built. The equations of the fitted regression line had a slope significantly different from zero and the intercept was not significantly different from zero. The regression coefficient value of 0.9841 confirmed the linearity throughout the range of dilutions tested, between 1.81*1012 molecules (with Ct = 18.03) and 5 target molecules (with Ct = 45.5) of the TOPO-TA-COI I A. tumida used. A recombinant plasmid containing the Real Time PCR target sequence was created to define the sensibility of the molecular methods, that was >99%. The amplification protocol resulted highly specific for the *A. tumida* stump present in Italy and did not show inaccuracy respect to *Galleria mellonella* larvae and adult coleoptera *Cychramus luteus*, *Brachypeplus glaber*, *Meliogethesaneus f* detected in Reggio Calabria apiaries. These two new DNA extraction methods from hive debris and swabs could be integrated in future surveillance programmes for timely, pre-clinic diagnosis of SHB.

Annex 16 Pietropaoli M., Jannoni Sebastianini R., Formato G. (2019). Apicoltura: sondaggi FAO. Partecipa! Apinsieme, December 2019, 8 – 11.

IL PUNTO

APICOLTURA: SONDAGGI FAO PARTECIPA!

A cura di Pietropaoli, Jannoni Sebastianini, Formato

Un'iniziativa a cui partecipare... e numerosi. I sondaggi sono finalizzati a raccogliere dati a livello internazionale sull'impiego dei medicinali veterinari e l'applicazione delle buone pratiche apistiche. L'apicoltura sostenibile è, infatti, quel tipo di attività dove, mediante una corretta gestione degli alveari attraverso le buone pratiche apistiche, si riduce al massimo l'impiego dei farmaci. La collaborazione di tutti gli apicoltori nella raccolta delle informazioni, rispondendo a tutti o ad almeno uno dei sondaggi, permetterà ai ricercatori di valutare in maniera attendibile le problematiche del settore apistico e aiuterà i governi a rispondere ai loro bisogni

L'Organizzazione delle Nazioni Unite per l'alimentazione e l'agricoltura (FAO) attua numerose iniziative per proteggere le api e gli altri impollinatori vista la loro fondamentale importanza nel garantire il servizio di impollinazione.

Un esempio è la giornata mondiale dell'ape (per maggiori informazioni vedere il numero di giugno 2019 di Apinsieme).

La FAO ha, inoltre, predisposto una piattaforma specifica dedicata all'apicoltura dal nome "TECA beekeeping", raggiungibile al sito web

www.fao.org/teca/forum/Beekeeping/en/

Su questa piattaforma (Figura 1) gli apicoltori possono confrontarsi tra loro, con esperti e con rappresentanti di organizzazioni e istituzioni che si occupano del settore al fine di condividere informazioni, conoscenze ed esperienze a livello internazionale.

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Figura 1 La homepage del sito TECA beekeeping

Gli obiettivi del "TECA beekeeping" sono:

- mettere a disposizione del settore apistico uno spazio in cui potersi confrontare e condividere esperienze e soluzioni;
- offrire informazioni tecniche affidabili e validate da esperti che possano aiutare gli apicoltori nelle loro attività;
- facilitare la condivisione della conoscenza ed il collegamento in rete tra le parti (associazioni, cooperative, apicoltori, ONG, Enti, Istituti di ricerca, ecc.);
- identificare soluzioni e opportunità per introdurre innovazioni, potenziare tecnologie e buone pratiche nel settore dell'apicoltura;
- raccogliere dati e informazioni attraverso sondaggi e/o discussioni tramite moderatori esperti del settore.

Relativamente all'ultimo punto, sono al momento disponibili tre sondaggi (anche in lingua italiana), indirizzati agli apicoltori, realizzati grazie alla collaborazione tra l'Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, la FAO, l'Università degli Appalachi (USA) ed Apimondia nell'ambito del progetto europeo BPRACTICES

http://www.izslt.it/bpractices/

I sondaggi sono relativi a:

- la gestione della varroa (Figura 2)
- la gestione delle malattie infettive delle api (Figura 3)
- la resistenza agli antibiotici (Figura 4)

I sondaggi sono finalizzati a raccogliere dati a livello internazionale sull'impiego dei medicinali veterinari e l'applicazione delle buone pratiche apistiche. L'apicoltura sostenibile è, infatti, quel tipo di attività dove, mediante una corretta gestione degli alveari attraverso le buone pratiche apistiche, si riduce al massimo l'impiego dei farmaci.

Il loro uso illecito od improprio, difatti, influisce sulla qualità dei prodotti dell'alveare (miele, pappa reale, polline, propoli e cera) per la presenza di residui, oppure comporta nel tempo l'inefficacia dei trattamenti a seguito dello sviluppo di forme di resistenza nei patogeni delle api.

La collaborazione di tutti gli apicoltori nella raccolta delle informazioni, rispondendo a tutti o ad almeno uno dei sondaggi, permetterà ai ricercatori di valutare in maniera attendibile le problematiche del settore apistico e aiuterà i governi a rispondere ai loro bisogni.

Ricordiamo, infine, che per completare ogni sondaggio occorrono circa 5 minuti e le risposte sono completamente anonime.

È previsto, infine, un premio per un apicoltore di ciascun continente estratto casualmente da un sistema informatico. Il premio, donato da Apimondia, FAO ed IZSLT, consisterà in attrezzature e pubblicazioni relative all'apicoltura.

Gli intervistati che desiderano partecipare all'estrazione del premio devono selezionare la relativa casella nei sondaggi e comunicare il loro indirizzo e-mail in modo da essere contattati entro il 20 dicembre 2019.

Figura 2 Sondaggio sulla gestione della varroa

Figura 3 Sondaggio sulla gestione delle malattie infettive delle api

Figura 4 Sondaggio sulla resistenza agli antibiotici

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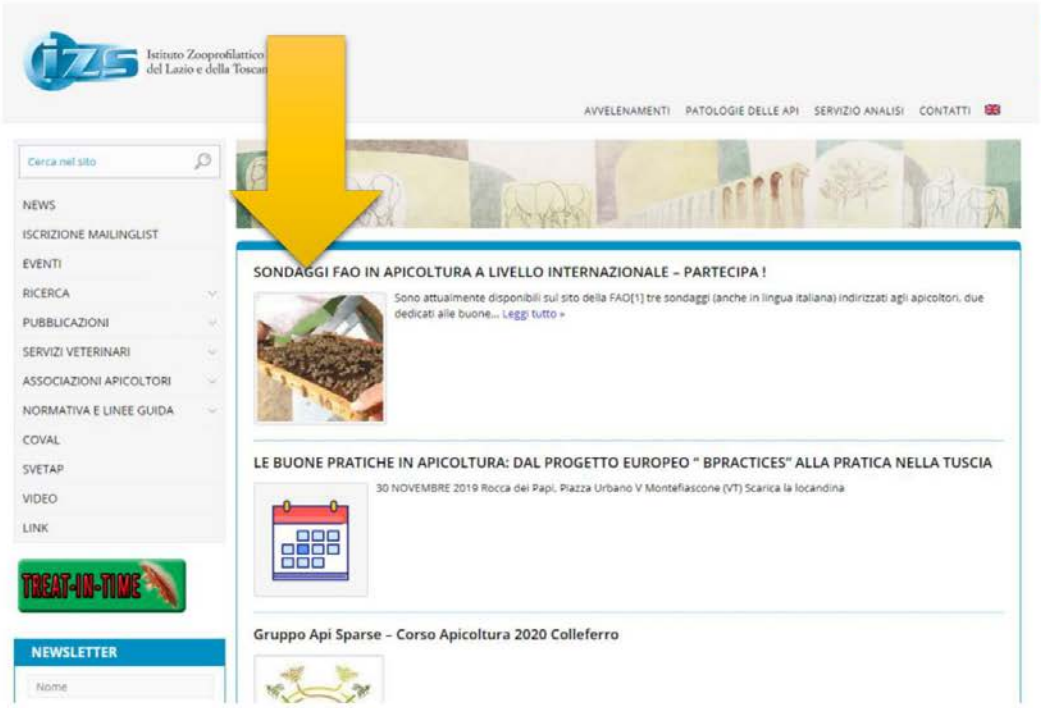
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del Lazio e della Toscana
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PATOLOGIE DELLE API
SERVIZIO ANALISI
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SERVIZI VETERINARI
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NORMATIVA E LINEE GUIDA
COVAL
SVETAP
VIDEO
LINK
TREAT-IN-TIME
NEWSLETTER
Nome
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Sono attualmente disponibili sul sito della FAO[1] tre sondaggi (anche in lingua italiana) indirizzati agli apicoltori, due dedicati alle buone... Leggi tutto »
LE BUONE PRATICHE IN APICOLTURA: DAL PROGETTO EUROPEO " BPRACTICES" ALLA PRATICA NELLA TUSCIA
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Exchange Group on Beekeeping
The TECA Beekeeping Exchange Group was created in 2010 in collaboration with the International Federation of Beekeepers' Associations (Apimondia) to address the need for a central online place for storing and sharing reliable information on beekeeping for smallholder beekeepers.
Beekeeping is widely practiced in the world as an income generating activity and for the benefits that bees and their products offer to mankind. Access to reliable and validated information can help producers to improve their activities and livelihoods.
The TECA exchange group gathers people, organizations and institutions with different expertise or interest in beekeeping to share information, knowledge, and experiences, to learn from each other and to the network.
Ongoing surveys
SURVEY ON VARROA MANAGEMENT

Figura 5 Come accedere al sondaggio dal sito del Laboratorio Apicoltura dell'IZSLT

Figura 6 Come accedere al sondaggio dal forum del TECA beekeeping

Figura 7 Come selezionare la lingua del sondaggio

Per accedere ai sondaggi è possibile visitare:

- il sito del Laboratorio Apicoltura dell'Istituto Zooprofilattico Sperimentale Lazio e Toscana *www.izslt.it/apicoltura* (Figura 5)
- oppure il Forum del TECA Beekeeping, all'indirizzo *http://www.fao.org/teca/forum/Beekeeping/en/* (Figura 6),

cliccando sulle immagini indicate dalla freccia gialla.

Una volta entrati nel sondaggio, è possibile selezionare la lingua grazie al menù a tendina in alto a destra (Figura 7 – freccia blu).

I risultati dei sondaggi saranno condivisi in forma aggregata sul forum del TECA beekeeping in una specifica "discussione con moderatore" e ne sarà data anche diffusione su articoli e siti web degli organizzatori.

Buon sondaggio a tutti!

Italiano

Questo sondaggio è stato elaborato e condotto dalla Appalachian State University nel contesto del progetto BPRACTICES (http://www.izslt.it/bpractices/), finanziato dall'UE, con il supporto tecnico di Apimondia, del Dipartimento Animal Production and Health dell'Organizzazione delle Nazioni Unite per l'alimentazione e l'agricoltura (FAO) e dell'Istituto Zooprofilattico Sperimentale del Lazio e della Toscana. Tutte le risposte rimarranno anonime per la sicurezza e la protezione delle vostre informazioni personali. L'intento di questo sondaggio è quello di comprendere meglio le conoscenze degli apicoltori sull'Apis melliferae sull'uso di antibiotici in tutto il mondo. Il sondaggio richiede tra 5 e 10 minuti ed è sempre possibile controllarne lo stato di avanzamento visionando la barra sulla

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Good farming practices in apiculture

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Summary

Modern European beekeeping is facing numerous challenges due to a variety of factors, mainly related to globalisation, agrochemical pollution and environmental changes. In addition to this, new pathogens threaten the health of European honeybees. In that context, correct colony management should encompass a wider vision, where productivity aspects are linked to a One Health approach in order to protect honeybees, humans and the environment. This paper describes a novel tool to be applied in beekeeping operations: good beekeeping practices (GBPs). The authors ranked a list of GBPs scored against their importance and validated by an international team, including researchers, national animal health authorities and international beekeepers' associations. These activities were carried out in the project 'BPRACTICES', approved within the transnational call of the European Research Area Network on Sustainable Animal Production

(ERA-NET SusAn) in the Horizon 2020 research and innovation programme of the European Union. This study, created through an international collaboration, aims to present an innovative and implementable approach, similar to applications already adopted in other livestock production systems.

Keywords

Apiculture – BPRACTICES – GBPs – GFPs – Good beekeeping practices – Good farming practices – Honeybee.

Introduction

Beekeeping, or apiculture, is the practice of managing honeybee colonies for farming purposes. Bees provide a wide variety of products: honey, pollen, royal jelly, propolis, wax and venom. Many beekeepers also sell colonies, rear queen bees and provide pollination services to farmers (1). According to the World Organisation for Animal Health (OIE) (2) and the European Union (EU) legislation (3) bees are classified as terrestrial animals and as such fall under veterinary care. The most common species used in beekeeping are the western honeybee (*Apis mellifera*) and the eastern honeybee (*Apis cerana*) (2).

Honeybees are vital pollinators of wild plants and crops. As pollinators, honeybees alongside other wild pollinators support biodiversity of wild plants and contribute to higher yields of important highly valued agricultural crops. Considering the recent 'pollination crisis' due to the decline in numbers of wild pollinators and occasional extensive losses of domestic honeybee colonies (4, 5, 6, 7, 8), the European Parliament (9) stated: 'the beekeeping sector throughout the world, and more particularly in Europe, is encountering very serious difficulties… [and] only bees, in sufficient numbers, can guarantee pollination, it is essential to respond without delay to the crisis in bee health in an appropriate manner and with effective weapons'.

International trade in bees and bee products continues to spread throughout the world. It has increased considerably over the past few

decades, and is likely to continue to grow as technology makes movements easier and lowers national barriers to trade (10, 11). This, however, also facilitates the spread of diseases, an example being the recent introduction from Africa of the honeybee pest *Aethina tumida* in Southern Italy (12).

The introduction of relatively recent pesticides, such as neonicotinoids, is also among the factors contributing to environmental pollution which predispose bees to increased stress and pathogen sensitivity; owing to their high toxicity these pesticides often cause death (13, 14).

The widespread establishment of varroosis has caused an increase in viral infections in hives due to the mite's role as a mechanical and biological vector (15). Viruses such as acute bee paralysis virus (ABPV), Kashmir bee paralysis virus (KBPV) and Israeli acute paralysis virus (IAPV), which once caused covert infections and had limited impact on bee health, are now seeing an increase in virulence, with clinically significant diseases affecting susceptible hives that have been weakened by various parasites or stress (16).

Finally, climatic change and the considerable heterogeneity of the European beekeeping industry and its managerial factors (11) are parameters to consider in prevention of honeybee losses.

Given the emerging challenges that beekeeping has to face, along with the more traditional ones (e.g. *Varroa destructor*, *Nosema* spp., American and European Foulbrood, etc.), an innovative, integrative approach that takes into account all steps of the beekeeping value chain, from breeding bees to harvesting hive products, is highly advisable. One Health is the modern denomination of the multi-sectorial worldwide-accepted strategy to design and implement programmes, policies, legislation and research in which different preventive areas communicate and work together to achieve better public health outcomes. The main sectors where the implementation of the One Health approach is particularly relevant are human, animal and environmental health protection (17) (Fig. 1).

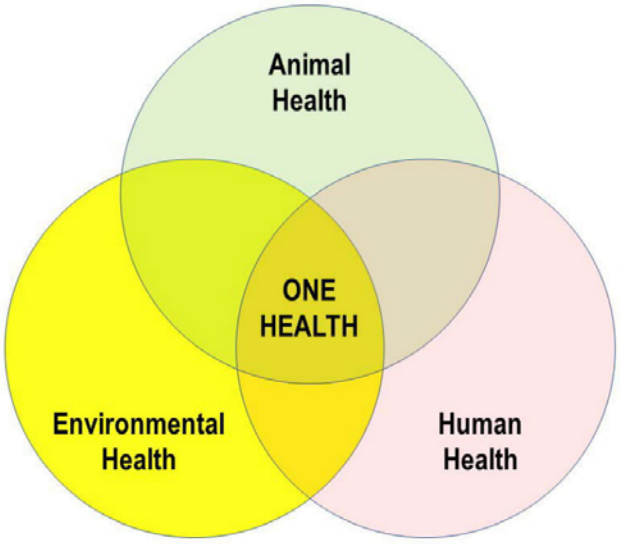

Fig. 1
Elements of the One Health approach

In the above-described scenario, good farming practices (GFPs) should be considered as a preventive tool able to control those factors that negatively affect honeybee health and have consequences for human health, the environment and farm productivity. Despite this, the scientific literature and relevant regulations covering the beekeeping sector contribute only a few general references to the definition of good beekeeping practices (GBPs).

This study was intended to identify and define GBPs through the process of definition, validation, classification, identification and evaluation, in order to obtain a list of validated and effective practices to be shared with all stakeholders. To fulfil these goals, the OIE–FAO (Food and Agriculture Organization of the United Nations) guidelines 'Guide to good farming practices for animal production food safety' were used as a starting point (18).

The study was performed in the framework of 'BPRACTICES', a transnational project funded within the Horizon 2020 research and innovation programme of the EU, called the European Research Area

Network on Sustainable Animal Production (ERA–NET SusAn). The project consortium is made up of a multidisciplinary group representing research institutes, the FAO and international beekeepers' associations (Table I).

Table I

The BPRACTICES project

Consortium partners
Research Institutes
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT), Italy
Namik Kemal University, Turkey
Agricultural Institute of Slovenia, Slovenia
Centro de Investigación Apícola y Agroambiental de Marchámalo (CIAPA), Spain
Austrian Agency for Health and Food Safety (AGES), Austria
Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE), Italy
University of Genoa, Italy
European Union Reference Laboratory (EURL) for Bee Health, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), France
Food and Agriculture Organization of the United Nations (FAO)
Beekeeping Exchange Group, Technologies and Practices for Small Agricultural Producers (TECA), FAO, Italy
Beekeepers' associations involved in the project
International Federation of Beekeepers' Associations (APIMONDIA), Italy
European Professional Beekeepers Association (EPBA), Germany

Materials and methods

Classification of good beekeeping practices

Good beekeeping practices were classified by taking into consideration the most relevant 'headings' of the OIE–FAO guidelines (18). In addition, GBPs were classified in 'categories' similar to those used by Formato & Smulders (19), adapted to consider productivity and the One Health approach: human health, animal health and ecosystem health.

Identification of good beekeeping practices

The 11 participant partners identified, listed and ranked GBPs by relevance using a consensus approach. Direct, simple and easily understandable language was used to write the list of GBPs, to ensure that comprehension by all beekeepers was facilitated.

Validation of good beekeeping practices

During the process, crucial relevance was attributed to the practical point of view of beekeepers. Some of the most important international beekeepers' associations (the International Federation of Beekeepers' Associations [APIMONDIA] and European Professional Beekeepers Association [EPBA]), together with the members of the BPRACTICES consortium, participated in the identification and evaluation of GBPs according to their importance in daily apiary activities, in order to validate them.

Assessment of good beekeeping practices

A transparent and documented prioritisation process for GBPs was duly conducted among the different partners and stakeholders of the BPRACTICES project, using as a reference previous attempts at prioritisation conducted in similar fields (20).

In order to allow the project partners to perform a relevance-based assessment of GBPs, they received a Microsoft excel (Excel® 2016, Microsoft Corporation, Redmond, WA, United States of America) file

for completion, correction, amendment and rating of the GBPs. Partners evaluated each GBP according to 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 with the experience of the participants, based on the magnitude of the impact they can have in the context of the One Health approach (colony health, human health, environmental protection). In order to avoid bias, all scores were provided without allowing individuals to view the scores given by other participants.

Statistical methods

To rate each GBP, the mean result was calculated from the scores received and the answers were sorted according to the 'relevant' and 'mandatory' criteria. All ratings were statistically processed to obtain a final list containing a reasonable number of GBPs to recommend to beekeepers. For the final ranking, only scores with means higher than the 75th percentile threshold were considered.

Results

Definition of good beekeeping practices

As a result of the process mentioned above, GBPs were defined as 'those integrative activities that beekeepers apply for on-apiary production to attain optimal health for humans, honeybees and the environment' (Fig. 2). The implementation of the GBPs, therefore, would have a positive effect on colony health and on society, and at the same time could favour high production standards.

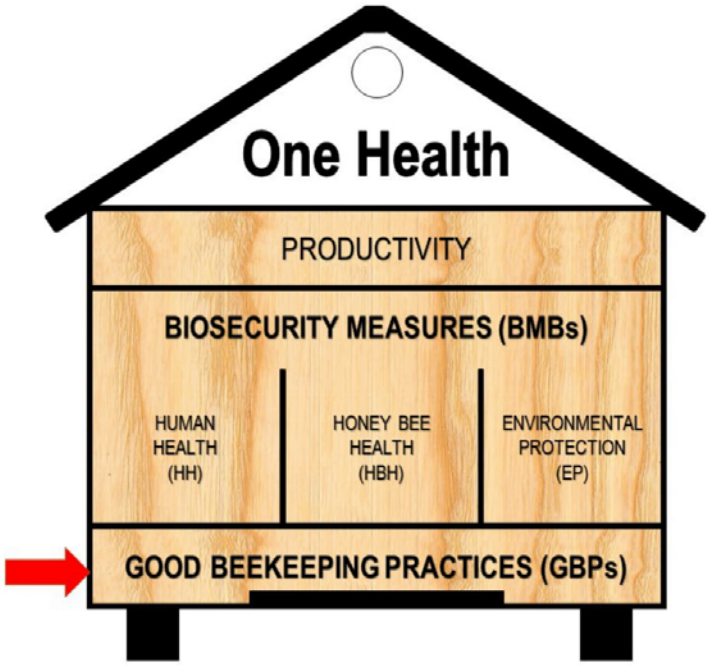

Fig. 2
Elements of the One Health approach in beekeeping
Arrow indicates good beekeeping practices

Classification of good beekeeping practices

According to the OIE–FAO classification of GFPs, GBPs were classified by considering the following headings: general apiary management, veterinary medicines, disease management (general), hygiene, animal feeding and watering, record keeping and training (Tables II and III).

Table II

General good beekeeping practices

1. General apiary management		Mean score (1–4)	Category
1.1. Transportation	Comply with legal obligations concerning restrictions on animal movements in the case of notifiable diseases	4.0	HBH
	Transport/move only healthy colonies	3.8	HBH
	Transport hives avoiding the warmer hours of the day, providing adequate openings for air ventilation in the hives	3.7	HBH
1.2. Hygiene	Respect hygiene rules (e.g. periodically clean suits, gloves, etc.)	3.8	HBH
	Practise good hygiene when dealing with dead colonies (combs, food stores, boxes, etc.)	3.8	HBH
	Disinfect levers and other potentially contaminated equipment (e.g. gloves) after inspection of hives affected by transmissible diseases	3.7	HBH
	Do not place honey supers directly on the ground (avoid contamination with *Clostridium botulinum*)	3.7	PS
	Avoid contact with dust during the transport of the supers from the apiary to the honey house	3.6	PS
	Do not place beehives directly on the ground	3.3	PS
	Use disposable gloves when handling diseased hives	3.3	HBH

1.3. Bee health	For nuclei use only bees and brood combs from healthy colonies	3.8	HBH
	Balance colony strength among colonies, transferring frames only in the case of healthy hives	3.7	HBH, PR
	Buy new bee colonies only after thorough inspection for bee diseases, preferably with a health certificate from a veterinarian	3.6	HBH
	Keep only healthy strong colonies in the apiary	3.5	HBH, PR
	Avoid areas with environmental pollutants (e.g. pesticides, heavy metals, etc.) to place apiaries	3.2	HBH, HH, PR
	Do not imbalance the proportion between nurse bees and brood while equalising the hives; preferably use combs with hatching bees to fortify weak colonies	3.2	PR, HBH
	Perform genetic selection in order to have queens that are more resistant to disease and adapted to local climatic conditions	3.0	HBH
	Keep newly introduced colonies separate from the existing stock for an appropriate period (at least one month) in order to monitor them against diseases to prevent transmission	2.9	HBH
	Avoid, as far as possible, the introduction of swarms of unknown origin, or colonies or queens from other apiaries	2.8	HBH
	Keep purchased or weak colonies in a quarantine apiary	2.8	PR
	Reduce bee stress (e.g. avoiding unnecessary winter inspections of the hives; limiting the use of the smoker; feeding the bees properly, etc.)	1.3	PR, HBH

1.4. Apiary management	Evaluate the melliferous and pollen capacity of the area and the availability of water resources	3.3	HBH
	Do not have beekeeping material abandoned in the apiary	3.2	PS
	Keep a good balance between the number of hives and the amount of melliferous plants/pollen sources in the area where the apiary is located	3.1	PR
	Avoid windy areas when placing apiaries	3.0	HBH
	Place apiaries in an accessible area	3.0	HBH
	Adjust the number of hives in the apiary according to season, pollen, nectar, honeydew resources	3.0	HBH, PR
	Adjust the number of hives within a flight range according to season, pollen, nectar, honeydew resources	3.0	HBH, PR
	Place apiaries on a firm area	2.8	HBH
	Prevent drift occurrence: avoid keeping too many colonies in a single row	2.7	HBH
	Place apiaries in an area accessible to vehicles	2.7	HBH
	Avoid having broken hives with openings or poorly maintained hives, to prevent robbing	2.6	HBH
1.5. Wintering	Before winter, reduce the empty space in the hive	3.0	PR
	Wintering: reduce the size of the hive entrance	3.0	HBH
	Wintering: perform beehive box maintenance (replacing parts or painting; verify the integrity of hive boxes, if needed)	2.8	PR
	Wintering: verify the external position of the frames with stores in the hive	2.5	PR

	Wintering: reduce the number of frames in the hive box	2.3	PR
	Wintering: insert a divider board to reduce the volume for the hive nest	2.3	PR
	Wintering: wrap the hive in black tar paper, if needed	1.3	PR
1.6. Human health	Have the support of an expert (e.g. veterinarian, technician, etc.) to provide assistance in case of need	3.3	PR, HBH, PS
	Use personal protective clothing and equipment when visiting honeybee colonies	2.8	HH
	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	2.5	PR, HH
	Keep corticosteroids or other appropriate medicines ready to use during apiary inspections to guarantee health of operators (for example, in case of anaphylaxis)	2.4	HH
	Limit the weight lift (e.g. when harvesting supers or when moving hives) and, if needed, use back protector devices	2.3	HH
	Avoid areas where allergenic plants (e.g. *Ambrosia trifida* and *Artemisia vulgaris*) can be found in a significant quantity	2.0	HH, PS
1.7. Colony management	Practise hive management according to region, season, strength of colony	3.7	PR
	Replace the queens at least every two or three years except for those of high genetic value	3.6	HBH, PR
	Prevent swarming by insertion of new wax foundations	3.1	HBH

Prevent swarming by colony splitting	3.0	PR
Comply with the planned schedule for beehive inspection	3.0	PR
Prevent swarming by placing of supers	2.8	PR
Prevent swarming by taking off the entrance reducer	2.8	HBH, PR
Prevent swarming by adopting genetic selection of the queens	2.8	PR
Use a queen excluder	2.8	HBH
Reduce the opening of the hive entrance during robbing and cold periods and increase the opening of the hive entrance during the hot season	2.8	HBH
Mark the queen bee according to the date of birth	2.7	PR, HBH
Orientate hive entrance so that the sun can reach the bees in the early morning hours	2.5	PS
Prevent swarming by insertion of drawn combs	2.2	PR
Prevent drift occurrence: paint/draw numbers or identification signs on the front and entrance of the hive	2.2	HBH
Indicate the age of the combs on the top bar of the frame (e.g. the year of placement of the frame with foundation)	1.6	HBH
Prevent swarming by removal of the beehive's bottom board	1.2	PR
Provide adequate openings in the hive for air circulation, if needed	1.2	PR, HBH

2. Veterinary medicines	**Mean score (1–4)**	**Category**
Use only veterinary medicines for honeybees registered in your country or medicines legally imported	4.0	HH, HBH, PS
Ensure that all treatments or procedures are carried out correctly as described in the instructions (respecting dosage and method of application)	4.0	HH, HBH, PS
Do not carry out illegal treatments	4.0	HH, HBH, PS
Use only pharmacological products registered for beekeeping use, follow the usage instructions and record the treatments	4.0	HH, HBH, PS
Observe the withdrawal period of veterinary products and ensure that products from treated hives are not used for human consumption until the withdrawal periods have elapsed	4.0	PS, HH
If using instruments for the application (formic acid dispenser, sublimators for oxalic acid treatment), ensure that they are appropriate and correctly calibrated for the administration	3.7	HH, HBH, PS
Respect the required storage conditions for veterinary medicines and feeds	3.6	PS, HBH
Dispose of used instruments and devices in a biosecure manner	3.5	HH, HBH

3. Disease management	**Mean score (1–4)**	**Category**
In the case of notifiable diseases follow the instructions from the veterinary regulations and competent authorities	4.0	HBH
In the case of infectious diseases clean all beekeeping material between uses (e.g. hive bodies, hive bottom boards, feeders, hive tools)	4.0	HBH, PR
Clean or disinfect (in the case of infectious diseases) the hive box before installing new colonies	4.0	HBH, PR
Carry out thorough inspections for clinical signs of bee diseases and presence of the queen in spring	3.8	HBH, PR
Carry out thorough inspections for clinical signs of bee diseases and presence of the queen at the end of the beekeeping season	3.8	HBH, PR
Quickly remove beehives with dead colonies	3.8	HBH
Take samples for laboratory analyses when sick or dead bees are found, if needed	3.8	HBH (Subcategory [PCI])

Clean equipment, scrape off wax and propolis, on a regular basis	3.8	HBH
Remove and process wax of all combs from dead, affected colonies	3.7	HBH, PR
Record the health status of the colonies: diseased/infected colonies (dates, diagnoses, ID of colonies affected, treatments and results)	3.6	PS, HBH
Renew 30% of the hive combs every year	3.5	HBH, PR
Record the health status of the colonies: mortality (dates, diagnoses, ID of colonies affected)	3.4	HBH
Verify promptly any signs of disease, asking a veterinarian (or a specialist)	3.3	HBH (Subcategory [PCI])
Do not move frames or any kind of biological material (for example, to balance hives) from one hive to another if their health status is not well known	3.3	HBH, PR
Inspect diseased hives only after inspections of healthy hives are ended	3.3	HBH
Select the best performing stocks of honeybees	3.2	HBH, PR
Burn dead colonies	3.2	HBH
Remove queens from colonies with clinical history of AFB disease	3.0	HBH, PR
Remove queens from colonies with clinical history of EFB disease	3.0	HBH, PR
Try to select and breed colonies that are more disease tolerant/resistant	3.0	HBH, PR
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)	3.0	PS, HBH, HH
Disinfect equipment (for example, with NaOH, hypochlorite) on a regular basis	2.8	HBH, PR
Carry out thorough inspections for clinical signs of bee diseases and presence of the queen before supering the hives	2.7	HBH, PR

4. Hygiene	**Mean score (1–4)**	**Category**
Torching (blue flame) used as a disinfection method for hives and beekeeping tools in the case of transmissible diseases	3.3	HBH
Bleaching (soda, NaOH, etc.) used as a disinfection method for hives and beekeeping tools in the case of transmissible diseases	3.2	HBH
Incineration of affected colony, if needed in the case of transmissible diseases	2.3	HBH

Always incinerate affected colony in the case of transmissible diseases	1.8	HBH
Water under high pressure and heated (90°C) used as a disinfection method for hives and beekeeping tools in the case of transmissible diseases	1.6	HBH
Autoclaving used as a method of disinfection of hives and beekeeping tools in the case of transmissible diseases	1.6	HBH
Gamma-irradiation as a method of disinfection of beekeeping tools in the case of transmissible diseases	1.5	HBH

5. Animal feeding and watering	**Mean score (1–4)**	**Category**
Do not feed the bees with honey, pollen or supplements, unless the absence of pathogens (spores of AFB, chalkbrood, *Nosema*, EFB, etc.) is certified	4.0	HBH
Provide artificial feeding during times of shortage or to build up winter stores, when needed	3.7	HBH, PR
Wintering: verify that there is a sufficient amount of stores in the hive	3.7	HBH
Provide nucleus and swarms with adequate food supply when needed	3.6	HBH, PR
Ensure the bees have access to safe water sources	3.3	HBH, PR
Do not feed bees openly in the field to prevent robbing and spread of diseases	3.3	HBH, PR
During transport provide adequate watering if needed	3.0	HBH

6. Record keeping	**Mean score (1–4)**	**Category**
Keep records of veterinary medicine treatments	4.0	PS, HBH
Registration of the beekeeper in the National Beekeeping Registry	3.8	PS, HBH
Record the exact position of the bee yards	3.8	PS, HBH
Identify with numbers/letters all the hives in each apiary	3.6	PS, HBH
Keep records of honeybee diseases and colony mortality or depopulation	3.5	PS, HBH
Set up a data-recording system that can be used to trace exactly which batches of commercial feed the colonies were fed with	3.5	PS, HBH
Keep all documents/certificates about the commercial feed used	3.5	PS, HBH
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	3.4	PS, HBH
Record all reared colonies	3.4	PS, HBH

Record all colony arrivals, with origin and date of arrival, to ensure that movements of incoming colonies are traceable to their source	3.4	PS, HBH
Keep records of movements of hives, swarms, queen bees	3.4	PS, HBH
Record period of collection of hive products from each apiary	3.4	PS
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	3.3	PS, HBH, HH
Keep all documents/certificates that indicate the raw materials used in feed manufactured by the beekeeper and given to the colonies	3.3	PS, HBH
Create a unique identification number for the apiary to easily trace the location of the hive (for stationary apiaries)	3.2	PS, HBH
Keep records of breeding activities (e.g. all breeding stock, queens' birth dates, their origin and arrival, the breeding dates and outcomes in cases of instrumental insemination, etc.)	3.2	HBH
Establish a data-recording system to ascertain the exact origin (batch) of bee products produced	3.2	PS, HBH
Keep all documents regarding self-checks and official controls on the proper management of the colonies and the sanitary and hygienic quality of the bee products	3.1	PS
Keep all documents proving that the bacteriological and physicochemical quality of the water used in the honey house, given to the colonies or used in feed preparation meets official national standards for tap water	3.0	PS
Record the origin and use of all feeds used, keep all records of any feed manufacturing procedures and records for each batch of feed	2.9	PS, HBH
Keep a list of certified suppliers	2.8	HBH
Record any other management changes that may occur	2.5	HBH
Record any change in feeding	2.4	PS, HBH
Keep all laboratory reports, including bacteriological tests and sensitivity tests	2.4	PS
Keep reference samples (–20°C) of all feeds administered to the bees	2.3	PS, HBH

7. Training	**Mean score (1–4)**	**Category**
Training/knowledge on honeybee diseases and clinical signs	3.5	PS
Follow a training programme in beekeeping and honeybee diseases	3.5	HBH
Attend personal training on beekeeping	3.1	HBH, PS, HH, PR

Record datasheets for each detergent/disinfectant used	3.0	HBH, PS, HH
Record disinfection procedures used	3.0	HBH, PS, HH
Record that disinfection procedures have been implemented	3.0	HBH, PS, HH
Keep the documents certifying qualification and training of persons working with bees	1.9	PS

AFB: American foulbrood
EFB: European foulbrood
HBH: honeybee health
ID: identification number
HH: human health
NaOH: sodium hydroxide
PCI: preclinical indicators
PR: productivity
PS: product safety

Table III

Headings for good beekeeping practices and number of most relevant good beekeeping practices identified

Headings	Number of GBPs identified in each heading
General apiary management	63
Veterinary medicines	8
Disease management (general)	23
Hygiene	7
Animal feeding and watering	7
Record keeping	25
Training	7

GBPs: good beekeeping practices

Identification of good beekeeping practices

An overall list of 251 GBPs was identified (www.izslt.it/bpractices/good-beekeeping-guidelines/) (21).

Assessment of good beekeeping practices

The 251 GBPs were arranged according to the average score attributed by the different partners. Then, in order to obtain a smaller, more practical and reasonable list of GBPs to provide to beekeepers, only the GBPs with a mean score within the 75th percentile were considered for each heading and category, reaching a total of 140 GBPs (Tables II and III).

Finally, a list of the 140 most relevant GBPs was obtained, classified as shown in Table III. For each heading, the following categories were considered: honeybee health (HBH), product safety (PS), human health (HH) and productivity (PR) (Tables II and IV). Some GBPs were included in more than one category (Table IV).

Table IV

Categories of good beekeeping practices with their abbreviations and number of most relevant identified good beekeeping practices

Categories for GBPs and abbreviations	Number of GBPs identified in each category
Honeybee health (HBH)*	109*
Product safety (PS)	44
Human health (HH)	16
Productivity (PR)	45

*including the subcategory 'Preclinical indicators' (PCI)
GBPs: good beekeeping practices

Discussion and conclusions

The aim of this work was to contribute to a first definition of GBPs in accordance with FAO and OIE guidelines (18) in close collaboration among scientists, Technologies and Practices for Small Agricultural Producers (TECA)–FAO and international beekeepers' organisations (APIMONDIA and EPBA).

Good beekeeping practices are intended to be implemented in the primary production of hive products. Moreover, GBPs are pre-requisites for the 'biosecurity measures in beekeeping' (BMBs). The latter are those operational activities aimed at limiting the spread of specific honeybee diseases. Only if GBPs are systematically implemented by the beekeeper can BMBs be properly tackled.

A clear definition of GBPs will ease the development of guidelines or recommendations to the beekeeping sector from international institutions involved in animal production, animal health and food safety (e.g. Codex Alimentarius, Joint FAO/World Health Organization [WHO] Expert Committee on Food Additives (JECFA), OIE, FAO, International Organization for Standardization [ISO]). Furthermore, this definition and the methodologies should be valuable for legislative bodies (e.g. EU Regulation 2016/429) (3), producers, Veterinary Services, capacity building activities, progressive management pathways in beekeeping, etc.

Daily implementation of GBPs in apiary management should result in multiple beneficial impacts:

a) for honeybee health, due to generally better management of hives (e.g. proper wintering, apiary position, feeding) and appropriate control of honeybee diseases (e.g. adopting preventive measures and integrated pest management with proper use of acaricides);

b) for human health, owing to appropriate use of antimicrobials and food safety of hive products;

c) for environmental protection, by preferring the use of organic compounds and avoiding the use of antibiotics;

d) from the economical point of view (22, 23, 24), healthier animals reduce the need for medicines (and the subsequent costs) and are able to increase the production (and the income) per hive.

The GBPs were classified by adapting the FAO and OIE guidelines 'Guide to good farming practices for animal production food safety' (18) to the beekeeping sector. In this study, the collaboration and the involvement of international associations of beekeepers (e.g. APIMONDIA and EPBA) represented an important, innovative approach that allowed consideration of the feasibility of applying the GBPs in everyday apiary activities.

Following the FAO and OIE guidelines for GFP (18), the same headings were adopted when collecting GBPs: general apiary management, veterinary medicines, disease management (general), hygiene, animal feeding and watering, record keeping and training (Table III).

Record keeping, in particular, has the potential to improve the other best practices through monitoring of the recorded practices. This can be useful for beekeepers on both small and commercial scales. Maintaining consistent and accurate records is an ongoing challenge for all sectors of the beekeeping community; however, various practical solutions have been devised to facilitate record keeping, including markings or indicators in the apiary, notebooks and spreadsheets, as well as recently introduced specialised apiary management and monitoring technology.

Of course, in order to extend the value of record keeping beyond the individual beekeeper to the global beekeeping community, it will be necessary to use standardised data (25, 26) and promote the sharing of relevant data to a common repository for analysis. At the same time, guidelines and policies that protect the beekeeper will also be needed to encourage data sharing. New technologies and best practices could

not only be recommended, but also built into software systems. Educational objectives could be enhanced through the application of smart software, which will also collect the data needed to finely tune and adapt current best practices to a customised solution.

The use of advanced analytical techniques will help beekeepers to recognise not only threats to the hive, but also what particular GBPs are best for that hive, with the help of a recorded history that will ensure the best outcome for that colony.

The list of 140 GBPs identified will allow more effective and harmonised training of beekeepers, veterinarians and paraprofessional technicians, as well as the application of effective biosecurity measures in beekeeping.

In conclusion, the authors have defined and listed those GBPs that, at the international level, represent universally accepted pre-requisites that will guarantee the sustainability, competitiveness and resilience of the apiculture sector and enable it to face the current challenges of modern beekeeping.

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Dear Dr Pietropaoli,
Dear Dr Formato,

Many thanks again for sending your paper entitled:

Biosecurity measures in European beekeeping
by M. Pietropaoli, A. Ribarits, R. Moosbeckhofer, H. Köglberger, O. Alber, A. Gregorc, M.I. Smodis Skerl, J. Presern, J. Bubnic, M. Necati Muz, M. Higes, B. Tiozzo, R. Jannoni-Sebastianini, J. Lubroth, J. Cazier, C. Lietaer, M. Bagni, R. Zilli & G. Formato

which you have submitted for publication in the *Scientific and Technical Review* of the OIE.

I am pleased to inform you that the reviewing process has started and your article has been sent to the reviewers chosen by the scientific adviser. I will contact you upon receipt of their comments.

Kind regards and have a nice week-end,
Séverine

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Annex 19

CONSUMERS' OPINIONS, PERCEPTIONS AND BEHAVIOURS RELATED TO THE PURCHASE AND CONSUMPTION OF HONEY IN ITALY

Report edited by the Health Awareness and Communication Department of the IZSVe

SUMMARY

INTRODUCTION

The Health Awareness and Communication Department of the Istituto Zooprofilattico Sperimentale delle Venezie was involved as a research partner within the ERA-NET SUSAN project titled 'New indicators and on-farm practices to improve honeybee health in the Aethina Tumida era in Europe'.

In line with subtask 3.1 of Work Package 7 (WP7), a semi-structured questionnaire (Appendix 1) was designed based on the existing literature (1–6). The questionnaire consisted of the following sections:

- Socio-demographic characteristics
- Purchasing behaviours
- Consumption behaviours
- Honey and production chain: Knowledge and perceptions

Before administration, the questionnaire was pre-tested on five honey buyers to identify and remove any unclear or dubious questions.

Between February 7th and 25th, 2019, a company specialized in opinion surveys administered the questionnaire through the computer-assisted web interviewing (CAWI) method to a sample of Italian honey buyers and consumers enrolled in the company's mailing list. The honey buyers were selected through a screening question placed at the beginning of the questionnaire: those who declared they had not bought honey in the last 12 months did not fill in the questionnaire.

The data were treated according to the General Data Protection Regulation (EU) 2016/679.

RESULTS

Socio-demographic characteristics

A total of 1,011 honey buyers completed the questionnaire. Among them, the majority are female (51.1%) and aged between 50 and 62 years old (25.5%). They live in South Italy and the islands (Sicily and Sardinia) (36.3%), have an upper secondary school diploma (50.7%), have an occupation (49.1%), and meet their financial needs with some difficulties (41.9%). The details of the respondents' socio-demographic characteristics are reported in Table 1.

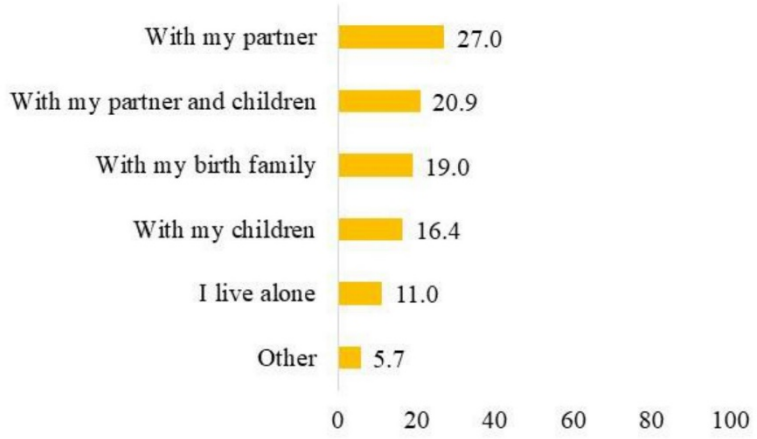
Table 1. Respondents' socio-demographic characteristics (n=1,011)

Characteristics	***n***	***%***
Gender		
Female	**517**	**51.1**
Male	494	48.9
Age (classes)		
18-35	247	24.4
36-49	250	24.7
50-62	**258**	**25.5**
63-80	256	25.3
Geographical area		
North West	269	26.6
North East	185	18.3
Centre	190	18.8
South and Islands	**367**	**36.3**
Educational qualification		
Elementary school diploma	8	0.8
Lower secondary school diploma	83	8.2
Vocational qualification	49	4.8
Upper secondary school diploma	**513**	**50.7**
Bachelor's degree	29	2.9
Master's degree	279	27.6
Ph.D. or other post-graduate qualification	50	4.9
Occupation		
Student	88	8.7
Looking for a first job	19	1.9
Homemaker	117	11.6
Employed	**496**	**49.1**
Unemployed	75	7.4

Retired	216	21.4
How well does your income meet your financial needs?		
Very easily	82	8.1
Quite easily	417	41.2
With some difficulties	**424**	**41.9**
With many difficulties	88	8.7

When asked, 'Who do you live with?', the majority of respondents (27%) stated 'with my partner' (Figure 1).

Figure 1. *Who do you live with?* (n=1,011, %)



The respondents were then asked if they were following a diet when they were surveyed; 10.7% stated 'yes'. Among them, the majority specified that they were following a low-calorie/weight loss diet (Table 2).

Table 2. *Specify which kind of diet you are following* (n=108[1])

Diets	**n**	**%[2]**
Low-calorie/ weight loss diet	42	36.5
Diet linked to the presence of pathologies (cholesterol, diabetes, allergies, intolerances, etc.)	27	23.5
Balanced diet	13	11.3

[1] 108 respondents, 115 types of diet classified
[2] Percentages are calculated starting from the 115 types of diets indicated by respondents

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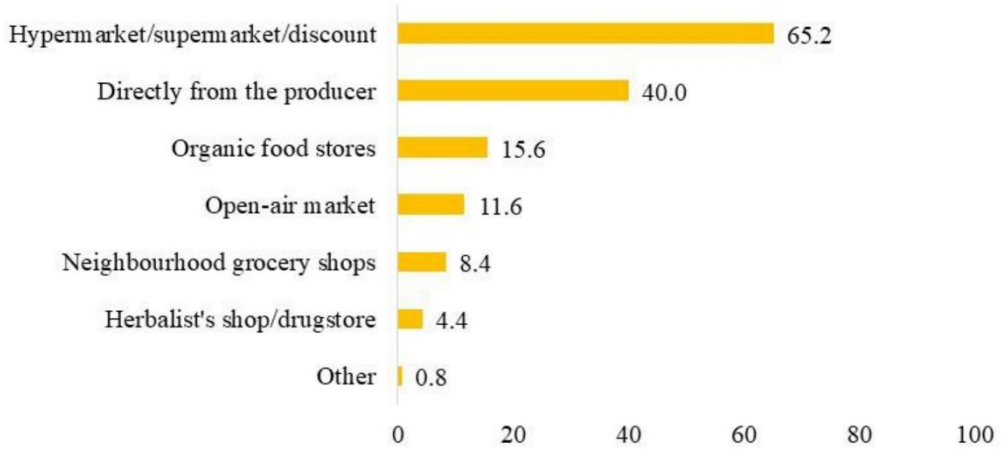
Vegetarian diet	8	7.0
Protein/ high protein diet	5	4.3
Vegan diet	2	1.7
Social diet[3]	2	1.7
Other diet	9	7.8
Not classified	7	6.1

Purchasing behaviours

In this section of the questionnaire, the purchasing behaviours adopted by honey buyers were investigated.

The respondents declared that they mainly buy honey in **hypermarkets/supermarkets/discount stores** and **directly from the producer** (Figure 2).

Figure 2. *Where do you mainly buy honey? Choose two options maximum* (n=1,011, %)



Those who selected the response option 'other' (8 respondents) specified that they buy honey through cooperatives, online stores, and other types of shops.

When asked 'Which types of honey do you prefer to buy?' 17.8% of respondents stated, 'I don't know'. Among the remaining 82.2%, the majority declared they consume 'acacia' and 'wildflower' honey (Figure 3).

[3] Collective path that consists of a healthy and conscious diet aimed at developing a correct relationship with food

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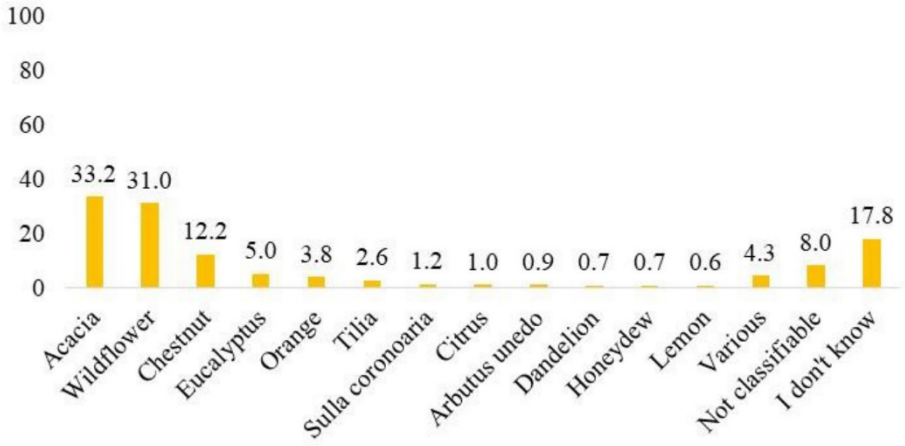
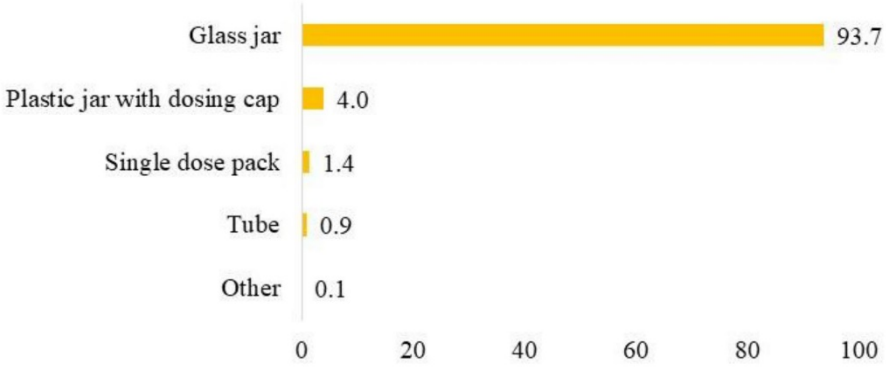
Figure 3. *Which types of honey do you prefer to buy?* (n=1,011, %)



Respondents were then asked to specify the kind of packaging of the honey they mainly buy. The **'glass jar'** is the most common packaging of honey purchased (Figure 4).

Figure 4. *What kind of honey packaging do you mainly buy?* (n=1,011, %)



Only one respondent selected the 'other' response option and specified that he mainly buys plastic jars without a dosing cap.

The respondents were then asked the importance of a series of aspects considered in the choice of honey they buy.

The most important aspect was found to be **'That the honey is produced in Italy'** (the percentage of people who selected the response options 'A lot' and 'Quite a lot' is equal to 93.2%), while the

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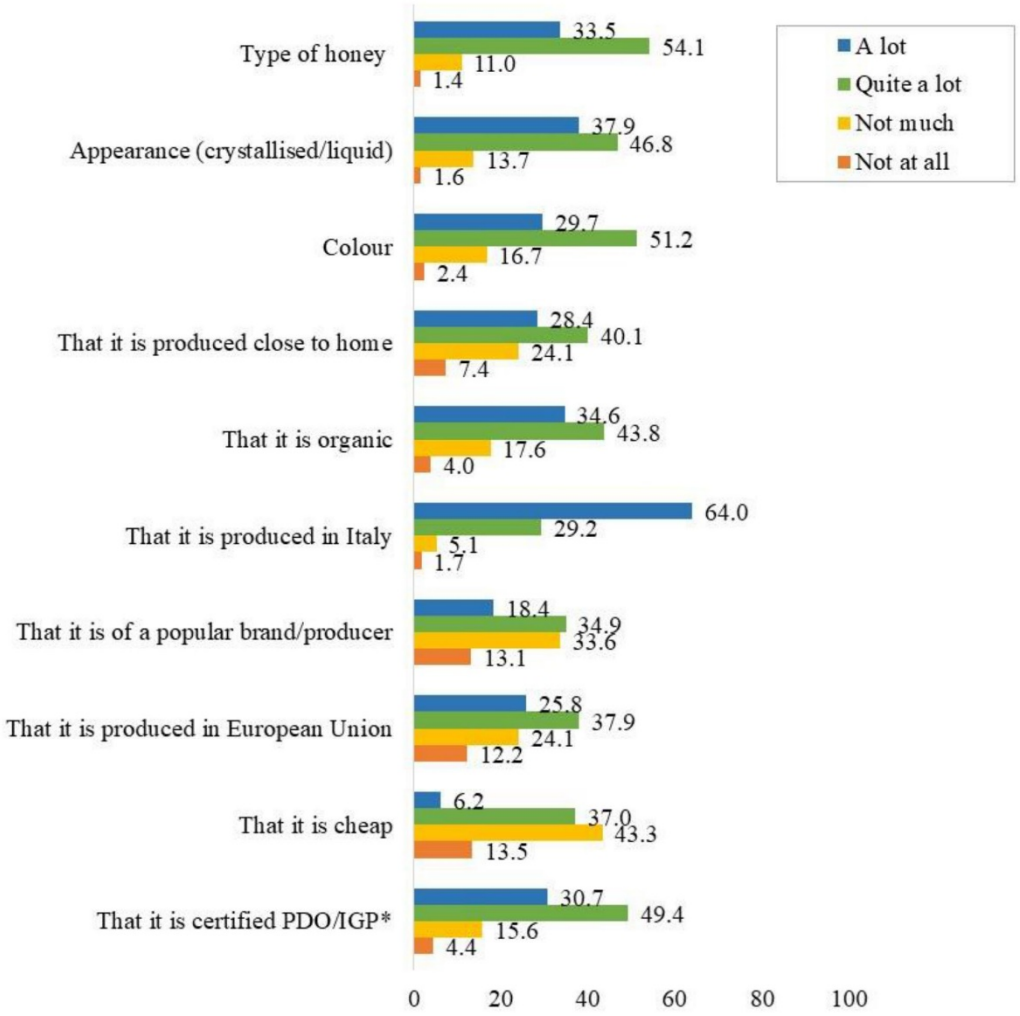
least important aspect is 'That it is cheap' (the percentage of people who selected the response options 'Not much' and 'Not at all' is equal to 56.8%) (Figure 5).

Figure 5. *How important are the following aspects when you are choosing which type of honey to buy?* (n=1,011, %)



* PDO, protected designation of origin, and PGI, protected geographical indication

When asked, 'Do you read the label on the honey you buy?' The majority of respondents (56.5%) stated 'Yes, always' (Figure 6).

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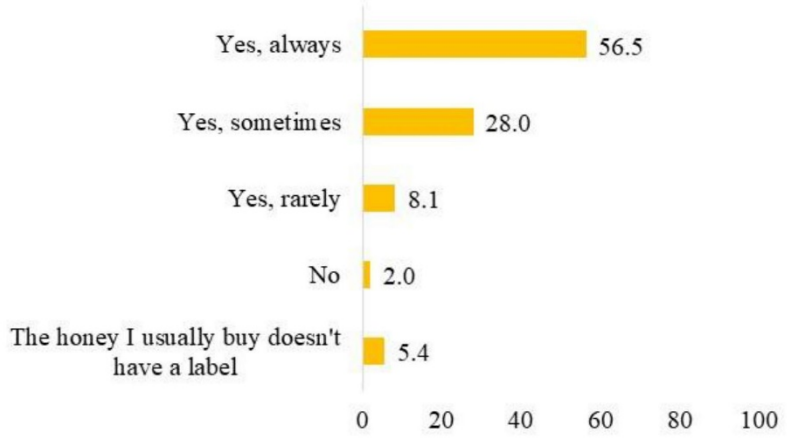
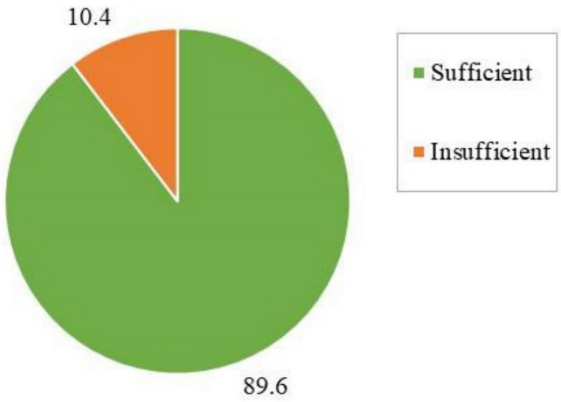
Figure 6. *Do you read the label on the honey you buy?* (n=1,011, %)



The 2.0% of the sample (equal to 20 respondents) who declared that they do not read the label were asked why. The majority of them (50%, n=10) stated that they trust the producer, 30% (n=6) that they do not read the label out of indifference, and 15% (n=3) that they do not read the label out of laziness. Finally, one person (5%) referred to the inability to evaluate the information contained in the label.

Only those who stated that they read the label on the honey they buy (92.6%, n=936) were asked to evaluate the completeness of information contained on the label. Of these respondents, **89.6% evaluated the information 'sufficient'** (Figure 7).

Figure 7. *Do you think that the information contained in the label is…?* (n=936, %)



Those who considered the information on the label 'insufficient' (10.4%, n=97) were asked to specify what further information they would like to receive. The responses given to this open-ended question were manually coded in non-mutually exclusive classes (Appendix 2). Among them,

- 78.4% claimed the need to be more informed on the exact origin of the honey and the entire supply chain; in particular, they want to know the honey production area, origin of the bees and position of the plants/flowers
- 9.3% asked for more information on the honey composition
- 6.2% would like to know how bees are bred
- 5.2% would like to know the period of honey extraction
- 3.1% asked for more information on the beekeepers
- 2.1% asked for more information on the nutritional values
- 1% would like to know the potential contraindications linked to honey consumption
- 9.3% did not provide any meaningful answer: they stated that they did not know what additional information is needed or that more information in general is needed without specifying what type of information.

Then, all respondents except those who stated that they do not read the label (2%, n=20) were asked how important it is for them see on the label the types of information reported in Figure 8. The **'Place of origin'** was considered the most important aspect, followed by the 'Presence of other ingredients' and the 'Expiration date', while the 'Brand' was considered the least important aspect.

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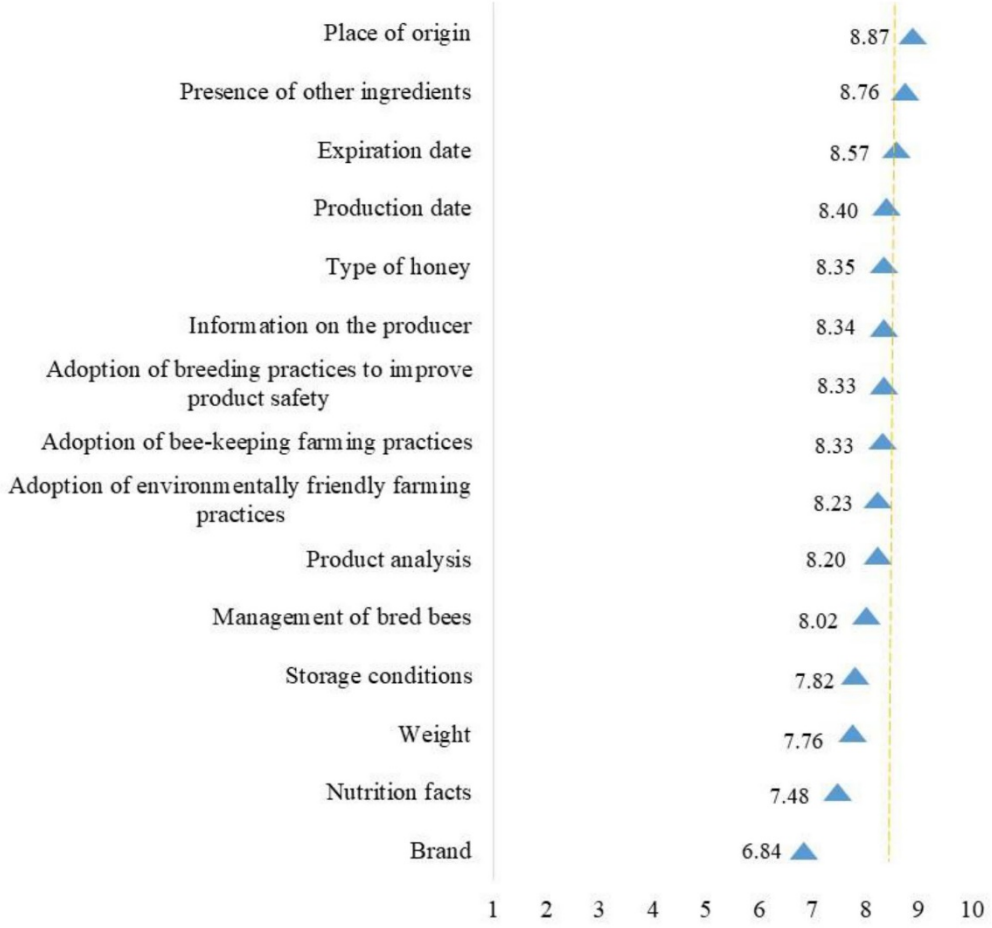
Figure 8. *How important is it for you see the following types of information on the label? Likert scale 1-10, where 1='not at all important' and 10='very important'* (n=991, average values)



At the end of this questionnaire section, the respondents' willingness to use the QR code on the label to access further information about honey was investigated. The QR code was described in the questionnaire as a two-dimensional barcode that contains machine-readable information (for example, by smartphone) about the item to which it is attached.

Among the respondents, more than 60% stated that they would use the QR code (Figure 9).

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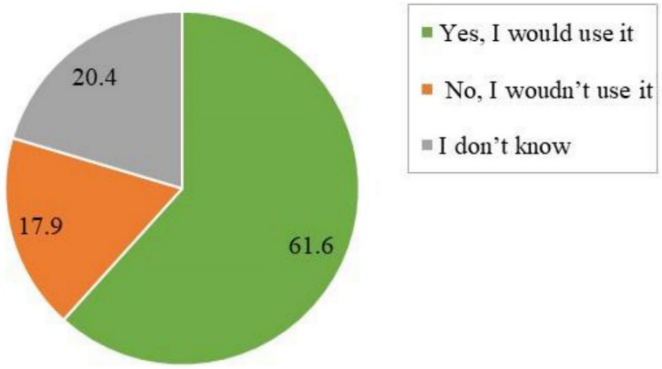
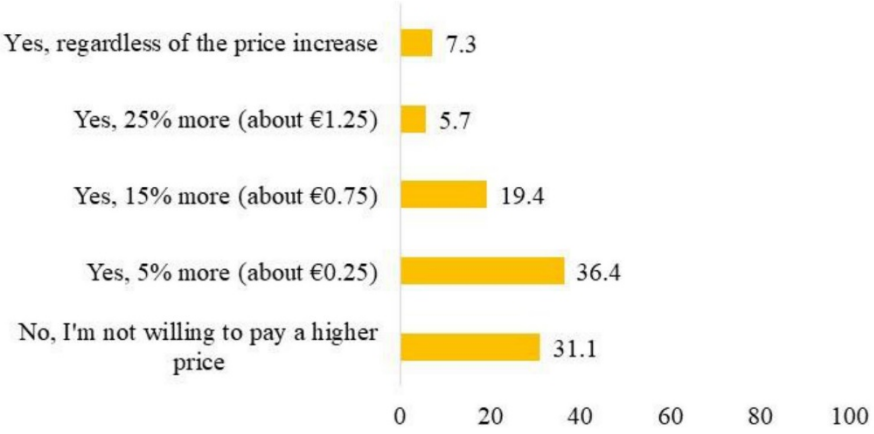
Figure 9. *Would you use the QR code via smartphone to access information about the honey you buy?* (n=1,011, %)



When asked, 'Would you be willing to pay a higher price for a package of honey if it offered you more information about the product? Choose the option you prefer by referring to a 500-gram package at a cost of 5.00 euros', the majority of respondents (36.4%) stated 'Yes, 5% more (about €0.25)' (Figure 10).

Figure 10. *Would you be willing to pay a higher price for a package of honey if it offered you more information about the product? Choose the option you prefer by referring to a 500-gram package at a cost of 5.00 euros* (n=1,011, %)



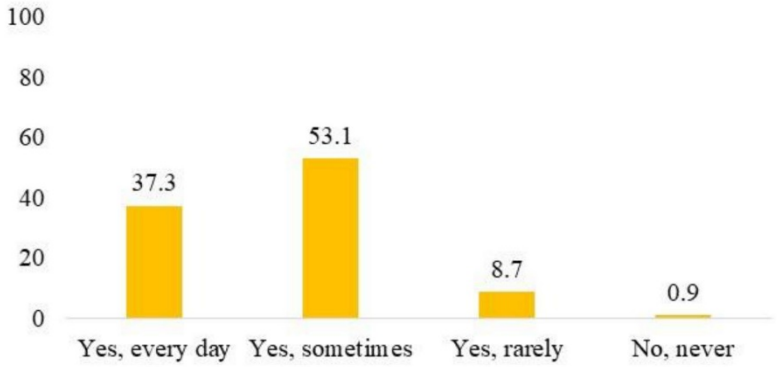
BPRACTICES

Consumption habits

In this section of the questionnaire, the frequency with which respondents eat honey and the reasons for honey consumption vs. non-consumption were investigated.

Almost all the respondents declared that they eat honey (Figure 11).

Figure 11. *Do you eat honey?* (n=1,011, %)



Those who stated that they eat honey (99.1%, n=1,002) were asked to explain why they consume it, ranking up to 4 response options in order of importance. **'It is good for health'** is the main reason (Table 3).

Table 3. *Why do you eat honey? Rank up to 4 options in order of importance* (n=1,002)

Reasons	Rank
It is good for health	1
It is a natural product	2
For its therapeutic properties	3
I like the taste	4
For its nutritional value	5
It has fewer calories than other sweeteners	6
It is a quality product	7
It is free of antibiotics	8
It is produced with respect for the environment	9
It is safe to eat	10
Other reasons	11

Additionally, those who stated that they do not eat honey (0.9%, n=9) were asked to explain this behaviour ranking up to 4 options in order of importance. **'I don't like the taste'** is the main reason (Table 4).

BPRACTICES

Table 4. *Why don't you eat honey? Rank up to 4 options in order of importance* (n=9)

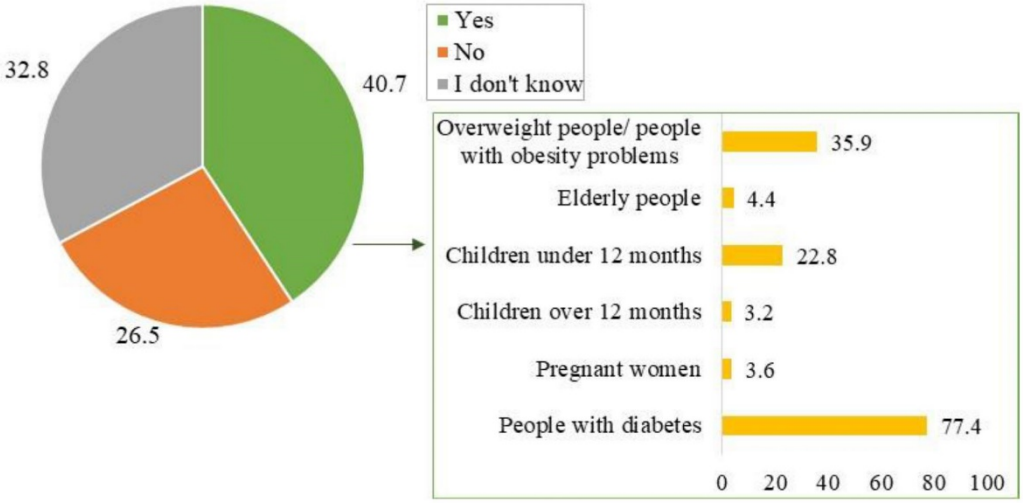
Reasons	Rank
I don't like the taste	1
I don't like the consistency	2
It is inconvenient to use	3
It is not suitable for my diet	4
I'm not used to consuming it	4
It may contain pesticides and other environmental chemicals	4
It may contain antibiotics	5
I prefer other sweeteners	6

Honey and production chain – Knowledge and perceptions

In the last section of the questionnaire, the perceptions and opinions of Italian honey buyers towards honey in general and its potential risks were investigated.

Respondents were asked whether, in their opinion, there are any categories of people for whom the consumption of honey is not recommended. The majority of respondents (40.7%, n=412) thought that honey is not recommended for one or more categories of people, especially for **persons affected by diabetes**.

Figure 12. *In your opinion, are there any categories of people for whom the consumption of honey is NOT recommended?*[4] (%, n=1,011)



[4] If yes, it was possible to select more than one category of people

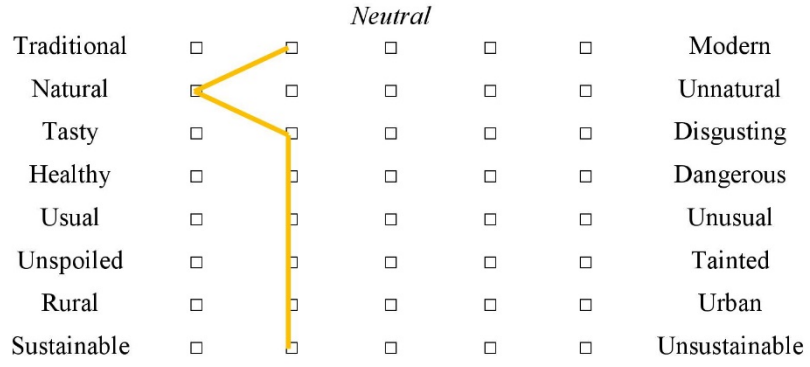
The respondents who selected at least one category of people (40.7%, n=412) in the previous question were asked 'Why is honey not recommended for these people'. The answers were manually classified. Please note that more than one category can refer to the same answer.

The performed analysis showed the following:

- The majority of answers (84%, n=346) referred to the high sugar content (e.g., 'it is high in sugar'; 'it is too sweet'; 'it increases blood sugar')
- 12.6% of the answers (n=52) referred to the high caloric intake
- In 9.5% (n=39) of the answers, the respondents stated that honey is dangerous for children. Of these, 24 mentioned – more or less directly – infant botulism
- In 3.4% (n=14) of answers, the respondents stated that it can cause allergic reactions
- 13.8% of the answers (n=57) were not classifiable since the respondents were not able to explain, or they repeated the situation of the selected category (e.g., 'because of diabetes'), or provided explanations that were too general (e.g., 'because it is dangerous')

When asked, 'In general, how would you describe honey? Put an 'x' closer to the adjective that better describes honey in your opinion', the respondents defined the honey as quite traditional, tasty, healthy, usual, unspoiled, rural, and sustainable. Moreover, they considered it very natural (Figure 13).

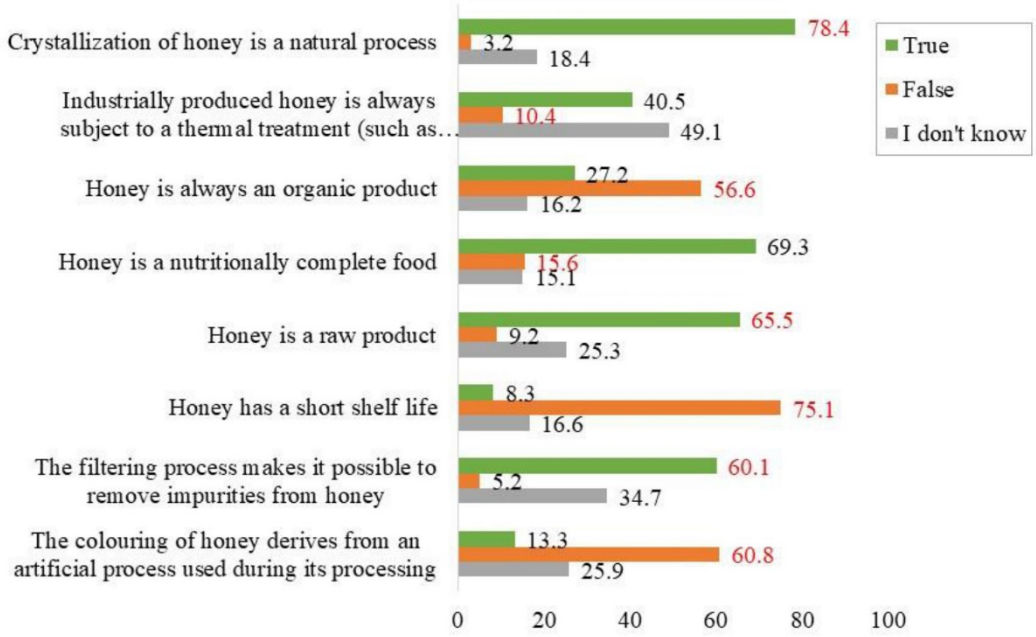
Figure 13. *In general, how would you define honey? Put an 'x' closer to the adjective that better describes honey in your opinion* (n=1,011)



The respondents' knowledge about honey and its production chain was then investigated. In particular, the respondents were asked to report whether a series of statements were true or false. The results are reported in Figure 14. The **red** colour identifies, for each statement, the percentage of correct answers.

BPRACTICES

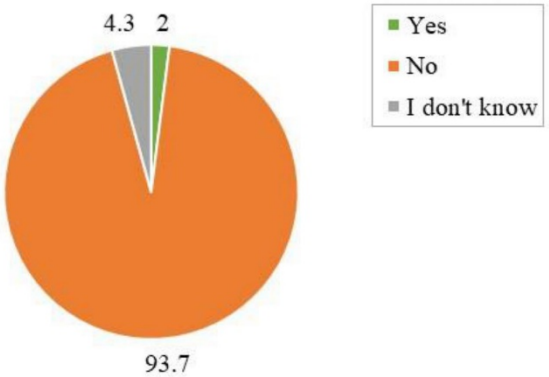
Figure 14. *Please report whether the following statements are true or false* (%, n=1,011)



To summarize what is shown in Figure 14, a variable was developed to count the correct answers of each respondent to the eight knowledge questions, thus providing a score range from 0 to 8. On average, the number of correct answers given by the respondents is equal to 4.2 (with a standard deviation of 1.6).

The respondents were then asked whether, in their opinion, honey is dangerous to health. More than 90% of the sample stated 'No' (Figure 15).

Figure 15. *In your opinion, is honey dangerous to health?* (%, n=1,011)



Only those who stated 'Yes' to the previous question (2%, n=20) were asked to specify the risks that they think are associated with honey consumption and to provide an example of each one. Their answers are reported in Table 5.

Table 5. *What risks do you associate with honey consumption? (Possibly more answers) For each selected risk, provide an example* (n=20).

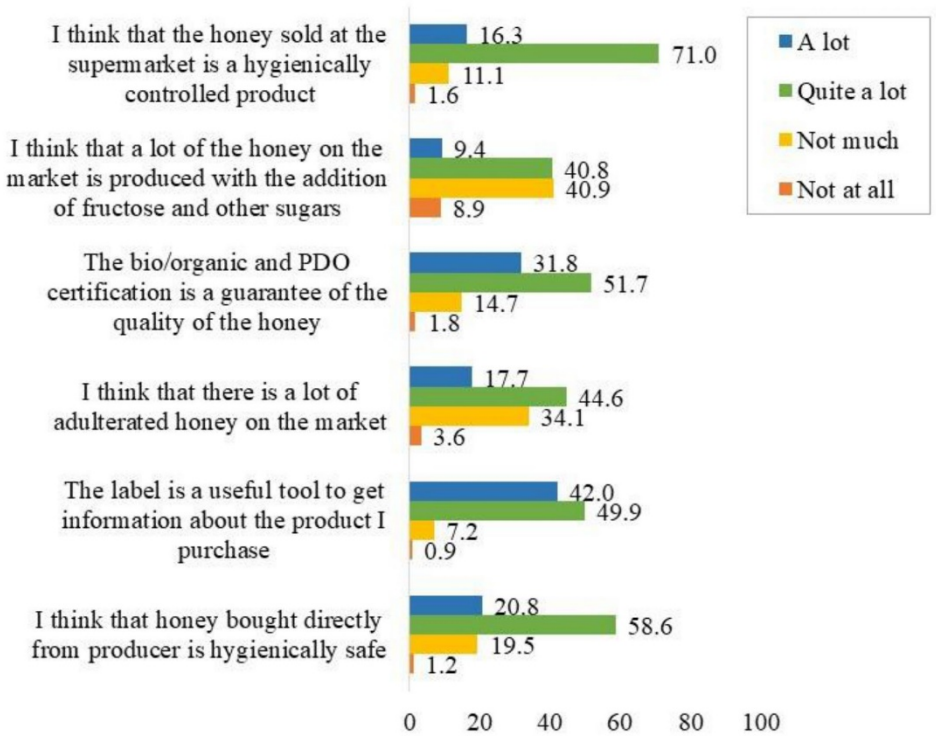
Risks	**%**	**Examples**[5]
Risks related to the presence of drug residues	40	Antibiotics
Risks related to the presence of environmental contaminants	35	Water; Pollution; Shortage of bees
Risks related to the presence of adulterants	35	Chemical; Colours; Addition of sugar in honey
Nutritional risks	20	Diabetes
Risks related to the presence of pathogenic microorganisms	10	-
Other risks	10	Sugars; Botulism in young children

The last question of the questionnaire investigated the respondents' level of agreement with the statements reported in Figure 16. The higher agreement (the percentage of the response option 'A lot' plus the percentage of the response option 'Quite a lot' was higher than 85%) emerged in relation to the statements **'I think that honey sold at the supermarket is a hygienically controlled product'** and **'The label is a useful tool to obtain information about the product I purchase'**.

[5] Only a few respondents provided an example; the others stated, 'I don't know'.

BPRACTICES

Figure 16. *Report your level of agreement with the following statements* (%, n=1,011)



CONCLUSION

The collected data allowed us to outline the purchasing and consumption behaviours adopted by Italian honey buyers and to deeply understand their opinions and perceptions towards honey in general and its production chain.

This survey must be considered as a pilot study that could be deepened/completed with other surveys that could be carried out in the countries to which the other project partners belong.

The main results obtained in the Italian context are summarized below:

- Italian honey buyers prefer to purchase honey in **hypermarkets/supermarkets/discount stores** and **directly from the producer**
- In several parts of the questionnaire, it was revealed that the **origin of the product** plays a very important role in the respondents' purchasing and consumption behaviours. For example, 'That the honey is produced in Italy' is considered the most important aspect in the choice of which honey to buy. Moreover, even if the majority of respondents evaluated the information contained on the label as '**sufficient'**, the need to have more information about the exact origin of honey was observed. Again, the '**Place of origin**' was considered the most important information on the label by those who declared that they usually read it
- More than 60% of the respondents stated that they would **use the QR code** to access further information about honey
- Most respondents stated that they were willing **to pay a higher price** for a package of honey if it offered them **more information** about the product
- '**It is good for health**' is the main reason why respondents consume honey, while 'I don't like the taste' is the main reason reported by those who stated that they do not eat it
- Approximately 40% of the respondents thought that honey is not recommended for some categories of people, especially for persons affected by **diabetes**
- In general, the respondents defined honey quite **traditional, tasty, healthy, usual, unspoiled, rural, sustainable, and very natural**
- A **lack of knowledge** about honey and its production chain was observed among the interviewees
- In general, honey is **not considered dangerous** to health
- **The label** is considered a useful tool to obtain information about the product.

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APPENDIX 1

QUESTIONNAIRE (*English version*)

0. Have you bought honey in the last 12 months?
   - ☐ Yes (continue the questionnaire)
   - ☐ No (stop the questionnaire)

A. PURCHASING BEHAVIOURS

1. Where do you mainly buy honey? (choose two options maximum)
   - ☐ Hypermarket/supermarket/discount
   - ☐ Neighbourhood grocery shops
   - ☐ Herbalist's shop/drugstore
   - ☐ Directly from the producer
   - ☐ Open-air market
   - ☐ Organic food stores
   - ☐ Other (specify _____)

2. Which types of honey do you prefer to buy?
   - ☐ Specify your preferred types of honey _____
   - ☐ I don't know

3. What kind of honey packaging do you mainly buy?
   - ☐ Single dose pack
   - ☐ Tube
   - ☐ Glass jar
   - ☐ Plastic jar with dosing cap
   - ☐ Other (Specify_____)

4. How important are the following aspects when you are choosing which honey to buy?

	Not at all	Not much	Quite a lot	A lot
Type of honey	☐	☐	☐	☐
Appearance (crystallised/liquid)	☐	☐	☐	☐
Colour	☐	☐	☐	☐
That it is produced close to home	☐	☐	☐	☐
That it is organic	☐	☐	☐	☐
That it is produced in Italy	☐	☐	☐	☐
That it is from a popular brand/producer	☐	☐	☐	☐
That it is produced in the European Union	☐	☐	☐	☐
That it is cheap	☐	☐	☐	☐

That it is certified PDO/IGP[a]	☐	☐	☐	☐

[a] PDO, protected designation of origin, and PGI, protected geographical indication

5. Do you read the label on the honey you buy?

- ☐ Yes, always (go to question 6)
- ☐ Yes, sometimes (go to question 6)
- ☐ Yes, rarely (go to question 6)
- ☐ No (go to question 5.1)
- ☐ The honey I usually buy doesn't have a label (go to question 7)

5.1. Why don't you read the label? *Specify the reason* (go to question 8)

_____

6. Do you think that the information contained on the label is…?

- ☐ Sufficient (go to question 7)
- ☐ Insufficient (go to question 6.1)

6.1 What other information would you like to receive? Please specify below.

_____

7. How important is it for you see the following types of information on the label?
Likert scale 1-10, where 1=not at all important, 10=very important

Type of honey	1 2 3 4 5 6 7 8 9 10
Expiration date	1 2 3 4 5 6 7 8 9 10
Brand	1 2 3 4 5 6 7 8 9 10
Weight	1 2 3 4 5 6 7 8 9 10
Nutrition facts	1 2 3 4 5 6 7 8 9 10
Information on the producer	1 2 3 4 5 6 7 8 9 10
Storage conditions	1 2 3 4 5 6 7 8 9 10
Production date	1 2 3 4 5 6 7 8 9 10
Presence of other ingredients	1 2 3 4 5 6 7 8 9 10
Place of origin	1 2 3 4 5 6 7 8 9 10
Management of bred bees	1 2 3 4 5 6 7 8 9 10
Product analysis	1 2 3 4 5 6 7 8 9 10
Adoption of environmentally friendly farming practices	1 2 3 4 5 6 7 8 9 10
Adoption of bee-keeping farming practices	1 2 3 4 5 6 7 8 9 10
Adoption of breeding practices to improve product safety	1 2 3 4 5 6 7 8 9 10

8. Would you use the QR code via smartphone to access further information about the honey you buy?
The QR code is a two-dimensional barcode that contains machine-readable information (for example, by smartphone) about the item to which it is attached.

- ☐ Yes, I would use it

- ☐ No, I wouldn't use it
- ☐ I don't know

9. Would you be willing to pay a higher price for a package of honey if it offered you more information about the product? Choose the option you prefer by referring to a 500 gram package at a cost of 5.00 euros.

- ☐ No, I would not be willing to pay a higher price
- ☐ Yes, 5% more (about €0.25)
- ☐ Yes, 15% more (about €0.75)
- ☐ Yes, 25% more (about €1.25)
- ☐ Yes, regardless of the price increase

B. CONSUMPTION HABITS

10. Do you eat honey?

- ☐ No, never (go to question 10.1)
- ☐ Yes, rarely (go to question 11)
- ☐ Yes, sometimes (go to question 11)
- ☐ Yes, every day (go to question 11)

10.1 Why don't you eat honey? *Rank up to 4 options in order of importance on the right*

I don't like the consistency	____
It may contain antibiotics	____
I don't like the taste	____
I prefer other sweeteners	____
It may contain pesticides and other environmental chemicals	____
It is not suitable for my diet	____
It could be easily adulterated	____
It is inconvenient to use	____
It isn't a natural product	____
It may contain pathogenic micro-organisms	____
It contains too many calories	____
I'm not used to consuming it	____

Other (Specify _____)

(After question 10 go to question 12)

11. Why do you eat honey? *Rank up to 4 options in order of importance on the right*

It is free of antibiotics	____
It is produced with respect for the environment	____
It is good for health	____
For its nutritional value	____
It has fewer calories than other sweeteners	____
For its therapeutic properties	____
It is a quality product	____
I like the taste	____
It is safe to eat	____
It is a natural product	____

Other (specify_____)

C. HONEY AND PRODUCTION CHAIN – Knowledge and perceptions

12. In your opinion, are there any categories of people for whom the consumption of honey is NOT RECOMMENDED? (more answers are possible)

- ☐ Yes, overweight people/people with obesity problems (go to question 12.1)
- ☐ Yes, elderly people (go to question 12.1)
- ☐ Yes, children under 12 months (go to question 12.1)
- ☐ Yes, children over 12 months (go to question 12.1)
- ☐ Yes, pregnant women (go to question 12.1)
- ☐ Yes, people with diabetes (go to question 12.1)
- ☐ No (go to question 13)
- ☐ I don't know (go to question 13)

12.1 Why is honey not recommended for these categories of people?

_____

13. In general, how would you describe honey? Put an "x" closer to the adjective that better describes honey in your opinion.

			Neutral			
Traditional	☐	☐	☐	☐	☐	Modern
Natural	☐	☐	☐	☐	☐	Unnatural
Disgusting	☐	☐	☐	☐	☐	Tasty
Healthy	☐	☐	☐	☐	☐	Dangerous
Usual	☐	☐	☐	☐	☐	Unusual
Tainted	☐	☐	☐	☐	☐	Unspoiled
Rural	☐	☐	☐	☐	☐	Urban
Sustainable	☐	☐	☐	☐	☐	Unsustainable

14. Please report whether the following statements are true or false:

	True	False	I don't know
Crystallization of honey is a natural process	☐	☐	☐
Industrially produced honey is always subject to a thermal treatment (such as pasteurisation)	☐	☐	☐
Honey is always an organic product	☐	☐	☐

Honey is a nutritionally complete food	☐	☐	☐
Honey is a raw product	☐	☐	☐
Honey has a short shelf life	☐	☐	☐
The filtering process makes it possible to remove impurities from honey			
The colouring of honey derives from an artificial process used during its processing			

15. In your opinion, is honey dangerous to health?
- ☐ Yes (go to question 15.1)
- ☐ No (go to question 16)
- ☐ I don't know (go to question 16)

15.1 What risks do you associate with honey consumption? (possible more answers)

- ☐ Risks related to the presence of pathogenic microorganisms (provide an example_____)
- ☐ Risks related to the presence of environmental contaminants (provide an example_____)
- ☐ Risks related to the presence of drug residues (provide an example_____)
- ☐ Risks related to the presence of adulterants (provide an example_____)
- ☐ Nutritional risks (provide an example_____)
- ☐ Other risks (specify_____)

16. Report your level of agreement with the following statements.

	Not at all	Not much	Quite a lot	A lot
I think that the honey sold at the supermarket is a hygienically controlled product	☐	☐	☐	☐
I think that a lot of the honey on the market is produced with the addition of fructose and other sugars	☐	☐	☐	☐
The bio/organic and PDO certification is a guarantee of the quality of the honey	☐	☐	☐	☐
I think that there is a lot of adulterated honey on the market	☐	☐	☐	☐
The label is a useful tool to get information about the product I purchase	☐	☐	☐	☐
I think that honey bought directly from producer is hygienically safe	☐	☐	☐	☐

D. SOCIAL AND PERSONAL DATA

17. Year of birth: _____

18. Gender
- ☐ Female
- ☐ Male

19. Who do you live with? (more than one answer can be given)
- ☐ I live alone
- ☐ With my partner
- ☐ With my children (aged 0-12 months)
- ☐ With my children (over 12 months)
- ☐ With my birth family
- ☐ Other (specify_____)

20. Right now, are you following a diet?
- ☐ Yes (specify which kind of diet _____)
- ☐ No

21. Geographical area

22. Educational qualification
- ☐ Elementary school diploma
- ☐ Lower secondary school diploma
- ☐ Vocational qualification
- ☐ Upper secondary school diploma
- ☐ Bachelor's degree
- ☐ Master's degree
- ☐ Ph.D. or other post-graduate qualification

23. Occupation
- ☐ Student
- ☐ Looking for a first job
- ☐ Homemaker
- ☐ Employed
- ☐ Unemployed
- ☐ Retired

24. How well does your income meet your financial needs?
- ☐ Very easily
- ☐ Quite easily
- ☐ With some difficulties
- ☐ With many difficulties

QUESTIONNAIRE (*Italian version*)

Domanda filtro

1. Hai acquistato miele negli ultimi 12 mesi?
   - ☐ Sì (prosegui il questionario)
   - ☐ No (termina il questionario)

B. ABITUDINI DI ACQUISTO

3. Dove acquisti principalmente il miele? *È possibile selezionare al massimo 2 risposte*
   - ☐ Ipermercato/supermercato/discount
   - ☐ Alimentari di quartiere
   - ☐ Erboristeria/farmacia
   - ☐ Direttamente dal produttore
   - ☐ Mercato
   - ☐ Negozi biologici
   - ☐ Altro _____

4. Quali varietà di miele preferisci acquistare?
   - ☐ Specifica la/le tue varietà preferite _____
   - ☐ Non so

3. Che tipo di confezione acquisti prevalentemente?
   - ☐ Confezione monodose
   - ☐ Tubetto
   - ☐ Barattolo di vetro
   - ☐ Barattolo di plastica con dosatore
   - ☐ Altro Specifica_____
   - -

4. Quanto sono importanti per te i seguenti aspetti per la scelta del miele da acquistare?

	Per nulla	Poco	Abbastanza	Molto
Varietà del miele	☐	☐	☐	☐
Aspetto del miele (cristallizzato/liquido)	☐	☐	☐	☐
Colore	☐	☐	☐	☐
Che sia prodotto vicino a casa	☐	☐	☐	☐
Che sia certificato biologico	☐	☐	☐	☐
Che sia prodotto in Italia	☐	☐	☐	☐
Che sia di una marca/produttore noto	☐	☐	☐	☐
Che sia prodotto in Unione Europea	☐	☐	☐	☐
Che sia economico	☐	☐	☐	☐

Che sia certificato DOC/DOP/IGP	☐	☐	☐	☐

5. Leggi l'etichetta del miele che acquisti?
   - ☐ Sì, sempre *vai alla domanda 6*
   - ☐ Sì, qualche volta *vai alla domanda 6*
   - ☐ Sì, raramente *vai alla domanda 6*
   - ☐ No *vai alla domanda 5.1*
   - ☐ Il miele che acquisto solitamente non ha etichetta *vai alla domanda 7*

5.1. Perché non leggi l'etichetta? *Specifica di seguito la tua motivazione (vai alla domanda 8)*

_____

6. Ritieni che le informazioni sul miele disponibili in etichetta siano...?
   - ☐ Sufficienti *vai alla domanda 7*
   - ☐ Insufficienti *vai alla domanda 6.1*

6.1 Quali altre informazioni vorresti ricevere? *Riporta di seguito la tua risposta*

_____

_____

_____

7. Quanto ritieni importante ricevere informazioni su questi aspetti attraverso l'etichetta? Scala 1-10, dove 1=per nulla importante, 10=molto importante

Varietà di miele	1 2 3 4 5 6 7 8 9 10
Data di scadenza	1 2 3 4 5 6 7 8 9 10
Marca	1 2 3 4 5 6 7 8 9 10
Peso	1 2 3 4 5 6 7 8 9 10
Tabella nutrizionale	1 2 3 4 5 6 7 8 9 10
Informazioni sul produttore	1 2 3 4 5 6 7 8 9 10
Modalità di conservazione	1 2 3 4 5 6 7 8 9 10
Data di produzione	1 2 3 4 5 6 7 8 9 10
Presenza di altri ingredienti	1 2 3 4 5 6 7 8 9 10
Provenienza	1 2 3 4 5 6 7 8 9 10
Gestione delle api in allevamento	1 2 3 4 5 6 7 8 9 10
Analisi effettuate sul prodotto	1 2 3 4 5 6 7 8 9 10
Adozione di pratiche di allevamento rispettose dell'ambiente	1 2 3 4 5 6 7 8 9 10
Adozione di pratiche di allevamento rispettose delle api	1 2 3 4 5 6 7 8 9 10
Adozione di pratiche di allevamento per migliorare la sicurezza del prodotto	1 2 3 4 5 6 7 8 9 10

8. Utilizzeresti il *Qr code* tramite smartphone per accedere ad ulteriori informazioni riguardo al miele che acquisti?
Il QR code è un codice a barre bidimensionale composto da moduli neri disposti all'interno di uno schema di forma quadrata. Viene impiegato per memorizzare informazioni generalmente destinate ad essere lette tramite un telefono cellulare o uno smartphone.

- ☐ Sì, lo utilizzerei
- ☐ No, non lo utilizzerei
- ☐ Non saprei

9. Saresti disposto a pagare di più una confezione di miele che offra la possibilità di accedere a maggiori informazioni riguardo al prodotto? Scegli l'opzione che preferisci riferendoti ad una confezione di 500 grammi con un costo di €5.00

- ☐ No, non sono disposto a pagare di più
- ☐ Sì, il 5% in più (circa €0.25)
- ☐ Sì, il 15% in più (circa €0.75)
- ☐ Sì, il 25% in più (circa €1.25)
- ☐ Sì, indipendentemente dall'aumento di prezzo

B. PREFERENZE DI CONSUMO

10. Ti capita di consumare miele?

- ☐ No, mai (vai alla 10.1)
- ☐ Sì, ma solo in rare occasioni (vai alla 11)
- ☐ Sì, qualche volta (vai alla 11)
- ☐ Sì, quotidianamente (vai alla 11)

10.1 Per quali motivi NON consumi il miele? *Sposta nel riquadro a destra al massimo 4 opzioni in ordine di importanza*

Non mi piace la consistenza	____
Può contenere antibiotici	____
Non mi piace il gusto	____
Preferisco altri dolcificanti	____
Può contenere pesticidi e altre sostanze chimiche ambientali	____
Non è adatto alla mia dieta	____
Potrebbe essere facilmente adulterato	____
È scomodo da usare	____
Non è un prodotto naturale	____
Può contenere microrganismi patogeni	____
Contiene troppe calorie	____
Non sono abituato a consumarlo	____

Altro Specificare _____

(Vai alla domanda 12, alla sezione C. Conoscenza e percezione)

11. Quali sono i motivi per i quali consumi miele? *Sposta nel riquadro a destra al massimo 4 opzioni in ordine di importanza*

È privo di antibiotici	____
È prodotto nel rispetto dell'ambiente	____
Fa bene alla salute	____
Per il suo valore nutrizionale	____
È meno calorico rispetto ad altri dolcificanti	____

Per le sue proprietà terapeutiche ____
È un prodotto di qualità ____
Mi piace il gusto ____
È un prodotto sicuro da consumare ____
È un prodotto naturale ____

Altro, specificare_____

C. IL MIELE E LA FILIERA PRODUTTIVA – Conoscenze e percezioni

12. Esistono, a tuo parere, delle categorie di persone alle quali il consumo di miele è SCONSIGLIATO? (più opzioni possibili)

- ☐ Sì, le persone in sovrappeso/con problemi di obesità (vai alla 12.1)
- ☐ Sì, gli anziani (vai alla 12.1)
- ☐ Sì, i bambini di età inferiore ai 12 mesi (vai alla 12.1)
- ☐ Sì, i bambini di età superiore ai 12 mesi (vai alla 12.1)
- ☐ Sì, le donne in gravidanza (vai alla 12.1)
- ☐ Sì, le persone con diabete (vai alla 12.1)
- ☐ No (vai alla 13)
- ☐ Non so (vai alla 13)

12.1 Per quale motivo il miele è sconsigliato a queste persone? *Riporta di seguito la tua risposta*

_____

13. In generale, come definiresti il miele? Metti una X più vicina all'aggettivo che pensi sia più adeguato a descriverlo.

			Neutro			
Tradizionale	☐	☐	☐	☐	☐	Moderno
Naturale	☐	☐	☐	☐	☐	Artificiale
Disgustoso	☐	☐	☐	☐	☐	Appetitoso
Salutare	☐	☐	☐	☐	☐	Dannoso
Abituale	☐	☐	☐	☐	☐	Occasionale
Contaminato	☐	☐	☐	☐	☐	Incontaminato
Rurale	☐	☐	☐	☐	☐	Urbano
Sostenibile	☐	☐	☐	☐	☐	Non sostenibile

14. Indica se le seguenti affermazioni sono vere o false:

	Vero	Falso	Non so
La cristallizzazione è un processo naturale del miele	☐	☐	☐

Il miele prodotto industrialmente è sempre soggetto ad un trattamento termico (come ad esempio la pastorizzazione)	☐	☐	☐
Il miele è sempre un prodotto biologico	☐	☐	☐
Il miele è un alimento completo dal punto di vista nutrizionale	☐	☐	☐
Il miele è un prodotto crudo	☐	☐	☐
Il miele è un prodotto che ha una breve durata di conservazione	☐	☐	☐
Il processo di filtrazione permette di ripulire il miele dalle impurità			
La colorazione del miele deriva da un processo artificiale ottenuto durante la sua lavorazione			

15. Secondo te il miele è un alimento rischioso per la salute?
- ☐ Sì (vai alla 15.1)
- ☐ No (vai alla 16)
- ☐ Non saprei (vai alla 16)

15.1 Quali rischi associ al consumo di miele? (Ruotare gli item – possibili più opzioni)

- ☐ Rischi legati alla presenza di microrganismi patogeni (fai un esempio_____)
- ☐ Rischi legati alla presenza di contaminanti ambientali (fai un esempio_____)
- ☐ Rischi legati alla presenza di residui di farmaci (fai un esempio_____)
- ☐ Rischi legati alla presenza di sostanze adulteranti (fai un esempio_____)
- ☐ Rischi di tipo nutrizionale (fai un esempio_____)
- ☐ Altri rischi (specifica_____)

16. Indica il tuo grado di accordo con le seguenti affermazioni.

	Per nulla	Poco	Abbastanza	Molto
Penso che il miele che si trova al supermercato sia un prodotto controllato dal punto di vista igienico sanitario	☐	☐	☐	☐
Penso che molto del miele in commercio sia prodotto con l'aggiunta di fruttosio e altri zuccheri	☐	☐	☐	☐
La certificazione (bio, dop, ...) è una garanzia della qualità del miele	☐	☐	☐	☐
Penso che ci sia molto miele adulterato in commercio	☐	☐	☐	☐
L'etichetta è un utile strumento per avere informazioni sul prodotto che acquisto	☐	☐	☐	☐
Penso che il miele che si acquista direttamente dal produttore sia sicuro dal punto di vista igienico sanitario	☐	☐	☐	☐

D. DATI SOCIOANAGRAFICI

25. Anno di nascita: _____

26. Genere
- ☐ Femminile
- ☐ Maschile

27. Vivi (sono possibili più risposte)
- ☐ Da solo (risposta esclusiva)
- ☐ Con partner
- ☐ Con figli (0-12 mesi)
- ☐ Con figli (da 1 anno in su)
- ☐ Con la famiglia di origine
- ☐ Altro (specificare)_____

28. In questo momento stai seguendo qualche dieta alimentare?
- ☐ Sì, specifica_____
- ☐ No

29. Provincia di residenza *Menù a tendina* _____

30. Qual è il tuo titolo di studio?
- ☐ Licenza di scuola elementare
- ☐ Licenza di scuola media inferiore
- ☐ Qualifica professionale
- ☐ Diploma di scuola secondaria superiore
- ☐ Diploma universitario
- ☐ Laurea
- ☐ Specializzazione post laurea/Master/Dottorato

31. Qual è la tua condizione occupazionale?
- ☐ Studente
- ☐ In cerca di prima occupazione
- ☐ Casalinga/o
- ☐ Occupata/o
- ☐ Disoccupata/o
- ☐ Pensionata/o

32. Per quanto riguarda le tue risorse finanziarie, come arrivi a fine mese?
- ☐ Molto facilmente
- ☐ Abbastanza facilmente
- ☐ Con qualche difficoltà
- ☐ Con molte difficoltà

APPENDIX 2

Label	Answers (in Italian language)
Exact provenience of the honey	Luogo esatto di produzione Paese di provenienza nello specifico Da dove arriva veramente Zona di produzione, composizione Paese di origine scritto chiaramente e 100% della stessa origine e qualità Specifica zona di produzione Luogo dei api Zona Zona di prelievo delle api Zona specifica di produzione Zona dove viene estratto il miele Paesi di origine Che si capisca in quale nazione è prodotto con facilità Non sempre si trova il luogo di raccolta del miele, ma solo il luogo di confezionamento Che sia ben leggibile il luogo di produzione Località di Raccolta e produzione Dove crescono esattamente i fiori dai quali è prodotto. Vicino a fabbriche? Strade? L'esatta provenienza del miele. Le calorie Maggiori indicazioni su dove sono state collocate le arnie, soprattutto in caso di spostamenti per cercare un polline particolare monotipo... Maggiori informazioni sui luoghi e i metodi di produzione Filiera di provenienza Zona e periodo di raccolta Zona di provenienza, indicazioni sulle proprietà, controindicazioni. Quando e dove è prodotto. Di solito non c'è Scritto Il luogo di provenienza specificato per bene Origine effettiva Il luogo della raccolta e produttore Origine mese di produzione zona di produzione La filiera L'origine, non sempre specificata, ed il tipo di filiera Dove, quando, come. Luogo di produzione, luogo di confezionamento, altezza luogo di produzione dal livello del mare Origine più evidente deve essere sul vasetto Che sia meglio specificata la provenienza e il produttore (confezionato presso non ha senso) Vorrei si identificasse meglio l'area geografica in cui è prodotto Da chi, dove è prodotto, se bio e se eticamente raccolto (no affumicatura api) La filiera di produzione Provenienza Zona di produzione non solo la nazione

	Filiera Il luogo preciso di produzione Maggior dettaglio sui paesi di origine e sulla qualità del miele Tutto il ciclo bisogna riportare Area, azienda e periodo della raccolta Area di raccolta Zona produzione non solo stato Provenienza calorie raccolta Reale zona di produzione Origine territoriale, manipolazione I campi dove si nutrono le api. Le qualità di alberi o fiori presenti nel campo. Eventuali trattamenti subiti dalle piante. Anno produzione, regione, ingredienti Informazioni sulla provenienza Zona di produzione Località di produzione Non mi basta sapere che è prodotto in Italia, voglio sapere dove. Chi lo produce, zona La zona di provenienza specifica Quale parte dell'Ue di dove sono prodotti Il luogo di allevamento delle api e gli anni
Composition	Zona di produzione, composizione Paese di origine scritto chiaramente e 100% della stessa origine e qualità Zucchero presente, se ci sono conservanti aggiunti Come è fatto Se naturale o con aggiunta di componenti non naturali Se è chimico o fatto con vere api Anno produzione, regione, ingredienti Mese di raccolta per poter valutare l'assenza di sciroppi. Se c'è zucchero aggiunto
Beekeeping	Produzione e metodo di allevamento non dannoso delle api Maggiori informazioni sui luoghi e i metodi di produzione Manca la mortalità delle api in zona dove è stato l alveare Dove, quando, come. Come vengono allevate le api Da chi, dove è prodotto, se bio e se eticamente raccolto (no affumicatura api)
Beekeeper	Che sia meglio specificata la provenienza e il produttore (confezionato presso non ha senso) Da chi, dove è prodotto, se bio e se eticamente raccolto (no affumicatura api) Chi lo produce, zona
Nutritional values	L'esatta provenienza del miele. Le calorie Calorie
Contraindication	Zona di provenienza, indicazioni sulle proprietà, controindicazioni.
Not meaningful answers	Di più Maggiori specifiche Non saprei Non tutti i tipi di miele hanno le etichette esaustive Non so

	Non so Filiera Vorrei ricevere informazioni più dettagliate e non le solite diciture generiche. Comunque acquisto raramente il miele nei negozi e quindi non mi pongo ulteriori questioni. Carenti

CONSUMERS' OPINIONS, PERCEPTIONS AND BEHAVIOURS RELATED TO THE PURCHASE AND CONSUMPTION OF HONEY IN AUSTRIA

Report edited by the Health Awareness and Communication Department of the IZSVe

SUMMARY

INTRODUCTION

The Health Awareness and Communication Department of the Istituto Zooprofilattico Sperimentale delle Venezie, in collaboration with the Austrian Agency for Health and Food Safety, realized the survey as part of the WP7 "New traceability system" of the BPRACTICES project.

The questionnaire consisted of the following sections:

- Socio-demographic characteristics
- Purchasing behaviours

The questionnaire was created online by means of the IZSVeSurvey application (created from the LimeSurvey software) and disseminated between October 7th and December 1st, 2019, through all the communication channels of the project and of the project partners involved (web sites, social media, newsletter…)

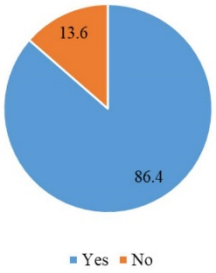
The honey buyers were selected through a screening question placed at the beginning of the questionnaire: those who declared they had not bought honey in the last 12 months did not fill in the questionnaire.

The data were treated according to the General Data Protection Regulation (EU) 2016/679.

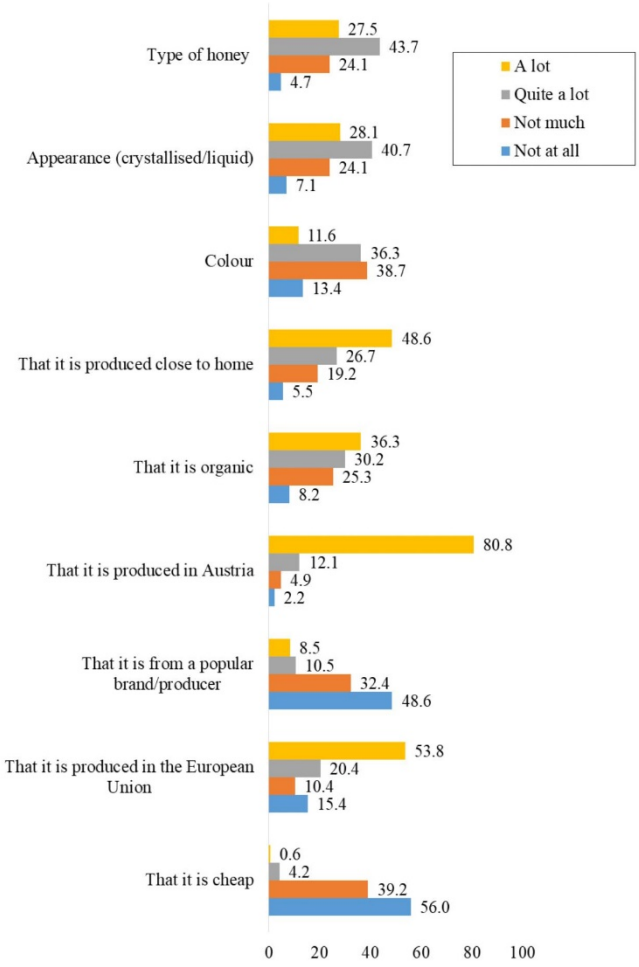
RESULTS

Purchasing behaviours

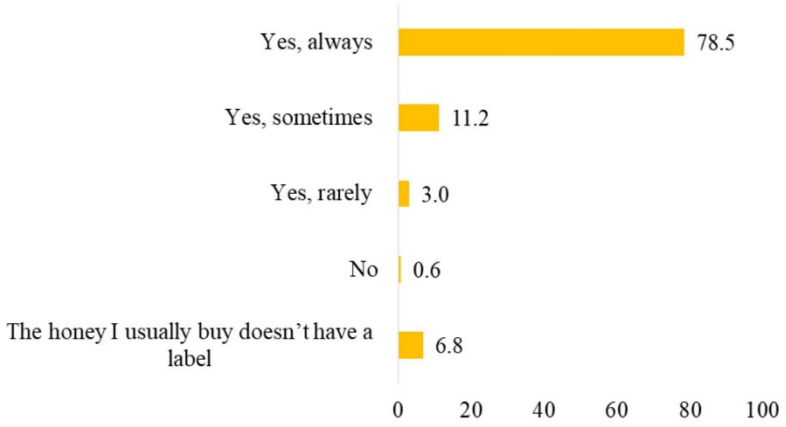
0. Have you bought honey in the last 12 months? (n=736, %)



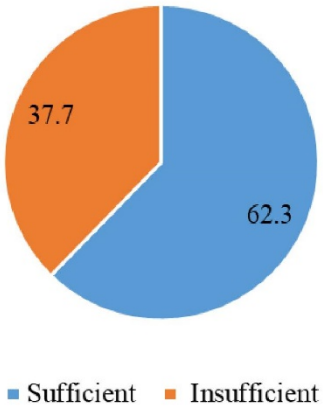
1. How important are the following aspects when you are choosing which honey to buy? (n=636, %)



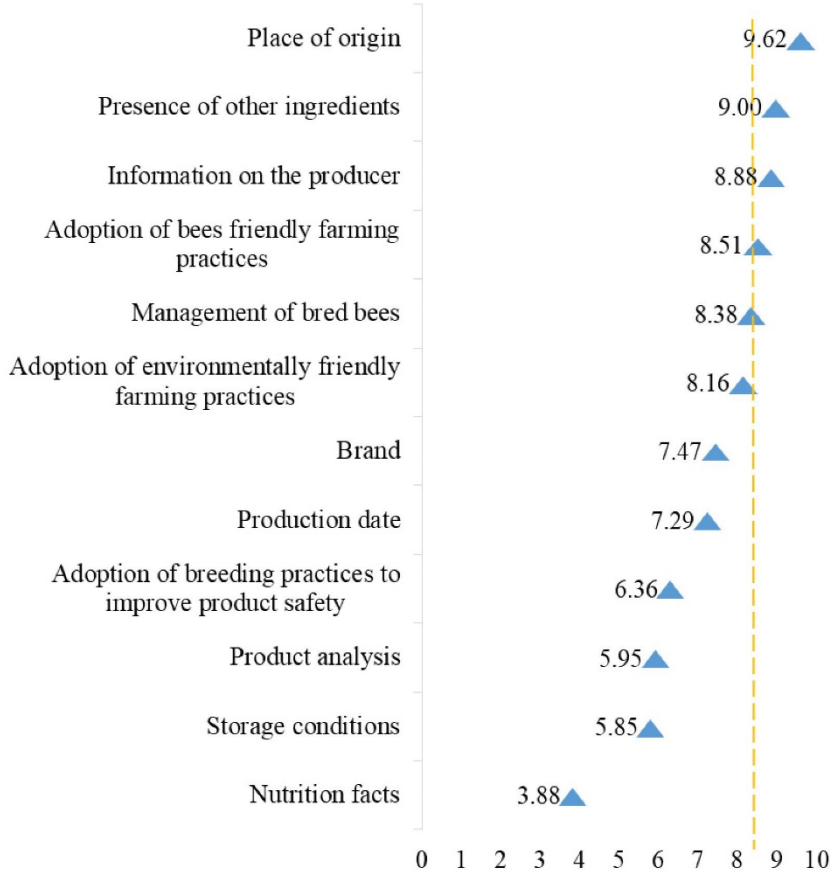
2. Do you read the label on the honey you buy? (n=636, %)



3. Do you think that the information contained on the label is…? (To this and the following question (4) answered only those who have selected "Yes, always", "Yes, sometimes" or "Yes, rarely" in the previous question, n=589, %)

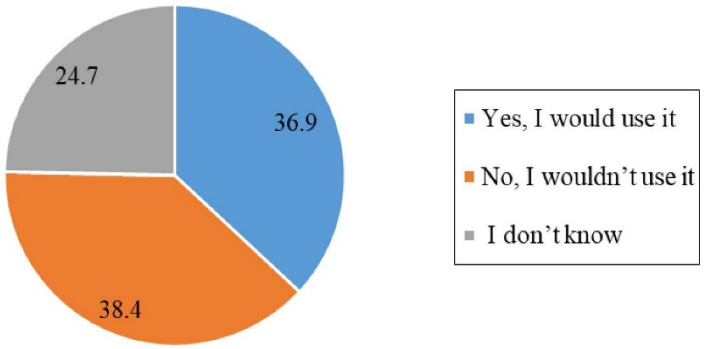


4. How important is it for you see the following types of information on the label? *Likert scale 1-10, where 1=not at all important, 10=very important* (n=589, mean value)

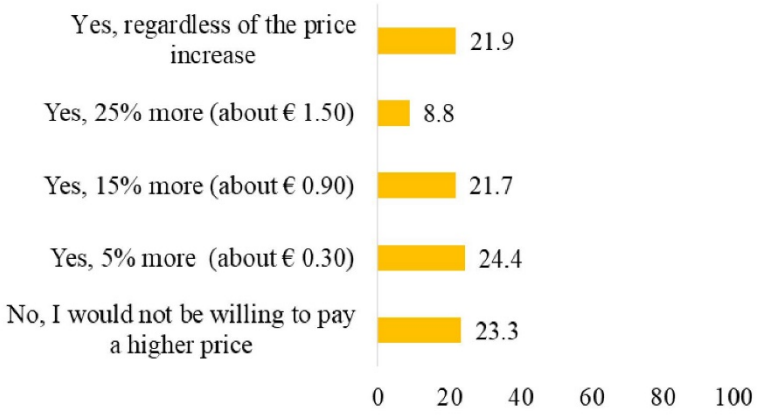


5. Would you use the QR code via smartphone to access further information about the honey you buy? (n=636, %)

The QR code is a two-dimensional barcode that contains machine-readable information (for example, by smartphone) about the item to which it is attached.



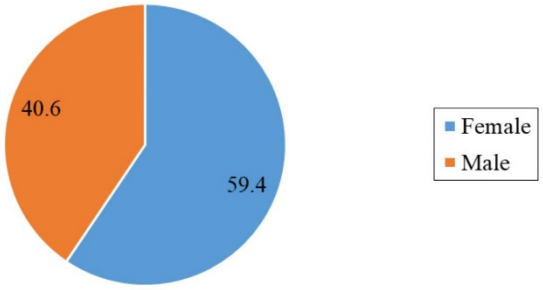
6. Would you be willing to pay a higher price for a package of honey if it offered you more information about the product? Choose the option you prefer by referring to a 500 gram package at a cost of 6.00 euros (n=636, %)



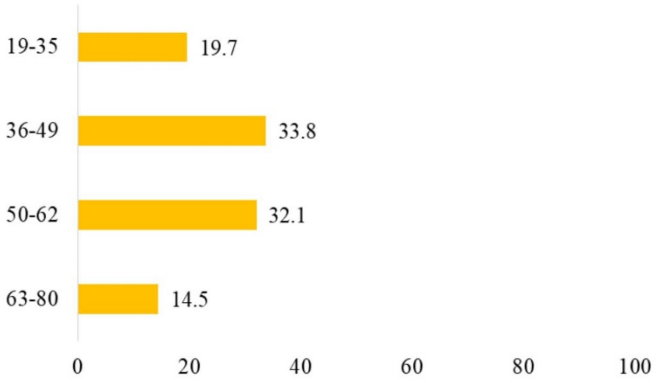
BPRACTICES

Socio-demographic characteristics

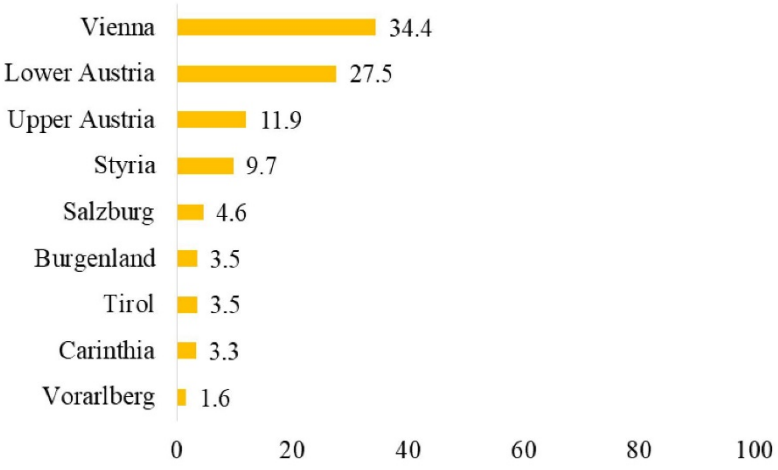
7. Gender (n=636, %)



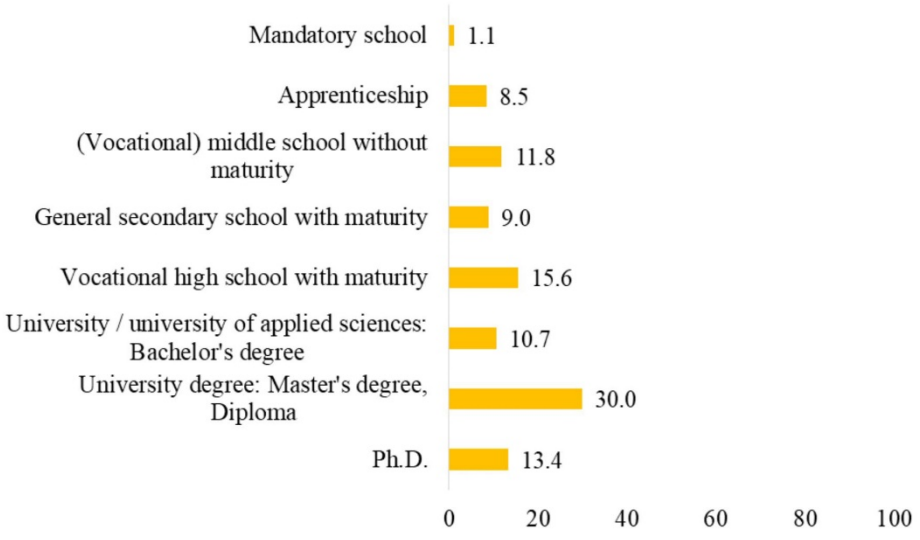
8. Year of birth (age classes) (n=636, %)



9. Geographical area (n=636, %)



10. Educational qualification (n=636, %)



APPENDIX 1

Questionnaire German version

0. Haben Sie in den letzten 12 Monaten Honig gekauft?
   - ☐ Ja (Weiter)
   - ☐ Nein (Fragebogen beenden)

1. Wie wichtig sind Ihnen die folgenden Eigenschaften beim Honigkauf?

	Unwichtig	Weniger wichtig	Wichtig	Sehr wichtig
Honigsorte	☐	☐	☐	☐
Zustand (kristallisiert/flüssig)	☐	☐	☐	☐
Farbe	☐	☐	☐	☐
Dass er aus der Umgebung des Wohnortes stammt	☐	☐	☐	☐
Dass er aus einer Bioimkerei stammt	☐	☐	☐	☐
Dass er aus Österreich stammt	☐	☐	☐	☐
Dass es sich um eine bekannte Marke/einen bekannten Lieferanten handelt	☐	☐	☐	☐
Dass er aus der EU stammt	☐	☐	☐	☐
Dass er billig ist	☐	☐	☐	☐

2. Lesen Sie das Etikett des Honigs, den Sie kaufen?
   - ☐ Ja, immer (weiter zu Frage 3)
   - ☐ Ja, manchmal (weiter zu Frage 3)
   - ☐ Ja, selten (weiter zu Frage 3)
   - ☐ Nein (weiter zu Frage 5)
   - ☐ Der Honig, den ich üblicherweise kaufe, hat kein Etikett (weiter zu Frage 5)

3. Halten Sie die Information auf dem Etikett für
   - ☐ ausreichend
   - ☐ nicht ausreichend

4. Wie wichtig sind Ihnen die folgenden Informationen auf dem Etikett? *Bewertungsskala 1-10, wobei 1=unwichtig, 10=sehr wichtig*

Marke / Abfüller	1 2 3 4 5 6 7 8 9 10
Nährwertangaben	1 2 3 4 5 6 7 8 9 10
Information zum Hersteller / ImkerIn	1 2 3 4 5 6 7 8 9 10
Lagerbedingungen	1 2 3 4 5 6 7 8 9 10
Datum der Herstellung	1 2 3 4 5 6 7 8 9 10
Andere Zutaten	1 2 3 4 5 6 7 8 9 10
Herkunft	1 2 3 4 5 6 7 8 9 10
Betriebsweise (konventionell/bio)	1 2 3 4 5 6 7 8 9 10
Analyseergebnisse zum Produkt	1 2 3 4 5 6 7 8 9 10
Anwendung umweltfreundlicher Imkereipraxis	1 2 3 4 5 6 7 8 9 10
Anwendung bienenfreundlicher Imkereipraxis	1 2 3 4 5 6 7 8 9 10

Anwendung züchterischer Methoden zur Erhöhung der Produktsicherheit 1 2 3 4 5 6 7 8 9 10

5. Würden Sie den QR-Code über Smartphone nutzen, um weitere Informationen über den Honig, den Sie kaufen, zu erhalten?

Der QR-Code ist ein zweidimensionaler Barcode, der maschinenlesbare Informationen (z.B. per Smartphone) zum Produkt enthält, auf dem er sich befindet.

- ☐ Ja, ich würde ihn verwenden
- ☐ Nein, ich würde ihn nicht verwenden
- ☐ Ich weiß es nicht

6. Wären Sie bereit einen höheren Preis für ein Glas Honig zu bezahlen, wenn es mehr Informationen zum Produkt bietet? Treffen Sie Ihre Wahl bezogen auf einen Honigpreis von 6.00 Euro pro 500 Gramm.

- ☐ Nein, ich bin nicht bereit einen höheren Preis zu bezahlen.
- ☐ Ja, 5% mehr (ungefähr €0.30)
- ☐ Ja, 15% mehr (ungefähr €0.90)
- ☐ Ja, 25% mehr (ungefähr €1.50)
- ☐ Ja, unabhängig von der Preissteigerung

7. Geschlecht

- ☐ Weiblich
- ☐ Männlich

8. Geburtsjahr: _____

9. Bundesland

- ☐ Vorarlberg
- ☐ Tirol
- ☐ Salzburg
- ☐ Oberösterreich
- ☐ Niederösterreich
- ☐ Burgenland
- ☐ Steiermark
- ☐ Kärnten
- ☐ Wien

10. Höchste abgeschlossene Ausbildung

- ☐ Pflichtschule
- ☐ Lehrabschluss
- ☐ (Berufsbildende) mittlere Schule ohne Matura
- ☐ Allgemeinbildende höhere Schule mit Matura
- ☐ Berufsbildende höhere Schule mit Matura
- ☐ Universität/Fachhochschule: Bachelor
- ☐ Universität/Fachhochschule: Master, Diplom
- ☐ Doktorat, PhD

CONSUMERS' OPINIONS, PERCEPTIONS AND BEHAVIOURS RELATED TO THE PURCHASE AND CONSUMPTION OF HONEY IN SLOVENIA

Report edited by the Health Awareness and Communication Department of the IZSVe

SUMMARY

INTRODUCTION

The Health Awareness and Communication Department of the Istituto Zooprofilattico Sperimentale delle Venezie, in collaboration with the Agricultural Institute of Slovenia, realized the survey as part of the WP7 "New traceability system" of the BPRACTICES project.

The questionnaire consisted of the following sections:

- Socio-demographic characteristics
- Purchasing behaviours

The questionnaire was created online by means of the IZSVeSurvey application (created from the LimeSurvey software) and disseminated between October 7th and December 15th, 2019, through all the communication channels of the project and of the project partners involved (web sites, social media, newsletter…)

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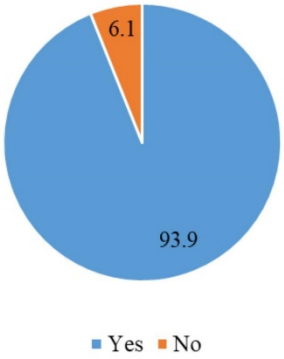
The data were treated according to the General Data Protection Regulation (EU) 2016/679.

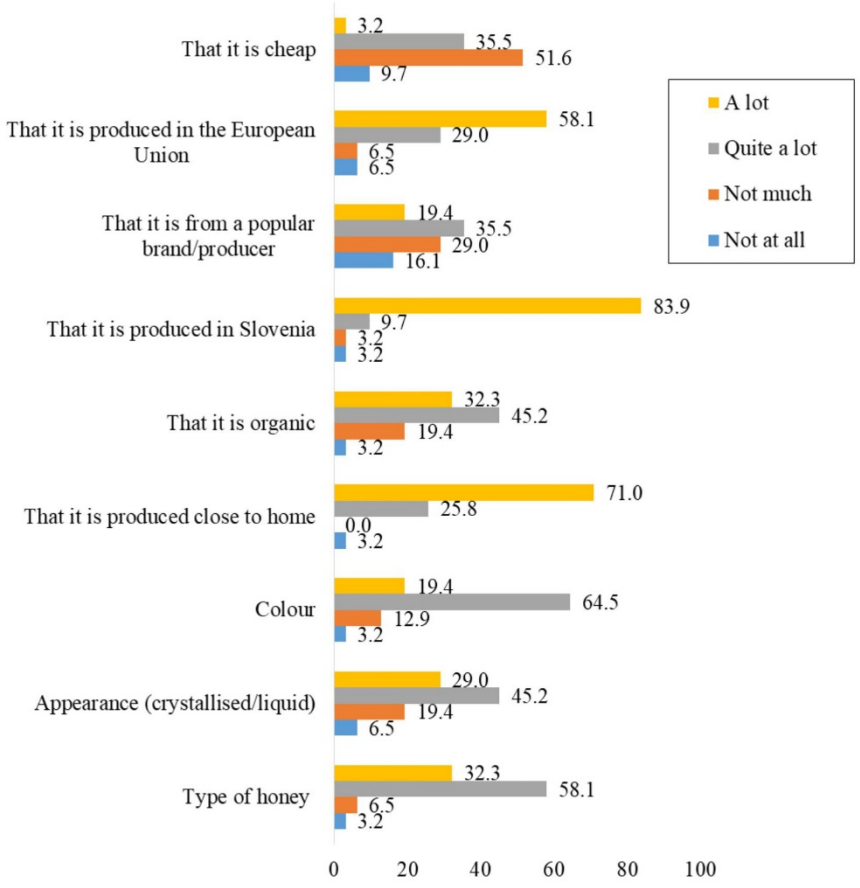
RESULTS

Purchasing behaviours

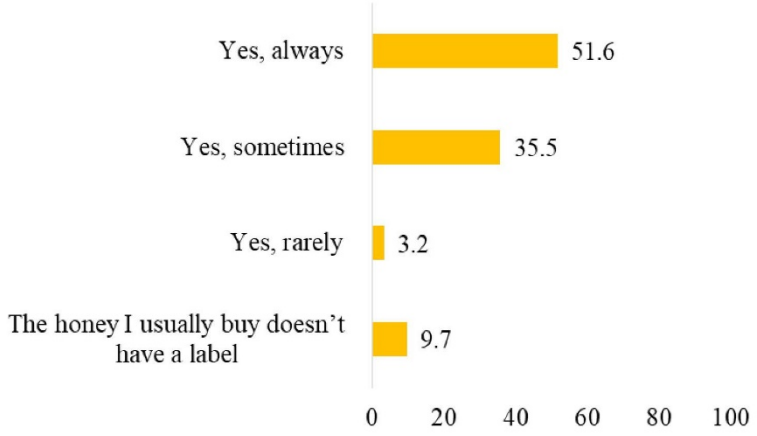
0. Have you bought honey in the last 12 months? (n=33, %)



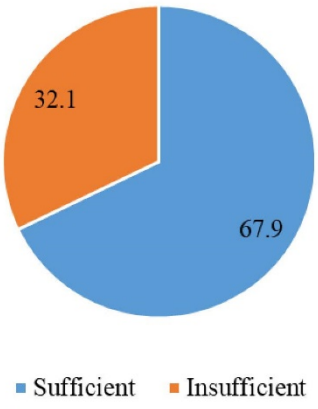
1. How important are the following aspects when you are choosing which honey to buy? (n=31, %)



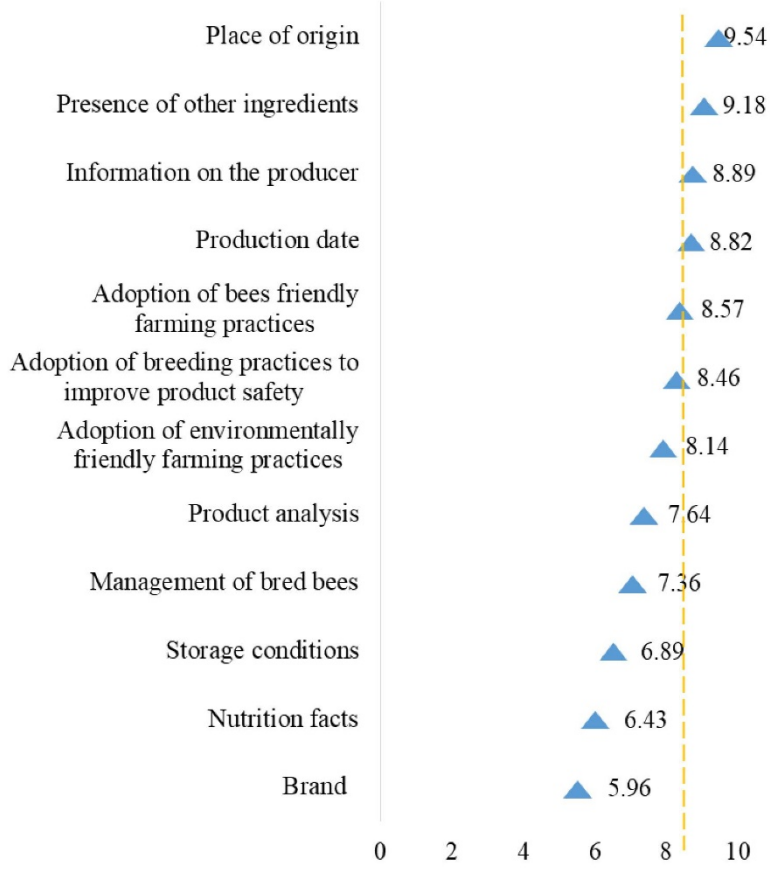
2. Do you read the label on the honey you buy? (n=31, %)



3. Do you think that the information contained on the label is…? (To this and the following question (4) answered only those who have selected "Yes, always", "Yes, sometimes" or "Yes, rarely" in the previous question, n=28, %)

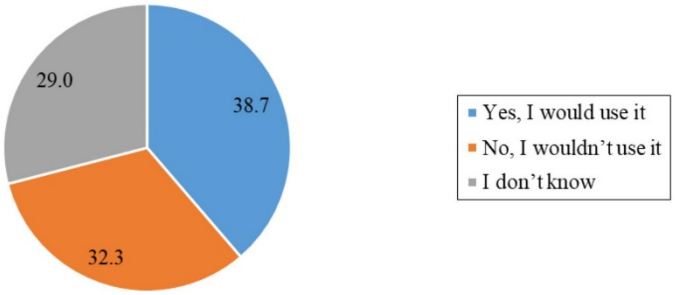


4. How important is it for you see the following types of information on the label? *Likert scale 1-10, where 1=not at all important, 10=very important* (n=28, mean value)

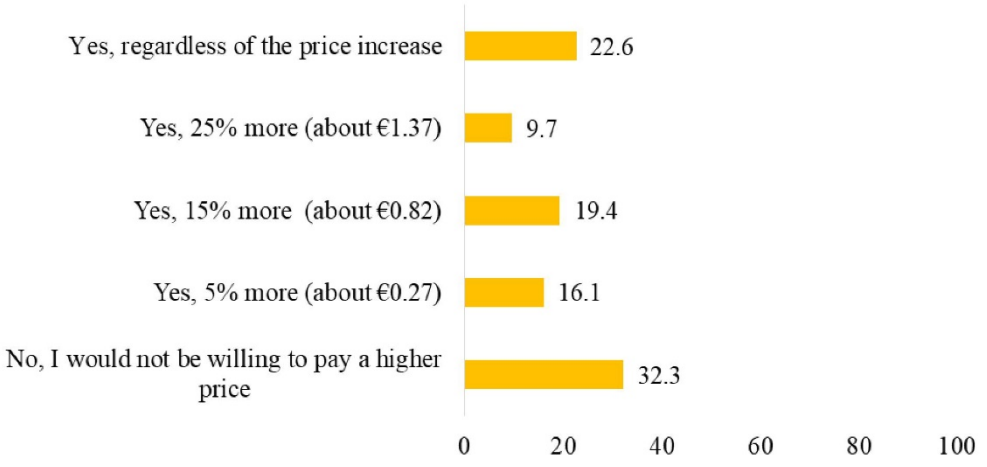


5. Would you use the QR code via smartphone to access further information about the honey you buy? (n=31, %)

The QR code is a two-dimensional barcode that contains machine-readable information (for example, by smartphone) about the item to which it is attached.



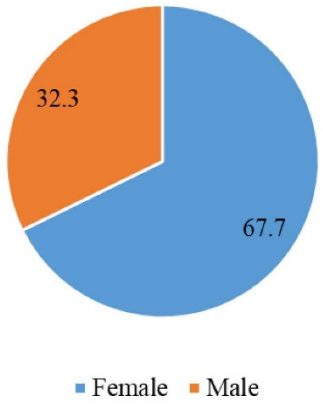
6. Would you be willing to pay a higher price for a package of honey if it offered you more information about the product? Choose the option you prefer by referring to a 450 gram package at a cost of 5.5 euros (n=31, %)



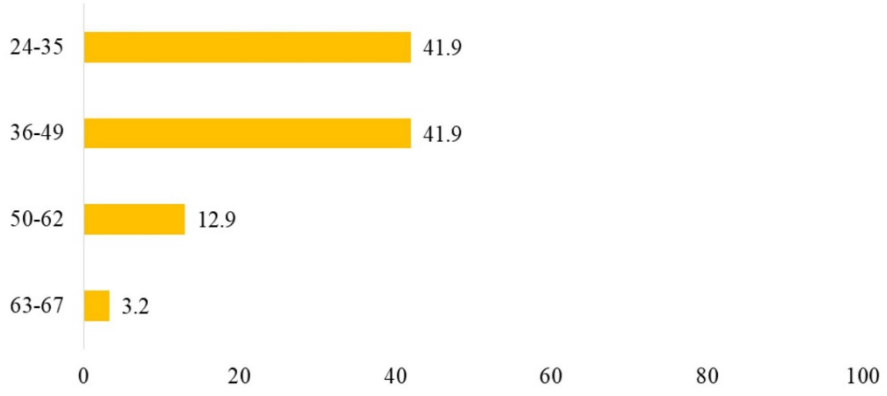
BPRACTICES

Socio-demographic characteristics

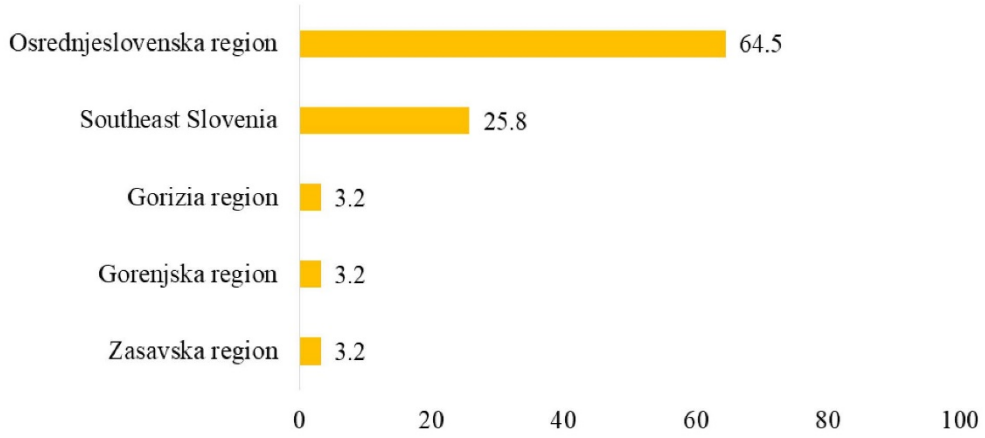
7. Gender (n=31, %)



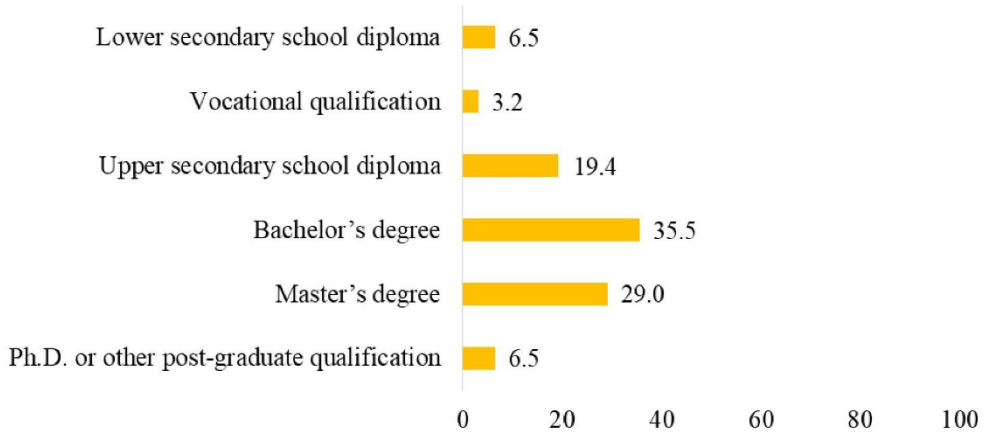
8. Year of birth (age classes) (n=31, %)



9. Geographical area (n=31, %)



10. Educational qualification (n=31, %)



APPENDIX 1

Questionnaire Slovenian version

Vprašalnik

0. Ste v zadnjem letu kupili med ?
   - ☐ Da (nadaljujte z izpolnjevanjem vprašalnika)
   - ☐ Ne (prosimo vas, da vprašalnika ne izpolnjujete)

1. Kako pomemba so vam naslednja dejstva, ko se odločate za nakup medu?

	Popolnoma nepomembno	Nepomembno	Pomembno	Zelo pomembno
Vrsta medu	☐	☐	☐	☐
Izgled (kristaliziran/tekoč)	☐	☐	☐	☐
Barva	☐	☐	☐	☐
Da je lokalno pridelan	☐	☐	☐	☐
Da je ekološko pridelan	☐	☐	☐	☐
Da je pridelan v Sloveniji	☐	☐	☐	☐
Da je priznane blagovne znamke/priznanega pridelovalca	☐	☐	☐	☐
Da je pridelan v Evropski uniji	☐	☐	☐	☐
Da je cena nizka	☐	☐	☐	☐

2. ali preberete deklaracijo na medu, ki ga kupite?

   - ☐ Vedno (pojdite na vprašanje 3)
   - ☐ Občasno (pojdite na vprašanje 3)
   - ☐ Redko (pojdite na vprašanje 3)
   - ☐ Ne (pojdite na vprašanje 5)
   - ☐ Med, ki ga običajno kupujem, nima deklaracije (pojdite na vprašanje 5)

3. So informacije na deklaraciji po vaše…?

   - ☐ Zadostne
   - ☐ Nezadostne

4. Kako pomembne so vam naslednje informacije iz deklaracije? *Likertova lestvica 1-10, kjer je 1=popolnoma nepomembno, 10=zelo pomembno*

Znamka	1 2 3 4 5 6 7 8 9 10
Hranilna vrednost	1 2 3 4 5 6 7 8 9 10
Informacije o pridelovalcu	1 2 3 4 5 6 7 8 9 10
Pogoji shranjevanja	1 2 3 4 5 6 7 8 9 10
Datum pridelave	1 2 3 4 5 6 7 8 9 10
Prisotnost drugih sestavin	1 2 3 4 5 6 7 8 9 10

Kraj porekla	1 2 3 4 5 6 7 8 9 10
Čebelarska praksa	1 2 3 4 5 6 7 8 9 10
Analize pridelka	1 2 3 4 5 6 7 8 9 10
Uporaba okolju prijaznih čebelarskih praks	1 2 3 4 5 6 7 8 9 10
Uporaba čebelam prijaznih čebelarskih praks	1 2 3 4 5 6 7 8 9 10
Uporaba čebelarskih praks, ki pripomorejo k večji varnosti pridelkov	1 2 3 4 5 6 7 8 9 10

5. ali bi uporabljali QR kodo, da bi prek pametnega telefona dostopali do več podatkov o medu, ki ga kupujete? QR koda je dvodimenzionalna koda, ki vsebuje informacije za branje z napravami (pametni telefon, tablični računalnik) o produktu, na katerega je pritrjena.

- ☐ Da, bi jo uporabljal
- ☐ Ne, ne bi je uporabljal
- ☐ Ne vem

6. Bi bili pripravljeni plačati višjo ceno za proizvod, če bi vam bilo na voljo več informacij o njem? Izberite možnost, ki vam najbolj ustreza ob predpostavki, da 450g medu stane 5,5, EUR.

- ☐ Ne nebi plačal višje cene
- ☐ Da, plačal bi 5% več (približno €0.27)
- ☐ Da, 15% več (približno €0.82)
- ☐ Da, 25% več (približno €1.37)
- ☐ Da, ne glede na ceno

7. Spol

- ☐ Ženski
- ☐ Moški

8. Leto rojstva: _____

9. Regija (Savinjska regija, Zasavska regija, Posavska regija, Jugovzhodna Slovenija, Osrednjeslovenska regija, Gorenjska regija, Primorsko-notranjska regija, Goriška regija, Obalno-kraška regija, Podravska regija, Koroška regija)

10. Vaša najvišja dosežena stopnja izobrazbe:

- ☐ Osnovnošolska izobrazba
- ☐ Srednješolska izobrazba
- ☐ Poklicna izobrazba
- ☐ Visokošolska izobrazba
- ☐ Višješolska izobrazba
- ☐ Magistrska izobrazba
- ☐ Doktorska ali druga podiplomska izobrazba

Annex 20

CONSUMERS' OPINIONS AND PERCEPTIONS RELATED TO THE TRACEABILITY SYSTEM FOR ACCESSING INFORMATION ON HONEY

Report edited by the Health Awareness and Communication Department of the IZSVe

SUMMARY

INTRODUCTION

The Health Awareness and Communication Department of the Istituto Zooprofilattico Sperimentale delle Venezie was involved as a research partner within the ERA-NET SUSAN[1] project titled 'New indicators and on-farm practices to improve honeybee health in the Aethina Tumida era in Europe'.

In line with subtask 3.2 of Work Package 7 (WP7), social research methods (focus groups and questionnaires) were applied to identify the weaknesses and strengths of a traceability system based on the QRCode/RFID technology that was developed and implemented during the project. The traceability system allows consumers to access a web page with information on honey features suggested by beekeepers.

Participants were asked to access the web page via the QRCode applied on the honey jar.

www.smielatura.it/lotto/lotto.php?lotto=01072018

Two focus groups were held in Bologna and Padova (Italy) on May 20th and 28th.

- ✓ *First focus group*: May 20th, 2019, Bologna (IT)
  Participants: 11 honey buyers

- ✓ *Second focus group*: May 28th, 2019, Padova (IT)
  Participants: 14 honey buyers

Moreover, a paper-and-pencil self-administered survey was carried out between June 11 and 12, 2019, at FICO Eataly World (Bologna) with the support of the CONAPI (Italian National Consortium of Beekeepers) Association. Two experts belonging to the research team showed the interviewees the traceability system and provided support while they tested the QRCode/RFID technology. Then, the interviewees were invited to complete a questionnaire composed of 10 questions.

The data were handled according to the General Data Protection Regulation (EU) 2016/679.

[1] European Research Area NETwork on Sustainable Animal Production

FOCUS GROUPS

Participants' socio-demographic characteristics

A total of 25 honey buyers were selected through the 'snowball sampling' method. No stratification by gender or age was used.

The focus group discussion was led by a moderator in the presence of an observer, who were both members of the research team. A draft of the discussion is included in Appendix 1.

After being introduced to the research project, the participants were invited to introduce themselves. Their socio-demographic characteristics are reported in Table 1.

Table 1. Socio-demographic characteristics of the focus group participants (n=25)

Characteristics	***n***	***%***
Gender		
Female	**21**	**84**
Male	4	16
Age		
18-35	**18**	**72**
36-49	7	28
Qualification		
High school diploma	8	32
University degree	**15**	**60**
Master or Ph.D.	2	8
Occupation		
Student	4	16
Employed	**21**	**84**
Housing situation		
Partner	6	24
Partner and children	6	24
Family of origin	6	24
Alone	6	24
Other (friends)	1	4

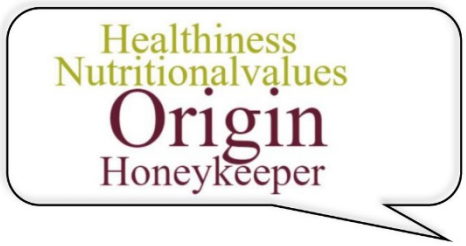
BPRACTICES

Buying habits and label preferences

At the beginning of the discussion, the participants were asked to describe their buying habits and preferences regarding the information that they look for on labels. From the two focus group discussions, it emerged that honey was most frequently purchased at 'local markets' and 'supermarkets' (Figure 1). Moreover, the 'origin' of honey was the most sought after information on the label (Figure 2).

Figure 1. *Where do you buy honey most frequently?* (n=25)

Figure 2. *What information do you search for on the label?* (n=25)



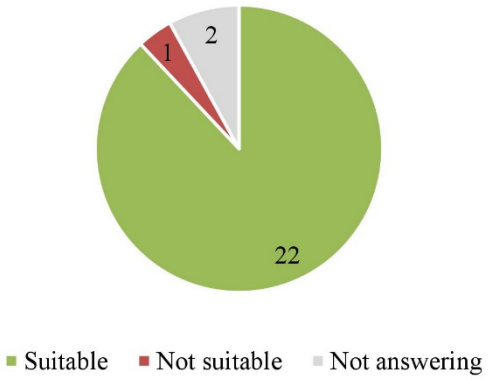
BPRACTICES

Assessment of the traceability system

Participants were then invited to use their smartphones to access the web application directly from the QRCode placed on the honey jar. After individually testing the traceability system and visiting the web platform linked to the QRCode, participants were asked to complete a brief questionnaire on the web platform (Appendix 2). Then, the design of the web application and the information available through the QRCode were discussed at length and evaluated to identify the weaknesses and strengths of the traceability system. The answers provided in the questionnaire and the main topics that emerged from the collective discussion are reported below.

In the questionnaire, the majority of respondents (n=22) reported that the QRCode was 'suitable' for accessing the web page (Figure 3).

Figure 3. *In your opinion, the choice to use the QR code to access the web page is* (n=25)



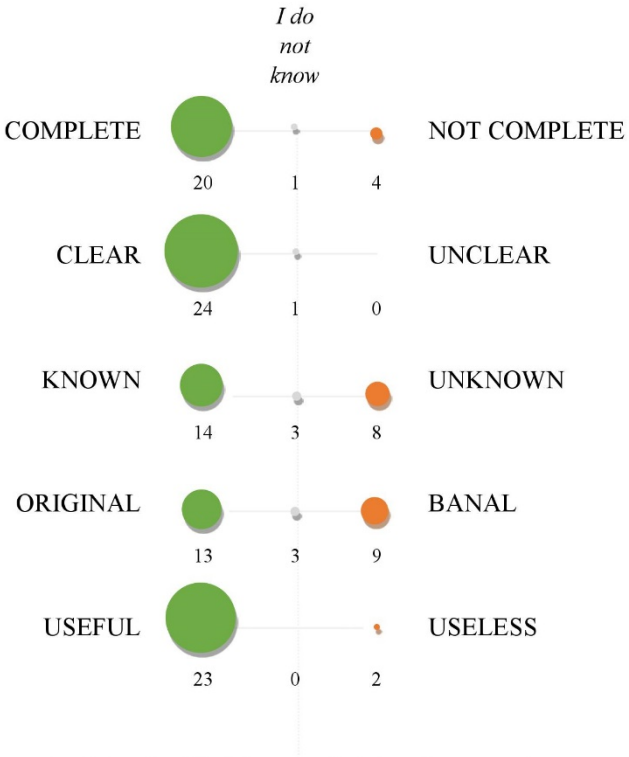
In the discussion, most of the respondents reported the possible difficulty of using the QRCode for those unfamiliar with the technology (e.g., elderly people). In the participants' opinion, the QRCode should not be the only way to access the product information; additional suggested ways are listed in Table 2.

Table 2. Participants' suggestions regarding ways to access information

Suggestions synthesis
The QRCode should be integrated into the label, not used as a substitute
The link to the website should be written on the label/jar
Access to information should be provided via a social network
Additional ways of accessing information should be based on the different target groups (elderly people)

When asked to 'Evaluate the honey content on the web page. Choose the most appropriate one for each pair of adjectives', the majority of respondents assessed the content as 'complete', 'clear', 'known', 'original', and 'useful' (Figure 4).

Figure 4. *Evaluate the honey content on the web page. Choose the most appropriate one for each pair of adjectives* (n=25)



During the discussion, potential and critical issues related to the content presented on the web page emerged, and some improvements were proposed. The collected comments are synthetized in Table 3.

Table 3. Participants' opinions of the web page content

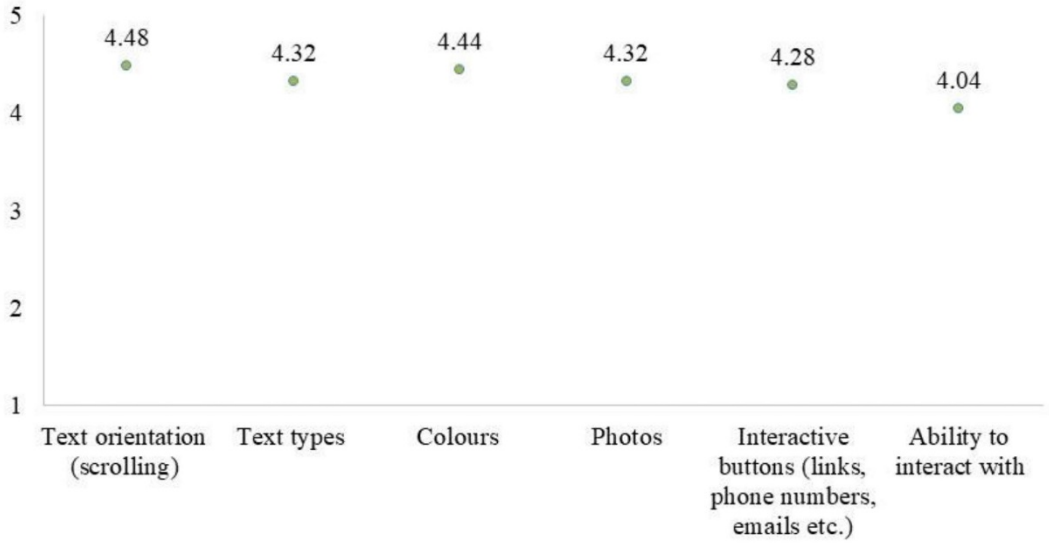
Topics covered	*Positive issues*	*Negative issues*	*Improvements*
Order of contents	- Synthetic	- Block of text too long at the beginning - Improve the order of information	- Put the technical and synthetic information of the product at the beginning, followed by the more descriptive information (e.g., honey characteristics, origin, expiration date and then honey description, beekeeper information)
Chemical analysis	- Transparency - Stimulates curiosity - Provides official certification	- Difficult to read by non-experts - The document is too long	- Improve readability by outlining the main results - Define the context - Explain the meaning of the analysis/substances analysed
Beekeeper biography	- Introduce the story of the producer/farm	- Redundant - Stereotypical	- Be more personal (Who are you? Who works on your farm?) - Synthesize and create a link with more details/ information (button 'Come to know us')
Photos	- Pleasant - Stimulates curiosity	Without personality Unrealistic, appear to be stock photos	- Be coherent with the geo-localization - Be realistic/authentic - Show the production phases, your apiary
Localisation map	- Useful information	- The google map shows unnecessary information (e.g., restaurants) - It is difficult to understand what the surrounding environment is like	- Improve the geo-localisation - Add more information on the area and a description of the environment - Indicate the bees' range of movement
Expiration date	- Useful information	Not clear	- Add information on: harvesting date, extraction date, canning date quantity produced (kg)

BPRACTICES

Respondents were then asked to evaluate the design and interactivity of the web page by assigning a score from 1 (minimum) to 5 (maximum) for each of the aspects reported in Figure 5.

All of the aspects received positive scores, especially 'text orientation' and 'colours'.

Figure 5. *Evaluate each aspect by attributing a value 1-5 where 1 = not adequate / satisfactory at all and 5 = very adequate* (mean value, n=25[2])



The comments collected during the discussion are reported in Table 4.

Table 4. Participants' suggestions regarding the graphical aspects of the web page

Aspects	***Comments synthesis***
Text orientation	- The display must be adaptable to various platforms/devices - The coloured text boxes are functional but a little outdated; the positioning of the text box should adapt to the screen rotation
Text types	- Underline the most important information (use bold) - Standardize the line spacing - Be coherent in the use of the fonts for titles and texts; a sans serif font is preferred
Colours	- Colour range is too varied; a pastel colour palette is preferred
Interactive buttons	- Make the button disappear when clicked - Differentiate the colours of the buttons - Insert a dropdown menu to improve searching for information
Interaction	- Insert links to social networks

[2] One interaction evaluation score was missing

Finally, in the last part of the focus group discussion, participants were invited to give useful suggestions to improve the web page. Their answers are synthetized in Table 5.

Table 5. Participants' suggestions for improvement of the web page

Topics	***Suggestions synthesis***
Honey experience	- Add tips on food pairings - Add tips for use/ suggested recipes - Add comparison with similar products of the beekeeper
Honey characteristics	- Give information on honey: benefits for health nutritional values preservation methods, temperature, time
Honey information	- Add price (kg/jar) - Add information on the various production phases (honey harvesting, extraction, etc.)
Bees	- Provide information on bees: bees health, bees welfare, beekeeping practices - Provide information on the importance of bees for the environment and the importance of beekeeping to sensitize consumers
Sharing	- In the label, add a claim to attract the consumer to the web page (ex. "Discover the best food pairing", "Do you want to know how I breed my bees?", etc.) - Add information on the smielatura project/contextualize the web page - Insert suggestions for similar types of honey (from the same producer) - Give consumers the possibility to rate the product (comments, stars, etc.) or to track/register their preferences and their purchases - Add a downloadable brochure with the beekeeper's products - Create an English version

PAPER-AND-PENCIL SELF-ADMINISTERED SURVEY

Respondents' socio-demographic characteristics

A total of 59 Italian honey consumers completed the questionnaire (Appendix 3). Among them, the majority were female (55.9%), between 18 and 35 years old (39%), lived in Central Italy (40.7%) and were employed (63.8%). The details of the respondents' socio-demographic characteristics are reported in Table 6.

Table 6. Respondents' socio-demographic characteristics (n=59)

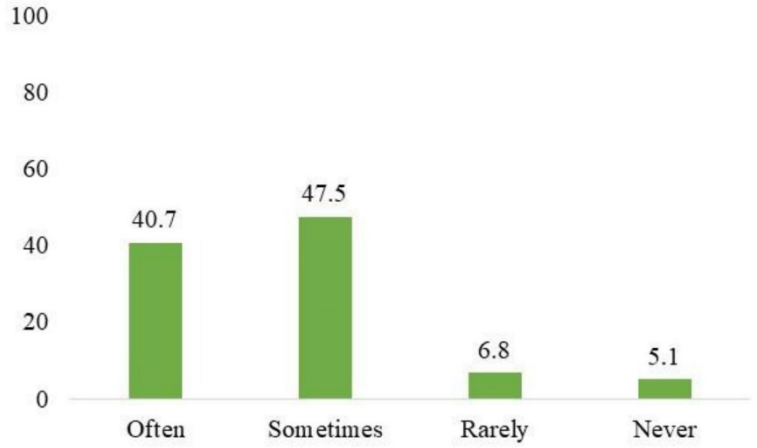
Characteristics	***n***	***%***
Gender		
Female	**33**	**55.9**
Male	26	44.1
Age (classes)		
18-35	**23**	**39.0**
36-49	14	23.7
50-62	11	18.6
63-80	11	18.6
Geographical area		
North West	9	15.3
North East	18	30.5
Centre	**24**	**40.7**
South and Islands	8	13.6
Occupation[3]		
Student	6	10.3
Homemaker	3	5.2
Employed	**37**	**63.8**
Unemployed	1	1.7
Retired	11	19.0

When asked, 'How frequently do you buy honey?', the majority of respondents (47.5%) stated that they buy honey sometimes (Figure 6).

[3] One missing value

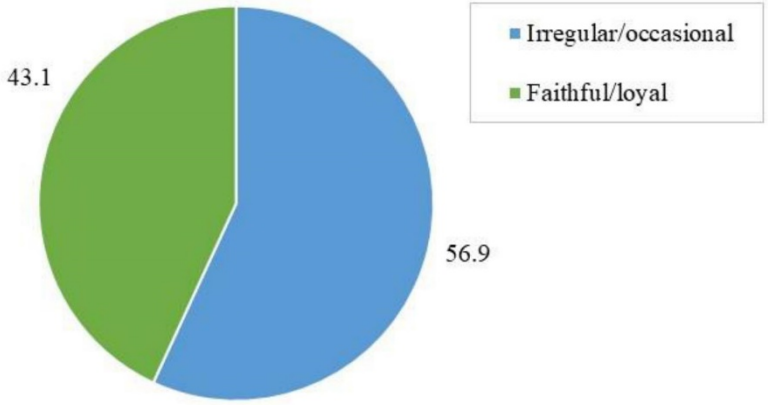
BPRACTICES

Figure 6. *How frequently do you buy honey?* (n=59, %)



Respondents were then asked to choose the type of honey consumer that best described them, irregular/occasional or faithful/loyal. The majority of respondents (56.9%) defined themselves as irregular/occasional consumers (Figure 7).

Figure 7. *What kind of honey consumer do you consider yourself?* (n=58, %)[4]



[4] One missing value

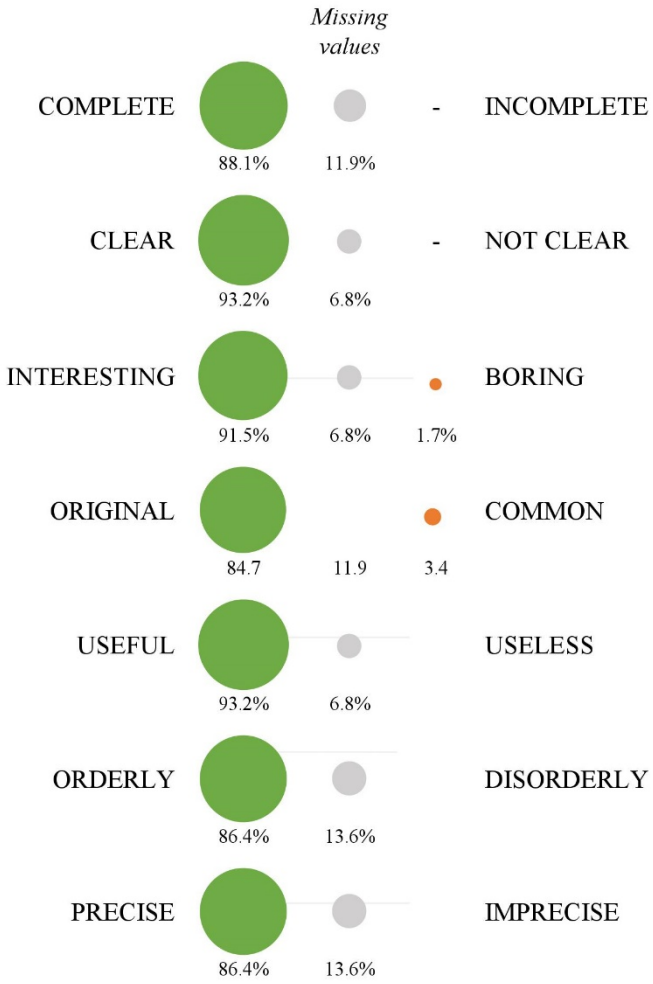
BPRACTICES

Assessment of the traceability system

At the beginning of the questionnaire, respondents were asked to assess the capability of the QRCode technology to provide access to the information on honey and producers. All of the respondents considered the QRCode technology to be 'suitable'.

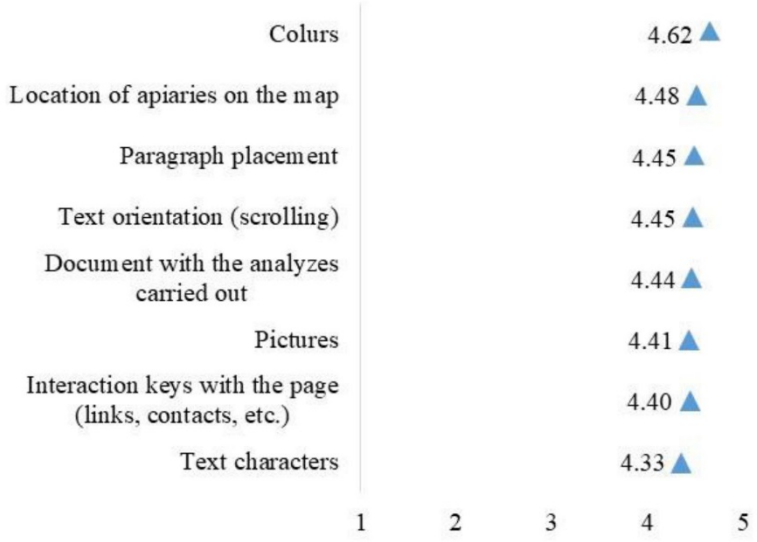
After looking at the web page linked to the QRCode, respondents were asked to evaluate the information on honey, choosing between positive and negative adjectives. The majority of respondents considered the information on honey to be 'complete', 'clear', 'interesting', 'original', 'useful', 'orderly', and 'precise' (Figure 8).

Figure 8. *After looking at the web page, assess the information on honey choosing the positive or negative adjective for each couple* (n=59, %)



Then, interviewees were asked to assess the appearance of the web page by assigning a value from 1 (not at all appropriate) to 5 (very appropriate) for a series of aspects. The 'colours' were considered the most suitable aspect of the web page (Figure 9).

Figure 9. *Assign a value from 1 (not at all appropriate) to 5 (very appropriate) to each aspect of the web page* (mean values)[5]



Finally, respondents were asked to provide potential suggestions that could improve the web page. Twenty-seven interviewees answered the question, and among them, 7 stated that they had no suggestions. According to respondents' answers, information about matching honey with other food should be added to the web page. Moreover, some information could be better summarized and simplified. Respondents' suggestions are summarized in Table 7.

[5] Missing values vary between 1 and 3 depending on the evaluated aspect

Table 7. *Do you have any suggestions for further improving the web page?* (n=21)

Topics	***Suggestions synthesis***
Content	- Information on migratory beekeeping - Sensitize consumers about bees' welfare and environmental pollution - Outline the chemical analysis - Tips on food pairings and best use for cooking - Advice on honey use - Advice on storage and preservation - More details on honey smell and taste - Information on other products of the same producer - Video of the honey chain - More pictures of the apiary/hives/environment
Structure and texts	- Better summarize the information/create an index/ drop-down menu - More engaging information on the descriptive texts - Do not use complex terms
Interactivity	- Increase the number of links with other web pages (producer website, etc.) - Development of an app for smartphone to make the purchase easier
Truthfulness of information	- Verify the information/data published by the beekeepers

Finally, please note that two respondents reported difficulty using the QRCode in the case of older people.

CONCLUDING REMARKS

This phase of the research project allowed us to identify the **weaknesses and strengths of the traceability system** based on QRCode/RFID technology by means of two different social research methods: focus groups and questionnaires.

The obtained results were consistent between them: No differences were observed between what was detected through the focus groups and what was observed with the survey. A synthesis of the main findings is provided below:

- Participants seemed to **positively welcome the proposal of the traceability system**, even though most of them were unfamiliar with the QRCode technology
- In general, the information on honey provided on the webpage was considered by most to be 'complete', 'clear', 'original', and 'useful'
- Regarding the webpage content, most of the participants asked for **more synthesis** (e.g., on the chemical analyses) and **interaction** (e.g., social network)
- The possibility of having more information on the beekeeper is greatly appreciated, particularly if that information is authentic
- Tips on **food pairings** and **honey usage** were requested several times
- Participants evaluated the graphical aspects highly (in particular, the '**colours**'), but they requested **more adaptability to different devices**

APPENDIX 1

a) Round of presentations and introduction of the project http://www.izslt.it/bpractices/the-traceability-system/ https://www.smielatura.it/

b) Opening questions

You have been selected as honey buyers:

Can you tell us where you buy honey most often? Which information do you mostly search for when you buy honey? *Round*

Do you usually find the information you are looking for on the label?

c) Individual platform test - jar distribution

We would like to test the site created for the project together with you and ask you to help us improve it and make it as accessible as possible for the consumer.

We invite you to access the platform using the QRCode that you can find on the jar cap and observe it individually for a few minutes.

(Do you know how the QRCode works? Have you ever used it on your mobile phone? If not, here is a guide to download the app)

When everyone is connected:

We ask that you look at the web site carefully and try to think if:

- The provided information is complete or if there is any information missing;

- The graphical display is adequate and pleasant.

(A few minutes to read the information on the platform)

d) Individual evaluation

Now that you have looked at the web site, we ask that you fill out a short questionnaire on the content and technical aspects of the site

Questionnaire distribution and compilation

e) Collective evaluation

We will write on the board the information that will emerge and ask that you indicate the that you agree with most or that you consider the most important

1. Accessibility:

- Comments on the QRCode use (*NOT SUITABLE – board list*)

2. Information:

- Let's focus on the negative aspects that you have indicated: What are they, and why did you mark them? (*Board list with motivations*) + Integrations (have you marked other aspects?)
- Did you expect to find other types of information? What is the missing information to add that the consumer would like to receive? (*board*)

3. Graphics:

- To which aspects did you assign low scores (1-3)? Why? (*Board list with motivations*)

- Notes to report?

4. **Let's try to focus on the main critical points that you found:** *(re-read the board and integrate)*

- Do you have any other comments and / or suggestions that could be useful for improving the web page? (*board integration*)

APPENDIX 2

"Smielatura.it"

Information on honey via a smartphone

1. Accessibility

In your opinion, the choice to use the QR code to access the web page is:

☐ Suitable and easily used by everyone
☐ Not suitable

Specify the reason or suggest other methods that seem more appropriate for accessing the webpage:

_____
_____
_____
_____

2. Information

Evaluate the honey content on the web page:

Choose the most appropriate one for each pair of adjectives

Positive:		*Negative:*	
☐	Complete	Not complete	☐
☐	Clear	Unclear	☐
☐	Known	Unknown	☐
☐	Original	Banal	☐
☐	Useful	Useless	☐

Notes / remarks to report (*below, please report your observations)*

_____
_____
_____
_____
_____

3. Graphics and interactivity

Evaluate each aspect by attributing a value 1-5 where 1 = not adequate / satisfactory at all and 5 = very adequate

Text orientation (scrolling)	1 2 3 4 5
Text types	1 2 3 4 5
Colours	1 2 3 4 5
Photos	1 2 3 4 5
Interactive buttons (link, phone numbers, emails, etc.)	1 2 3 4 5
Ability to interact with	1 2 3 4 5

Notes / remarks to be reported

_____
_____
_____
_____

General suggestion

What would you change or add to improve the page / website?

_____
_____
_____
_____

APPENDIX 3

"Smielatura.it"
Information on honey via a smartphone

What information on honey that you buy and eat would you like to have?
How would you like to get that information?
Help us to improve our project! Access the QRCode, view the web page and fill out the short questionnaire below.

1. What do you think of the use of **QRCode** technology for accessing information on honey and its producers?
   - ☐ Suitable
   - ☐ Not suitable

2. After looking at the web page, assess the information on honey, choosing, for each pair of adjectives, the positive or negative one

1	☐	Complete	Incomplete	☐
2	☐	Clear	Not clear	☐
3	☐	Interesting	Boring	☐
4	☐	Original	Common	☐
5	☐	Useful	Useless	☐
6	☐	Orderly	Disorderly	☐
7	☐	Precise	Imprecise	☐

3. Help us to assess the appearance of the web page! Assign to each aspect a value from 1 (not at all appropriate) to 5 (very appropriate)

	Not at all appropriate				Very appropriate
Text orientation (scrolling)	1	2	3	4	5
Text characters	1	2	3	4	5
Colours	1	2	3	4	5

Pictures	1 2 3 4 5
Interaction keys with the page (links, contacts, etc.)	1 2 3 4 5
Location of apiaries on the map	1 2 3 4 5
Paragraph placement	1 2 3 4 5
Document with the analyses carried out	1 2 3 4 5

4. Do you have any suggestions for further improving the web page? Please write them below!

_____
_____
_____
_____

Personal data

Gender:

- ☐ Male
- ☐ Female

Year of birth: _____

Area of residence: _____

Occupation:

- ☐ Student
- ☐ Employed
- ☐ Unemployed
- ☐ Homemaker
- ☐ Retired

How frequently do you buy honey?

- ☐ Often
- ☐ Sometimes
- ☐ Rarely
- ☐ Never

What kind of a honey consumer are you?

- ☐ Irregular/occasional
- ☐ Faithful/loyal

Varroa Management

Start of Block: Informational Block

Q1 This survey is elaborated and conducted by Appalachian University within the context of the EU-funded project BPractices, with the technical support of Apimondia, the Animal Production and Health Division of the Food and Agriculture Organization of the UN, and the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana. All responses will remain anonymous for the safety and protection of the respondent's personal information. The intent of this survey is to better understand beekeepers' knowledge on Apis mellifera (honey bee) and the use of antibiotics around the world. The survey should take between 5 and 10 minutes to complete, and you can always check your progress with the bar at the top of the screen. Thank you for your honesty and time in responding to this survey which will be used to identify priorities for supporting beekeepers and making beekeeping more sustainable worldwide.

Q2 **Informed Consent**

The study has been explained to me in a language that I comprehend. All the questions I had about the study have been answered.

I have been informed that it is my right to refuse to participate today and that if I choose to refuse I do not have to give a reason, and there will be no negative consequences for me.

I have been informed that anything I say during the discussion today will remain completely confidential: my name will not be used in any materials produced from this study nor any other information that could be used to identify me. I have been informed that I can request access to, moderations to, and/or deletion of my personal data.

I release Appalachian University and its employees dealing with the use of my personal data as described above.

Q3 I agree to take part in this study

O Yes (4)

O No (5)

End of Block: Informational Block

Start of Block: Demographic Information

Q4

Q5 Select the country in which you primarily house your bees:

▼ Algeria (1) ... New Zealand (212)

Q6 Select the region of the country you selected that you primarily house your bees in:

▼ Argentine Northwest (2) ... Wyoming (53)

Q7 Year you were born:

▼ 1920 (1) ... Other (82)

Q8 Mark your gender:

O Male (1)

O Female (2)

O Prefer not to answer (3)

Q9 Mark your **highest** education level:

O High School (Secondary) or less (1)

O Vocational or Technical Degree, Associates Degree, or Some College (2)

O University Degree (3)

O Post-graduate qualification (4)

Q10 How many years have you been a beekeeper?

▼ 0 (4) ... 50 (54)

Q11 Estimate the number of hives you are currently managing:

_____

Q12 What type of hive are you using? (check all that apply)

- ☐ Top-Bar Hive (2)
- ☐ Langstroth Hive (4)
- ☐ Warre Hive (6)
- ☐ Dadant Blatt (10)
- ☐ Other(s) (11) _____

Q13 Do you consider yourself a professional beekeeper?

- ○ Yes (4)
- ○ No (5)

Q14 Do you move your bees at all throughout the year?

O Yes (10)

O No (11)

Q15 How often do you inspect your hives **during the active** season: (Please, select the closest to your situation)

O Never (1)

O Once a month (3)

O Two to three times a month (4)

O Four times a month (8)

O More than four times a month (6)

End of Block: Demographic Information

Start of Block: Block 6

Q16 Which of the following photos is an example of Varroa mites?

O Image:87bcf174 20d6 44f7 b167 71efced85258 (1)

O Image:Afb (2)

O Image:European foulbrood (4)

O Image:Varroa mites (5)

O Image:Chalkbrood 7 (6)

End of Block: Block 6

Start of Block: Block 7

Q17 **Beekeeping Practices for Varroa**

Q18 How **knowledgeable** are you regarding Varroa:

- O No knowledge (7)
- O Little knowledge (8)
- O Moderately knowledgeable (9)
- O Very knowledgeable (10)
- O Extremely knowledgeable (11)

Q19 Please tell us your experience in recognizing Varroa:

- O Never seen it (1)
- O Saw a live example of it (5)
- O Seen it multiple times (6)

Q20 How **useful** do you think each example below is at Varroa prevention/control?

	I don't know (1)	Not at all useful (2)	Moderately useful (3)	Extremely useful (4)
Adopt/provide hives with screened bottom boards (1)	O	O	O	O
Nuclei and swarms should originate from colonies with no clinical signs of diseases related with Varroa (2)	O	O	O	O
Maintain the number of Varroa below the harmful threshold in each colony (3)	O	O	O	O
Adopt diagnostic tools for measuring Varroa infestation levels (for example, icing sugar method, CO2 test, mite fall etc.) after treatments and during the year (for example, in the spring at the beginning of beekeeping season or before harvesting) (4)	O	O	O	O
Provide sufficient number of healthy spare bees at the right time (7)	O	O	O	O
Have good knowledge of the signs of varroosis and virosis (8)	O	O	O	O
Select and breed queens that are more Varroa tolerant/resistant (9)	O	O	O	O

Treat swarms (no brood) just after harvest (10)	O	O	O	O

End of Block: Block 7

Start of Block: Acarides

Q21 Have you treated your bees with medicine against Varroa in the last 2 years?

O Yes (1)

O No (2)

Q22 List any medicines against Varroa you use regularly: (if you don't know, leave it blank)

_____

Q23 Indicate where you get your medicines against Varroa that you use: (check all that apply)

- ☐ Agro Chemical Supply-House (5)
- ☐ Veterinarian (1)
- ☐ Pharmacy (2)
- ☐ Other beekeeper (3)
- ☐ Internet (4)
- ☐ Extension Services (8)
- ☐ Other(s), please explain (6) _____

Q24 Do you normally need to get a prescription for medicines against Varroa?

- ○ Yes (1)
- ○ No (2)
- ○ Depends on these conditions: (3) _____

Q25 If/when you use medicines against Varroa how do you proceed?

	Yes (1)	No (2)
Treat simultaneously all colonies of the apiary (1)	O	O
Treat only the diseased hives in an apiary (2)	O	O
Perform at least 2 treatments per year (3)	O	O
Rotate the products (4)	O	O
Use preferably medicines allowed in organic farming (5)	O	O
Monitor efficacy of treatments: verifying Varroa fall after treatment (6)	O	O
Monitor efficacy of treatments: verifying Varroa mite presence on adult bees after treatment (7)	O	O

Q26 How often do you think beekeepers use medicines against Varroa without following the label instructions?

O Never (1)

O Sometimes (2)

O Often (3)

O Usually (4)

O Always (5)

End of Block: Acarides

Start of Block: Block 6

Q27 Would you be interested in bee health training?

O Yes (4)

O No (5)

Q28 Would you be interested in an online training course?

O Yes (4)

O No (5)

Q29 Please list any professional beekeeping associations/groups related to bees that you belong to/know about:

_____

Q30 Please list any bee-specific training or courses that you've attended:

_____

Q31 How interested are you in a nationwide service connecting beekeepers with veterinary experts specialized in bees?

O Not at all interested (1)

O Somewhat interested (2)

O Interested (3)

O Very interested (4)

O Extremely interested (5)

Q32 If you are willing to be available for a few follow up question or more information, please leave your email address below:

_____

Q33 Share any additional comments you have:

_____

Q34 This is the end of the survey, and by clicking the next button you're submitting the survey. Thank you for your response.

For more information you can go to: www.fao.org/antimicrobial-resistance

Contact

End of Block: Block 6

Antibiotic Resistance

Start of Block: Informational Block

Q1 This survey is elaborated and conducted by Appalachian University within the context of the EU-funded project BPractices, with the technical support of Apimondia, the Animal Production and Health Division of the Food and Agriculture Organization of the UN, and the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana. All responses will remain anonymous for the safety and protection of the respondent's personal information. The intent of this survey is to better understand beekeepers' knowledge on Apis mellifera (honey bee) and the use of antibiotics around the world. The survey should take between 5 and 10 minutes to complete, and you can always check your progress with the bar at the top of the screen. Thank you for your honesty and time in responding to this survey which will be used to identify priorities for supporting beekeepers and making beekeeping more sustainable worldwide.

Q2 **Informed Consent**

The study has been explained to me in a language that I comprehend. All the questions I had about the study have been answered.

I have been informed that it is my right to refuse to participate today and that if I choose to refuse I do not have to give a reason, and there will be no negative consequences for me.

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I release Appalachian University and its employees dealing with the use of my personal data as described above.

Q3 I agree to take part in this study

O Yes (1)

O No (2)

End of Block: Informational Block

Start of Block: Location

Q4

Q5 Select the country in which you primarily house your bees:

▼ Algeria (1) ... New Zealand (212)

Q6 Select the region of the country you selected that you primarily house your bees in:

▼ Argentine Northwest (2) ... Wyoming (53)

Q7 Year you were born:

▼ 1920 (1) ... Other (82)

Q8 Mark your gender:

O Male (1)

O Female (2)

O Prefer not to answer (3)

Q9 Mark your **highest** education level:

O High School (Secondary) or less (1)

O Vocational or Technical Degree, Associates Degree, or Some College (2)

O University Degree (3)

O Post-graduate qualification (4)

Q10 How many years have you been a beekeeper?

▼ 0 (4) ... 50 (54)

Q11 Estimate the number of hives you are currently managing:

_____

Q12 What type of hive are you using? (check all that apply)

- ☐ Top-Bar Hive (2)
- ☐ Langstroth Hive (4)
- ☐ Warre Hive (6)
- ☐ Dadant Blatt (10)
- ☐ Other(s) (11) _____

Q13 Do you consider yourself a professional beekeeper?

- ○ Yes (5)
- ○ No (6)

Q14 Do you move your bees at all throughout the year?

O Yes (10)

O No (11)

Q15 How often do you inspect your hives **during the active** season? (Please, select the closest to your situation)

O Never (1)

O Once a month (3)

O Two to three times a month (8)

O Four times a month (6)

O More than four times a month (7)

End of Block: Location

Start of Block: Antibiotics

Q16 What are antibiotics intended to do? (check all that apply)

- [ ] Medicines that prevents diseases (1)
- [ ] Medicines that cure diseases (2)
- [ ] Medicines that kill germs (3)
- [ ] Medicines that kill bacteria (4)
- [ ] Medicines that increase production (5)
- [ ] Other (6)
- [ ] I don't know (7)

Q17 Have you treated your bees with antibiotics in the last 2 years?

- O Yes (1)
- O No (2)

Q18 List any medicines or treatments you use regularly: (if you don’t know, leave it blank)

_____

_____

_____

_____

_____

Q19 Do you use antibiotics for any of the following? (check all that apply)

- [ ] Nosema (1)
- [ ] Varroa (7)
- [ ] American Foulbrood (2)
- [ ] European Foulbrood (3)
- [ ] Small Hive Beetle (8)
- [ ] None (6)
- [ ] Other(s), please explain (4) _____

Q20 Indicate where you get your antibiotics: (check all that apply)

- [ ] Agro Chemical Supply-House (9)
- [ ] Veterinarian (1)
- [ ] Pharmacy (2)
- [ ] Other beekeeper (3)
- [ ] Internet (6)
- [ ] Extension Services (8)
- [ ] Other(s), please explain (5) _____

Q21 Do you normally need to get a prescription for antibiotics?

- O Yes (1)
- O No (2)
- O Depends on these conditions: (3) _____

Q22 Describe how you use antibiotics: (check all that apply)

- ☐ Prevention of an infection (1)
- ☐ Treatment of an infection (2)

Q23 Where do you get information on the use of antibiotics? (check all that apply)

- ☐ Agro Chemical Supply-house (7)
- ☐ Veterinarian (1)
- ☐ Pharmacy (8)
- ☐ Other Beekeepers (2)
- ☐ Internet (3)
- ☐ Books (4)
- ☐ Extension services (5)
- ☐ Other(s), please explain (6) _____

Q24 How often do you think beekeepers use antibiotics without following the label instructions?

- O Never (1)
- O Sometimes (2)
- O Often (3)
- O Usually (4)
- O Always (5)

Q25 How **knowledgeable** are you in issues of antibiotics intended to be used on bees?

O No knowledge (5)

O Little knowledge (1)

O Somewhat knowledgeable (2)

O Moderately knowledgeable (3)

O Extremely knowledgeable (6)

Q26 How much do you agree with the statement that "honey/honeycomb from bees just treated with antibiotics should not be consumed?"

O Agree (1)

O Indifferent (2)

O Disagree (3)

Q27 Do you know what antibiotic residues are?

O Yes (4)

O No (5)

Q28 Do you know what drug-resistant infections are?

O Yes (4)

O No (5)

Q29 How often do you see antibiotics fail to treat bees?

- O Never (1)
- O Sometimes (2)
- O Almost always (3)
- O Always (4)
- O I don't know (5)

Q30 How much do you agree with the statement that "if medicines are used too often then they might stop working?"

- O Agree (1)
- O Indifferent (2)
- O Disagree (3)

Q31 Has a veterinarian ever told you about the risks of either using medicines too often or the wrong type of antibiotics?

- O Yes (4)
- O No (5)

Q32 How much do you believe drug resistant infections will impact you, your family/friends and your bees?

O No impact (1)

O A little impact (2)

O A large impact (3)

O I don't know about drug resistant infections (4)

Q33 Please tell us your experience in recognizing bee resistance to medicines:

O Never seen it (1)

O Saw a live example of it (2)

O Seen it multiple times (3)

End of Block: Antibiotics

Start of Block: Demographic Information

Q34 **Drug Resistant-Infections**

Microbes (germs) causing infections can develop the ability to tolerate the antibiotics and other antimicrobials we used to treat and cure specific infections. This phenomenon is called "antimicrobial resistance" and is causing medicines to fail. This puts the health of people and animals everywhere at risk because these resistant infections can be spread. We all have a responsibility to use appropriate medicines, only when needed, and under expert advice and prescription so we can keep medicines working.

Q35 Would you be interested in bee health training?

O Yes (4)

O No (5)

Q36 Would you be interested in an online training course?

O Yes (1)

O No (2)

Q37 Please list any professional associations for beekeeping/groups related to bees that you belong to/know about:

_____

Q38 Please list any bee-specific training or courses that you've attended:

_____

Q39 How interested are you in a nationwide service connecting beekeepers with veterinary experts specialized in bees?

- O Not at all interested (6)
- O Somewhat interested (7)
- O Interested (8)
- O Very interested (9)
- O Extremely interested (10)

Q40 If you are willing to be available for a few follow up questions or more information, please leave your email address below:

_____

Q41 Share any additional comments you have:

_____

Q42 This is the end of the survey, and by clicking the next button you're submitting the survey. Thank you for your response.

For more information you can go to: www.fao.org/antimicrobial-resistance

Contact

End of Block: Demographic Information

Infectious Disease Management

Start of Block: Informational Block

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I release Appalachian University and its employees dealing with the use of my personal data as described above.

Q3 I agree to take part in this study

O Yes (4)

O No (5)

End of Block: Informational Block

Start of Block: Location

Q4

Q5 Select the country in which you primarily house your bees:

▼ Algeria (1) ... New Zealand (212)

Q6 Select the region of the country you selected that you primarily house your bees in:

▼ Argentine Northwest (2) ... Wyoming (53)

Q7 Year you were born:

▼ 1920 (1) ... Other (82)

Q8 Mark your gender:

O Male (1)

O Female (2)

O Prefer not to answer (3)

Q9 Mark your **highest** education level:

O High School (Secondary) or less (1)

O Vocational or Technical Degree, Associates Degree, or Some College (2)

O University Degree (3)

O Post-graduate qualification (4)

Q10 How many years have you been a beekeeper?

▼ 0 (4) ... 50 (54)

Q11 Estimate the number of hives you are currently managing:

_____

Q12 What type of hive are you using? (check all that apply)

- ☐ Top-Bar Hive (2)
- ☐ Langstroth Hive (4)
- ☐ Warre Hive (6)
- ☐ Dadant Blatt (10)
- ☐ Other(s) (11) _____

Q13 Do you consider yourself a professional beekeeper?

- ○ Yes (4)
- ○ No (5)

Q14 Do you move your bees at all throughout the year?

O Yes (10)

O No (11)

Q15 How often do you inspect your hives **during the active** season: (Please, select the closest to your situation)

O Never (1)

O Once a month (4)

O Two to three times a month (8)

O Four times a month (6)

O More than four times a month (7)

End of Block: Location

Start of Block: Block 6

Q16 Which of the following photos is an example of Nosema?

☐ Image:87bcf174 20d6 44f7 b167 71efced85258 (1)

☐ Image:European foulbrood (8)

☐ Image:Afb (9)

☐ Image:Varroa mites (10)

☐ Image:Chalkbrood 7 (11)

Q17 Which of the following photos is an example of American Foulbrood?

- [ ] Image:87bcf174 20d6 44f7 b167 71efced85258 (1)
- [ ] Image:European foulbrood (8)
- [ ] Image:Afb (9)
- [ ] Image:Varroa mites (10)
- [ ] Image:Chalkbrood 7 (11)

Q18 Which of the following photos is an example of European Foulbrood?

- [ ] Image:87bcf174 20d6 44f7 b167 71efced85258 (1)
- [ ] Image:European foulbrood (8)
- [ ] Image:Afb (9)
- [ ] Image:Varroa mites (10)
- [ ] Image:Chalkbrood 7 (11)

End of Block: Block 6

Start of Block: Beekeeping Practices for Nosema

Q19 How **knowledgeable** are you in the following bee diseases:

	No knowledge (1)	Little knowledge (2)	Moderately knowledgeable (3)	Very knowledgeable (4)	Extremely knowledgeable (5)
Nosema (1)	O	O	O	O	O
European Foulbrood (2)	O	O	O	O	O
American Foulbrood (3)	O	O	O	O	O

Q20 Please tell us your experience in recognizing the following diseases:

	Never seen it (1)	Saw a live example of it (2)	Seen it multiple times (3)
Nosema (1)	O	O	O
American Foulbrood (2)	O	O	O
European Foulbrood (3)	O	O	O

Q21 How **useful** it is to be able to recognize the signs of each of the following bee diseases:

	Not at all useful (1)	Slightly useful (2)	Moderately useful (3)	Very useful (4)	Extremely useful (5)
Nosema (1)	O	O	O	O	O
European Foulbrood (2)	O	O	O	O	O
American Foulbrood (3)	O	O	O	O	O

Q22 **Beekeeping Practices for Nosema**

Q23 Please indicate how **useful** each of the following practices are in preventing/managing Nosema, according to your experience:

	Not useful (3)	Slightly useful (4)	Moderately useful (5)	Very useful (6)	Extremely useful (7)
Remove combs that show signs of dysentery (1)	O	O	O	O	O
Take samples of forager bees for diagnostics (2)	O	O	O	O	O
Take samples of hive debris for diagnostics (8)	O	O	O	O	O
Treat for Varroa (3)	O	O	O	O	O
Feed colonies (5)	O	O	O	O	O
Replace the queen (6)	O	O	O	O	O
Treat with antibiotics (9)	O	O	O	O	O

Q24 Please indicate how **feasible** each of the following could be in your beekeeping activities, according to your experience:

	Not feasible (3)	Slightly feasible (4)	Moderately feasible (5)	Very feasible (6)	Extremely feasible (7)
Remove combs that show signs of dysentery (1)	O	O	O	O	O
Take samples of forager bees for diagnostics (2)	O	O	O	O	O
Take samples of hive debris for diagnostics (7)	O	O	O	O	O
Treat for Varroa (3)	O	O	O	O	O
Feed colonies when needed (5)	O	O	O	O	O
Replace the queen (6)	O	O	O	O	O
Treat with antibiotics (8)	O	O	O	O	O
Select and breed Nosema resistant bees (12)	O	O	O	O	O

End of Block: Beekeeping Practices for Nosema

Start of Block: Beekeeping Practices for AFB and EFB (American and European Foulbrood)

Q25 **Beekeeping Practices for AFB and EFB (American Foulbrood and European Foulbrood)**

Q26 Please indicate how **useful** each of the following practices are in preventing/managing AFB/EFB, according to your experience:

	Not useful (3)	Slightly useful (4)	Moderately useful (5)	Very useful (6)	Extremely useful (7)
Inspect hives more frequently to detect the disease earlier. (1)	O	O	O	O	O
Be aware of the odor opening the hive. (2)	O	O	O	O	O
Perform the ropiness test to confirm clinical outbreak of AFB. (3)	O	O	O	O	O
Find AFB and EFB typical scales. (4)	O	O	O	O	O
Adopt commercial on-field kit for self diagnosis. (5)	O	O	O	O	O
Disinfect or incinerate the inferred bee tools, facilities and equipment. (6)	O	O	O	O	O
Process wax safely in order to control the disease. (8)	O	O	O	O	O
Monitor the presence of the disease even from apparently healthy hives sending to the lab samples as a preventative measure. (12)	O	O	O	O	O
Send samples from hives showing signs of the disease to a lab. (13)	O	O	O	O	O

Do a shook swarm of the infected hives (moving bees to fresh new comb foundations and destroying the old combs). (14)	O	O	O	O	O
Do the shook swarm of the whole apiary. (15)	O	O	O	O	O
Treat with antibiotics. (16)	O	O	O	O	O
Destroy only infected colonies. (17)	O	O	O	O	O
Destroy the whole apiary. (18)	O	O	O	O	O
Take steps quickly to manage the disease. (19)	O	O	O	O	O
Select queen breeders free of AFB/EFB. (20)	O	O	O	O	O

End of Block: Beekeeping Practices for AFB and EFB (American and European Foulbrood)

Start of Block: Demographic Information

Q28 Would you be interested in bee health training?

O Yes (4)

O No (5)

Q29 Would you be interested in an online training course?

O Yes (4)

O No (5)

Q30 Please list any professional beekeeping associations/groups related to bees and bee products that you belong to/know about:

_____

Q31 Please list any bee-specific training or courses that you've attended:

_____

Q32 How interested are you in a nationwide service connecting beekeepers with veterinary experts specialized in bees?

O Not at all interested (1)

O Somewhat interested (2)

O Interested (3)

O Very interested (4)

O Extremely interested (5)

Q33 If you are willing to be available for a few follow up questions or more information, please leave your email address below:

_____

Q34 Share any additional comments you have:

_____

Q35 This is the end of the survey, and by clicking the next button you're submitting the survey. Thank you for your response.

For more information you can go to: www.fao.org/antimicrobial-resistance

Contact

End of Block: Demographic Information