Istituto Zooprofilattico Sperimentale
del Lazio e della Toscana *M. Aleandri*

Ministero della Salute

APIMONDIA

HONEY BEE HEALTH SYMPOSIUM 2019

NEW APPROACHES TO HONEY BEE HEALTH

Rome 13th - 15th Feb 2019

HONEY BEE HEALTH
SYMPOSIUM 2019

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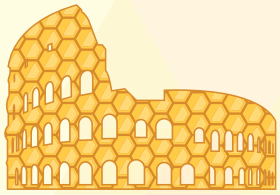
Index



HONEY BEE HEALTH SYMPOSIUM 2019

PRESENTATION

HONEY BEE HEALTH
SYMPOSIUM 2019

Scientific Committee

Jeff Pettis – Apimondia
Andrea Maroni Ponti, Luigi Ruocco and Daniele Scricciolo – Ministry of Health
Andrea Leto, Giancarlo Ferrari and Giovanni Formato – IZSLT
Juan Lubroth – FAO
Alberto Contessi – Osservatorio Nazionale Miele
Alberto Masci – Ministry of Agriculture
Valter Bellucci, Pietro Massimiliano Bianco and Lorenzo Ciccarese – ISPRA
Franco Mutinelli – NRL and Luciano Ricchiuti – II.ZZ.SS.

Organizing Committee

Peter Kozmus and Riccardo Jannoni-Sebastianini – Apimondia
Silvio Borrello and Gaetana Ferri – Ministry of Health
Ugo Della Marta – IZSLT
Berhe Tekola – Director of Animal Production and Health Division. FAO
Alessandra Pesce – Ministry of Agriculture
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Rita Marcianò – Latium Region
Emanuela Balocchini – Tuscany Region

Scientific Secretariat

Filippo Jannoni-Sebastianini and Riccardo Jannoni-Sebastianini – Apimondia;
Antonella Bozzano, Giovanni Formato, Patrizia Gradito, Marco Pietropaoli, Jorge Rivera-Gomis, Marzia Romolaccio and Alessandra Tardiola – IZSLT.

Organizing Secretariat

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Speakers, Moderators and Committees' members

Michele Amorena – Professor of Farmacology and Veterinarian Toxicology, University of Teramo – Italy

Emanuela Balocchini – Head of the Prevention, Life and Work Safety, Food and Veterinary Sector of Tuscany Region – Italy

Valter Bellucci – Department of environmental monitoring and biodiversity conservation, "Istituto Superiore per la Protezione e la Ricerca Ambientale" (ISPRA) – Italy

Pietro Massimiliano Bianco – Department of environmental monitoring and biodiversity conservation, "Istituto Superiore per la Protezione e la Ricerca Ambientale" (ISPRA) – Italy

Silvio Borrello – General Director of the Animal Health and Veterinary Medicines, Ministry of Health – Italy

Emanuele Carpana – Researcher of CREA (CREA – Centro di ricerca Agricoltura e Ambiente

CREA - Research Centre for Agriculture and Environment – Italy

Joseph Cazier – Chief Analytics Officer for Hivetracks.com and Director for the Centre of Analytics Research and Education, Department of CIS & Supply Chain Management, Appalachian State University – USA

Giuseppe Cefalo – President of the National Union of Italian beekeepers' Associations - Unione Nazionale Associazioni Apicoltori Italiani (UNAAPI) - Italy

Antonella Cersini – Biotechnology Department, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" – Italy

Lorenzo Ciccarese - Department of environmental monitoring and biodiversity conservation, "Istituto Superiore per la Protezione e la Ricerca Ambientale" (ISPRA) – Italy

Raffaele Cirone – President of the Italian Federation of Beekeepers - Federazione Apicoltori Italiani (FAI) - Italy

Alberto Contessi – President of the "Osservatorio Nazionale miele" – Italy

Antonio D'Angeli – President of the National Association of Italian Beekeepers, Associazione Nazionale Apicoltori Italiani (ANAI). Italy

Benjamin Dainat - Agroscope Swiss Bee Research Centre – Switzerland.

Ugo Della Marta – General Director of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) – Italy

Gaetana Ferri – General Director of Food Hygiene, Safety and Nutrition, Ministry of Health – Italy

Giancarlo Ferrari – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) – Italy

Giovanni Formato – Head of the laboratory of "Apiculture, Honey Bee Productions and Pahtologies", Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT); President of the Scientific Society of Veterinarians in Apiculture (SVETAP) – Italy

Gianluca Grandinetti – Dipartimento tutela della salute Regione Calabria. Regione Calabria, Italy

Mariano Higes - Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA) – Spain.

Riccardo Jannoni-Sebastianini – Secretary-General of *APIMONDIA, International Federation of Beekeepers' Associations* – Italy

Peter Kozmus – Acting President of Apimondia – Slovenia.

Andrea Leto – Sanitary Director of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) – Italy

Giulio Loglio – Veterinarian – Italy

Juan Lubroth - Chief of Veterinary Officers, Chief Animal Health Service, Animal Production and Health Division (AGAH), Food and Agriculture Organization of the UN (FAO) – Italy

Salvatore Macrì –Veterinarians, Animal Health and Veterinary Medicines, Ministry of Health – Italy

Rita Marcianò – Health and Social Policies Directorate, Health promotion and preservation. Latium Region – Italy

Andrea Maroni Ponti – General Directorate of Animal Health, Ministry of Health – Italy

Raquel Martín-Hernández - Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA) – Spain

Alberto Masci - Ufficio DISR 5, Servizio fitosanitario centrale, produzioni vegetali, Direzione Generale dello sviluppo rurale, Dipartimento delle politiche europee ed internazionali e dello sviluppo rurale, Ministero delle politiche agricole alimentari forestali e del turismo – Italy

Piotr Medrzycki – Researcher of CRA-Api (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria) – Italy

Michele Mortarino – Professor of parasitology and parasitic diseases, Department of Veterinary Sciences and Public Healht, University of Milan – SVETAP - Italy

Franco Mutinelli – National Reference Laboratory for Apiculture, Italy

Peter Neumann - President of Colony Losses International Association – COLOSS; Vinetum professor of the Institute of Bee Health, Institute of Bee Health, Vetsuisse Faculty, University of Bern – Switzerland

Diego Pagani – President of the Apimondia Scientific Commission on Economy and President of CONAPI (National Consortium of Beekeepers) – Italy

Marco Pellegrini - Ministero delle politiche agricole alimentari forestali e del turismo – Ministery of Agriculture, Italy

Alessandra Pesce – Vice-Secretary of Ministry of Agriculture. Ministero delle politiche agricole alimentari forestali e del turismo – Ministery of Agriculture, Italy

Jeff Pettis – President of the Apimondia Scientific Commission on Bee Health and United States Department of Agriculture's Beltsville Bee Laboratory – USA

Marco Pietropaoli – Apiculture Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) - Italy

Emilia Reda - Council for Agricultural Research and Agricultural Economy Analysis - Centre for policies and bio-economy (CREA-PB), Italy

Luciano Ricchiuti - Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" (IZSAM) - Italy

Jorge Rivera Gomis - Apiculture Unit, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) - Italy

Pasquale Rombolà – Osservatorio Epidemiologico Regionale, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) - Italy

Luigi Ruocco – General Directorate of Animal Health, Ministry of Health – Italy

Cristina Salvioni - Professor of Agricultural Economics. Department of Economics. University of Chieti-Pescara. Italy

Marc Oliver Schaefer - Aethina tumida/Small hive beetle, OIE Reference Laboratory – Germany

Daniele Scricciolo – General Directorate of Food Hygiene, Safety and Nutrition. Ministry of Health – Italy

Asger Søgaard Jørgensen – Apimondia and Danish Beekeepers Association

Berhe Tekola - Director of Animal Production and Health Division, Food and Agriculture Organization of the UN (FAO) – Italy

Jozef van der Steen – Alveus AB Consultancy, INSIGNIA project coordinator – Nederland

Rens van Dobbenburgh – Vice-President of the Federation of Veterinarians of Europe (FVE)

Flemming Vejsnaes – Consultant at Danish Beekeepers Association (DBA) – Denmark

James Wilkes –Co-founder of the Bee Informed Partnership, HiveTracks Founder, Professor of computer science, Appalachian State University – USA

Carlo Zaghi – Head of IV Division, Evaluation, risk mitigation due to chemicals and genetically modified organisms, Ministry of Environment – Italy

HONEY BEE HEALTH SYMPOSIUM 2019

Under the patronage of

FNOVI
FEDERAZIONE NAZIONALE ORDINI VETERINARI ITALIANI

ISPRA
Istituto Superiore per la Protezione e la Ricerca Ambientale

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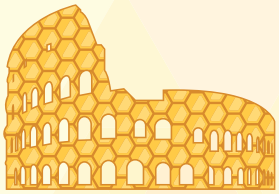
PROGRAMME



HONEY BEE HEALTH SYMPOSIUM 2019

13 FEBRUARY 2019

07.45 Registration and set-up of Poster Session 1: "Good Beekeeping Practices"

OPENING SESSION

Chairs: *Riccardo Jannoni-Sebastianini* – Apimondia *and Giovanni Formato* – IZSLT

08.30 **Welcome from organizers**
Italian Ministry of Health – *Silvio Borrello, Gaetana Ferri*
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) – *Ugo Della Marta*
Apimondia – *Peter Kozmus*

08.50 **Welcome from supporting organizations**
Food and Agriculture Organizatio) (FAO) – *Berhe Tekola*
Italian Ministry of Agriculture – *Alessandra Pesce*
Italian Ministry of Environment – *Carlo Zaghi*

PLENARY SESSION
AN OVERVIEW OF THE HONEY BEE PATHOLOGIES IN 2019

09.10 EU Honey Bee Health perspectives
Michele Mortarino – Veterinary Faculty, University of Milan

09.30 Extra-EU Honey Bee Health perspectives
Jeff Pettis – Apimondia

09.50 FAO perspectives on honey bees in terms of animal health and food security
Juan Lubroth – FAO - Animal Production and Health Division (AGA)

10.10 Discussion

10.30 **Coffee break**

SESSION
GOOD BEEKEEPING PRACTICES - GBPS

11.00 Pre-clinical indicators as innovative tools in beekeeping, in the context of the BPRACTICES project
Giovanni Formato – IZSLT

11.20 HIVELOG and the Internet of Things – IoT
Flemming Vejsnaes – Danish Beekeeping Association DBA, Denmark

11.40 Why Veterinary prescription in beekeeping
Rens van Dobbenburgh – FVE

12.00 Discussion on "GBP"

12.00 *Scientific contributions on* "Good Beekeeping Practices" (part I)
- Blocks for Bees: Using Blockchain Technology to Keep Honeybee Enterprises Healthy
- Investigation on the use of veterinary medicinal products and best practices in beekeeping
- First attempt of standardization of official control procedures at the apiary level
- Honey bee in the history: a golden standard to train human being
- Best management practices for every beekeeper

- The control of beekeeping in Italy, between problems and perspectives: certainly more than just a challenge
- Determination of a new efficient biomarker to evaluate the health and productivity of honey bees

13.00 **Lunch**

14.30 *Scientific contributions on* "Good Beekeeping Practices" (part II)

SESSION

AETHINA TUMIDA – THE SMALL HIVE BEETLE (SHB)

Chair: *Marc Oliver Schaefer* – *Aethina tumida* -Small hive beetle- OIE Reference Laboratory *and Andrea Maroni Ponti* – Ministry of Health

14.50 An update on small hive beetles
Peter Neumann – University of Bern

15. 10 Small hive beetle invasion in EU
Franco Mutinelli – National Reference Laboratory for Apiculture, Italy

15. 30 Comparison of two colony inspection methods for the detection of Small Hive Beetle (SHB) in Calabria region (Italy)
Jorge Rivera Gomis – IZSLT

15.50 Discussion on "*Aethina tumida* – The Small Hive Beetle (SHB)"

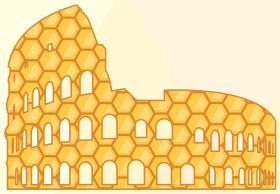
16.10 **Coffee break**

16.30 PRACTICAL DEMONSTRATIONS

Using An Open Apiary Management System to Help Meet Key U.N. Sustainable Development Goals
Joseph Cazier and *James Wilkes* – Appalachian State University – USA

17.10 End of Poster Session 1: "Good Beekeeping Practices"

Note: first 2 days of symposium in the Hotel Palatino – Via Cavour 213, Rome; the 3rd day (optional, registration is needed) of technical tour (to visit a professional beekeeper's facilities, nearby Rome)



HONEY BEE HEALTH SYMPOSIUM 2019

14 FEBRUARY 2019

08.45 **Set up of Poster Session 2 "Honey Bee Diseases" and Poster Session 3 "Honey Bees, Environmental Pollution and Pesticides"**

SESSION
MAIN HB DISEASES

Chair: *Jeff Pettis* – Apimondia and *Benjamin Dainat* – Agroscope

09.00 Varroa resistant Honey Bees
Benjamin Dainat – Agroscope

09.20 Updates on *Nosema ceranae*
Mariano Higes and *Raquel Martín-Hernández* – Centro de Investigación Apícola y Agroambiental de Marchamalo

09.40 Diagnosis and control of AFB and EFB
Emanuele Carpana – CREA

10.00 Use and management of veterinary medicines in beekeeping
Salvatore Macrì – Ministry of Health

10.20 Discussion on "Main HB diseases"

10.30 **Coffee break**

11.00 *Scientific contributions on* "Main HB diseases"
- Bee Varroa Scanner
- First molecular clone of Chronic Bee Paralysis Virus (CBPV)
- Hive debris (ring tests) to diagnose AFB, EFB, Nosema spp. and SHB
- Recombinant expression and purification of VP1 of Sacbrood virus
- Recent findings of parasitic phorid flies in honey bee
- Beeheal: Monitoring microsporidia and viruses in honey bee colonies in Spain

12.30 Discussion on "Main HB diseases"

13.00 **Lunch**

14.30 **End of Poster Session 2 "Honey Bee Diseases" and Poster Session 3 "Honey Bees, Environmental Pollution and Pesticides"**

14.30 **PARALLEL SESSIONS**

1 – SESSION
HONEY BEES, ENVIRONMENTAL POLLUTION AND PESTICIDES

or

2 – SESSION
SHB IN CALABRIA REGION: ECONOMIC IMPACT AND FINANCING

1 – SESSION
HONEY BEES, ENVIRONMENTAL POLLUTION AND PESTICIDES

Chairs: *Diego Pagani* – Apimondia and *Alberto Masci* – Ministry of Agriculture

14.30 Bees protection and sustainable land management
Lorenzo Ciccarese – ISPRA

14.50 Using pollen to monitor pesticide environmental pollution with a citizen scientist approach: the INSIGNA project
Jozef van der Steen – Alveus AB Consultancy

15.10 Honey bee (*Apis mellifera spp.*) and apiculture: essential components for a sustainable global growth
Michele Amorena – University of Teramo

15.30 *Discussion on "Honey bees, environmental pollution and pesticides"*

15.50 **Coffee break**

15.40 Scientific contributions on "HB, environmental pollution and pesticides"

16.20 Oral presentations on "HB, environmental pollution and pesticides"

- Does thiamethoxam effect honey bee queen (*Apis mellifera carnica*) development?
- Development of Some Residue Free Honey Bee (*Apis mellifera*) Colonies
- A meta-analysis to quantify toxicity of binary mixtures in bee species: evidence for deviation from dose addition and mechanistic implications

2 – SESSION
SHB IN CALABRIA REGION: ECONOMIC ASPECTS AND FINANCING OF THE BEEKEEPING SECTOR

14.30 SHB in Italy: economic considerations and financing
Cristina Salvioni – University of Chieti-Pescara, Italy

14.50 Round Table* part I

15.50 **Coffee break**

14.50 Round Table* part II

*with the participation of: Asger Søgaard Jørgensen (Apimondia), Dr. Marco Pellegrini (Ministery of Agriculture), Dr. Andrea Maroni Ponti (Ministery of Health), Emilia Reda (CREA-PB), Dr. Gianluca Grandinetti (Calabria Region), Raffaele Cirone (FAI – Italian Beekeepers' Federation), Giuseppe Cefalo (UNAAPI –National Union of Italian Beekeepers), Antonio D'Angeli (ANAI – National Association of Italian Beekeepers), Diego Pagani (CONAPI – Italian Beekeepers' National Consortium).

16.50 PRACTICAL DEMONSTRATIONS

Innovative methods to assist beekeepers in controlling *Varroa destructor*: "Treat in time"
Pasquale Rombolà and *Marco Pietropaoli* – IZSLT

Innovative, non-invasing pollen sampling methods
Giulio Loglio – Veterinary Services ASL BG; *Sjef van der Steen* – Alveus AB Consultancy)

17.20 Conclusions of the Honey Bee Health symposium 2018 from organizers
Ugo Della Marta – IZSLT
Peter Kozmus and *Riccardo Jannoni-Sebastianini* – Apimondia

15 FEBRUARY 2019

TECHNICAL TOUR IN COLLABORATION WITH PROFESSIONAL BEEKEEPERS

Local organizer: *Marco Pietropaoli* – IZSLT

GENERAL INFORMATION

Congress Venue

Hotel Palatino
Via Cavour, 213/M, 00184 Roma RM

How to get to the congress venue

From the Highway (30 km):

- From A1, for those coming from Milan, exit at Roma Nord and, after about 20 km, take the Settebagni exit. Continue along Via Salaria towards Roma Centro.
- From the A1, for those coming from Naples, exit at Rome East, take the Grande Raccordo Anulare and take Via Appia in the direction of S. Giovanni. Continue along Via Cavour. From Leonardo da Vinci Airport (35 km).
- Leonardo Express: With departures every 30 minutes Leonardo Express connects Rome Termini station with Fiumicino airport with no intermediate stops. Departures from Termini Station, buses 23 and 24. Service is also guaranteed in case of strike.
- Bus Shuttle: Departures every 30 minutes, Bus Shuttle connects Termini Station to Fiumicino and Ciampino Airports. Duration of the journey is approximately 1 hour and 15 minutes.
- Terravision: Departures every 20 minutes during the day, less frequent at night. Terravision connects Termini Station to Fiumicino Airport. The journey takes 55 minutes.

From Termini station: The Hotel is just 700 meters from Termini Station. You can take a taxi or Metro, Line B, Laurentina Direction. The Hotel is only one stop from Termini: get off at Cavour, the Hotel is only 50mt away.
By Taxi: From the two Airports, Fiumicino and Ciampino, the journey takes about 40 minutes.
GPS COORDINATES
41°53'44.3"N 12°29'32.2"E

Congress Languages

The languages of the Symposium 2019 are English and Italian. Simultaneous translation will be provided.

Coffee Breaks and lunches

Lunch is at participant's expenses and can be purchased directly at the bar and restaurant of the hotel.

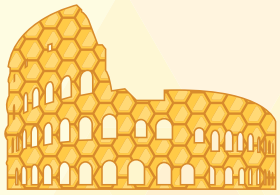
Name Badge

Upon arrival, all participants will receive a name badge, which must be worn visibly for the entire duration of the congress.

Audiovisual Instructions

Presentations can be downloaded directly in the meeting room at least 30 minutes before the presentation. Please bring the presentation on a Pen Drive, Hard Disk USB, CD-Rom and DVD-Rom. Video presentations need to have the following format .avi, .wmv, .mpeg. If Speakers have their presentation using a system different from MS Windows (such OS MAC or Linux), they are kindly requested to come earlier at the slide centre, in order to have enough time to solve possible compatibility issue. Speakers using their own MAC are kindly requested to bring also their Apple/VGA adapter.

KEYNOTE SPEAKERS



1. Session: An overview of the Honey Bee Pathologies in 2019

EU Honey Bee Health perspectives

Michele Mortarino
Dipartimento di Medicina Veterinaria, Università degli Studi di Milano - Società Scientifica Veterinaria per l'apicoltura (SVETAP)

Reports by public authorities and independent networks together with scientific evidences suggest that the health of honeybees is mainly affected by a number of factors: 1) poor nutrition status of the colonies, mainly linked to modified interaction between honeybees and their main sources of food, 2) new pathogens and predators not yet controllable by natural adaptation or emergence of resistance in the host, 3) increasing exposition to toxic chemicals, mainly insecticides, herbicides and fungicides applied to crops and remaining in the environment over long periods, and finally 4) winter losses of honeybee colonies where a combination of the above stressors is usually involved.
From a perspective point of view, some of the above threats may not be easily removed in the short term, as the lower availability of flower resources and alteration of blooming periods are mainly a consequence of large-scale crop monocultures and climate change, and the invasivity of alien pathogens and predators can quickly lead to endemic situations like experienced in the past decades about the mite varroa. Thus, in the next years the preservation of honeybee health will mostly rely on highly-managed colonies provided with external feeding and effective chemical and/or organic acaricides. At this last regard, scientists and veterinarians active in apiculture should keep working closely with beekeepers and beekeeper associations to fill existing knowledge gaps regarding the status of Varroa resistance to the approved active principles, the efficacy of new active principles and integrated varroa management practices, and how to provide honeybees with certain genetic traits that may be less vulnerable to specific pathogens. Fostering technological innovation and development of non-invasive approaches to monitor the health of the hive would also help to face the ethical challenges underlying artificial breeding of livestock, like apiculture can be perceived.
Different tools are available at a European level for present and future initiatives to improve the sanitary status of the apiculture sector. Direct payments for technical support of hive health are available to beekeepers and their associations under specific Common Agriculture Policy (CAP) measures activated by each Nation and/or other local initiatives. Financial support of research programs is also possible at the EU level through the Horizon 2020 research framework and related aims. Specific professional skills for the prophylaxis and management of colony health disorders are also increasingly supported, e.g. through the promotion of training opportunities for veterinary students in EU Universities. As in the recent past, also for the next future professional associations and scientific networks together with institutional and production bodies involved at various levels in beekeeping should establish constructive debates on specific honeybee health issues. Effective cooperation among stakeholders would allow to pursue harmonization of the priorities and to start operative strategies for the promotion of hive health across EU in the next years. Among these, Good Beekeeping Practices (GBPs) are a comprehensive set of protocols recommended to beekeepers for a proper management of the apiary and developed in the last years through the synergy of research, professional and istitutional bodies. The implementation of GBPs at the farm level should be encouraged as it can ensure food safety of the hive products and prevent spread of honeybee diseases. Accordingly, specific accreditation programmes are currently running in some EU Member states/regional areas to relieve the administrative burden for beekeepers and/or their associations that partly or fully comply with GBPs. This process should be boosted in the next years, also through the adoption of a common GBP-based policy at EU level. Besides, finalization of EU guidelines for proper beehive identification systems in accordance with the peculiarities of the apicultural production in each member State is foreseen to help the prevention of honeybee pathogens spread and the control of diseases. Finally, the effects of the new European regulation on veterinary medicines, with special regard to the harmonization of pharmaceutical regimes and product availability across Europe, shall also be verified in the next future.

Extra-EU Health perspectives on Honey Bees

Dr. Jeff Pettis
President, Apimondia Bee Health Commission

Honey bees are managed worldwide for honey, pollination and the production of bee products such as wax and royal jelly. The threats that bees face differ by region and the species of honey bee managed. I will restrict most of my comments to the European honey bee *Apis mellifera* but *Apis cerana* and other *Apis* species play key roles in Asia. What is true is that many of our beekeeping problems arise because we are trying to manage a bee outside its natural range (e.g. *A. mellifera* in Asia). While some problems are specific to beekeeping in the EU, in general beekeepers globally face many of the same threats. An EU specific threat to *A. mellifera* is the Asian hornet that is spreading across Europe following introduction via a shipment of pottery into France in 2004. While this pest is confined to Europe, at the moment, it may spread to new areas of the globe and reinforce the reality that we live in a world of common shared pests and diseases of honey bees.

If you are old enough you may remember a time when all you worried about as a beekeeper was; swarming, American foulbrood (AFB) and overwintering. Times have changed. Humans and trade are to blame for most of the worldwide movement of new pest and diseases of honey bees. There are regulations designed to limit the spread of all live animal pest and diseases. The World Organization for Animal Health (also known as OIE) sets these regulations in place to protect bees and beekeepers. This international body sets out diagnostic procedures and safe transport protocols for movement of live animals (honeybees) across borders. OIE list six diseases and pests that OIE regulates and most of these are globally distributed but have differing impacts depending on what species or subspecies of bee one manages. A major problem is that many pest and diseases are not covered by OIE guidelines and thus we have limited means to prevent or intercept their introduction to new areas (e.g. Asian hornet in the EU). I will discuss issues in bee health in the Americas and Australasia where *A. mellifera* is exotic and contrast that with problems faced by beekeepers in Africa. By no means will this be an all inclusive discussion of bee health issues but I hope to highlight the challenges we face and how beekeepers can help to prevent new introductions of pest and diseases.

European honey bees were introduced to the Americas and Australasia to produce honey and bees wax and pollinate crops. Managed honey bees have become vital to large-scale production agriculture in many areas. As such, hives are often managed on a commercial scale with an individual beekeeper managing more than 2,000 hives that both pollinate crops and produce honey. This type of beekeeping has its own set of problems. Such as the spread of AFB and other diseases as bees are managed in large apiaries and transported for pollination. In the U.S., pollination contracts for bees to pollinate almonds requires that colonies have 8 frames of bees in February, a time when they should still be in hibernation mode: this is unrealistic. However, beekeepers are finding creative ways to winter bees and build them up to meet this pollination demand. It does come at a cost in terms of feeding and transportation and more than 1.7 million colonies come together in California for a 4-6 week period which leads to increased disease spread. The revenue from pollination fees helps to offset the negative aspects of this early season pollination but by no means is it normal for bees to be that active in temperate regions in February. Australasia also has large scale commercial beekeeping with many of the challenges that this form of beekeeping produces. Contrast this high input beekeeping and pollination with smaller more diverse beekeeping in Africa.

The majority of bees managed in Africa are done on the local level and without movement of hives to new areas. The average beekeeper may keep bees in traditional log or bark hives or in movable frame hives that they monitor in set locations. The challenges the beekeeper faces may be more in terms of market access for honey and beeswax as opposed to damage from pests and diseases. The many honey bee subspecies in Africa are well adapted to dealing with pests and diseases. A good example of this is the small hive beetle; considered a background pest that rarely causes problems in Africa but is problematic as it has spread worldwide. Additionally, Varroa appears to be held in check by African bee sub-species, likely due to a variety of resistance mechanisms from grooming and hygienic behavior to small colony size and swarming. This is not to say that pests and diseases are not present and can cause harm to bees in Africa,

they can. But in general, the bees in Africa are managed in a more sustainable manor than are bees in other parts of the world where larger colonies are encouraged for honey production and pollination. Globally, we need to continue to select for locally adapted bees that can fight of pests and diseases. Better bees mean fewer control efforts by beekeepers and better health for our bees.
We face an everchanging challenge to manage productive and health bee hives. Beekeepers play a key role in keeping bees safe from invasive new pest as they are indeed the first line of defense. Regulations and quarantines play important roles, but it is the beekeeper who is most likely to detect a new problem. Thus, beekeeper education is an important tool to limit the spread of exotic problems. Your observations of changes in your colonies or new symptoms within your colonies can be vital to prevent new pest and diseases from spreading. Knowing what is normal in your hives and being able to recognize new threats is an important step to protect all beekeepers. Bee hives are susceptible to pest and diseases that do not respect borders, it is our job to try and limit the spread and impact of these pest on honey bees.

FAO perspectives on honey bees in terms of animal health and food security

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Bees are classified as as terrestrial animals by the World Organisation for Animal Health (OIE) (1) and likewise considered as such by European Union (EU) legislation (2). The position and importance of honey bees in terms of their contributions to environment biodiversity, quantity and quality of agro-livestock production systems have been highlighted elsewhere (3, 4, 5). The contribution of pollination provided by honey bees (agro-environmental service) is crop/orchard-dependent. For instance, strawberry fruits pollinated by bees have a highest commercial value (+54.3%), weight (30.3%) and shelf- life (11.0%) compared with those that are self-pollinated (6). Absence of bees for pollination can mean a loss to the farmer of 75 percent of the crop (7, 8). Moreover, hive products itself may represent an important source of food and energy for human consumption and a relevant economic input to improve livelihoods of farmers in developing rural areas all over the world.
More than 75 percent of the world's food crops rely to some extent on pollination for yield and quality. The absence of bees and other pollinators would obliterate the production of coffee, apples, almonds, tomatoes and cocoa, for instance. While bees and other pollinators are vital for animal production systems and food security by guaranteeing the agricultural production of animal feed [terrestrial and aquatic], vegetables, fruits, and crops they are facing several threats affecting directly or indirectly their health and considerable contributions to a biodiverse ecosystem, such as: land-use changes, disease and pests, poor management practices, indiscriminate use of veterinary medicines and pesticides, climate change, spread of monocultures that reduce the variety of food for bees, and globalization (an important driver that allows pests and diseases to spread over long distances).
Therefore, honey bees, like other large animals, must be protected. In fact, ensuring healthy hives, will contribute to the fulfillment of the FAO Sustainable Development Goals:
SDG 1 - No poverty: increasing honey bee populations and beekeeping production systems can be a possible to boost for economic growth and reduce poverty and inequalities among communities and nations.
SDG 2 - Zero hunger: increasing honey bee populations and beekeeping production systems contributes to the reduction of hunger and malnutrition.
SDG 3 - Good health and wellbeing: Increasing quality food intake by including honeybee products in the diet, promotes health by boosting the immune system, quality nutrition.
SDG 4 - Quality education: Balancing animal-source food intake (like honey, pollen, royal jelly) to increase children's cognitive development, prevention of stunting, and school attendance and performance.
SDG 5 - Gender equality: Specific hive productions (e.g. royal jelly and pollen) traditionally involve women. These activities will foster women's participation and decision-making powers in the livestock sector.
SDG 6 - Clean water and sanitation: Hive breeding does not need high water-use. This livestock production

systems could contribute in areas where water is scarce. Breeding hives do not produce animal manure and do not pollute the environment. Monitoring the environmental impact of pesticides and residues is required.
SDG 7 - Affordable and clean energy: Beekeeping production is a highly energy-efficient sector, with a low energy consumption related to high quality food production.
SDG 8 - Decent work and economic growth: Promoting the inclusion of beekeeping activities in agricultural livelihoods to increase food production and the income of smallholders and family farmers.
SDG 13 - Climate action: Combating the effects of climate change through improving a general environmental health and forestation. Bees are a sensitive indicator to environmental changes.
SDG 14 - Life below water: Reducing the impact of livestock on marine ecosystems by preventing pollution and containing the use of fish products in animal feed.
SDG 15 - Life on land: Breeding hives increase pollination and environmental biodiversity. Breeding hives increase pollination that is able to increase quantity and quality of crops. Breeding hives thus means to guarantee the productions of the other agro-livestock sectors.
SDG 12 - Responsible consumption and production: Producing more with less, while balancing consumption, and reducing losses. Enhancing the provision ecosystem services through sustainable grassland management and improvements in feed-use efficiency.
SDG 17 - Partnerships for the goals: Building inclusive partnerships in the livestock sector to support the achievement of the Agenda 2030. Translating the key role of livestock in the FAO Sustainable Development Goals into national policies and strategies. Contributing to a sustainable livestock management in agriculture and advancing towards an integrated livestock sustainable development approach.
Shifting the focus of the debate from fostering sustainable production to measuring progress to achieve the UN Sustainable Development Goals by enhancing the livestock sector's contribution, FAO is committed to beekeeping as a result of specific country requests and instances raised by its Member States. As an example:

1. The Animal Production and Health Division is enshrining beekeeping in its Domestic Animal Diversity Information System to accommodate also domesticated honey bees;
2. FAO is currently supporting a study on the use of antimicrobials in beekeeping with the objective to produce guidelines on the best management practices to reduce and potentially eliminate the use of antimicrobials in beekeeping adopting a sistematic progressive management pathway (PMP). Such guidelines can help to minimise the risk of residues in honey bee products and antimicrobial resistance in the "One Health" approach and will ensure the improvement of honeybee health though prevention of threats, improved hygiene and consequently increase the performance of the beehives, the profitability of the beekeeping operation and the pollination service provided by honeybees.
3. This could open new markets for honey bee products worldwide, even in those regions where the use of antimicrobials in apiculture is allowed and applied.
4. Another area of direct involvement is entrusted with the Agricultural Research Unit (AGDR) through the Technologies for Agriculture (TECA; http://www.fao.org/pollination/resources/news/detail/en/c/1129671/) platform that, in its dedicated section of the Beekeeping Exchange Group, offers an articulated repository of information material, manuals, technical sheets, equipment construction plans and best management practices for small holders engaging in beekeeping especially in rural areas and communities.
5. FAO is also actively collaborating at various levels with other external partners such as the World Organisation for Animal Health, Apimondia and the network of Italian Regional Institutes for Animal Health and Food Safety to complement its action in beekeeping.

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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

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"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);

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);

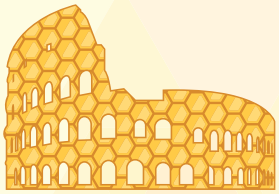
Disinfect beekeeping tools and equipment between uses: torching (*Nosema ceranae* spores are inactivated by over 60 °C); gamma irradiation; fumigation of combs with glacial acetic acid, sodium hydroxide 5% (caustic soda); sodium hypochlorite 0.5% (bleach). Prerequisite of any use of disinfectants is 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.



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

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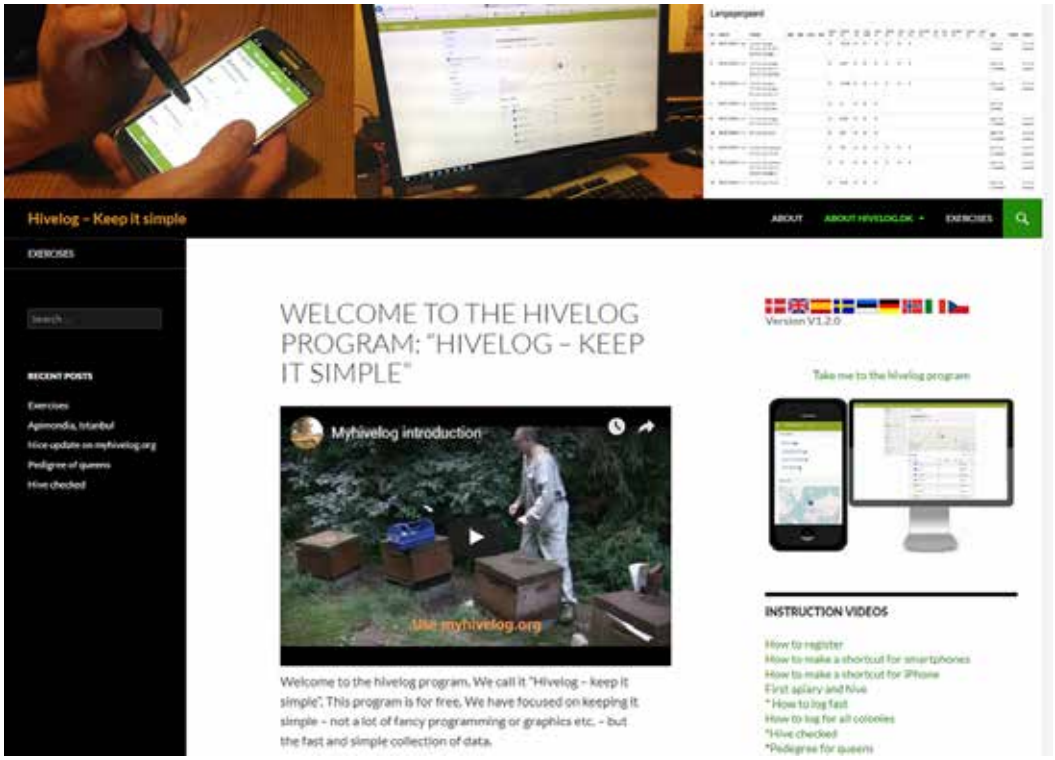
Danish Beekeepers Association

Hivelog - Keep it Simple. An easy way to keep track on your colonies
Become a better beekeeper with this Danish, freely available Hivelog program. Hivelog is made by beekeepers, for beekeepers. It is a hive log/note program that can be used on your smartphone, iPhone, tablet etc. This program is not an app, so it is easy to access and use on all platforms.
Today in modern farming it is getting more and more common to collect data during the season to improve/optimize the use of fertilizer, pesticides, feeding, soil use, etc. The opposite is the case within "common" beekeeping. In general no or very few data are collected and stored on single colony level for later use/experience/development. Using this program, you will be able to get an easy and fast overview of your beekeeping. You can compare queens, colonies, behavior, feeding, varroa treatment, varroa counts, bee diseases etc., but also recall how and when you did your activities. The program sticks to simple statistics, that is needed by most beekeepers. For beekeepers with special needs, you can export the data to excel spreadsheets and here personalize your own statistics.
We have focus on only the most important information that is needed for improving beekeeping. The

backbone is the Danish queen breeders paper hivelog. We focus on making data collection fast and easy. Beekeepers do not have time for time-consuming logging. We try to keep the program simple and user friendly. There is no fancy setup or layout.
The program is now translated into 9 languages – Danish (www.stadekort.dk), English (www.myhivelog.org), Spanish (www.RaApi.org), Norwegian, Swedish (www.skötselkort.se), Estonian, Italian and Czech. To encourage the use of this non-commercial program, it is for free, no matter how many colonies you have. Within the next 3 years, we plan to expand the program with a bee disease module, giving the beekeepers "hands on" bee disease information directly in the apiary. Go to www.myhivelog.org, register and try it out. During the conference, feel free to approach me if you have questions, I can solve most of them on location. More information on www.myhivelog.org.



3. Session: Aethina tumida – The Small Hive Beetle (SHB)

Small hive beetle invasion in EU

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Larvae of Aethina tumida Murray, the small hive beetle (SHB), were firstly notified in the European Union in Portugal in 2004 following an importation of queen bees from Texas (US). The stamping out measures immediately adopted prevented any further infestation and spreading of this exotic beetle. Ten years later adults and larvae of SHB were reported in Italy in September 2014 in honey bee nucleus colonies near the Gioia Tauro port in the Calabria region (southern Italy). In November 2014, an infested apiary was found in eastern Sicily. Genetic analyses revealed the African origin of SHB introduced into Italy. Early reaction measures adopted in Italy required immediate notification of SHB detection to the local veterinary services, movement restriction of the concerned colonies and apiaries, destruction of the entire infested apiaries followed by ploughing and pyrethroids soil drench application. In Calabria region, 132 positive sites and a single one in Sicily were officially reported and destroyed between 2014 and 2018. The Ministry of Health granted compensation to beekeepers according to the law in force. Furthermore, a 20 km radius protection zone and a surveillance zone covering the entire territory of Calabria and Sicily regions were established. Compulsory visits to all apiaries in the protection zone with georeferentiation and visual colony inspection according to 5% expected prevalence (95% CI) were applied. In the surveillance zone, apiaries to be inspected were selected according to risk analysis or randomly and colonies were inspected according to 2% expected prevalence (95% CI). Sentinel honey bee nucleus colonies were installed to improve SHB detection and facilitate the activity of official veterinarians in the protection zone. In spring 2017 restriction measures applied to Sicily region were lifted while the surveillance program was continued and intensified. Additionally, the national SHB surveillance program established in spring and autumn 2015 was extended until 2018. No SHB has been detected outside the two concerned regions. Future perspectives of containment are discussed.

Comparison of two colony inspection methods for the detection of Small Hive Beetle (SHB) in Calabria region (Italy)

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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

82 colonies divided in two groups recording the number of SHBs found. The colonies were housed in 10 frame Dadant-Blatt hives and homogeneously distributed in two apiaries in a SHB infested area in the Calabria region. All groups were homogeneous in terms of strength and amount of brood. The average time needed to apply the official inspection protocol was 11 minutes and 43 seconds per hive, while the "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 of the inspection time. The difference between the methods was statistically significant (p=0.014). Adopting the official inspection protocol, 2.05±3.00 SHBs were found, while with the time-saving protocol 2.86±3.77 SHBs were found. There was not a statistically significant difference between the two methods (p=0.151). The time-saving method reduces the inspection time by 31.86% while the efficacy remains the same of the official inspection method. Using an automatic instrument to capture the beetles could reduce the inspection time. The time-saving inspection method represents a useful detection tool, easing the application of SHB control measures.

4. Session: Main Honey Bee diseases

Varroa resistant Honey Bees: where do we stand?

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The mite *Varroa destructor* remains the main biotic threat to *A. mellifera* worldwide. This parasite has eradicated most wild *A. mellifera* populations and generates important economic losses in the beekeeping industry. Until now, the most widespread Varroa control strategies rely on synthetic acaricide such amitraze, whereas a more marginal but increasingly used strategy relies on organic compounds such as oxalic and formic acids. However, none of these medication-based strategies is sustainable in the long term due to the accumulation of residues in hive product and concerning the synthetic acaricide to the appearance of resistance in their targets. It is therefore becoming crucial to find alternative, more satisfying control methods. Breeding Varroa resistant honeybees could represent such an alternative. In this presentation, we will discuss approaches to select and breed for such honeybees, and highlight challenges that need to be overcome to reach this goal, as well as perspectives for the current initiatives.

Updates on *Nosema ceranae*

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Nosema ceranae is the most prevalent Microsporidia of *Apis mellifera* and it is a major health problem for bees worldwide. Microsporidia are a truly diverse lineage of extremely reduced obligate intracellular parasites which are related to Rozellida (=Cryptomycota) or that diverged as the branch below the fungi, within the Holomycota clade of Opisthokonta (James et al., 2013; Bass et al., 2018; Galindo et al., 2018).
Since its first report in *Apis mellifera* in 2006, several studies have been developed to address its epidemiology and routes of transmission (faecal-oral) and to determine the effects in honey bees both at individual and colony levels. Nowadays, it is known that *N. ceranae* infection modifies the honey bee's metabolism, immune response and other vital functions. It has been also described that the interactions with other nosogenic agents (as pesticides and other pathogens), the environmental conditions, the beekeeping practices and the host genetics will have a direct influence on the development and evolution of the infection and the effects of the resulting disease. Due to the absence of clear clinical signs, the infection can be unnoticed by the beekeeper for long periods. However, there are some effects of infection that can be perceived in the colonies as a loss of adult bee population, a reduced honey production and higher susceptibility to other diseases. Due to the absence of specific treatment methods, the control techniques should be directed to reduce the number of bees infected in the colony, in order to keep the parasitic charge at low levels compatible with a productive activity that could fit beekeeping and pollination requirements.

Diagnosis and control of American foulbrood and European foulbrood

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American foulbrood (AFB) and European foulbrood (EFB) are serious and widespread bacterial diseases of honeybees that cause considerable economic losses to beekeepers worldwide.
They affect the larval stages but can kill the entire diseaded colony, if untreated AFB is caused by the spore-forming bacterium *Paenibacillus larvae*, practically present in pure culture in the infected larvae. EFB is caused by *Melissococcus plutonius*, generally associated to secondary invaders like *Enterococcus faecalis* and *Paenibacillus alvei*.

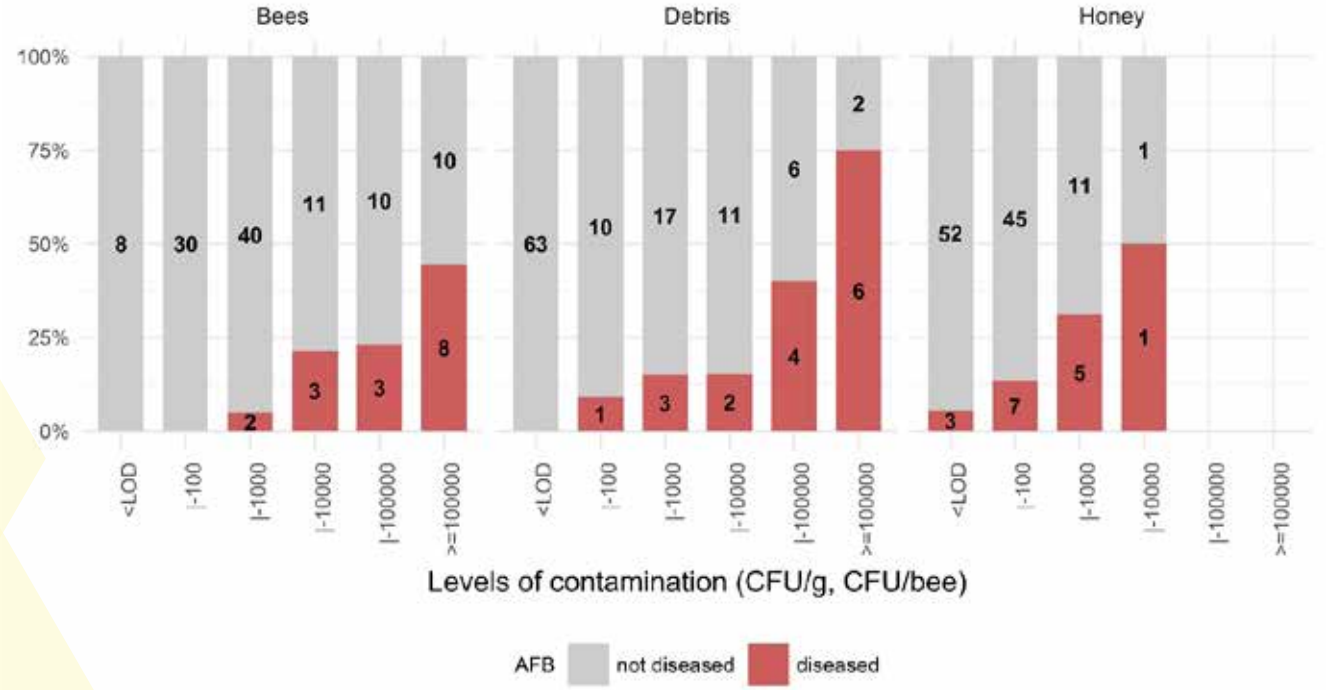
AFB and EFB are notifiable diseases in most countries, as considered very contagious and dangerous. In fact diseased colonies are considerably weakened and can die, if not managed with appropriate hygienic practices. Moreover contaminated beekeeping equipment generally must be destroyed or sanitized to prevent further spread of the infection.
The use of antibiotics for the control of these infections involves the risk of honey contamination of beehive products and can led to the appearance of resistant bacterial strains. Furthermore antibiotics do not ensure curative effect, especially in the case of *Paenibacillus larvae*, which produces resistant spores. . Nowadays, the use of antibiotics in beekeeping is not allowed by health legislation in European Union, and the affected colonies have to be destroyed by burning the hives or sometimes submitted to technical actions, like the shaking method, if infected but not yet clinically diseased.
Because of the above mentionated problems, the control of AFB and EFB is essentially entrusted to preventive measures, which first of all include early diagnosis of the infection. Moreover, in future perspective, the development of alternative methods to conventional antibiotitcs could be advantageous to reduce the impact of the infections by means of environmentally sound strategies.
The following paragraphs report some results of the studies I have carried out relatively early diagnosis of AFB and the effectivness of natural compounds in the control of AFB and EFB.

Quantification of *Paenibacillus larvae* spores in samples of bees and hive debris as a tool for American foulbrood risk assessment

The diagnosis of AFB based on the recognition of clinical signs is a practice with some clear limitations. It can be laborious and time consuming, particularly for large beekeeping operations or for territorial monitoring. Moreover, the disease may remain undetected by visual inspection in the initial stages (Lindström and Fries, 2005). Furthermore, clinical examination does not enable the detection of colonies with asymptomatic infections.
Actually subclinical infections are very common and may lead to recurrences of the clinical forms in apiaries and contribute to the horizontal transmission of the infection from one hive to another (Lindström and Fries, 2005). Hence, identifying colonies with high spore loads allows the implementation of preventive measures to control the onset and spread of the disease (von der Ohe, 1997).
Today, standard methods based on microbiological and biomolecular techniques are available for the detection and quantification of the spore load in adult bees and hive materials such as honey, wax and wax debris (de Graaf *et al.,* 2013; Anonymous, 2016).
The level of *P. larvae* spores in adult bees and honey stored near sealed brood can provide information about the presence of disease symptoms in honey bee colonies. This relationship has been investigated by or the honey. Several authors found correlations between certain spore values in adult bees or in honey stored in the brood chamber and the presence of AFB, and they related levels of *P. larvae* spores to observations of disease symptoms (Goodwin *et al.,* 1996; Ritter, 2003; Fernandez *et al.*, 2010; Gende *et al.,* 2011). Methods for the detection of *P. larvae* spores in wax debris collected at the bottom of the hive have been developed in the Czech Republic (Titera and Haklova, 2003; Bzdil, 2007; Ryba *et al.*, 2009). These methods have shown a good ability to identify colonies infected by *P. larvae*.
The research here reported is a synthesis of a study which I have previously published in cooperation with other authors (Bassi et al, 2018). It concerns the relationship between the level of the wintry contamination by *P. larvae* in materials taken from the hive and the onset of the AFB in the following spring. For this purpose, culture-based techniques were applied to samples of adult bees, honey and hive debris, collected in winter from 165 hives distributed in ten apiares. Analytical performances were compared to the results of clinical examinations carried out in the spring on the same colonies.
To collect debris, plastic sheets were placed at the bottom of the hive at the beginning of December. At the end of January, the sheets with debris were picked up from each colony, together with 50 living bees and 50 g of honey.
From the beginning of March to the end of May, the colonies were checked at three-week intervals by visual inspection for the presence of clinical symptoms of AFB. The results of the clinical examination were

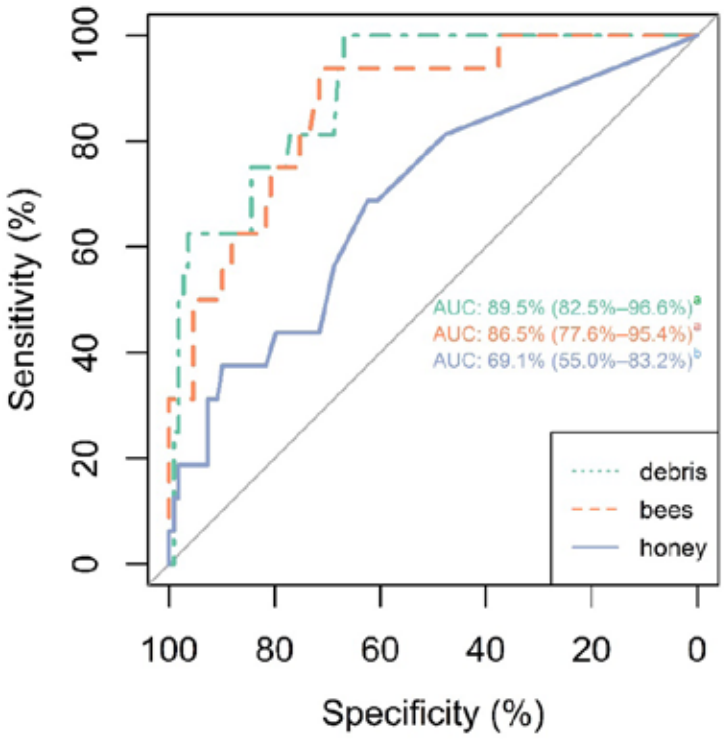
recorded simply as presence or absence of disease symptoms. The diseased colonies were destroyed immediately after the diagnosis.
The analytical results of bees, honey and hive debris, grouped in six classes of contamination (one negative class [< LOD] and five positive classes) and categorised by clinical outcome, are shown in Fig. 1. The proportion of diseased colonies therefore varies depending on the level of contamination. In particular, all the colonies with debris contamination < LOD were negative for AFB, while some positive colonies were found at each level of contamination; the colonies with bee contamination < LOD or with low contamination (< 100 CFU/bee) showed no symptoms of disease; finally, at each level of honey contamination, some AFB-positive colonies were found, even in cases with negative analytical results.

Fig. 1



To compare the performance of the three diagnostic tests, we used receiver operating characteristic (ROC) curves and positive predictive value (PPV) calculated at specific cut-off thresholds.
The ROC curves provide a synthetic description of the ability of the test to distinguish between diseased and non-diseased colonies (accuracy). The extension of the area under the ROC curve (AUC) is an indicator of the accuracy of the diagnostic test: the greater the AUC, the greater is the discriminant power of the test. In Fig. 2, the ROC curves show sensitivity (Se) and specificity (Sp) values of each material at various cut-off thresholds. AUCs were calculated with 95% confidence intervals; AUC can be seen as the probability that a diseased colony has a greater test value than a non-diseased colony.
The AUCs of debris and bees had similar values (89.5% and 86.5%, respectively) and did not show significant differences (DeLong's test, $p = 0.45$). Conversely, the AUC value of honey (69.1%) was significantly smaller than those of the other two tested materials (DeLong's test, $p<0.01$).
Because a diagnostic test is considered appropriate if the AUC is ≥ 70% (Swets, 1988), we decided to exclude honey from the subsequent data analysis.
Se and Sp of bees and debris against the possible cut-off thresholds are graphically shown in Fig.3.
The high proportion of positive samples (117/125) makes the examination of adult bees a more sensitive method, but the specificity of this method is poor at all cut-off thresholds. In general, compared with bees, debris had a lower Se and a higher Sp.

Fig. 2



In table 1, some values of Se and Sp obtained at selected cut-off thresholds are shown . With the LOD as the cut-off threshold, the Se is 100% for both debris and bees, while the Sp is 58% for debris and 7% for bees. The positive predictive value (PPV) is another parameter considered to evaluate and compare the performance of bee and debris examination. The PPVs reported in the table are related to the disease prevalence detected in our study (12.8%). Debris showed better performance in terms of PPV, due to the higher Sp of this material. When the cut-off threshold increases, the PPV also increases. In particular, this effect is evident in the debris, for which the PPV is 75% at a cut-off threshold of 50,000 CFU /g.

Table 1

Cut-off threshold	Debris			Bees		
CFU/g – CFU/bee	*Se (%)*	*Sp (%)*	*PPV (%)*	*Se (%)*	*Sp (%)*	*PPV (%)*
LOD	100	58	26	100	7	14
100	94	67	29	100	35	18
1000	75	83	39	87	72	31
10000	62	93	56	69	82	35
50000	56	97	75	62	88	43

The results obtained show that the risk of disease is related to *P. larvae* spore levels in the hive; the higher the number of spores detected in winter, the greater is the probability that the colony develops AFB symptoms in spring. However, other important factors are involved in determining the outcome of *P. larvae* infection in honey bees colonies, like the colony resistance, mainly due to the level of hygienic behaviour (Spivak and Reuter, 2001), and the different virulence of *P. larvae* strains or genotypes (Genersch *et al.*, 2005).

According to the classification proposed by Sweets (1988), the AUC values demonstrate a good test accuracy for both bees and debris, in contrast, the honey examination shows a moderate accuracy (Fig. 2). For this reason the honey has limited usefulness for disease risk assessment.
In theory, the optimal cut-off can be considered the one that maximises the sum Se + Sp. In our case, this value corresponds to 76-80 CFU/g for debris (Se = 100%, Sp = 66%) and 764-875 CFU/bee for bees (Se = 94%, Sp = 71%).
The results obtained from bees and debris do not show statistically significant differences and both are suitable to foresee the disease onset. Nevertheless, the debris examination seems more suitable for identifying honey bee colonies at risk of disease, because of its higher Sp and consequently higher PPV. The debris sampling is not destructive, is easy to perform and requires little time; additionally it is a non-invasive sampling and can be performed without opening the hives even in the winter.
These preliminary results are promising and should therefore be confirmed on a larger sample size of infected colonies, including colonies with *P. larvae* genotype ERIC II infections and colonies with mixed infections (ERIC I + ERIC II).
The possibility of identifying colonies with an increased risk of developing the disease in the short to medium term represents a useful tool for controlling the spread of AFB, because it allows beekeepers to take appropriate measures to prevent the onset of the disease and the spread of the infection.

Natural strategies for the control of American foulbrood and European foulbrood

The development of alternative methods for the control and prevention of AFB and EFB has been receiving considerable attention of the researchers in the last decades. Different natural strategies based on the application of essential oils, plant extracts, propolis, royal jelly, non-conventional natural molecules, bacteria and bacteriocines have been studied in vitro and in vivo for the prevention and control of *P. larvae* and *Melissococcus plutonius.* This topic has been recently reviewd by Kusyšinová *et al*. (2016) and Alonso-Salces *et al*. (2017). Although many natural substances have shown inhibiting activity against bacteria responsible of honey bee diseases, few field experiments have been performed so far, with not encouraging results.
Here are summarized the results of some tests I carried out in the past on the antibacteral activity of essential oils and fatty acids.
Table 2 shows the antibacterial activity of essential oils obtained from *Polygonum bistorta,* an autochtonous plant of Italian Alps, provided with medicinal properties. The volatile axtracts of the plant were assayed with strains of *Paenibacillus larvae* (AFB), *Melissococus plutonius* (EFB) and against a reference bacterial species, *Bacillus subtilis*. Moreover data were compared to those obtained with reference standard of known effectiveness, such as the essential oils from leaves and bark of *Cinnamomum zeylanicum* and the antibiotic oxytetracyclin (Cecotti et al., 2012).
Antimicrobial activity noticeably varied depending on the phenological stage in which the plant material was collected. *P. bistorta* oils gave comparable values of inhibition to those of *C. zeylanicum* in vegetative and flowering phases, while it proved to be even more active against both *P. larvae* and *M. plutonius* in the fructifying phase. It is worth to note that inhibition values for the fructifying phase were comparable to those observed for oxytetracyclin. The standards of the two most abundant compounds of *P. bistorta* oil were also tested: lauric acid and its methyl ester. Lauric acid is likely to be the most active antimicrobial component of the essenstial oil and these data confirm the high activity of linear-chain acids, particularly against *P. larvae*. It is worth to note that this is a different chemical class of compounds from that reported for the antimicrobial substances from other plant species like *C. zeylanicum*, namely aromatic cinnamates.

Table 2. Inhibition area diameter of the *Polygonum bistorta* L. volatile fractions, mean value (in mm.) in an agar-well diffusion test. Values relative to concentrations of 2000 µg/ml per well (in ethanol)

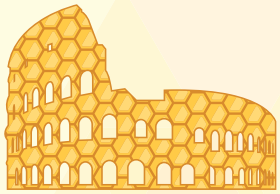
Bacterial strain	Analyte								
	Pb.1[a])	*Pb*.2[a])	*Pb*.3[a])	*Cz*.1[b])	*Cz*.2[b])	Ot.[c])	Lauric acid[d])	Methyl laurate[e])	Blank[f])
Paenibacillus larvae									
ATCC 9545	7.5	7.5	20.0	9.5	4.0	36.0	22.5	2.0	3.0
CRA-API 10/8	8.5	13.0	24.5	8.0	3.0	36.0	29.5	3.0	3.0
CRA-API 09/1	8.0	7.5	24.0	12.0	5.0	39.0	24.0	2.5	3.0
Melissococcus plutonius									
ATCC 35311	0.5	0.5	4.5	0.5	0.5	39.0	2.5	0.5	0.5
CRA-API 08/1	0.0	0.5	2.5	2.0	0.0	37.0	2.5	0.5	0.0
CRA-API 09/2	0.5	0.5	6.0	2.0	0.5	39.0	4.0	1.0	0.5
Bacillus subtilis									
ATCC 31324	5.5	4.0	11.0	13.0	0.0	20.0	8.0	3.0	0.0

[a]) *Pb*.1, *Pb*.2, *Pb*.3: *Polygonum bistorta* essential oils from vegetative, flowering and fruiting stages, respectively.
[b]) *Cz*.1, *Cz*.2: Essential oils from *Cinnamomum zeylanicum* bark and leaves, respectively, tested as references.
[c]) Ot.: Oxytetracyclin, tested as reference antibiotic compound.
[d]) Blank: 100% Ethanol.

The inhibition power of fatty acids against *P. larvae* was highlighted also in my previous studies (Carpana et al. 2005). In table 3 are reported the results of bioassays performed with the disk diffusion method on linoleic acid and several essential oils. The activity of oxytetracyclin as reference conventional antibiotic is also reported. Among the natural compounds, the greatest inhibiting action was shown by linoleic acid, followed by essential oils of *Cinnamomun zeylanicum*, *Aloysia triphylla* (lemon verbena), *Cymbopogon citratus* (lemongrass) and *Eugenia caryophyllata* (eugenia).
The Minimum inhibiting concentration against *P. larvae* was then determined for linoleic acid and cinnamon oil, in comparison to some conventional antibiotics, using a broth dilution method. Results confirm the remarkable activity of linoleic acid:

oxitetracyclin	0,11 µg/ml
tylosina	0,11 µg/ml
amoxicillin	0,26 µg/ml
linoleic acid	2,25 µg/ml
olio essenziale di cannella	64,0 µg/ml

Afterwards, field tests were carried on to verify the effectivness of linoleic acid in controlling American Foulbrood. Linoleic acid was administered to 10 infected bee-colonies. Its action has been evaluated in comparison with a group treated with oxytetracycline hydrochloride and with a control untreated group. Chemicals were administrated with sugar candy and simultaneously infected brood was removed and destroyed. During the 12 weeks following the treatment, the disease recurrence rate was 0/10 in the oxyteytracycline group, 4/10 in the linoleic acid group and 8/10 in the control group. Despite some colonies shown a relapse, disease recurring was significantly less in the group treated with linoleic acid in comparison to untreated group.
These data confirm that fatty acids are among the most active natural substanses against bacteria affecting honey bees, as it results from several other studies (Feldlaufer *et al,* 1993; Hornitzky, 2003; Kusyšinová, *et al* 2016). In addition, they are important in honey bee development, nutrition and reproduction. The pollen, which is fed to bee larvae together with royal jelly, is an important source of fatty acids and this fact suggests that natural immunity of honey bees could increase through food (Feldlauder *et al*., 1993).
In conclusion fatty acids constitute a promising alternative for fighting AFB and EFB. They are, other than potentially effective, safe and environmentally-sound as well as non toxic to man.



However, to introduce natural compounds in beekeeping, further research is necessary to solve some practical problems. The effectiveness of these antimicrobials at field conditions must be verified. As well, studies on the distribution and effects of these products on beehive products are necessary Another important issue is the development of proper delivery methods of the natural products inside the beehives for the in vivo treatments. Furthermore, the willingness of bees tu take the compounds should be evaluated.

Finally, it is important to underline that the use of natural compounds for the control of honey bee infections should be intended as subsidiary to the good breeding practice, for maintenance of health and production of bee colonies.

Table 3-. Diameters (mm) of zones of inhibition for natural substances tested against *P. l. larvae* and *B. subtilis*, using disk diffusin method. Average values and standard deviation of three measurements are reported for each dose. 0 = no activity. a) Test results with linoleic and botanical compounds b) test resdults with Oxytetracyclin as reference antibiotic

a	Cultures	Paenibacillus larvae larvae												Bacillus subtilis			
		MO/03				934/03				ATCC/9545				ATCC/10783			
mg/disk		5000	500	50	5	5000	500	50	5	5000	500	50	5	5000	500	50	5
Linoleic acid		47,0	21,7	17,3	9,0	55,3	41,3	25,7	11,0	49,7	22,3	13,7	8,7	10,3	7,3	0	0
		± 9,8	± 2,9	± 2,5	± 1,0	± 5	± 4,2	± 0,6	± 1,7	± 4,5	± 2,5	± 1,2	± 1,2	± 0,6	± 0,6		
Cinnamomum zeylanicum		21,7	16,7	0		35,0	10,3	0		31,7	0	0		21,3	12,3	0	
		± 2,9	± 2,9			± 13,2	± 2,1			± 2,9				± 2,1	± 2,5		
Citrus paradisi		0	0	0		12,0	0	0		11,3	0	0		11,3	0	0	
						± 2,0				± 1,5				± 0,6			
Citrus sinensis		0	0	0		10,7	0	0		0	0	0		11,3	0	0	
						± 1,2								± 0,6			
Citrus aurantium		8,7	0	0		8,3	0	0		0	0	0		8,7	0	0	
		± 1,2				± 0,6								± 1,2			
Citrus reticulata		12,7	0	0		19,3	0	0		8,3	0	0		0	0	0	
		± 1,2				± 3,1				± 0,6							
Citrus limonum		0	0	0		0	0	0		0	0	0		11,3	0	0	
														± 0,6			
Aloysia triphylla		36,7	0	0		43,3	0	0		24,7	0	0		30,7	0	0	
		± 7,0				± 5,8				± 4,6				± 1,2			
Cymbopogon citratus		33	0	0		32,3	17,0	0		21,3	0	0		17,3	0	0	
		± 3,0				± 0,6	± 2,6			± 1,2				± 1,2			
Melaleuca alternifolia		9,3	0	0		8,7	0	0		8,3	0	0		9,3	0	0	
		± 1,2				± 1,2				± 0,6				± 1,2			
Melaleuca leucadendron		8,7	0	0		8,3	0	0		0	0	0		10,7	8,3	0	
		± 1,2				± 0,6								± 1,2	± 0,6		
Eugenia caryophyllata		44	13,0	0		38,0	14,7	0		29,7	12,0	0		20,7	8,3	0	
		± 8,5	± 4,0			± 2,0	± 2,1			± 6,8	± 4,4			± 1,2	± 0,6		

b	Cultures	Paenibacillus larvae larvae												Bacillus subtilis			
		MO/03				934/03				ATCC/9545				ATCC/10783			
mg/disk		30	3	0,3	0,15	30	3	0,3	0,15	30	3	0,3	0,15	30	3	0,3	0,15
Oxytetracyclin		60,3	44,3	0	0	61,7	43,3	13,3	9,3	38,0	24,3	9,0	7,7	27,0	18,7	9,7	8,3

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Use and management of the veterinary medicinal product in beekeeping

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The veterinary medicinal product is not a derivative of the medicinal product for human use but has its own identity and is studied and developed according to the animal species to which it will be destined. Veterinary medicinal product means any substance or combination of substances presented as having curative and prophylactic properties of animal diseases, which can be used on the animal or administered to the animal for the purpose of restoring, correcting or modifying physiological functions through a pharmacological action, immunological or metabolic, or to establish a medical diagnosis. No veterinary medicinal product may be marketed without obtaining marketing authorization. It is forbidden to administer to the animals pharmacologically active substances such as oxalic acid if not in the form of authorized veterinary medicinal products. Pharmaceutical companies producing veterinary drugs have the task of guaranteeing the quality, safety and clinical efficacy of the drug The competent authority carefully verifies the studies and authorizes the marketing of the veterinary medicinal product The veterinarian must inform the farmers about the correct management of veterinary medicinal products in order to prevent common diseases. It must also ensure that the medicines are used only as prescribed. Most veterinarians are responsible for the proper management of stocks of drugs on the farm, registration of therapies in the register of treatments and use in derogations to veterinary medicines which is its exclusive prerogative.Compliance with laws should not be considered by the breeder mere bureaucracy but a practice of protection that, based on scientific data, guarantees the health of bees and consumers and allows the company to be placed at a low level of risk with reduced controls by the competent authorities. The legislation therefore becomes not an obstacle but a means to operate in quality. Farmers must be aware that often few simple measures are needed to improve the environmental, nutritional and sanitary conditions of the animals assisted in order to guarantee them the physical conditions and well-being necessary for the development of a solid immunity that protects them from pathogenic agents from the external environment to reduce the use of drugs in general as much as possible. The concepts mentioned above can be schematically translated into the following general practices:

- Improper use of the veterinary medicinal product in the treatment of certain animal diseases has led to the development of organisms resistant to them. For example, inappropriate use of acaricides in the treatment of varroasis could lead to birth resistance and increase the risk of transmission of infectious diseases.
- The presence in the honey of prohibited substances or with residual limits higher than those allowed represents a serious risk for the health of consumers, negatively affecting the productivity and profitability of the apiaries.
- The use of veterinary medicinal products or other unauthorized substances may pose a risk to the veterinarian or beekeeper if used otherwise than authorized and posing a risk to the environment. It is necessary a reasonable relationship between authorized drugs purchased and hives in the company.
- The commitment over time to deepen the knowledge about the veterinary drug, was appreciative. All this led to the development of products for specific use with formulations suitable for use in different animal species.
- The use of specific products for veterinary use is always advisable ensuring quality, safety and effectiveness.

5. Session: Honey bees, environmental pollution and pesticides

Bees protection and sustainable land management

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Bees, both social and solitary, are the most important pollinators in natural and semi-natural ecosystems; they include many species (about 20,000 in the world, almost 1,000 in Italy) and contributing to about one third of the entire world food production. Yet, we need to apply proper landscape management practices in order to boost native bees density by increasing habitat-carrying capacity (Kremen C., *et al*. 2007). For this purpose we suggest to integrate the following sustainability practices into specific national management plans (PAN) [Potts S.G. *et al.,* 2010]: -connecting habitats with flowering strips and hedgerows around arable fields, small forest patches or even single trees as '*stepping stones*' in order to enhance opportunities for pollinators colonization, and to reduce the risk of population crash in the field and surrounding habitats by foregoing use of broad-spectrum pesticides during bloom. This is especially true for those chemicals with systemic or micro-encapsulated insecticide formulations that can easily contaminate nectar, pollen and others hive products; -increase nesting opportunities of different bees species including gaps in surface vegetation and holes in the terrain, or modifying cultivation practices (Biesmeijer JC, et al. 2006),, for example retaining neighboring forest nesting sites for ground-nesting bees or leaving dead wood and providing holes for nesting-cavity; -increase different suitable floral resources in the local area and the broader landscape during the season of pollinator activity for enhancing biodiversity and feeding availability for bees (Decourtye A, Mader E, Desneux N. 2010).Crop rotation by appropriate flowering plants should be also applied, especially in intensively managed farmlands, as they would improve vital ecosystem services, and improve pest control by breaking cycles of damaging pests or soil erosion control (Mallinger R. E. and Gratton C., 2015). Financial burdens of these recommendations could be compensated through agro-environmental schemes, such as those in Europe, to encourage farmers who apply management strategies aimed to the safeguard of soil integrity and the enhancement of ecosystem biodiversity.

The Insignia project: Environmental monitoring of pesticides use through honey bees (PP-1-1-2018; EC SANTE)

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[1] Alveus AB Consultancy, Oisterwijk, Netherlands (coordinator Insignia project)
[2] www.insignia-bee.eu

Insignia is a pilot project initiated and financed by the European Commission. It aims to design and test an innovative, non-invasive, scientific substantiated citizen science environmental monitoring protocol, for the detection of pesticides by honey bees. The study is being done by a consortium of specialists in honey bees, apiculture, statistics, analytics, modelling, extension, social science and citizen science from Austria, Belgium, Denmark, France, Greece, Ireland, Italy, Latvia, the Netherlands, Portugal, Spain and the UK.
Honey bee colonies are excellent bio-samplers of floral material of biological origin like nectar, pollen and plant pathogens as well as that of non-biological origin, such as pesticides or airborne contamination. In an apiary, foraging honey bee colonies spread themselves over a circle of 1 km radius, increasing to several kms if required depending on the availability and attractiveness of food. All material collected is concentrated in the hive.
The honey bee colony provides four main matrices for monitoring: bees, honey, pollen and wax. For pesticides, pollen and wax are the focal matrices. During the season, the majority of pollen is consumed within days, so beebread can provide recent, random sampling results. On the other hand wax acts as a passive sampler, building up an archive of pesticides that have entered the hive. Alternative in-hive passive samplers will be tested to replicate wax as a "pesticide-sponge". Trapped pollen will be sampled as well every two weeks to record foraging conditions. The data on pollen and pesticides will be combined to obtain

information on foraging conditions and pesticide use, together with evaluation of the Corine database for land use and pesticide legislation to model the exposure risks to honey bees and wild bees.
All monitoring steps from sampling through to analysis will be studied and tested in four countries in year 1 (2019), and the best practices will then be ring-tested in nine countries in high and low risk areas in year 2 (2020). Monitoring issues addressed will be amongst others the best matrices for pesticide adsorbance, storage and transport of samples, molecular detection of pollen origin and social acceptance of the protocol by the apiculturist citizen scientists.
The result will be a scientific substantiated, practical and easily applicable, citizen science protocol for monitoring pesticide use through honey bees. Information about the course of the project and its results and publications will be accessible via the insignia website www.insignia-bee.eu.

Honey bee (*Apis mellifera* spp.) and apiculture: essential parts for a sustainable global growth

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University of Teramo

The assessment of the environmental health status is a requirement that can be carried out following several methods: chemical, physical, electronic and biological. Bioindicators, an integral part of an ecological system, warn us with obvious or hidden "signals" of what happened or is happening in the ecosystem. The ability to observe these signals, their organization and interpretation is an essential condition for implementing actions aimed at preventing, reducing or eliminating the impact of pollution on the environment. Long-term biomonitoring programs are very useful both for increasing scientific knowledge and providing critical information for environmental policies. These programs should be considered as essential parts of economic policies.
Biomonitoring with honeybees is a well-established technique that could be adopted for detecting several environmental contaminants or pollutants. In fact, the honeybee is a good bioindicator as it is inextricably linked to the natural environment in which it lives. The close interaction of the bee with the environment makes it possible to detect the presence of pollutants and contaminants both on the bee itself and via the residues in honey, pollen, wax, propolis and larvae. In order to make effective monitoring with bees becomes important knowledge of the chemical-physical characteristics of the pollutants and the matrices in which to look for them.
The bee as biological indicator may reveal essential aspects of the environment that surrounds us. Its presence or absence in the environment may be related to the presence or absence of high concentrations of pollutants or stressor agents. Bees show functional or structural changes correlated with chemical agents. Bees are natural bio-collector of pollutants. The presence of these compounds can be detect on the honeybee body or inside the hive, in the beehive products.

6. Session: SHB in Calabria region: economic impact and financing

Public support for Aethina Tumida eradication programs: insights from Southern Italy

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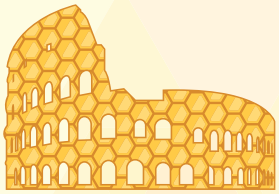
Invasive alien species (IAS) are recognized as a key pressure on biodiversity and a priority for action by the European Commission. The internationally agreed hierarchical approach to reduce and control IAS includes three distinct types of measures: prevention, early detection, and rapid eradication. A goal of the European Union is to avoid any further the spread of those IAS that are already well-established in the EU to minimize the harm they can cause.
In this paper we explore the case of the introduction *in 2014 in Calabria region (Italy)* of the invasive alien species *Aethina tumida (Small Hive Beetle – SHB).* The invasion of *SHB* in Italy is causing considerable financial losses to beekeepers and the Government. The decline in pollinators also poses a threat to crop yields. A surveillance system has been put in place to detect the presence of SHB. Eradication measures have been applied since 2014 with the destruction of all colonies at apiary sites whenever a single infested colony was found, and compensations paid to beekeepers for the destruction of infested apiaries.
In this paper we review existing measures, as well as legal and institutional frameworks to identify gaps, weaknesses and inconsistencies arisen so far, and suggest how to improve the strategy for the control of SHB.

PRACTICAL DEMONSTRATIONS



Using An Open Apiary Management System to Help Meet Key U.N. Sustainable Development Goals

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Introduction

The United Nations (U.N.) Sustainability Goals were born at the 2012 Sustainable Development conference in Rio de Janeiro with the idea of having a positive impact on the world around 17 key areas[1]. While no one program is likely to address every goal, there are some, like the one proposed here, that have the potential to help make progress in multiple, relevant areas. Building quality apiary management software and releasing it to the world's beekeepers has this potential. In this presentation and paper, we will show several ways in which sponsoring and releasing this type of software can help achieve a number of these key goals. Some of the impacts will be direct and measurable, others more diffuse and harder to measure, but still important.

In this presentation, we:

- Share what Apiary Management Software is;
- Discuss the importance of it being open and accessible to beekeepers everywhere;
- Discuss how this and other data collection systems can be harmonized through a data standard such as BeeXML;
- Illustrate how such a system or collection of systems can be used to directly aid with progress towards U.N. Sustainable Development Goals by reducing poverty (Goal 1), reducing hunger (Goal 2), and fostering economic development (Goal 8) while indirectly benefiting other goals; and
- Present an example of one such system (HiveTracks.com).

Apiary Management Systems

An Apiary Management System is a collection of software, sensors, data, knowledge, and best practices designed to help beekeepers and those that depend on them to manage their bees better. Some of these systems are built for hobbyist beekeepers and some for commercial beekeepers. The commercial systems are generally built to help manage business operations as well as bee health.

In today's world, it is hard to imagine an organization of any scale operating without some form of software and record keeping to assist. Generally, the better the records the better the organization can learn and grow over time, solidify best practices, and transfer learning to new branches of the enterprise to scale.

More than record keeping, Apiary Management Systems, especially commercial ones, build accountability, traceability, and best practices into the software to assist beekeeping operations. Additionally, they lay the groundwork for growth and improvements over time as data can be analyzed to assess which management actions or circumstance led to the best results and systems can be set up to build those processes into beekeeping operations.

Remote sensors (IoT), including hive scales, temperature sensors, video monitoring, and others, can be part of a good Apiary Management System. Additionally, they generally record data such as management actions, health status, and outcomes in terms of honey production, hive survival, and pollination effectiveness.

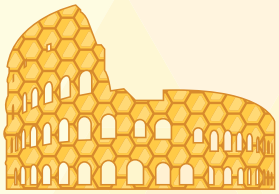
HiveTracks.com (hobbyist and commercial versions) and HiveLog are examples of Apiary Management Systems.

Openness

One important component of an Apiary Management System is its openness. An open platform is one that puts the users in charge of their data and process and allows them to selectively share parts of their data in a standardized way so it could be merged and aggregated with other data from other users of the same or similar systems. This data could then be mined, using analytics techniques including statistics, machine learning, and artificial intelligence, creating useful insights that could then be incorporated into the system, helping everyone.

[1] http://www.undp.org/content/undp/en/home/sustainable-development-goals/background.html

Additionally, this open data, with appropriate privacy protections, could help policy makers and others make decisions to imrpove honeybee health and the crops that depend on pollinators.
An open system could also be modified and adapted to suit the needs of various organizations and shared with its members, while the core part of the system could remain consistent to allow for aggregation and analysis across platforms. For example, organizations such as Apimondia, the U.N. FAO office, or a regional bee club, could all offer a version of this software, with their logo and other information as a type of sponsor, to their members as a benefit.
This "white label" version of the system could be adapted to their members' needs - with an organization like the *Chilean Association of Organic Beekeepers*, for example - maintaining a version with the common core features, and perhaps a few extra features needed by organic beekeepers for their members (and maybe a few less features of the kind they would not need, such as for antibiotic treatments).
Another key feature of an open system is that members of this group could share data with each other. This feature would allow leaders of this group to see how their members are helping each other. They could share updates and status reports at meetings, club mentors could monitor and help new beekeepers -- even remotely -- and give timely assistance. Also, threats could be spotted early and shared with members to enable preventive action. Members would also learn best practices over time, adjusted for their philosophy (i.e. organic vs conventional), their climate, bee genetics, type of hives, and regional crops and flora.
This openness could also increase knowledge. For example, perhaps the Club de Apicultores de Santiago (a more conventional beekeeping club in Chile) also white labels a version of the open software for their members, maybe even co-branded with a larger organization such as FAO or Apimondia. While they both might keep bees in the same region of Chile, they may have different approaches (organic vs conventional), but because they are in the same climate and face similar stressors, and all are collecting a common core of data, outcomes of their efforts could be compared to learn which practices under which circumstances provide the best results and could be shared internally as well as with others.
Thus, learning could be enhanced and knowledge built back into the system with *Decision Support Systems* (DSS) to help beekeepers while maintaining the choice of which approach to take among members. Further, some portion of this data, with privacy protections in place, could be collected at a larger scale. Since the core has a common base it can be analyzed with Big Data Analytical Techniques to understand broader trends and provide valuable, but still private, data to organizations that need to make better policy decisions. This could include the analysis of honey bee health and diseases, best practices for pollination or honey production GIS location optimization for better hive placements or other factors.
Please note that open is not the same as free. While the goal would be for the Apiary Management System to be free to most end users, especially in developing areas, there is a cost to build and maintain such a system. Ideally, the core system would be developed and maintained collaboratively with help from the community. Improvements would be shared with organizations benefiting from the tools, data, or goodwill from helping sponsor these kinds of systems so all can benefit.
Data Harmonization
Other Apiary Management Systems, including Hive Log, IoT vendors such as Arnia and Solution Bee, organizations such as COLOSS and the Bee Informed Partnership (BIP,) as well as citizen science beekeepers and researchers across the globe are also doing good work with bees and collecting data.
To really address the problems that beekeepers and those depending on them face, data from multiple apiary management systems, IoT devices, researchers, and others need to be harmonized. Walter Haefeker, President of the European Professional Beekeepers Association and Chair of the Apimondia Working Group on Data Standardization (Authors Joseph and James are also committee members), has led an effort to build a standard for collecting bee data.
The recommended platform, BeeXML is a concept for sharing data across platforms and sources in a structured way so that relevant (and privacy-protected) data can be aggregated and analyzed. As more systems adopt this standard, or in some cases as the standard adopts to the data collected, the breadth and depth of the data will grow and enable deeper and more in-depth analysis that can then be deployed to assist beekeepers. Beyond training, the best practices can be built right into the software system to help mentor beekeepers in its use and application. This data can also provide valuable insights for policy makers and researchers alike.



In addition to the breadth of data that could be collected from general beekeepers, software providers, veterinary labs, and IoT manufacturers, we also call for the establishment of a *Bee Data Journal* to collect, store, and harmonize data from honeybee researchers. It would be a peer-reviewed, open access journal where researchers could submit relevant high quality data sets, making them available to the world for secondary and meta-analysis and receiving academic credit for their important contribution. Such a journal, focused on honeybee data, would provide high quality depth and scientific rigor to complement other data collection efforts from contributing beekeepers using the various tools and systems available.

United Nations Sustainability Goals

The system and infrastructure described above would aid in progressing several of the United Nations Sustainability Goals, either directly or indirectly as they are all connected.

Beekeeping has been described as an ideal, low-capital venture, accessible and empowering to both male and female rural entrepreneurs in economically depressed areas (Mburu, et al., 2015). However, despite the increased initiatives focused on promoting beekeeping in these areas, a lack of training and knowledge has been cited as a significant barrier in efficient honey production and thus improved household well being (Amulen, et al., 2017). By increasing access to data-driven solutions, rural beekeepers can benefit from increased income and independence through greater efficiency in their beekeeping efforts, directly impacting United Nations Sustainability Goals 1 (No Poverty), 5 (Gender Equality), and 8 (Decent Work and Economic Growth). In addition, efficient honey production provides a low cost, high calorie and nutrition-dense food, which can aid with Goal 2 (Zero Hunger).

There is also likely a multiplicative effect. Having pollinators near farmers and others who tend crops that need pollinating, benefits not only the beekeepers but the farmers by potentially increasing the yields of both (Goal 8), reducing hunger by providing more food in the region (Goal 2), and helping poverty by reducing food cost (Goal 1) to people in areas with more plentiful food. Another factor is that the foods bees pollinate, 87/115 (Bauer and Wing, 2010) commercially grown crops, tend to be the healthier foods, mostly fruits and vegetables. This factor adds the potential to also help with Goal 3 (Good Health and Well being) by providing better quality food in likely food deserts. Similarly, many honey products have natural medicinal benefits further aiding with this goal.

Finally, in addition to aiding with economic development in economically-depressed regions, it is also likely that having a greater diversity of beekeepers in more areas, with mixed sizes of operations, could lead to more resiliency in the pollination and food systems, helping to reduce the likely impacts of climate change (Goal 13: Climate Action). By making beekeepers aware of the impact of climate change on an ecological system they monitor closely, it allows them to put appropriate pressure on policy makers to build more resilience into the system. Similarly, beekeeping efforts in economically-depressed regions have been linked with greater awareness and preservation of natural habitats and watershed conservation due to the economic incentive provided by beekeeping (Gemeda, 2014). By promoting an increase in the number and effectiveness of rural beekeepers through data-enabled education and decision guidance, we may enable greater stewardship of the environment, potentially aiding with United Nations Sustainability Goals 13 (Climate Action) and 15 (Life on Land).

The need for technological intervention has been recently summarized by Lietaer (2019), "Despite the favourable natural environment existing in almost all developing countries and the potential for building sustainable livelihoods in rural areas, beekeeping often lacks the necessary financial, extension and technological support required to fully exploit its great potential in conserving forests and natural ecosystems and in reducing poverty." Significant development in exploiting this potential can be realized by promoting maximum honey production through technological, data-enabled solutions that increase beekeeping efficiency longevity.

Finally, with a changing climate, practices that worked well in the past may not be optimal in a world with more volatile climate patterns. Having decision support systems built into software can speed up the adoption of more up to date practices that are proven to work in different environments and lead to greater climate resiliency. This could also help with Goals 13 (Climate Action) and 15 (Life on Land).

An Example of an Apiary Management System

HiveTracks.com is one example of an open software platform that could meet these needs. A brief overview of the software, features, and benefits will be shared at the conference, time permitting.

We sincerely hope that organizations like Apimondia and the United Nations FAO office and others will support efforts to use open apiary management systems to help meet our world's sustainable development goals.

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Innovative methods to assist beekeepers in controlling *Varroa destructor*: "Treat in time"

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Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri"

Varroa management requires the use of veterinary medicines. The efficacy of some treatments is related to environmental conditions like temperature and humidity. In this practical demonstration we will show the Treat In Time model. Considering the best temperature ranges of available drugs in Italy, suggested in the leaflets, and temperature data on a spatial basis, we developed a model able to indicate the best treatment to be applied in relation to the climatic conditions.
More in detail, model considers temperatures data of the last 40 years provided by Worldclim spatial dataset (www.worldclim.org) and attributes them to specific cells of 1 square kilometre. The attribution of suitability of the use of a specific treatment is given if temperature ranges of that product do not fall below the average minimum temperature or do not rise above the maximum average temperature. Moreover, considering that real-time and 7-days forecast temperatures are fundamental to plan an effective treatment, we added into the model a free forecast service in order to help the beekeepers in the decision-making process. Further developments will consider other variables like wind speed and humidity in order to increase its reliability and it will try to develop a model of the varroa population development considering data from:

- Nectar and pollen sources and blooming periods
- Density of colonies
- Micro-climatic conditions

Such model will permit to identify macro-zones where beekeepers will be informed about the infestation level and plan coordinated treatments in order to reduce reinfestation and the development of drug resistant traits.

Beebread collector: an innovative tool that allows you to use beebread as a food and to carry out laboratory analyzes to protect the consumer and the environment.

Giulio Loglio
Veterinarian;
Abstract.

Beebread is the product obtained from the fermentation of fresh pollen stored by bees in the combs after kneading it with honey and enzymes. Unlike fresh pollen, it is a very digestible, nutritious food with a low allergenic action: 30 gr are sufficient. of beebread to meet the daily protein needs of an adult man. Due to the difficulty of extraction, beebread has always been little used. To allow the extraction of bread from the combs, without damaging the cells of the honeycombs, has been devised a very simple innovative tool: the "beebread collector" With this tool it is now possible to use beebread not only as a food but also to perform laboratory analysis. In fact, beebread is an excellent "bioindicator" that can be used to identify substances that have deposited on the land and vegetation of a territory (pesticides, crop protection products, heavy metals, radioactive substances, etc.) and to detect the atmospheric pollutants. Moreover, beebread can

be used as a new matrix to identify the presence of bacteria responsible for the American foulbrood and European foulbrood in the hives.

Introduction.

The beebread: differences compared to the pollen The beebread is an excellent food product obtained from the fermentation of fresh pollen in the honeycomb cells. It has never been enhanced by beekeepers for self-consumption due to the lack of a tool that would allow an easy harvest.

The "beebread collector" it is a simple and inexpensive tool that allows the extraction of bees bread from honeycombs in the form of pallets, a product that can be used as a food by both the beekeeper and the local consumer. Due to its high nutritional value, the beebread, combined with honey, can be used as food in developing countries. Since the tool does not ruin the cells of the combs can be used by observers to sample in the apiary a bees bread, perfectly clean, to be used to detect the presence of hepatotoxic pollen and to search for agrochemicals, insecticides, environmental contaminants and micro-organisms responsible for bee diseases.

Compared to beebread, fresh pollen is rich in water (20-30%): in order to preserve it it must be dehydrated or refrigerated. Conservative interventions that unfortunately negatively affect some nutritional properties of fresh pollen (photo 1).

Photo 1: dehydrated pollen.

The cells of the honeycomb containing pollen are never completely filled and are never operculated (photo 2).

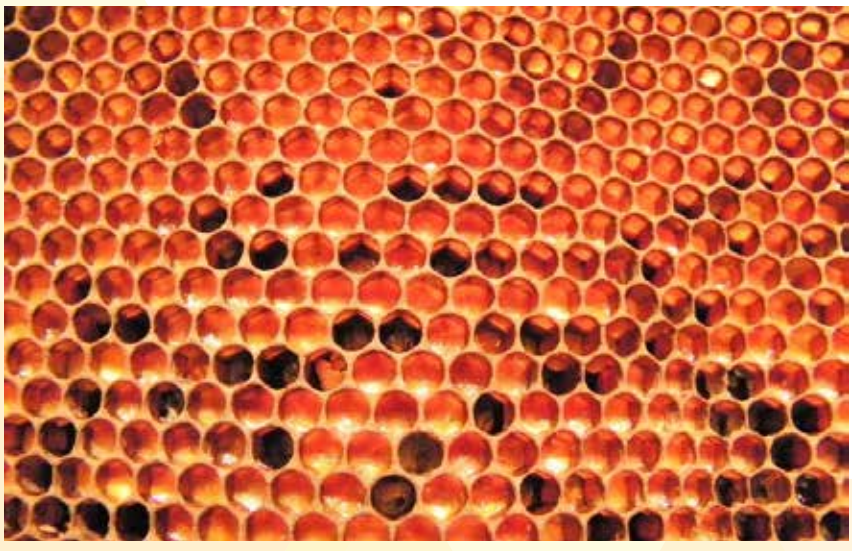


Photo 2: cells of a honeycomb containing beebread.

From the moment for each trip a bee carries 15 mg of pollen and which in a day makes about 20 trips carrying 300 mg of pollen in the hive, it is estimated that in a year a colony of medium size is able to collect almost 50 kg of pollen.
The consumption of pollen allows nurse bees (between the ages of 5 and 15 days) to process and secret the royal jelly with the hypopharyngeal and mandibular glands.
In the same cell of honeycomb are stored pellets of pollen of different plant essences: this mixing allows to obtain a beebread that has a fairly constant protein concentration (about 22% -30%).
In this way, nurse bees can be used to feed the larvae of a product that has a "good" quality protein value able to avoid those protein deficiencies that are responsible for the birth of "underweight" bees with a reduced "fat body".
While the raw pollen is collected outside the hive using special traps, the beebread is extracted from the combs . While raw pollen is collected outside the hive using special traps, currently beebread is extracted by crushing the combs with expensive machinery.
From the point of view of food, man tolerates beebread better than fresh pollen, so much so that it is advised to use it in comparison to the raw one, to those allergic to pollen. Because of its acidity, beebread is easily assimilated from the intestine, being rich in simple sugars, enzymes, fibers and amino acids.
Regarding the biological value there is a substantial difference between fresh pollen and beebread. A part of the fresh pollen, taken as food by man, passes through the intestinal lumen without being digested because the gastric juices can not dissolve the exina, the cell wall of the pollen. In beebread the various fermentative processes that take place in the cells by the enzymes and honey added by the bees lead to the production of lactic acid which, in about 2-3 months, degrades the exina, making the various nutritional elements more digestible and more assimilable.
The raw pollen collected with the traps is a product of vegetable origin that is more and more often contaminated by agropharmaceuticals used in the fight against fungal, bacterial and parasitic diseases of various plant essences. If the fresh pollen is contaminated it will be consequently also the beebread produced with this pollen. In beebread it is possible to find not only the pollen contaminants but also the synthetic acaricides used for the fight against varroa.
This work illustrates in detail the "beebread collector", a very simple and very low cost tool designed to allow easy extraction of beebread from the cells of honeycombs to be used for food use (self-consumption) or for laboratory tests (photo 3).
This tool is not intended for companies that industrially provide for the extraction and marketing of beebread because they have expensive and sophisticated machines that are very efficient.

Photo 3: the beebread collector.

Components of the beebread collector .
- a small rigid tube with a diameter of about 4.8 mm and very thin walls. The rigid tube must have a diameter of about 4.8 mm because it is the same diameter as a bee cell.

- the small rigid tube is inserted into the plastic conical support
- a plunger, with a diameter of about 4,5 mm, which can slide into the rigid tube.
- a small spring 2-3 cm long inserted into the plunger.

Photo 4. Components of the beebread collector: spring, plunger, little rigid tube, plastic conical support.

The beebread collector must be made of material that can come into contact with food The plunger must have a length such as to spill from the rigid tube for 1-2 mm when the plunger is pressed fully.
The spring has the task of keeping the plunger in a back position when it is not pressed.
To extract the bees bread it is sufficient to grasp the beebread collector as if it were a pen to be written and insert it vertically in a cell containing the bees bread exerting at the same time both a light pressure and rotary movements so as to allow the rigid tube to reach the bottom of the cell (photo 5).

Photo 5. It is important to insert the beebread colector vertically into the honeycomb cell. The rigid tube does not ruin the cells of the honeycomb because it has the same diameter.

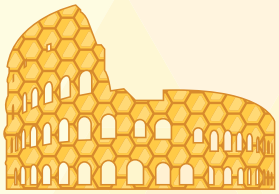
In doing so, the outer part of the tube, sliding in contact with the inner walls of the cell, detaches the bee-bread from the cell walls and conveys it into the central part of the tube which at the time of sampling must

always have the light free and do not hold the plunger end.
To collect the beebread, simply press the plunger: the beebread is pushed out of the tube in the form of a small pellet.
The function of the spring is to keep the plunger retracted so that the inner lumen of the rigid tube is always free and ready to receive other beebread.
Usually each cell of a honeycomb contains about 75 mg of beebread and with the beebread collector they can sample about 50 mg in the form of a small pellet (photo 6).

Photo 6. The pellet of fresh beebread.

To collect 10 gr of beebread, it is necessary to insert the beebread collector into about 200-250 honeycomb cells.
Since in 10 dm2 of honeycomb there are on average 415-425 cells it means that from these it is possible to extract about 20 gr of beebread that provide the same quantity of proteins (high quality amino acids) of 100 gr of meat or of 2 eggs.
For laboratory analysis, depending on the number and type of substances to be searched, usually 10 gr of beebread are required. For each analysis, the analysis laboratories normally use from 0.5 gr to 1 gr of beebread.
When finished work, it is essential to clean the plunger of the bees' bread collector with hot water while it is advisable to replace the rigid tube to avoid cross contamination or when it is worn out.
It is possible to make the "beebread collector" much more efficient by connecting the rigid tube directly to a vacuum pump so that the beebread, after being sucked out of the honeycomb cell, is deposited in a hermetic container: but this technique is more expensive. (photo 7)

Photo 7: the differences between the beebread extracted with the manual bee collector (pellets) (on the left in the photo) and the motorized beebread collector (crumbled) are evident (on the right in the photo).



Beebread is an excellent food. The recommended daily dose is around 10-15 gr.
Its intake immediately after extraction from the cells of the honeycomb allows the consumer to ingest an intact natural food, rich in all those food substances (carbohydrates, carbohydrates, proteins, lipids, micro and macroelements, vitamins) that are partially destroyed and deactivated by various processes to which industrial beebread is subjected to ensure proper conservation and commercialization. Moreover, the beebread contained in a honeycomb, if stored in a cool and dry environment, keeps its nutritional qualities unaltered for a long time.
An enterprising beekeeper can allocate part of his beehives to the production of beebread during the year using genetically selected bees for the collection of pollen: as is done with honey in honeycomb, it can yield to the consumer small honeycomb of beebread after placing them in food containers.
With the beebread collector, the beekeeper can use the quantity of beebread necessary for his needs and that of his family members for self-consumption.
Not only. You can also decide to start the sale of "beebread in honeycomb" to consumers in the area as already done with "honey in honeycomb". A new way to valorise a product of the hive at zero Km from undisputed food quality poorly or not used at all for the difficulty of extraction on site.
The benefits for researchers, veterinarians and beekeepers who can use beebread to carry out research for pesticides, pollutants and infectious agents at any time of the year are undeniable, even when the bees have long since ceased to collect pollen.
The hives depopulation and the increasingly frequent contamination of pollen with agropharmaceuticals, acaricides, heavy metals, etc. they will involve the execution of samplings at apiaries with increasing frequency in order to monitor the environmental situation.
With the beebread collector, used directly in the apiary, it is possible to take and send to the laboratory a perfectly clean sample, without residuals of exuvia and wax, which can be immediately processed without further loss of time.
The importance of beebread collector from a health point of view:

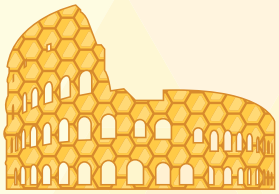
- the raw pollen collected with the traps is a product of vegetable origin that is more and more often contaminated by agropharmaceuticals;
- laboratory analyzes have highlighted the risks of this situation that exposes the consumer to serious dangers as the toxicity of each individual pesticide is increased due to the synergistic effect.

Veterinarians and researchers often need to have samples of beebread for:

- exclude the presence of dangerous substances that could be contained in beebread intended to be marketed as food
- detect residues of crop protection products that may be responsible for chronic damage to the brood and which prevent a harmonious development of the bee family
- highlight the presence of heavy metals, in particular lead
- identify pollutants that are dispersed in the atmosphere and fall back to the ground
- evaluate the qualitative and quantitative composition of beebread, in particular its protein content (amino acids).
- true pollens that, containing hepatotoxic alkaloids, can be harmful to the liver of man: Echium vulgare (Viperina azzurra, common viper), Echium plantagineum (Viperina piantaginea), Senecio jacobaea (Senecio di San Giorgio), Senecio ovatus (Senecio di Fuchs, Senecio silvano, Eupatorium cannabinum (aquatic hemp).
- to detect the presence and the infective load of the main pathogens of bees (American foulbrood, European foulbrood, nosemissens, etc.)

It is important to remember that the bee can be used as a bio-indicator: the myriad of hairs that cover its body are able to capture not only the pollen grains but also the powdery substances present in the atmosphere. Substances that, brushed and harvested from the bee along with the pollen, become a component of beebread within which they can be searched. The difficulty of extracting beebread from the honeycombs has meant that for very few scientific researches have taken this matrix into consideration for years.
In addition, those sampling, whether cognitive or official, must have the warning to take the beebread at different points of the honeycomb or honeycombs in order to ensure a homogeneous collection. At the end

of the operations it is essential to clean the " beebread collector " by washing the plunger with hot water and replace the rigid tube.
In special cases, depending on the research to be carried out, it may be necessary to replace the "beebread collector " for each beehive, like a single-use syringe, to avoid cross contamination.
Conclusions.
Currently, veterinarians and researchers, to extract the beebread from the honeycombs and be able to submit it to laboratory tests, usually use small metal or plastic spatulas. Spatulas which, breaking the walls of the cells, also remove small portions of wax and exuvia which make the execution of laboratory analyzes more difficult.
The " beebread collector " is a "work tool" that allows veterinarians and researchers to operate in a professional way and to exploit a hive matrix that has not been fully exploited to date.
With this new tool they will be able to carry out cognitive and official sampling collecting beebread without residuals of exuvia and wax. Since the " beebread collector " does not ruin the honeycombs, once the quantity necessary for laboratory analysis is collected, it will be possible to insert the honeycomb to the beehive again so that the bees clean it to use it according to the their need.
The depopulation of the hives and the increasingly frequent contamination of pollen with agropharmaceuticals, acaricides, heavy metals, will increase the number of sampling by qualified personnel that can be carried out at any time of the year, even when the bees have stopped for some time to collect pollen.
In beebread it is possible to find not only the pollen contaminants but also the synthetic acaricides used for the fight against varroa. In particular, the veterinarians, in case of suspicion, will be able to make official samples to find the residues of those insecticides illegally employed in the hives
Always with the " beebread collector " it will be possible to sample the beebread from beehives to detect the presence of P. larvae and to be able to express an assessment on the health level of the apiary. In the future, beebread could be used, together with bees, honey and residues present on hive funds, as a new matrix to implement prophylaxis plans and to be able to attribute to the beekeeping farms the status of "free from American foulbrood".
The results of laboratory analysis performed on beebread will help improve bee health and protect the consumer.
Not only. By exploiting the bee as a bio-indicator, it will be possible to look for the environmental pollutants that accumulate in beebread to identify the local sources responsible for their diffusion: in this way it will be possible to improve the ecosystem and consequently safeguard the health of citizens.
The world population continues to increase. In the future, the land currently being cultivated, due to climate change and soil depletion, will not be able to meet the food needs of billions of people. At the same time it was understood that it is impossible and absurd to continue destroying forests to create new lands to cultivate. To solve this problem, we are trying to change the eating habits of the populations living in industrialized countries by teaching to reduce the intake of animal proteins and by favoring the use of plant-based foods or proteins obtained from insect breeding. The vegetarian diet, currently considered an expensive fashion, will become the "obligatory" food style of the world population in the future.
The "beebread collect " can be used in industrialized countries for research purposes and for local consumption of beebread. But this simple tool could find practical utility especially in developing countries where currently populations suffer severe food shortages. I am convinced that in the future rationally managed beekeeping could play an important role in food, ecology and the environment worldwide.
Since beebread has a high nutritive power (100 gr of beebread is equivalent to 500 gr of meat or 7 eggs and contains 20 of the 22 amino acids present in nature) it would be important to teach how to breed bees in the countries in development in order to obtain both an energy food from honey and proteins from beebread. At the same time, through the pollination of local plant essences or seeded and planted with the contribution of international projects, bees could help reduce desertification, improving the living conditions of local populations and our ecosystem.
The photographs are all by the author.



Innovative, non-invasive sampling of the honey bee colony

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Honey bee colonies are big sources of data about the environment they forage in. To obtain these data, the colony must be sampled. This can be done invasively / sacrificially or non-invasively. Invasive sampling means killing bees and / or brood. Non-invasive sampling implies the conservation of the bio-sampling unit, the honey bee colony; no bees are killed and if food is sampled, it is done in a way and in amounts that the bees can easily replenish.

The main matrices in the honey bee colony that can be sampled for data collection are bees, pollen, wax and honey. As we are dealing with non-invasive sampling, taking bees or collection of the contents of the honey stomach is not relevant here.

Non-invasive sampling of honey can provide information on the botanical origin of the nectar, chemical composition and medicines but is an unreliable data source of pesticides and other contaminants such as particulate matter or heavy metals. Non-invasive sampling of honey necessitates leaving sufficient nectar or honey for the short- and long term needs of the bees.

Pollen is an excellent data source of the foraging area, the quality of the environment as a food source and of pesticide burden. Pollen can be trapped using a pollen trap in front of the hive as beebread in the hive. One can discuss whether taking pollen or beebread is invasive or non-invasive, but sampling pollen and beebread in amounts where the colony does not suffer can be considered as non-invasive.

Beebread is stored in the vicinity of the brood nest. Sampling beebread cells requires emptying these cells and generally results in damaging the wax combs. Recently, however, a beebread sampler has been developed, and using this device leaves the frames intact and enables beebread to be collected non-invasively.

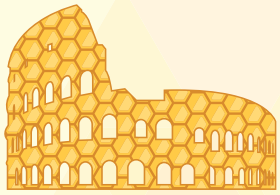
Non-invasive sampling of bees can be done by forcing them to enter the hive through a tube, lined with sticky or other specific material that adsorbs particles from the bee's hairs and feet. This can be used for the early detection of plant pathogens, pollen diversity and pesticides.

Alternatives for wax sampling are passive samplers with specific e.g. pesticide adsorbent material, inserted in the hives as e.g. strips between the combs or disks mounted in the frames. This innovative way of non-invasive sampling will be tested and evaluated in the Insignia project.

Bee-pathogens circulate in the hive due to trophallaxis, physical exchange and auto- and allogrooming. Non-invasive sampling of these pathogens is not yet common practice but is potentially possible with sticky or specific in-hive passive samplers.

New innovative devices and implications for the colony of non-invasive sampling in terms of amounts, location and timing will be discussed.

ORAL PRESENTATIONS



1. Session: Good Beekeeping Practices – GBPs

Blocks for Bees: Using Blockchain Technology to Keep Honeybee Enterprises Healthy

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In addition to the biological and ecological problems that honeybees are facing, there are a number of business problems that beekeepers are confronting. Addressing these business problems will help the honeybees by keeping the beekeepers in business. Specifically, we discuss the emerging role that Blockchain technologies can play in helping beekeepers. We explore applications in honey adulteration and traceability, pollination services, and with insurance pricing and claims verification. Blockchain technology has the potential to help address these problems in novel ways. Importantly, by utilizing the cryptographically-secure nature of blockchain to store accurate and distinguishable data about individual beehives, it is possible to remove many of the information asymmetries that exist in markets related to beekeeping. First, we proffer that blockchain can provide traceability solutions for honey adulteration. Through secure production and supply tracking, honey producers are enabled to make provable assertions about the quality of their honey and related bee products. This effectively allows beekeepers to separate the market for pure honey, from the market of honey which may be adulterated, increasing price efficiency and largely mitigating the depressive effects adulteration has on honey prices. This also has the potential to increase price premiums by proving a given local origin or varietal or organic honey features. Next, we illustrate how smart contracts can increase efficiency in the pollination contracting process by enabling contract standardization, paying for pollination performance, and offering an automatic fulfillment system that cannot be forged. Finally, we discuss hive insurance and claim verification. Using the security of the data to prove insurance claims are not fraudulent and that beekeepers have fulfilled all of their obligations allows a robust insurance market to thrive. These business model improvements are promising because they have the potential to impact many beekeeping practices with data-driven technological advances in ways beyond what has been possible or practical in the past. Our framework lays the groundwork for blockchain-driven improvement in the beekeeping industry in honey production, bee health and survival, and pollination, while also increasing access to financial safeguards through apicultural insurance.

Investigation on the use of veterinary medicinal products and best practices in beekeeping

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The beekeeping sector is facing increasing worldwide challenges that range from climate change/global warming to a plethora of new pathogens/invaders and environmental stressors deriving from pollution or agricultural activities. In this complex situation, the application of good farming practices, the access to Veterinary Medicinal Products (VMPs) and proper training of beekeepers could help mitigate the risks. However, there is a lack of information about the measures that are routinely applied by beekeepers to tackle some of these problems and the amount of assistance and education they receive from beekeepers' associations or veterinarians. Therefore, a global survey was set up, in collaboration between the Technologies and practices for small agricultural producers (TECA - FAO) platform and Istituto Zooprofilattico Sperimentale Lazio e Toscana with the engagement of the International Federation of beekeeper's Associations, in order to investigate about some aspects related to

these issues. In particular, an online questionnaire was prepared and participants from target professions (e.g. beekeepers, veterinarians, beekeeping inspectors...) were invited to participate. The questionnaire was promoted in 4 languages in 3 consecutive editions between 2015 and 2017. It consisted of 3 parts that aimed to gather information about the perception of beekeepers on the main diseases that afflict bees, the beekeepers knowledge about the best farming practices and the level of technical assistance they receive. In total 403 questionnaires were collected and analyzed. Most participants belonged to the European countries and to the beekeepers and veterinarian categories. Preliminary results showed that most beekeepers are well informed about the main diseases that affect their bees (American Foul Brood, European Foul Brood and Varroa). Not all beekeepers apply the best practices in their day to day activities, especially with regards to varroa monitoring prior and/or after the treatments. Most beekeepers rely on their personal experience and self-education to diagnose the diseases. Technical assistance derived mainly from beekeepers' associations while the lack of veterinarians' involvement together with the availability of registered VMPs for various honeybee diseases was an issue highlighted by more than half of beekeepers. The study revealed the need to improve the level of assistance that beekeepers should be able to receive in their apiaries notably in regards to the application of the good beekeeping (best farming) practices. The role of veterinarians in the beekeeping sector is still generally misperceived. A more efficient training and VMPs specific for honeybees are more than ever needed to confront the challenges that beekeepers are increasingly facing.

First attempt of standardization of official control procedures at the apiary level

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From 2016 to 2018, the Italian Ministry of Health supported a national project entitled: "Feasibility study to reduce the prevalence of honey bee diseases through the application of good beekeeping practices (GBPs)". Activities involved 6 Regions, the competent Istituti Zooprofilattici and several Veterinary Services of Local Health Units, the Trento Province and the National Reference Centre for Apiculture. Aims of the project were: -set up a standard check-list to assess honey bee's health; -set up a new approach for sampling to be adopted at the apiary level by officiers; -evaluate the ability of good beekeeping practices in preventing honey bee diseases; -standardize innovative laboratory methods; -publish training material for beekeepers and veterinarians; -develop a rewarding system for beekeepers respectful of GBPs. During the project, the check-list and a new sampling method have been tested on 57 apiaries, visited twice, in

order to establish a precise protocol to assess honey bee's health. The most suitable matrices have been identified to verify the prevalence of the main honey bee diseases: *Varroa destructor*; *Paenibacillus larvae*; *Melissococcus plutonius*; *Nosema spp.*; 3 honey bee viruses and *Aethina tumida*. Depending on the consistency of the apiary, a specific number of colonies were inspected assuming a prevalence of 10%. After colonies identification with a specific ID, hives were visually inspected, and a first module was compiled with beekeeper's ID, the location of the apiary, the colony ID, the date and the outcome of the clinical visit. A procedure was developed to sample three matrices: 300 adult honey bees, icing sugar and hive debris. Specific laboratory analysis were carried out on those samples to identify the best matrices to assess the colonies health status. In the poster are reported details of this first attempt of standardization of official controls at the apiary level.

Honey bee in the history: a golden standard to train human being

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honey bees/ symbol/society/ mythology/art

It is possible to travel with the bees through history, mythology, literature, philosophy and ethics, the visual arts and cinema. The bees have been frequently considered a gold standard for many aspects by the human being through history, mythology, literature, sociology, philosophy and ethics, visual arts and cinema. The aim of this work is to illustrate some of the many traces of bees and beekeeping in the different aspects of human life. History. Bees and beekeeping were important in many historical periods: ancient Greece, ancient Rome, Middle Ages, Renaissance and other times until now. The first trace of honey bees was found in some painting in the Araña cave near Valencia, dated 5000 years B.C. In ancient Egypt the bees were considered sacred: Egyptians believed that honey had therapeutic and magical properties. The symbol of the New Kingdom of Lower Egypt was a bee. It was found carved in the columns of some temples. Napoleon adopted the bees as a symbol of his empire: an expression of immortality and resurrection. Mythology. Bees was frequently found in the mythology: the tears of the Egyptian god Ra turned into bees; in the Odyssey, Ulysses used the honey to evoke the souls of the dead; Krishna depicted as a bee on a lotus flower. In literature there are many references to bees through the centuries. Virgil in the *Georgics* describes the conditions suitable for beekeeping and the social life of bees; Shakespeare in *Romeo and Juliet* refers to the dual nature of honey, sweet and cloying, like love. Pablo Neruda wrote the poetry *Ode to the bee*, and Federico García Lorca *The song of honey*. Sociology. Are the bees monarchical or democratic? Conservatives or revolutionaries? Aristotele, Proudhon, T. D. Seeley and others deal with this topic. Visual arts. There are several examples of bees even in visual arts. Albrecht Dürer in 1514 painted the famous *Cupid, honey thief*; Pablo Picasso is the author of the *L'Abeille* etching (1938), where bees fly among the flowers; the surrealist artist A. G. Regner painted *Look in the hive*. Cinema. The bee society has been an interesting starting point for several movies too, like: The Deadly Bees (1967); Killer Bees (1974 e 2002); The Savage Bees (1976); The Swarm (1978); Bees (1998); Bee movie (2007); The secret life of bees (2008); Il tempo delle api (2017); Maya the bee (2018).

Best management practices for every beekeeper

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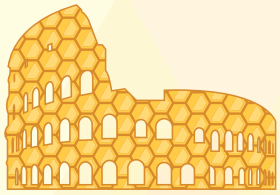
Beekeeping practices have hundreds year old traditions which have been changing throughout times. Best beekeeping management decisions need to include tradition as well as plethora of professional activities and regulations. Important policy requirements for specific professional topics need to be available for every beekeeper, whether they are small scale, professional or an enterprise. Best prac-

tices include instructions for keeping bees in rural, urban areas, at rooftops, or in gardens. The density of managed honey bees in geographic area and forage abundance are also factors of consideration. There are professional practices needed for queen breeders, nucleus or honey producers and keepers of bees for pollination. The management of honey bee colonies in a variety of environmental conditions and honey processing practices include several topics that need to be followed and applied as 'good bee keeping practices'. Disease and pest control is one the most critical for beekeepers to manage. We will discuss some aspects of control varroa mite, currently the most devastating parasite for honey bees. Varroa control can be achieved by integrating broodless conditions, through either total brood removal or queen caging, in combination with oxalic acid (OA) applications. We observed significant varroa mortality after total brood removal or caging the queens and OA applications in broodless colonies, as well as in colonies with brood that received four consecutive OA applications. On the other side, we have also found that amitraz applications after harvesting season can considerably reduce numbers of varroa. Formic acid applications have limited effect on varroa population reduction. In laboratory tests, we recorded higher mortality of caged bees exposed to acaricide Apistan® compared to oxalic acid or untreated controls. Therefore, in conclusion, there is a need for an integrated varroa control approach. We recommend combining OA applications with artificial broodless colony conditions achieved either by brood removal or queen caging as an effective management strategy. It is laborious and inconvenient for small or large scale beekeepers to keep strong and healthy colonies. There is therefore a need to perform greater analyses and propagation of more tolerant honey bee colonies to varroa infestation for every native subspecies or local honey bee population.

The control of beekeeping in Italy, between problems and perspectives: certainly more than just a challenge

Filippo Bosi
Local health unit of the Romagna, DSP Ravenna, Italy

In the past, beekeeping was often considered a smaller animal husbandry, but since it has been realized that honey bees are fragile, threatened by so many dangers, and that their pollination function is lacking, which is essential for agricultural productivity and for the maintenance of ecosystems, this reality has gained a lot of visibility and collective interest. If for the European beekeeping therefore the future represents a challenge (European Parliament resolution on prospects and challenges for the EU apiculture sector, 01/03/18), the same is particularly true in Italy, where apiculture recognizes many strengths, but also many critical issues, also seen the SHB. The same goes for the health control of this activity, which along with significant positive elements still has large gaps. The reasons for these problems are many and have distant origins due to the epochal distance of the veterinary profession from the apistic reality, which is still reducing, the underestimation of its peculiarities and important functions, the obsolete and almost unmodifiable veterinary legislation, the overlapping of other secondary rules that have favored the discrepancy of official controls, and so much more. A little at a time, however, the competent authority, at various levels, has taken note of this state of affairs and is trying to remedy it. It is not easy, however, to take possession of a large part left open or to complete the control according to the dictates of European food law. A notable restriction also derives from the considerable reduction of official health personnel. A decisive turning point to overcome the obstacles, apparently insurmountable, can derive from a prompt global assessment of the problems, from the reformulation of the strategies adopted so far and from the application of Reg. (EU) N° 2016/429 which should rewrite the health measures to be applied, according to the criteria based on prevention. To achieve the goal many other ingredients will be required, many of which are already available, but the sincere (sine cera ...) sharing of intent and strategies with all the other components in the field cannot be missing; it is worth the risk, it is time to roll up our sleeves, we cannot wait any longer! Italy can become an experimental laboratory, useful for the rest of the continent.



Determination of a new efficient biomarker to evaluate the health and productivity of honey bees

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honeybee, vitellogenin, gene expression, insecticide resistance

In healthy colonies, the interaction of the Vitellogenin (Vg) protein with the endocrine factor, juvenile hormone (JH), acts as a pacemaker guiding the sequence of behaviors that worker bees perform during their lifetime. Nurse bees have high Vg and low JH; older ones have inverse parameters. Pathogens and parasites can alter physiological values1. Vg has multiple roles in bee health besides being essential for the development of the egg. It is involved in the immune response and has antioxidant properties2 and influences both the production of food for the brood and of substances, with a consequent increase in life expectancy3,5. The search for biochemical markers can be useful for evaluating the health and productivity of a beehive: for example, poor nutrition could correlate with the low overall levels of Vg6,7. The examined bees were collected in July and classified in four groups of nurse and worker bees from strong apiaries (highly productive) and weak apiaries (poorly productive). From every apiary, about 30 bees were collected, and ten apiaries per group were involved. The bees were collected and frozen at -80°C within 24 hours until the analysis8. The extraction of total RNAs was carried out by adapting RNeasy Power Soil Total RNA Kit. DNase treatment and cDNA synthesis were obtained by QuantiNova Reverse Transcription Kit (Qiagen). Primers used for quantification of Actin as housekeeping: Forward TGCCAACACTGTCCTTTCTG, reverse AGAATTGACCCACCAATCCA9; primers used for quantification of Vg: Forward GCAGAATACATGGACGGTGT, reverse GAACAGTCTTCGGAAGCTTG10. Sybr Green used in Real-Time RT-PCR analysis was SensiFASTTM Sybr Lo-ROX Kit. Our RT-PCR results show that in Strong Nurse Bees group the Vg expression was about 16-fold respect to Weak group. In the Strong Worker Bees group, there was a Vg level higher than in the Weak group, the increase was about 1.8-fold. The results will allow to map the productivity of the apiaries and identify the apiaries at risk with significant economic feedback. We found a correspondence between the Vg level and the health and productivity of both nurse and worker honey bees. Although more experiments are in progress, present results confirm Vg as an essential marker of apiaries health. Further improvements and experimental strategies will be discussed.

2. Session: Main Honey Bee diseases

Bee Varroa Scanner

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Varroa destructor and the viruses it transmits are considered the first cause of honey bee colony losses. The control of this parasite is a difficult task for beekeepers and requires repeated determinations of infestation level throughout the year to decide if and how to treat honey bee colonies. Counting the number of varroa fallen on the hive bottom board is a reliable and not invasive way to do so, but is rather time consuming and prone to observer's errors. The Bee Varroa Scanner (BeeVS) is an integrated and really innovative system that will allow to reduce the time required to carry out the checks and that highly improves the precision of the determination. This highly technological prototype tool is able to recognize and count the number of varroa on the "fall tray" of the hive using a sophisticated algorithm of artificial intelligence fully automating the count. Furthermore, the system with an online database that allows geo mapping of the mite distribution in the land and accurate hot spot analysis of the state of varroa destructor. The University of Turin tested in 2017 the tool BeeVS in his experimental apiaries and also some of the main Italian beekeepers associations have been able to verify its operation. The trials made it possible to verify precision, accuracy, and reliability of the instrument. BeeVS allows timely varroa control treatments, thus reducing the damages caused by the mites and the use of acaricides. Its use by beekeepers will allow saving bee colonies from collapsing due to varroa and virus infestation adopting an integrated approach, moving towards a more sustainable future of beekeeping.

First molecular clone of Chronic Bee Paralysis Virus (CBPV)

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The chronic bee paralysis virus (CBPV) causes a pronounced paralyzing disease in adult bees and was one of the first isolated honey bee viruses. CBPV grows to high titers in infected honeybees and produces non-enveloped virions containing two RNA molecules of positive polarity. In this study, we generated an infectious molecular clone of CBPV using standard techniques. Furthermore, we established an in vitro system for CBPV rescue as a tool to unravel the factors responsible for its pathogenicity. Following RT-PCR amplification, both RNAs of CBPV were inserted in a bacterial plasmid backbone. An artificial Bgl-II site was introduced as a molecular marker to distinguish between wild type virus and the synthetic genome. The resulting plasmids containing RNA1 and RNA2 sequences were transcribed and the capped synthetic cRNAs were injected into bee pupae. The recombinant CBPV (rCBPV) was harvested after an incubation period for 72 hours at 35°C. The injection of the cRNAs led to viral replication, the production of infectious virus progeny, and a sharp increase in mortality in bee pupae. In electron microscopy viral particles similar to wtCBPV could be observed after transfection of rCBPV. Several passages of the rescued virus confirmed the genetic stability of the introduced marker gene. Even a topical infection with rCBPV led to viral replication to a mean viral titer of 2 x 10^11 GEs/bee, demonstrating the virulence of rCBPV in adult bees. In this study, we describe the first reverse genetics system for CBPV, a major pathogen of honeybees. Reverse genetics is an important prerequisite to study the viral life cycle and to get insights in the pathogenicity of this virus. Using the toolbox of molecular virology, we will be able to genetically modify the virus and identify the virulence factors of CBPV in the future.

Hive debris (ring tests) to diagnose AFB, EFB, *Nosema* spp. and SHB

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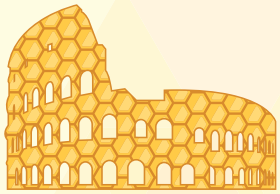
Within the project BPRACTICES (approved within the transnational call of ERA-Net Net SusAn- European Research Area on Sustainable Animal Production Systems- in Horizon 2020 research and in the European



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 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 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 method 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 them were selected several methods on the basis of the experimental specificity and sensibility.

Test Performance study for American Foulbrood (AFB) and European Foulbrood (EFB):

About the American Foulbrood detection, were selected protocols still using from AGES (Austria) and IZSLT (Italy) partners, and these includes both End Point PCR and Real Time PCR protocols. Regarding the End Point PCR for the detection of American Foulbrood, were selected two protocols: 1) End Point PCR protocol aimed for the metalloprotease target (Kilwinski et al.,2004), using the PII MP F3/PII MP B1 primer pair and with a PCR product of 273 bp; 2) End Point PCR specific for the 16S target (Bakonyi et al., 2003), using the AF6/AF7 primer pair and with a PCR product of 237 bp. For both protocols were used the same DNA polymerase: 5X FIRE Pol® Master Mix Ready to Load, with 7,5mM (Solis Biodyne). There is only one Real Time PCR protocol for American Foulbrood, which aimed to *tnp*60 target (glucose oxidase target) (Dainat et al., 2018). In this PCR are used PL-F/PL-R primer pair and a TaqMan labeled with HEX degenerata (PL-P) probe. Th eMaster mix used is Perfecta™qPCR ToughMix™ (Quanta Biosciences). About teh European Foulbrood, were selected protocols still using from AGES (Austria) and IZSLT (Italy) partners, and these includes both End Point PCR and Real Time PCR qualitative protocols. The End Point PCR protocols fo EFB is only one: in this PCR the target is the 16S (Govan et al., 1998), using the EFB-1/EFB-2 primer pair and obtainig a PCR product of 831 bp. In this method was used the 5X HOT FIRE Pol®Blend Master Mix Ready to Load, with 7,5mM $MgCl_2$ (Solis Biodyne). The selected Real time PCR protocols for EFB are two: 1) Real Time PCR aimed to *napA* pseudogene target (nitrate reductase) (Dainat et al., 2018), using MP-F/MP-R primer pair and a TaqMan probe labeled with FAM; 2) Real Time PCR aimed to sodA gene target (manganese-dependent superoxide dismutase)(Roetschi et al., 2018), using Melisso F/R primer pair and a MGB probe labeled with FAM. For both Real time PCR the was used the Perfecta™qPCR ToughMix™ (Quanta Biosciences) Master Mix. The six molecular protocols selected for EFBand AFB were tested on DNA samples extracted form hive debris collected from hives with specific symptoms for both foulbroods.

The samples were prepared and provided by AGES (Austria) (the organising laboratory for the Performance Study Tests) to each project partners. 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 partecipant partners. This partecipants were six in total:AGES (Austria); IZSLT (Italy), CIAPA (Spain); AIS (Slovenia), NKU (Turkey), EURL (France). For each of six protocols were calculated the general specificity and sensibility on a total amount of 20 blind samples. In particular, the positive samples were divided in 6 samples with a high positivity level and 6 samples with a low positivity level. The negative samples were 7 in total. In addition, for each positive sample subgroup were estimated the sensibility again. All data about Performance Study Test for EFB and AFB were elaborated by AGES (Austria) and at the moment must be elaborated the speficic report.

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 were used 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 selected only one Real Time PCR protocol for *Nosema apis:*tharget 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 *Aethin 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 partecipant partners. This partecipants were six in total:AGES (Austria); IZSLT (Italy), AIS (Slovenia) and NKU (Turkey). For each protocols is ongoing the sensibility and sensitivity estimation, on a total of 40 blind samplas, 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*). Were tested a total of 16 negative samples. Therefore, for each sugroup were estimated the sensibility again. All data about Performance Study Test for *N. ceranae* and *N. apis* were elaborated by AGES (Austria) and at the moment must be elaborated the speficic report.

Test Performance study for *Aethina tumida*:

About the *Aethina tumida* detection, was selected the protocol in use at the time in the IZSLT (Italy) partner. This method is accreditated (ACCREDIA Lab. N° 201) and consist 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 concentration (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 partecipant partners. This partecipants were six in total:AGES (Austria); IZSLT (Italy), CIAPA (Spain); AIS (Slovenia), NKU (Turkey), EURL (France). For the moleculr protocols is ongoing the estimation of sensibility and sensitivity out f a total of 28 blind samples. 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*). Were tested a total of 13 negative samples. Therefore, for each sugroup were estimated the sensibility again. All data about Performance

Study Test for *N. ceranae* and *N. apis* were elaborated by AGES (Austria) and at the moment must be elaborated the speficic report.

Recombinant expression and purification of VP1 of Sacbrood virus

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Sacbrood is a disease of honey bees that affects the brood of *Apis mellifera* and *Apis cerana*.Unlike many other viral diseases of honey bees, it has typical clinical signs: affected larvae fail to pupate, accumulate ecdysial fluids under the unshed cuticula, and are finally converted into a dark dried sac. The disease is caused by the Sacbrood virus (SBV); a single-stranded, positive-sense RNA virus belonging to the genus Iflavirus, family Iflaviridae.The genome of the virus encodes a single polyprotein that is posttranslationally processed by virus proteases into mature structural and non-structural proteins. VP1(342 amino acids) is the largest structural protein with a calculated molecular weight of 38,5 kDa. The aim of this study was to express and purify recombinant VP1 (rVP1) in E. coli. The coding sequence of VP1 was inserted into a pET11a vector containing a histidine tag. After induction of expression using IPTG, the production of rVP1 was monitored over time. At the optimal time-point, the E. coli cells were harvested, lysed, and the protein was purified. The bacterial lysate was cleared by ultracentrifugation and soluble as well as insoluble fractions were purified using Ion Metal Affinity Chromatography (IMAC).Protein purifications were analyzed by SDS-PAGE and western blot. Detection of rVP1 at all steps was achieved using a primary mouse anti His-tag antibody and goat anti-mouse IgG HRPO-conjugate. Our analyses showed that rVP1 is mainly expressed as a soluble protein with maximum expression yields between 2h and 2.5h. The purified protein was subsequently used to immunize mice to generate serological reagents against SBV. The mice sera were tested for their reactivity against the rVP1. However, polyclonal immune response of all immunized mice against rVP1 was rather weak. We conclude that rVP1 of SBV has poor immunogenic properties in mice and that a strong adjuvant or different immunization protocols have to be used in order to obtain hyper-immune sera.

Recent findings of parasitic phorid flies in honey bee

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New findings on parasitoids of *Apis mellifera* L., in various parts of the world, mainly involve the Phoridae family and the genera *Megaselia* and *Apocephalus* in particular. These phorids create a threat to honey bees causing an abnormal behaviour leaving the hive in the night, attracted by the lights, and serious infestations of honey bees colonies. The first finding concerns *Apocephalus borealis* Brues, 1924, known as a potential vector of deformed wing virus (DWV) and *Nosema ceranae* in the United States, and considered as one of the possible causes of the Colony Collapse Disorder. *A. borealis* or a similar phorid was also detected during a large screening of pathogens in Belgium. Opportunist *Megaselia* genus in the *Megaselia rufipes* (Meigen, 1804) species has been identified in north-western Italy as a facultative parasitoid of honey bees with deformed wings, therefore presumably affected by DWV. This species has thus been reported for the first time as an effective parasitoid of *A. mellifera*. Afterwards *Megaselia scalaris* (Loew, 1866), already recorded on reared in laboratory or dead bees, has been found in central Italy in bees captured with bright traps and in apparently healthy foragers. In Algeria this species, detected on *Apis mellifera intermissa* and positive to DWV, appears to be a possible cause of DWV transmission. Additional observations of *M. scalaris* in Cameroon parasitized honey bees and *M. rufipes* and *M. preacuta* (Schmitz, 1919) in Macedonia on

dead bees were reported. The growing prevalence of the honey bee infestation by these phorid flies and the widespread diffusion in the world of such parasitoids lead us to believe that Phoridae family has an important link, not enough highlighted, on the health and survival of the domestic bee.

Beeheal: Monitoring microsporidia and viruses in honey bee colonies in Spain

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BEEHEAL is a project that aims to determine the phenology of *Nosema ceranae*, one of the most prevalent pathogens within the honeybee in four Mediterranean countries: Spain, France, Portugal and Israel. The possible synergisms among *N. ceranae, N. apis* and some bee infecting viruses will be also investigated. To do this, Nosema spp. infection levels and viral loads for ABPV, IAPV, CBPV, BQCV and DWV are being monitored in a long-term study. In Spain, at least 15 colonies of A. mellifera iberiensis are sampled every two months for pathogen screening including varroa levels. The sampling period started in February 2018 and will be performed until the end of 2019. According to the analysis of the samples collected until June 2018, the most prevalent microsporidium detected was N. ceranae, while N. apis was rarely found. The level of intra-colony infection for N. ceranae differed in the sampling time-points, with a higher proportion of bees being infected within the colonies in April (4-96%) than in February (<4-56%) or in June (<4-36%). Regarding viruses, the DWV infection level was low, at apiary level (highest in February: 23%) and at colony level (3.13%). At apiary level, the prevalence for ABPV was circa 40% being slightly higher in April. IAPV was only detected in June (prevalence around 10%) and BQCV was detected in all colonies apart from 15% that were found negative to this virus in February. All colonies were negative to CBPV. The study of correlation among pathogens detected and the comparisons among participating countries will be performed in future analysis when more data are available. This work has been developed under the BEEHEAL project. In Spain, BEEHEAL is funded through the ARIMNet2 2016 Call by the INIA (Spain) funding agency. ARIMNet2 (ERA-NET) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 618127.

3. Session: Honey bees, environmental pollution and pesticides

Does thiamethoxam effect honey bee queen (*Apis mellifera carnica*) development?

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Thiamethoxam, honey bee queen, ovaries, spermathecae-stored sperm, artificial queen rearing

Systemic pesticides used in agriculture settings can contribute in high honey bee colonies losses worldwide. Neonicotinoids with long-term residual effects present in the environment can have an adverse effect on non-target organisms. They can also accumulate in hive products, especially in comb wax, thus chronically exposing developing honey bees to sublethal doses. In light of the current situation, the aim of this study was to investigate the effects of thiamethoxam on the development of the reproductive system and physiology in the honey bee queens. Experiments were performed in honey bee nurse colonies and in the laboratory. Two groups of honey bee queen larvae were treated with thiamethoxam during rearing in the colony, via artificial feed in two sequences. In the first rearing round, queen larvae received a single treatment dose (4.28 ng thiamethoxam/queen larva), 4th day after grafting, while queen larvae in the second queen rearing round received a double treatment dose (total of 8.56 ng thiamethoxam/queen larva), 4th and 5th day after larvae grafting. After emerging, queens were anesthetized and weighed, and after mating with drones were anesthetized, weighed, and dissected. Ovary mass and number of stored sperm were determined. Body weight differed between untreated and treated honey bee queens. The results also show a decrease in the number of sperm within honey bee queen spermathecae that received the double thiamethoxam dose. Mating nuclei were examined and 70.59% of queens were found mated from the single dose thiamethoxam treatment; and 46.16% queens were from the double dose thiamethoxam treatment group. In both untreated, control mating nuclei groups single and double dose treatments, 81.80% and 75.00% queens were found mated. Data of our experiment provide insights into the sublethal effects of low doses of thiamethoxam on different physiological characteristics of queens. Reduced emerging queen body weights, reduced ovary weight, and lower sperm counts are the main observed effects at the individual queen bee level or the organ level. It is obvious that the use of standard, high-quality queens is a prerequisite for any research on colony development and behaviour, and economically successful beekeeping.

Development of Some Residue Free Honey Bee (*Apis mellifera*) Colonies

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Honeybees (*Apis mellifera*) have ecological and economic impact for sustainable biodiversity, natural or commercial pollination, production of honeybee products and apitherapy. Honey Bee colonies should be enough healthy before everything to be able to do the above duties. There are biotic and abiotic factors that threaten honey bee colony health. The spreading mechanisms of these factors are mostly due to the failures of the beekeepers, farmers, companies and governmentals. Among these mechanisms the farmer originated mistakes have more serious risks. For example the dosage of pesticides, the amount and type of improper adjustment of the pesticides, such as errors in spraying times. International queen bee trade mostly lack of a medical supervision and like that the drone semen exchanges between uncontrolled producers for usually uncontrolled, inadequate and unconscious artificial insemination. Medical risks and pathogens which were originate migratory beekeeping practices and failures. Uncontrolled drug resistance development problem, unconscious drug use, pesticide residues, pesticide remedies, immune system problems, One way feeding failures like exaggerated dosage of high fructose corn products. Insuf-

ficient quality of the beewax, insufficient sterilization, residue and pesticide source beewax and climatic changes. So many different variables have emerged especially in recent years. The above all medical reasons threatens the hundreds of thousands of years of honeybee evolutionary success. Honey bee colony health is collectively influenced by the above-mentioned factors and occurs as a syndrome called CCD in a complex manner. There are 8.5 million of colonies registered officially, financially supported and 70% of them are used in migratory beekeeping practices in the Turkey. There are 85,000 families who can be enrolled in beekeeping. Since 2006, millions of colonies was collapsed in Turkey. People take the causes of death one by one and do not look at the issue by CCD. We aimed to develop colonies free of chemical residues and some pathogens in order to obtain full colonies and to use them in areas such as CCD, immune system, drug development, vaccination projects, behavior and breeding researches.For this reason, we finished the first phase of our project supported by the ministry. In this congress, we will give informations about how will be free of chemical residues within the sustainable colony management.

A meta-analysis to quantify toxicity of binary mixtures in bee species: evidence for deviation from dose addition and mechanistic implications

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There is growing evidence that the weakening or death of bee colonies is mainly caused by the combined effects of multiple stressors rather than by one-off sudden attacks by a single factor. In particular, bees can be exposed to a wide range of multiple chemicals "chemical mixtures" as compounds from anthropogenic origin (e.g. plant protection products or veterinary drugs) or natural origin (e.g. mycotoxins, flavonoids and alkaloids). Therefore, quantifying the impact of multiple chemicals on bees has been identified by EFSA as a priority to understand their relative contribution to bee health in comparison to other stressors (e.g. varroa, viruses) in order to support the development of holistic risk assessment methods. Here, a meta-analysis of available combined toxicity data for binary mixtures was performed for honeybee, bumblebee and solitary bee species to test assumptions of combined toxicity (dose addition, response addition and interactions such as synergism and antagonism) in a quantitative manner. 92 case studies, including dose response data (51 case studies) were tested for estimating deviations from dose addition (i.e. interactions) by applying Toxic Unit (TU) approach. Interactions were observed for 42% of cases as synergism and 9% cases as antagonism. Sterol-biosynthesis-inhibiting (SBI) fungicides – insecticide/acaricide were the most investigated interactions (55%) for which synergisms were observed. Our findings suggest that most synergistic effects result from toxicokinetic (TK) interactions through inhibition of Cytochrome P450 or other drug metabolising enzymes in bees resulting in toxicodynamic (TD) consequences enhancing the toxicity of the binary mixture. Further research is ongoing in order to develop alternative methods to animal testing such as Quantitative structure–activity relationship models (QSARs) for predicting i) mode of action (MoA) of PPPs and ii) toxicity of PPPs binary mixtures in honeybees. In particular, when applying a tiered approach for ecological RA of multiple chemicals, QSARs models can be of value in order to predict (missing) information on individual compounds (tier 0) and to predict directly or stepwise the combined effects and interactions of chemicals in the mixture (tier 1). The model(s) will be implemented on the open source and standalone platforms VEGA (www.vegahub.eu) and CORAL (http://www.insilico.eu/coral/).

POSTERS

Poster Session 1: Good Beekeeping Practices

The beekeeping School of Etruria at the Naturalistic Museum of Lubriano for the diffusion of an eco-friendly apistic culture.

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The School of Beekeeping of Etruria is developed within the experience of the School of Beekeeping of the Naturalistic Museum of Lubriano (VT), named after Prof. CD Michener, Entomologist University of Kansas (USA) who in 60 years of scientific research has studied bees worldwide. Established by the Municipality of Lubriano with D.G.C. n. 105 of 29/10/2012, the School of Apiculture was created to meet the need for training in beekeeping. From 2011 to 2018 the training activities, with the support of teachers with proven experience (naturalists, agronomists, beekeepers, veterinarians, researchers) led 249 members from 15 different regions of Italy to deepen their knowledge on *Apis mellifera ligustica*: biology, breeding methods, beehive products (honey, propolis, wax, pollen, royal jelly), pollination service, management problems of pathologies and bees parasites. The preparation of the small experimental apiary of the Naturalistic Museum allows to continue the studies on the life cycle of the bees and relations with the ecosystem of the Valle dei Calanchi, an area of high naturalistic value between the Bolsena lake and the Tiber river. Since 2016, a series of single and associated subjects have joined the School of Apiculture of Etruria, which is proposed as a reference point for bee-keeping training for all, and for beekeeping teaching in schools of all levels: Lubriano Municipality and Municipality of Celleno (VT); Municipality of Porano (TR); CRM - Honey Research Center Dept. of Biology University of Tor Vergata - Rome; IRET-CNR - Research Institute on Terrestrial Ecosystems - Porano (TR); DIBAF - Dept. of Organic, Agri-Food and Forestry Systems University of Tuscia - Viterbo; ITAS - Ist. Agrario F.lli Agosti - BAGNOREGIO (VT). The operational offices of the School of Apiculture of Etruria are currently at the Naturalistic Museum of Lubriano (VT) and at the Ecomuseum of Landscape od Etruscans in Porano (TR).

Development of a standardized check-list on beekeeping management: a new risk assessment tool

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Colony losses in Europe are mainly related to beekeeper's background and apicultural practices adopted, as reported by the EPILOBEE project. A risk assessment tool considering management practices (Good Beekeeping Practices - GBPs) is, therefore, a useful tool in order to identify specific risks at the apiary level. Within the activities of a national project entitled: "Feasibility study to reduce the prevalence of honey bee diseases through the application of good beekeeping practices", from 2016 to 2018, 581 colonies were inspected to check their health status. New sampling procedures were applied, and a checklist was created to evaluate: the level of risk perception, formal aspects, the adoption of general and disease-related GBPs. Data deriving from the clinical visit and the laboratory outcomes were used as the "response" (Y) variables, while the data obtained from the checklist were used as an "independent" variable (X), by means of a linear regression model. Each item has been previously tested to verify its predictive potential, through simple odds ratio or GLM and only those that were useful for the description of the dependent variable, were used to contribute to form the degree of ordinal level that will characterize the independent variable. The linear model developed allowed us to quantify the weight of each variable and any interactions between different variables, indicating which are the beekeeping practices that best fit into a risk assessment tool to be used at the apiary level.

The guidelines for a proper use of antimicrobials in apiculture

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Like all living organisms, honey bees can be infested with diseases and pests. Some honey bee diseases of bacterial or fungal nature, like American foulbrood (AFB), European foulbrood (EFB) and nosemosis can be treated with antibiotics. However, the lack of awareness concerning the proper adoption of good beekeeping practices to prevent or control honey bee diseases, and the lack of relevant registered veterinary drugs leads to an uncontrolled use of antibiotics at the apiary level. As a result, this could lead to severe consequences for the human health, like the presence of veterinary medicines' residues in honey bee products and the development of antimicrobial resistance (AMR) at the apiary level. Hence the need to develop proper guidelines to help countries and beekeepers to tackle those issues. The stepwise approach used with the "Progressive Management Pathways" (PMP) is a useful and systematic framework for planning and monitoring risk reduction strategies for control of major livestock diseases. It is used to implement appropriate and sustainable measures for risk management in livestock production systems to assist countries and farmers. The PMP approach for apiculture (PMP-API) represents a useful tool if internationally adopted for planning and monitoring risk reduction strategies for control of major honey bee diseases, like in other animal species. To bring this new approach to the beekeeping sector and mitigate the risk of AMR emerging at the apiary level, the Food and Agriculture Organization of the United Nations (FAO), together with the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) signed in 2018 a Letter of Agreement to prepare a common document, "Guidelines for a proper use of antimicrobials in apiculture", to be published in 2019. The study will include a global survey to investigate the practices and the use of antibiotics at the international level to control the infectious honey bee diseases. This will help in addressing future training and guidelines to be provided to the operators of the apicultural sector. Outcomes of the guidelines will be:

- beekeepers of the different countries will improve their knowledge on good beekeeping practices and will be able to adopt them, preventing the major honey bee diseases;
- beekeepers of the different countries will improve their capacity to evaluate the risk related to the use of antimicrobials and they will use these medicines more properly;
- both governments and beekeepers will be able to adopt a Progressive Management Pathway approach in apiculture (PMP-API).

Good Farming Practices in Apiculture (Good Beekeeping Practices – GBPs)

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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.

Biosecurity Measures in Beekeeping

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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.

Veterinary use of Apitherapy in Beekeeping

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Propolis is used by bees for various purposes such as nest construction and defence against predators and pathogens. Well known for its antimicrobial properties, propolis promotes social immunity in the colony by controlling pathogen loads and promoting colony health. The chemical composition of propolis and its antimicrobial activity is complex and variable and depends on the local flora and the plant sources from which it origins. In the nest architecture propolis envelope acts as an important antimicrobial layer around the colony that helps protect the brood from infections, resulting in a lower colony-level pathogens load. Its mode of action is probably via volatile compounds or direct contact on the hive walls, the nest entrance and the rims of comb cells. This defense strategy may have evolved to reduce the need to maintain activated a costly individual immunity when the insect is not pathogen challenged, representing an example of self-medication behaviour. The therapeutic activity of propolis from various regions of the world has been studied for honey bees both in vitro and in vivo against Paenibacillus larvae, the causative agent of American foulbrood (AFB) and Ascosphaera *apis*, the fungal agent of Chalkbrood diseas. In vitro studies have demonstrated that specific compounds within propolis inhibit the growth of P. larvae and A. apis. When colonies in the field were challenged with P. larvae and subsequently treated with propolis per os via an aqueous, alcohol or sugar solution, the treatment reduced spore loads and disease signs from the hive. Some researches indicate that colonies with a propolis envelope have shown significantly reduced levels of AFB clinical signs, though it did not eliminate the disease completely. Clinical signs of AFB in field colonies and levels of *P. larvae* spores in honey stores were reduced when propolis was fed to the bees in sugar solution. Other field tests showed that colonies treated with added freshly collected propolis were significantly stronger than propolis-removed colonies, and titers of *V. Destructor* and Deformed Wing Virus (DWV) were lower. Further studies are needed to clarify the relationship between propolis chemical composition and honeybee colony health but it can be considered an important first step in identifying new possible active compounds to treat some diseases and mitigate losses in honey bee colonies by promoting their natural defences in a sustainable way.

The honey of sulla, traditional agri-food product made in Sannio

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The Sannio region is widely known as the place where nougat was born. This product, which appears in the list of the traditional agri-food products of the Campania Region, is prepared according to the traditional recipe: egg white, hazelnuts, almonds and honey of sulla. In the last decade, new varieties of sulla were introduced on the territory of the Province of Benevento and, at the same time, the traditional variety's production decreased drastically. During the control activity at the honey extraction laboratories, 11 samples of honey of sulla have been selected for a tasting event. The tasting evaluation criteria had all been acquired during the course "Introduction to sensory analysis of honey". The results obtained showed an homogeneity concerning the characteristics of said honey, in spite of the different territorial areas where the samples came from. Moreover, the research has showed that the average consumer does not tend to choose honey of sulla because of its peculiar characteristics, which do not meet the expectations of the public. That is the reason why many producers prefer either to mix it with millefiori honey, or to allocate it for the nougat industry, instead of making jars devoted exclusively to it.

Poster Session 2: Honeybee diseases

Honeybees surveillance in Piedmont region 2012-2018

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In Piedmont beekeeping is a practice of ancient tradition. The presence of mountains account for a wide variety of botanical species and valuable honey production. The average annual production is approximately 1400-1900 tons. Beekeepers are around 5605 and apiaries are 18914 mainly located in the areas of Cuneo (26.9%) and Turin (25.8%). The 71.9% of apiary are nomad, the number of honeybee colonies is nearly 194707. The over wintering colony mortality rate during 2013-2014 was 1.43%. A surveillance plan for mortality in honeybee colonies is active in the region. Samples of honeybee colonies, pollen, honeycomb, wax, hives and honey are collected to be processed by lab for presence of pests, diseases and chemical residues. From 2012 to 2018, 134 apiaries were investigate for honeybee colony mortality. In 66 out of 134 apiaries was detected at least a problem. The deformed wing virus and chronic bee paralysis virus were extensively present and found in 27 apiaries. Varroa, detected in 28 apiaries was widespread, while Nosema was detected in Biella (4), Turin (4) and Vercelli (2). American foulbrood, as well European foulbrood were detected respectively in 6 (Alessandria, Turin and Verbania) and 2 apiaries (Turin and Verbania). Presence of chemical residues were also detected. Phosphorated pesticides (Dimethomorph, Fludioxonil, Clothianidin,Thiamethoxam) were identified in one apiary in Asti province while Propiconazolo in one apiary in Verbania. Fluvalinate was discovered in one apiary in Cuneo province. Permethrin was found in Vercelli (1) and in Turin (2). Although the areas that reported more problems were Turin and Cuneo, no association was found between province and presence of Pests and diseases (chisq 9.5, pvalue= 0.22). Only in 50% of the analyzed apiaries the survey provided evidence relate to mortality in bee colonies. A long-term epidemiological survey on honeybees is necessary in order to design an active surveillance plan in the region.

Preliminary evidences of gut microbiota composition of a particular population of honey bee from Marche region

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Gastrointestinal microbiota shows a fundamental role in prevention of diseases, increase resistance and stimulate immune system development also in *Apis mellifera* (honey bee). The social insect honey bee is the most important pollinator globally, and the health of colonies has been a major concern following colony losses in the last decade. A few years ago was identified a bee population, which was isolated from several decades by other populations of *Ape mellifera*, in an area far from any human activities (Roti Abbey area, Matelica, Marche Region), without pollutants, and away from "genetic contamination" by other populations of honey bees. Our study was directed to characterize the "core" gut microbiome of this particular bee population by quantitative PCR along with deep sequencing of amplicons of the V4 region of the bacterial 16S rRNA gene to characterize both the size and the composition of the microbiome of bee workers. Preliminary results demonstrate a significant difference in Bifidobacteria and *Lactobacillaceae* composition of some stages of worker bees belonging to Roti bee population. These results open the possibility of isolating and expanding bacterial strains present in this honey bee population, and using these bacterial strains as "probiotics" for common honey bees.

Microbic flora obtained with cultural method from honey and debris taken from SHB positive hives of Calabria region-Southern Italy

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Between 2017 and 2018 the Apiculture laboratory of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri "of Rome carried out the cultural examination of samples taken from SHB positive beehives from Calabria region (southern Italy). The aim was to observe the microbial and mycotic flora present in these samples, specifically Kodamaea ohmeri, yeast that could be considered an indicator of the presence of SHB, as it is transported by *Aethina tumida* inside the infected hives, being able to recall further beetles mimicking the attraction action of the alarm pheromone of bees. A total of 33 samples have been analysed: 15 samples of honey in a jar, 8 samples of comb honey and 10 samples of debris taken from the hive bottom boards. The culture test was carried out by plating the above mentioned samples diluted with sterile saline solution on blood Agar, Sabouraud and DG18 media and incubating in aerobiosis at 37 ° C for the microbial flora and at room temperature for the molds. Subsequently, the isolated colonies were characterized with biochemical micromethod (bacteria and yeasts) and microscopically (molds). The results showed from the comb honeys: Bacillus spp. in all samples (above all *Bacillus licheniformis*), *Pseudomonas luteola, Pantoaea sp. Klebsiella spp, Bordetella spp.*, Kodamaea ohmeri, Aspergillus spp., Mucor and *Ascosphaera apis*. From honeys in jar it was possible to detect: Candida spp., Rhodotorula spp. and Mucor. Finally, the "debris" matrix was frequently contaminated with *Candida guilliermondii* (that is the imperfect form of Kodamaea ohmeri) and Bacillus spp.; with less frequency we found: *Kodamaea ohmeri, Rhodotorula mucillaginosa, Pseudomonas luteola*, Pantoea sp., *Pasteurella pneumotropica*, Mucor spp., *Ascosphaera apis* and Aspergillus spp. In conclusion, we found a recurrent microbial and mycotic flora, especially Bacillus spp. and *Candida guilliermondii. The last* was detected from 90% of debris, 28% honeycomb and 20% of jar honey samples.

Software vs Survey: Mining Data From Real Time Software Applications to Map and Monitor Honey Bee Health

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As the world's population continues to rise and hunger is widespread, bees are more important than ever in guaranteeing a sufficient food supply. However, honey bees' survival is at risk due to pathogens, pests, pesticides, mismanagement, poor nutrition, among other threats. Understanding how diseases move, where they are, and how they impact colony health can help us manage the situation more intelligently. Currently, a significant portion of our knowledge about the spread of honey bee diseases comes from surveys or other post hoc investigations. Time is an extremely important factor when monitoring honey bee health; therefore, a system able to provide real time feedback is necessary. In this project, we have compiled disease, pest, and mite data from a cloud-based software system (hivetracks.com). The software contains data on honeybee health, which we have used to prepare honeybee, disease maps over time. While the visual reports took time to design and develop, once built they can be updated in

near-real time as beekeepers use the software, allowing for quicker and more effective interventions and monitoring. Our analysis has been categorized into the three groups of diseases, pests, and varroa mites "Diseases" includes records of American and European Foulbrood, and Chalkbrood; "Pests" includes Tracheal mites, Nosema, and Small Hive Beetle; and "Varroa Mites" are exclusively inspections of Varroa. Currently, user data identifying trends just within the United States are the most reliable. Some methods can be applied on a global scale if information is collected properly, in a shared intentional approach. In the near future, with enough data from enough sources, it will be possible, to also apply machine learning techniques to not only monitor honeybee diseases in real time, but to predict their spread and take actions and preventive measures to mitigate them before they become a problem. This study will compare and contrast advantages of collecting and analyzing data from real time software applications vs post hoc techniques such as surveys. Recent analysis and visualizations will also be shared as an example of this approach.

New *Aethina tumida* detection methods using Real Time PCR from hive debris and swab samples

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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.

Evaluation of Varromed® performances in winter and autumn treatments of honey bee colonies (*Apis mellifera*) in a temperate area

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Varroa destructor is one of the most important pests of honey bees worldwide. The management of this pest is carried out by using acaricides, which usually present a high variability in efficacy and side effects due to variable factors like mite resistance, climatic or colony conditions. The development of new products based on "soft" acaricides, which have a low risk of residues and so far never demonstrated mite resistance, is an asset to beekeeping. During 2017 and 2018 we carried out a study to evaluate the performances of Varromed®, a product based on formic acid and oxalic acid in winter and autumn colony conditions. The trials were carried out in Central Italy (Rome). We tested both the acaricide efficacy and the honey bee toxicity given by the product. We quantified the mite fall dividing the hives in two experimental groups (treated and control), homogeneous in strength and in varroa infestation level. To perform the winter treatment, two groups of nine hives each were set up. Due to the presence of brood in our climatic conditions even in winter, we caged the queen during the whole period of the trial in order to obtain broodless condition as required by the label's instruction of Varromed®. Once obtained the broodless condition, we applied a single application of the product in the treated group. To perform the autumn treatment, we set up two groups of 10 (treated) and 9 (control) colonies. Considering the presence of brood, five applications of Varromed® were performed in the treated group. In winter conditions we recorded a mean acaricide efficacy of the treatment of 96.1% ± 3.5% (against a natural mite fall in the control group of 23.4% ±14.2%); while in autumn the final acaricide efficacy was 88.2±9.3% (with a natural mitefall of 44.6±16.3%). No mortality of queen bees was observed in the trials. No statistically significant reduction of the adult bees nor brood was found. As a conclusion, according to our studies, we can consider Varromed a valid acaricide for the use in temperate areas. Further studies should be carried out in order to evaluate its performances in different climatic conditions.

Emerging pathogens in honey bee: *Crithidia mellificae* and *Lotmaria passim*. An ongoing project for prevalence estimation and impact assessment on honey bee health in Italy

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Crithidia mellificae, Lotmaria passim, honey bee trypanosomatids, prevalence, Italy
During the last years, honey bee colonies suffered from depopulation worldwide without any apparent cause. Many factors seem to be involved in colony losses working in combination or synergistically but researchers worldwide didn't succeed in finding an unambiguous explanation. Honey bee trypanosomatids were considered emerging pathogens suspected to be involved in honey bee colony losses in association with other factors (other pathogens, natural immunity impairment, microbiota, metabolic stress, etc.). These parasites were increasingly reported worldwide as prevalent in managed honey bees. Since the first

isolation of *Crithidia mellificae* in the 60s, this species was considered the only trypanosomatid in *A. mellifera*. In 2015, an in-depth molecular and ultrastructural analysis conducted by Schwartz et al. determined that the majority of accessioned sequences belonged to a new species named *Lotmaria passim* that seems to be currently the most prevalent trypanosomatid species in Europe and United States. No comprehensive survey about *L. passim* and *C. mellificae* has been carried out in Italy until now and their prevalence and impact on honey bee health is still unknown. The research project "Emerging pathogens in honey bee: *Crithidia mellificae* and *Lotmaria passim*. Prevalence estimation and impact assessment on honey bee health in Italy" founded by the Italian Ministry of Health (grant IZS LT RC 0718) and coordinated by Istituto Zooprofilattico Sperimentale del Lazio e Toscana *M. Aleandri* was developed to fill this gap of knowledge. The aim of this research project is to check for the presence and eventually to estimate the level of occurrence of trypanosomatids in Italian honey bee; to isolate and characterize the circulating strains using Whole Genome Sequencing; to evaluate the possible association with colony losses. The project, started in January 2019, will contribute to increase the information about the diffusion of these emerging pathogens in Italy, to assess their impact on honey bee health and to identify the needs for proper preventive or therapeutic measures.

Efficacy of Varroa mite treatment using strips containing amitraz in bee colonies with high Varroa infestation level

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In June 2017, two apiaries were created: apiary with Cariolan (N = 14) and apiary with Caucasian (N = 12) queens. Bee colonies were prepared in hives with movable bottom board and beewax comb foundation populated with c.a. 15,000 worker bees and fumigated with tablet containing 12.5 mg of amitraz. Then no other varroa control treatment was applied to the end of season. In spring 2018 during Salix spp. flowering, when capped brood was in the colonies, the daily natural mites fall was on average 0.29 mite per colony. The colonies' strength and general condition did not indicate high level of mites in that period. In June, the mites were already visible, the colonies clearly weakened and the wingless workers with varroa mites on bodies crawled at hive entrances. At that time, the natural daily fall was 5.64 of mite per colony and the bee infestation (powder sugar method) reached on average 3.05 mite per 10g of bees. After main honey harvest (July 5), at least 1 month earlier than in other years, the regular varroa mite control was performed applying two plastic strips containing 500 mg of amitraz. During the 8 weeks of the strips exposure, an average of 3329.4 dead mites per colony were counted. Based on the last week's result, 370.5 mites per colony, it was decided to extend amitraz exposure period for the following 21 days with caging queens to prevent laying eggs. At that period, an average of 594.2 mites per colony fell, of which 62% after the first week, then 26 and 12% after the 2 following weeks. During the broodless period, additional control treatments were applied: trickling bees with 3.6% oxalic acid solution followed by fumigation with a 12.5 mg amitraz. During these treatments, an average of 30.8 dead mites per colony fell within the next three weeks. The effectiveness of treatment after an 8-week exposure of the strips was on average 84.7% (56.6-96.5). Finally, the 11 weeks exposure of strips, combined with broodless period in bee colonies resulted in a satisfactory treatment effectiveness level of 99.2%. High infestation of bee colonies at the end of 2018 an average of 3954.4 mites and especially the spring occurrence of varroa symptoms indicated a high level of mites in the colonies before previous winter. The above data indicate about mite re-infestation and the extremely high reproductive potential. On the other hand, alarming is also low efficacy of a formulation containing amitraz.

Bacterial flora obtained with cultural methods from gut of honey bees in central Italy

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In the period between April and November 2018 the Laboratory of Apiculture Unit of the Experimental Zooprofilattico Institute of Lazio and Tuscany "M. Aleandri "of Rome examined 28 pools of live adult bees sampled at the apiary of Blera in the province of Viterbo, in central Italy. With the aseptic technique, the intestines of these bees were taken and seeded on suitable culture media incubated for the appropriate time in aerobic, microaerophilia and anaerobiosis, in order to observe and identify the colonies of the microbial flora present in this intestinal environment and able to develop with the cultivation examination. The biochemical identification in micromethod has taken into account only the reliable results deduced through the calculation of the identification percentage and the typicality index and revealed a fairly wide and heterogeneous microbial panorama, where a greater frequency of: Enterobacteriaceae (especially with the genera *Hafnia, Enterobacter, Pantoea, Providencia, Klebsiella, Aeromonas and Serratia*), Lactobacillaceae, Bacillaceae (*Bacillus spp.*) Pasteurellaceae, Vibrionaceae, Corynebacteriaceae. Also present several families of environmental germs (Flavobacteriaceae, Pseudomanadaceae), present in the soil (Streptomycetaceae), symbionts of the plants (Rhizobiaceae), commensals of mammals, man and birds (Moraxellaceae, Pasteurellaceae). Also isolated the family of the Orbaceae, whose genes have already been isolated from the intestine of honey bees and bumblebees (*Gilliamella*).

The Mini-FLOTAC technique for the field diagnosis of Nosema spp. in honeybees (*Apis mellifera*).

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Nosema apis and *Nosema ceranae* are gastrointestinal microsporidia (1) and causative agents of nosemosis, one of the most common diseases of honey bees(1; 2).It is well known that nosemosis mainly caused by N. ceranae silently impairs honeybees' health, leading to reduction of colony population and potentially causing colony collapse(2).As the parasite represents a major hazard for beekeepers, a sensitive, rapid and affordable technique for a proper diagnosis is necessary(3).Usually, the field diagnosis of nosemosis is performed using standard microscopic examination of abdomens or fecal material taken from clinically or subclinically affected bees, to evaluate the presence of spores(5).In this study, for the first time the Mini-FLOTAC technique, a new quantitative very sensitive, accurate and precise copromicroscopic method(6), has been used for the diagnosis of Nosema spp..For this purpose, the abdomens of 10 adult forager bees, for each analyzed colony (n.10), were observed under a stereomicroscope for identification of anatomical changes and homogenized in the Fill-FLOTAC(6) with 10 ml of sodium chloride flotation solution (specific gravity 1200); then the two chambers of the Mini-FLOTAC apparatus were filled.After 10 minutes, the Mini-FLOTAC was translated and examined under a microscope.This technique was cheaper, easier, faster and more user-friendly than standard microscopic examination.In fact the reading field was clear and spores were bright and refractive.Therefore, the Mini-FLOTAC can be very useful for an initial screening to evaluate positive honeybees for Nosema that will be further analyzed for species identification.

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Total RNA sequencing, a molecular approach to improve honey bee health

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Honey bees have recently experienced a considerable decline in colony health, a complex phenomenon that can be attributed to biotic and abiotic factors. The situation is exacerbated by the interaction between multiple aspects, including habitat loss and fragmentation, climate changes, exposure to cocktails of ag-

rochemicals and novel parasites. To address this growing problem, the synergy of different scientific and technological approaches is advantageous in order to contribute to the protection of Honey bees. In particular, unravelling the molecular mechanisms of natural behaviours that promote colony resistance is fundamental for improving disease tolerance such as their ability to recognize and eliminate a damaged brood. In this study, Next Generation Sequencing (NGS) technologies were applied to deepen the understanding of the molecular mechanisms of hygienic behaviour. The hygienic behaviour test was carried out by treating with liquid nitrogen the areas containing the brood of 30 hives located in Reggio Emilia, Italy. The percentage of brood removed was measured 24 hours later. In order to study differential gene expression, the bees were taken before 24 hours, while performing the removal, and snap frozen in liquid nitrogen. For control, non-active bees were taken. The RNA sequencing was performed with HiSeq 2000 Illumina®, which allowed extensive profiling and a thorough transcriptome investigation. This technology allows the detection of a wide variety of RNA species, including mRNA and non-coding RNA. Alignment of the *Apis mellifera ligustica* sequences and the study of gene expression allowed to identify 36 differentially expressed genes with FDR p value correction lower than 0.05 and 119 microRNAs. Among these genes, the coding genes for the class of Odorant Binding Protein (OBP), Carboxylesterase and Troponin C type 1 were identified. The study contributes at increasing the knowledge on the molecular mechanisms underlying hygienic behaviour.

Optimization of Real Time PCR based detection of honey bee pathogens and parasites in hive debris

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Hive debris is a material of diagnostic value for monitoring honey bee pathogens or parasites. In particular the quantification in this matrix of Paenibacillus larvae, the aetiological agent of American foulbrood (AFB) has been recently proven to allow risk assessment for the disease. The composition of hive debris is highly heterogeneous and comprises wax, bee and parasitic insects or mites fragments, pollen, propolis and honey. When applying our recently described protocol for direct detection of Paenibacillus larvae by Real Time PCR in this material we found that DNA extractions from dark samples resulted in brownish extracts for which amplification was completely inhibited. Inhibition could be relieved by diluting DNA samples in 1:5 ratio with elution buffer, but this decreased the sensitivity of detection. Therefore, different DNA extraction procedures and DNA extraction kits from soil or stool, were introducing extra precipitation and washing steps, were also evaluated as well as separation of DNA from suspension by magnetic beads. Moreover, the addition of compounds such as polyvinilpyrrolidone (PVP) and bovine serum albumin (BSA) in the extracted samples or in the amplification reaction were tested. The different extraction procedures adopted are described in this report and their results are compared. The best performing protocols can be extended to other honey bee pathogens and parasites.

Evaluation of different hive matrices for honeybee virus detection.

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Several viruses, such as Kashmir Bee Virus (KBV) and Sacbrood Virus (SBV), were detected in honey, pollen, royal jelly and in bees in all developmental stages, suggesting the involvement of honeybee stored food supplies in the spread of viral infections (Mutinelli *et al.,* 2011). Three different matrices were con-

sidered during this study: homogenized bees, hive debris and powder sugar. Those sample matrices are not only suitable for virus detection, but also to find other bee pathogens, such as *Aethina tumida*, *Nosema* spp., American Foulbrood (AFB) and European Foulbrood (EFB). In this work, we evaluated the presence of Acute Bee Paralysis Virus (ABPV), Deforming Wing Virus (DWV) and Chronic Bee Paralysis Virus (CBPV) (Tantillo *et al.,* 2015) from 66 homogenized honeybee solutions, 51 hive debris and 65 powder sugar samples. The samples were collected from apiaries located in the Latium region (Central Italy). This made possible to evaluate the suitability of the matrices to carry out analysis to detect viruses and the prevalence of bee viruses in the Latium region. The molecular protocol included RNA extraction (Viral RNA Mini Kit, QIAGEN), cDNA synthesis (High-Capacity cDNA Reverse Transcription Kits, A. Biosystems) and Real Time PCR (Taqman Universal PCR Master Mix, Life Technologies). The Real Time PCR results showed that powder sugar and hive debris are excellent matrices for virus detection. The DWV was the most detected virus (54%), followed by the ABPV (39%) and the CBPV (7,4%). In conclusion, these results show an increase in the prevalence of ABPV in the Latium Region.

Loglio's jar": a useful diagnostic tool for keeping hives healthy

Giulio Loglio
Verinarian

Beekeepers and researchers need efficient, low-cost and credible working tools aimed at being aware of health situation of hives. "Loglio's jar" has been designed for this purpose. Intended for single use, it has been conceived to implement research plan and remediation of infected hives. "Loglio's jar" consists of a cylindrical container made of polyethylene terephthalate which is closed at one end by plastic cap and at the other end by a large-mesh filter suited for sealing the cylinder. The flexible mouthpiece allows to quickly collect about 300 bees from honeycombs without damaging them. To calculate the level of infestation with Varroa it is enough to dust the bees collected in the jar with powdered sugar. After vigorously shaking the jar to remove Varroe from bees, the powdered sugar is removed through the large-mesh filter and distributed on a sheet of white paper. Varroes appear as dark spots between the powdered sugar and their number, compared to the number of the bees collected, allows to calculate the level of infestation. The same powdered sugar, collected in a clean container, may be examined by a laboratory to diagnose diseases (i.e. American foulbrood and European foulbrood). The bees can be introduced back into their hive or sent, in whole or in part, to an analytical laboratory for the detection of bacterial, viral, protozoal and fungal diseases typical of the species.

Varroa mites resistant to pyrethroids in Spain

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Varroa destructor is an invasive parasitic mite that is now widespread around the world in *Apis mellifera* colonies after it shifted from its original host, the Eastern honey bee *Apis cerana*. It feeds on host's haemolymph and fat body through a puncture in the exoskeleton. It is considered as a major problem for beekeeping because of its role as a vector of several viruses, and Varroa-infested colonies in temperate climates may die in 2-3 years if not correctly treated with acaricides. Nowadays, beekeepers use a wide range of different chemical substances, application techniques and methods to keep mite populations

under control. In Spain, the most used methods are based in the application of veterinary medicaments consisting on acaricide impregnated strips, mainly pyrethroids and organophosphates. However, after several decades using these treatments, mite-resistance to some active substances has been described. Resistance to pyrethroid acaricides (such as fluvalinate or flumethrin) is the most widespread and it is due to a mutation in the voltage-gated sodium channel gene. The most common gene mutation in European Varroa populations is a modification of leucine to valine position (L925V) in the voltage-gated sodium channel. Recently two novel mutations in the same gene: methionine (L925M) and isoleucine (L925I) were described in North America. In this work, we present the results of the detection of pyrethroid-resistant mites in an apiary from Spain using a method previously described. This apiary was not treated with pyrethroids in the previous 24 months, but resistant mites were still found. Different hypothesis to explain this finding will be discussed.
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Deformed Wing Virus (DWV) in honey bee colonies *Apis mellifera* intermissa and sahariensis in Southern Algeria

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The honey bee is threatened by many pathogens. Of these, the deformed wing virus is one of the most prevalent and could be responsible for colony mortalities. The purpose of this work is to determine the prevalence of this virus in some apiaries in southern Algeria. Sampling was carried out in the southern regions of Algeria represented by Djelfa, El Bayadh, Laghouat, Ain Seffra and Bechar on bee colonies of the race *Apis mellifera* intermissa and Sahariensis. 45 adult bee samples were taken in all of these areas at apiaries with a mortality rate greater than 10%. For the detection of the virus, the RNA is extracted using the NucleoSpin® RNA II Kit (ACHEREY-NAGEL). Reverse transcription of the RNA and amplification of the DNA are performed using a continuous process by the RT-PCR Method with the kit RT-PCR kit (Qiagen) according to the manufacturer's recommendations. The results obtained show a variation in the prevalence of viruses between the apiaries and the zones studied. Apiaries in the Bechar area have the highest contamination rate (45%). The least contaminated area is that of Djelfa with a rate of 25%. No difference was recorded between the two subspecies of bees *Apis mellifera* intermissa and sahariensis. The study shows a difference between rates of varroa infestation in brood and bees, but no relationship was detected between these rates with the increase in the prevalence of the virus. No correlation was also detected between recorded mortalities and the prevalence of this virus.

Fieldtest to evaluate the shookswarm method for the elimination of *Paenibacillus larvae* in honeybee colonies in a subclinical state

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American Foulbrood is a notifiable disease and one of the most damaging honeybee brood diseases with very severe economic impacts. The BPRACTICES project aims at identifying good beekeeping practices as well as new diagnosis and control measures to prevent clinical outbreaks. Hive debris is a matrix for various PCR-based detection methods and sampling is non-invasive and time saving. Food store and hive debris samples were collected, and food store samples were tested for spores of Paenibacillus larvae employing the culture method. Apiaries with P. larvae-positive food store samples from some or all colonies but negative results for clinical symptoms were rated as "subclinically infested" by P. larvae. Such apiaries

were selected for the field trial. Seven beekeepers were recruited as participants for the trial to perform the shook swarm method in 2017 and 2018, respectively. The food store samples taken after the shook swarm procedure revealed that five of the seven beekeepers were successful in reducing P. larvae below the limit of detection of the applied culture method.

Preliminary results of different protocols for varroa control (queen caging plus oxalic acid treatment; formic acid treatment) in Austria

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Varroosis can cause massive losses of honeybee colonies in *Apis mellifera*, especially in temperate climates. The BPRACTICES project seeks to develop hive management strategies for varroa control with low input of medicines. The goal of the experiment was to assess if a treatment concept of summer queen caging plus oxalic acid treatment of broodless colonies or colonies without capped brood in combination with the standard winter treatment in the broodless stage is sufficient for a safe wintering and for achieving strong and productive colonies in the following season. The efficacy of three different treatment protocols for varroa control was evaluated. Two protocols are based on the temporary caging of the queen (24 and 19 days, respectively) plus treatment with oxalic acid; one protocol foresees two applications of formic acid without queen caging. In addition, an oxalic acid treatment was carried out during the hibernation period in all variants, and the beekeepers were recommended to keep their usual colony management if possible. In 2017/18, 200 colonies were included in the experiment. The proportion of colonies that exceeded the varroa infestation thresholds of 1% in July and 3% in September did not differ significantly between the three test groups. There was also no significant difference between the three groups in the proportion of productive colonies, defined as addition of least at least one honey super at the beginning of the spring honey flow. Queen failure was observed in all experimental groups. However, the frequency differed significantly between the three groups. In group A (24 days of queen caging), queen failure occurred in one third of the colonies, while in the other two groups the frequency was below 10%. Participating beekeepers associated the queen failures in group A with the method of queen caging or, in group C (no queen caging), with the formic acid treatment.

Trypanosomatids affect the survival of bees in experimental infections

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Trypanosomatids are flagellated protozoan known to infect *Apis mellifera* since the last century but have not been studied in depth until recent years, due to their higher prevalence and their possible implication in the colony losses phenomenon. However, its pathogenicity for bees remains unclear, being this aspect the main interest of our investigation. The first phase of our research was test different culture media to obtain the best growth standard curves of *Lotmaria passim* (ATCC PRA403) and *Crithidia mellificae* (ATCC 30254). We obtained the best growth results using Brain Heart Infusion (BHI) medium. Afterwards, to determine if the infective morphotypes of both species had an effect on the survival of bees, two experiments were designed infecting 5 days-old *Nosema*-free and trypanosomatid-free honeybee workers from

three different colonies. Experiment 1 was performed during spring, and evaluated possible differences of pathogenicity of promastigotes populations (96 h and 144 h of growth) of the two trypanosomatids. Experiment 2 was carried out during early autumn with the aim of determine if a major dose of parasites affect the survival of bees. Histological studies and SEM analysis showed that the epithelial cells of the hindgut of infected bees were covered by a layer of parasites, mostly promastigote-like, but there were no detectable alterations in the epithelium structure comparing with uninfected bees. However, the hindgut of 96 h *L. passim* infected bees present spheroid forms that left a "hole-erosion" on its initial location in the epithelium. Statistical analysis revealed higher mortality in all groups of infected bees compared with the control bees, especially the ones infected with *L. passim* 96 h promastigotes. Higher doses of parasites increase the mortality in both infected groups at an earlier time, especially in *L. passim* infected bees.
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A new plan for bee pathogens in Marche Region, Italy

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In Italy the development of beekeeping has been strongly affected by health problems: since the diffusion of *Varroa destructor* in the '80, to the recent introduction of *Vespa velutina* in Liguria (2013) and *Aethina tumida* in Calabria (2014), to the wide endemics of *Nosema ceranae.* All this pathogens cause loss of hives and low productivity with significant economic damage. Nowadays, with the implementation of the National registry, we can have knowledge the consistency of hives in bee-keeping farms, their location, as well as others information are available and then more detailed epidemiological evaluations are allowed. In the Marche Region, a surveillance plan has be developed by IZSUM in collaboration with the Regional Authority and the Bee Keepers Associations for the named pathogens. This plan, which will start in March 2019, has as aims: a)to assess the amount of infestation (March-April) by *V.destructor* and the prevalence of Nosemiasis, in healthy hives, using standard methods of diagnosis (OIE, Manual of Diagnostic Tests); b)to realize a detection network for the introduction of *V.velutina* and *A.tumida* (April-November), by using specific traps. The sampling is assessed, not only by a statistical approach, but also with a geographic ones; so we can detect the relationship between Varroa and environmental parameters, but also to improve the surveillance for invasive species. The whole region will be divided into squares (10x10kilometers); within each square a sentinel farm will be chosen on the basis of its own experienced management. In addition, the Plan will be provide a laboratory assistance for all the farms signed up in the National registry, in order to ensure standardized diagnostic methods for bee health issues. This Plan, in addition to an assessment of the distribution and geographical spread of the beekeeping problems, will allow the creation of a network for health control based by standard analytical methods for both endemic and exotic pathogens.

Brood interruption and oxalic acid treatment: effects on colonies and virus population

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Varroa destructor is a well-known pest and a significant stressor of honey bees. Mites feed on the fat body of honey bees and, through this gate, they transmit viruses that infect pupae and adult bees. Acaricide treatments against varroa mites can alter the host susceptibility to DWV infections by preventing the related damages of *V. destructor* infestation. "Soft" acaricides combined with brood interruption techniques are

highly effective acaricide treatments against varroa mites when correctly performed. By applying such techniques before the colony starts to produce the overwintering bees, the amount of mites and the viral load of the colony should be reduced. In this poster we present the protocol adopted to evaluate efficacy and effects on population dynamics of honey bees and virus titre (ABPV, DWV) of different brood interruption techniques (queen caging or trapping comb) combined with an oxalic acid treatment. Another aim of this study is to determine, if the selection pressure that Varroa mites undergo while brood is interrupted and they cannot reproduce, have any effect on the virus titre in adult bees after the queen is released from the cage. We will compare virus titre in bees before and after the queen was confined in the cage or the trapping comb. Trials were conducted in late summer 2018 in Slovenia, Italy and Turkey on 86 colonies.

Poster Session 3: Honey bees, environmental pollution and pesticides

Food safety of pollen: Identification by PCR of pollen containing alkaloids or allergenic species

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Pollen is a food from animal origin which is used by honeybees as they main protein source. In the recent years, pollen has become an important element both in human and animal nutrition. However, there is a lack of knowledge about the safety of this product, especially regarding possible allergenic and alkaloid properties. The objective of this work (IZSLT 06/14 RC "Local pollen production: hazard analysis and study of a model for its prevention") was to develop molecular methods for the characterization of pollen containing alkaloids or allergenic species. These analysis methods represent a valid tool to map agricultural areas and to reduce the collection of toxic pollen in specific periods of the year, avoiding the presence of dangerous substances in the final product. The work flow included: 1) DNA extraction from pollen granules (Invisorb Spin Food kit II, INVITEK), 2) development of End-Point PCR protocols to detect the presence of six species of pollen known to contain allergenic and alkaloid substances (*Ambriosia artemisiifolis, Borrago officinalis, Echium vulgaris, Eupathorium cannabinum, Senecio vulgaris, Salix alba*); 3) development of four Real Time PCR protocols to detect toxic or allergenic pollen from four plant species (*Arthemisia vulgaris, Fraxinus ornus, Alnus glutinosa, Betulla pendula*). The pollen was sampled within the Massa Macinaia area, Lucca province, Italy. A map indicating the plant species present depending on the season was created. Then, three samples of pollen were collected on March 2017, and three more samples of pollen were collected on April 2017. In the March 2017 samples the plant species found were *Borrago officinalis, Eupatorium cannabinu, Seenecio vulgaris, Echium vulgaris, Ambrosia artemisiifoli*a and *Salix alba*. *Arthemisia vulgaris, Fraxinus ornus* and *Alnus glutinosa* were not present. In the April 2017 samples we found the same pollen species as from March 2017 except *Salix alba.* The absent pollen species in the sample of March 2017 were also absent in the April 2017 samples. These results show that the pollen species harvested in a given area change with time, thus affecting the harvesting of pollen and the safety of the final product.

Trend of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDFs) in beehive matrices: a pilot study to evaluate possible application of "honey bees monitoring stations" as a preventive alert system.

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During their foraging activity, honeybees collect around 8000 samples from air, soil, plants and water, transporting into the hive all the environmental contaminants. Beeswax, consisting primarily of a mixture of fatty acids, is a matrix of particular toxicological interest because most of the fat soluble, non-volatile and persistent chemicals can accumulate in this matrix. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDFs) are groups of dangerous persistent organic pollutants (POPs) released in the environment primarily as by-products of chemical manufacturing processes and during the combustion of municipal and chemical wastes. Atmospheric transport and deposition processes lead to dispersion of PCDD/PCDFs into soils, plant surfaces, bodies of water and sediments. Due to their lipophilic properties, these molecules may accumulate in fatty tissues and bioconcentrate through food chain, showing higher amount in animal tissues than in environmental matrices. Considering the well-known role of honeybees as bioindicators for pesticides, heavy metals and other contaminants, the present study has been developed to evaluate the possible application of honeybees as a preventive alert system to monitor PCDD/PCDFs levels. Honeybees, honey and beeswax samples were collected in June and in September 2017 from three beehives located in the headquarter of Ducati Motor Holding S.p.A. (Borgo Panigale, Bologna, Italy). Higher concentrations have been registered in September, probably as a consequence of longer exposition time. Honeybees and honey samples have shown WHO-TEQ values lower than beeswax samples in both time of

sampling. The PCDD/PCDFs residues found in the beeswax samples indicate that this lipidic matrix is the principal accumulator for PCDD/PCDFs such as fat tissue of mammals, birds, fish and shellfish. Obviously, more studies are necessary to assess the use of honeybees and their products in biomonitoring projects about PCDD/PCDFs. However, the present data represent an important safety concern for consumers of honeybee products and suggest the effectiveness of the use of "honey bees monitoring stations" as an inexpensive alarm and investigation system for researching the human impact on an ecosystem.

Monitoring pesticide contamination and *Aethina tumida* infestation in honeybee products: a biosensing approach

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Key words: *Aethina tumida*, pesticides, biosensing, honey bees

Among the most dangerous risks for honeybee health and, as a consequence, for the quality of their products there are, undoubtedly, pesticides and parasite insects. Both these harmful agents require therefore a strict and timely monitoring of their presence in wax and honey in order to quickly react and save the colonies. In order to achieve such a monitoring, we have been developing two different kinds of biosensing approaches based of electrochemistry and microgravimetry. Particularly, we have implemented an approach based on Cyclic Voltammetry measurements for sensing the presence of organophosphates in honey and wax. Our strategy involves the use of the enzyme acetylcholinesterase (AchE) that is inhibited by most organophosphates used as insecticides. The presence of organophosphates is detected electrochemically (CV and conductimetry) following the activity of the enzyme that has been previously immobilized of the working electrode surface. *Aethina tumida* is a hive beetle parasite that moved from its native to sub-Saharan Africa location to the rest of the world becoming a major problem also in Europe. In order to monitor the infection by *A. tumida*, we have implemented a microgravimetric approach to detect the presence of the yeast *Kodamaea ohmeri*, that is present in honey as a consequence of beetle infestation. The proposed strategy involves the use of ad hoc produced polyclonal antibodies against a particular peptide of Kodamaea ohmeri found by RT-PCR analysis of ribosomal RNA sequence extracted from honey samples.

Feeding effect study on bees colonies development in biological mode

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In case of a food deficit, honeybees need a feed supplement as organic sugar syrup in biological mode. We studied the feeding effect on the development of honeybees colonies by comparing the initial and final average weight and number of bees and brood frames of strong, middle, low and very low hives batches. The average duration of feed consumption differed from one batch to another and sprode with the decrease of the hive strength.

A little guide of pollen stock color in a Tunisian apiary in spring season.

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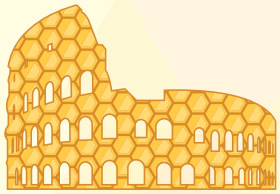
The study of pollen stock from 12 hives between the first of April and the end of June 2016 allowed us to achieve a micro guide to the layers color of pollen stored by the bees and the actual size of the corresponding pollen.



Natural biocide disrupts nestmate recognition in honeybees

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Honeybee colonies are under the threat of many stressors, biotic and abiotic factors that strongly affect their survival. Recently, great attention has been directed at chemical pesticides, including their effects at sub-lethal doses on bee behaviour and colony success; whereas the potential side effects of natural biocides largely used in agriculture, such as entomopathogenic fungi, have received only marginal attention. Here, we report the impact of the fungus *Beauveria bassiana* on honeybee nestmate recognition ability, a crucial feature at the basis of colony integrity. We performed both behavioural assays by recording bee guards' response towards foragers (nestmate or non-nestmate) either exposed to *B. bassiana* or unexposed presented at the hive entrance, and GC-MS analyses of the cuticular hydrocarbons (CHCs) of fungus-exposed versus unexposed bees. Our results demonstrated that exposed bees have altered cuticular hydrocarbons and are more easily accepted into foreign colonies than controls. Since CHCs are the main recognition cues in social insects, changes in their composition appear to affect nestmate recognition ability at the colony level. The acceptance of chemically unrecognizable fungus-exposed foragers could therefore favour forager drift and disease spread across colonies.

Bees decline and Global Change

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Solitary and social bees (Apoidea) insects provide substantial benefits and services to ecosystems and human society through their activity. The significant changes that are taking place in terms of climate and soil have a heavy impact, often negative, on the abundance, distribution and reproduction of the Apoidea, in both natural and semi-natural environment. There are numerous environmental drivers that pose relevant threats to biodiversity and the health of Apoidea. Climate changes in particular may affect the reproduction, spread and activity of the Apoidea, for example by modifying flowering time and making food, mainly nectar and pollen, unavailable for bees. For their peculiarities, bees play a strategic role as bioindicators of the quality of the environment and the state of the natural ecosystems. In the last two decades a large scientific evidence has been produced on the decline of populations of Apoidea because multiple drivers. According to the IPBES (2016) report, direct threats include land-use change, habitat loss, intensive agricultural management, pesticides use, environmental pollution, invasive alien species, pests and deseases, and climate change. In June 2018, the European Commission adopted a Communication, the first-ever EU initiative on safeguard of pollinators. The Initiative sets strategic objectives and a set of actions to be undertaken by the EU and its Member States to address the decline of pollinators and thus to contribute to global conservation efforts. It sets the framework for an integrated approach to the issue and a more effective use of existing tools and policies by defining three priorities:

1. improving knowledge of pollinators decline, especially bees and its causes and consequences;
2. tackling the causes of pollinator decline and finding /implement possible solutions:
3. raising awareness, engaging society-at-large and promoting collaboration among stakeholders.

ISPRA actively participates in research projects with the aim of find out what are the factors that determine the mortality of bee colonies, in relation to pesticides uses in natural and agricultural areas (ISPRA, 2011; Bellucci et al., 2010) and to identify better methods to protects all pollinators.

Note

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