



World Organisation
for Animal Health

Official Health Control of Bee Diseases

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Nairobi, Kenya

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Sector FAO Reference Centre for Apiculture, Health and Biosecurity ·
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Each slide will provide an example of practical application of the Terrestrial Animal Health Code, **Chapter 4.15**

Official health control of bee diseases: the WOAH standard

Terrestrial Animal Health Code, Chapter 4.15 — the framework this presentation follows

Chapter 4.15 — seven articles

- **4.15.1** Purpose — control endemic diseases, detect exotic incursions, allow safe international trade
- **4.15.2** Overview — the five components of official health control
- **4.15.3** Official registration of the apiaries in the whole country or region
- **4.15.4** Organisation for permanent official sanitary surveillance of apiaries
- **4.15.5** Conditions for approval of breeding apiaries for export trade
- **4.15.6** Sanitation, disinfection or disinfestation of apicultural equipment
- **4.15.7** Preparation of the international veterinary certificate for export





Content of the following slides

Presentation of IZSLT CC laboratory activities

- As an example of organisation for permanent health surveillance and diagnostic activities

New matrices and improved methods for on-field detection of Small Hive Beetle

- To optimize collection of diagnostic samples and annual risk-based visits

New matrices and improved methods for on-field detection of *Tropilaelaps*

- Early detection of *Tropilaelaps* spp.
- Official health controls at import of queen bees
- Management of an outbreak of SHB in Latium Region



Art. 4.15.2 organisation for permanent health surveillance;
Art. 4.15.4 collection of diagnostic samples and despatch to a laboratory,
hygiene measures

IZSLT activities for beekeeping sector

- Diagnosis of honey bee diseases
- Laboratory analyses in collaboration with the other laboratories: bacteriology, virology, parasitology, morphometry, melissopalynology, chemistry
- Research activities
- Technical support and training of operators
- National and international collaboration and cooperation
- Support to Governmental Institutions and policy-makers





Art. 4.15.1 purpose of the official health control of bee diseases — the framework within which the WOAH Collaborating Centre and the FAO Reference Centre hosted by IZSLT operate

International recognitions

IZSLT hosts two international reference structures dedicated to the apiculture sector



WOAH — World Organisation for Animal Health

- Collaborating Centre «Good Beekeeping Management Practices and Biosecurity Measures in the Apiculture Sector»



FAO — Food and Agriculture Organization of the United Nations

- Reference Centre «Animal Health and Food Security Discipline — Apiculture, health and biosecurity»

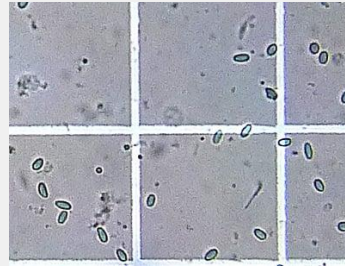
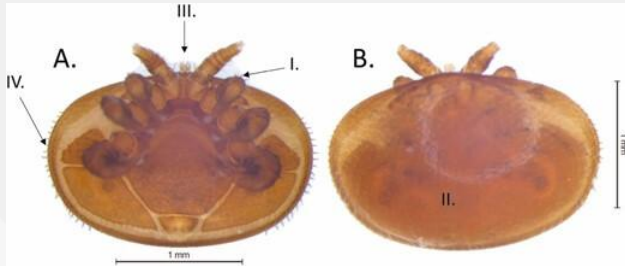




Analytical service

Analyses performed according to the WOAHP Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

- *Varroa destructor* infestation level assessment
- **Nosema* spp. microscopy
- American and European foulbrood cultural method
- American foulbrood PCR end-point analyses
- European foulbrood PCR end-point analyses
- *Aethina tumida* identification
- *Aethina tumida* PCR end-point analyses
- **Nosema apis* PCR end-point analyses
- **Nosema ceranae* PCR end-point analyses





Analytical service

Further laboratory analyses performed at IZSLT

- Visual inspection of affected samples
- Morphometrical identification of *Apis* species
- *Acarapis woodi* PCR end-point analyses
- *Lotmaria passim* PCR end-point analyses
- *Crithidia* spp. PCR end-point analyses
- Deformed Wing Virus PCR end-point analyses
- Israeli Acute Paralysis Virus PCR end-point analyses
- Kashmir Bee Virus PCR end-point analyses
- Chronic Bee Paralysis Virus PCR end-point analyses
- Acute Bee Paralysis Virus PCR end-point analyses
- Black Queen Cell Virus PCR end-point analyses
- Sac Brood Virus PCR end-point analyses
- Lake Sinai Virus PCR end-point analyses
- Slow Paralysis Virus PCR end-point analyses
- Honey and pollen analyses: melissopalynological, palynological and humidity





The European context

Honey bee diseases are classified according to different categories (Art. 9, Reg. UE 2016/429)

- **A Category:** no HB diseases (e.g. African Swine Fever, High Pathogenicity Avian Influence).
Diseases that do not normally occur in the Union and that, as soon as they are detected, require the immediate implementation of eradication measures.
- **B Category:** no HB diseases
Diseases that must be subject to control in all Member States with the aim of eradicating them throughout the Union.
- **C Category:** *Varroa*
Diseases that are relevant to certain Member States and for which measures are necessary to prevent their spread to parts of the Union that are officially free from those diseases or have eradication programmes in place.
- **D Category:** *Varroa spp.*, *Aethina tumida*, *Paenibacillus larvae*, *Tropilaelaps spp.*
Diseases for which measures are necessary to prevent their spread due to their introduction into the Union or movements between Member States.
- **E Category:** *Varroa spp.*, *Aethina tumida*, *Paenibacillus larvae*, *Tropilaelaps spp.*
Diseases for which surveillance is required within the Union.

Improved methods for on-field detection

The case of *Aethina tumida*



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Spread of SHB



Surveillance Plan based on Risk Analysis

A. Clinical inspection on stationary apiaries (low risk): random criterion

B. Clinical inspection on apiaries (high risk): risk criteria

- Migratory Beekeeping
- Receiving Biological Material
- Owning Multiple Stationary Apiaries In Different Regions
- Other Territorial Or Production Criteria
(E.G. Area Of High Interest For Pollination Or Honey Production)
- Honey Extraction Facilities





Sentinel honey bee colonies: from field detection to official confirmation

A standardised protocol to manage SHB-positive sentinel colonies — Calabria and Sicily, Italy

Set-up and field management

- One pair of sentinel colonies per surveillance site, covering an area of 2,660 m side, inside and at the borders of the protection zone
- 3 brood frames + 2 honey and pollen frames, queenright, established from SHB-free territories and moved under a 2 mm mesh net
- Managed directly by the Veterinary Services of the Local Health Unit: full inspection every ~20 days, queen cells removed, no supers, in-hive traps
- On detection, adults and larvae are killed in 70% ethanol and the colony is immediately closed and sealed



Advantages: faster and cheaper than full-colony inspection, freely accessible to the Veterinary Services at any time, biosafe killing and packing. **Limits:** lower attractiveness than full colonies and a need for specifically trained official veterinarians.



Art. 4.15.4 annual visits to an appropriate sample of apiaries based on the estimated risk;

Three years of SHB monitoring in Calabria: practical identification methods

Field experience 2014–2016 translated into operational recommendations for Member States at risk

Epidemiological picture

- 8,502 colonies destroyed as of 8 October 2016 under the eradication measures
- Prevalence in Calabria is very low: 3.59% (2014), 1.89% (2015), 2.80% (2016) 0.83% (2024) and season-related
- Need of adoption of innovative protocols and methods to detect SHB

Time-saving inspection protocol

- Divider placed between the last comb and the hive wall at least 48 h before the inspection
- Inspection started on the side opposite the divider, with slow movements; the last three combs and the divider are checked last
- Pollen and honey combs inspected thoroughly, brood combs only quickly scanned
- 7'59" vs 11'43" per hive: a 31.86% reduction of inspection time (Mann-Whitney, $p = 0.014$)

Sentinel apiaries: 2 to 5 strong, healthy and queenright colonies without supers, placed in sunny and windy locations. **Practical tips:** tight-fitting latex gloves for sampling, no shaking of bees off the combs, frames observed at arm's length.



The Mobile Divider

A method for concentrating SHB adults to improve detection

The device

- PVC sheet coated with aluminium on both sides: one opaque face to detect adults, one reflecting face to ease monitoring of the hive bottom
- 440 × 350 × 2 mm for a Dadant Blatt hive, easily adapted to any hive type; final price about EUR 5.00
- Bottom edge kept 2 cm above the hive floor so that bees can remove SHB eggs and larvae
- No increase of the in-hive temperature and no chemical compounds, hence no residues in bee products
- Mean number of SHB detected: 2.95 with the MD vs 2.05 with the official method
- Mean inspection time: 5'37" with the MD vs 6'02", i.e. 25 seconds faster



The MD detects the parasite even when only a few adults are present and infestation is very low. **Limit:** the device has no locking system, so capture depends on the operator's skill.



New matrixes to diagnose *Aethina tumida* presence at apiary level

Hive debris and swabs analysed by real-time PCR as an alternative to visual inspection of single hives

Sampling and analysis

- Swabs from the hive bottom (895.5 cm² sampled, moistened in 3 mL of PBS) and from the frame top bars (1 mL of PBS)
- 5 g of hive debris collected from screened bottom boards, with the inspection panel inserted one week before sampling
- Real-time PCR on a 109 bp fragment of the COI gene (TaqMan), confirmed by Sanger sequencing
- Three apiaries in Calabria with very low, medium and very high infestation: 0.04, 6.13 and 13.57 mean adult SHB ($p < 0.0001$)

Results

- Apiary 1 — very low (1.9% of hives positive at inspection): 5.8% of debris samples PCR positive, no positive swab
- Apiary 2 — medium (75.0%): 50% of frame swabs and 37.5% of hive bottom swabs positive
- Apiary 3 — very high (100%): 85.7% of hive bottom swabs positive
- Sequences with 99% identity to clade B, cluster B1 (AfricCam3); PCR specificity 100% and sensitivity > 99%

Fast, easy to apply and feasible when hive inspection is not possible (bad weather, aggressiveness, robbing): a pre-clinical indicator that could be integrated into the official diagnostic procedures and contingency plans.



Art. 4.15.1 detection of incursions of exotic diseases, safe international trade;
Art. 4.15.4 permanent official sanitary surveillance, additional visits for trade or transfer to other regions

Contingency plan for *Aethina tumida*

A proactive strategy to prevent the spread of the Small Hive Beetle (SHB) in EU - so far endemic in southern Italy only

Risk assessment

- Entry via honey bee trade, contaminated beekeeping material (wax) and other traded goods, migratory beekeeping and illegal movements
- *SHB endemic in Southern EU*
- Hives/nuclei trade increase the risk of establishment (forbidden)
- Only import of queen bees with 10 worker bees each are allowed

Diagnosis and identification

- Morphological identification and on-field detection methods
- Real-time and conventional PCR protocols (even from debris and earth on the bottom of the hives)

Prevention and preparedness

- Training and awareness for beekeepers, veterinarians and competent authorities; hygiene and quarantine measures; continuous monitoring
- Defined responsibilities, resource allocation, plan updates, communication and reporting, legal and regulatory compliance

Surveillance and response

- EU surveillance plans and enhanced border controls at ports and international airports
- Eradication only as a first attempt in case of new invasion; when endemic: IPM combining traps with insecticides (no VMP so far authorized in EU), good apicultural practices, and monitoring of infestation levels.

Three interconnected pillars — prevention, training and control — supported by a coordinated and flexible response network with clearly defined roles in each Member State.

Innovations in laboratory analysis

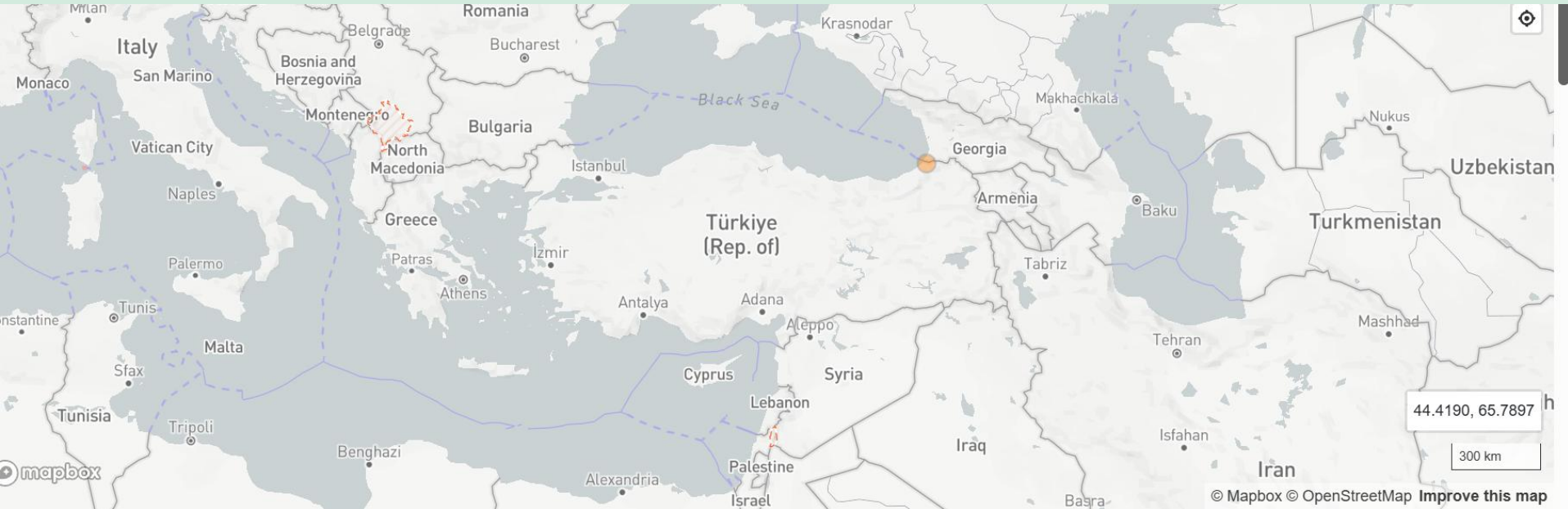
The case of *Tropilaelaps spp.*

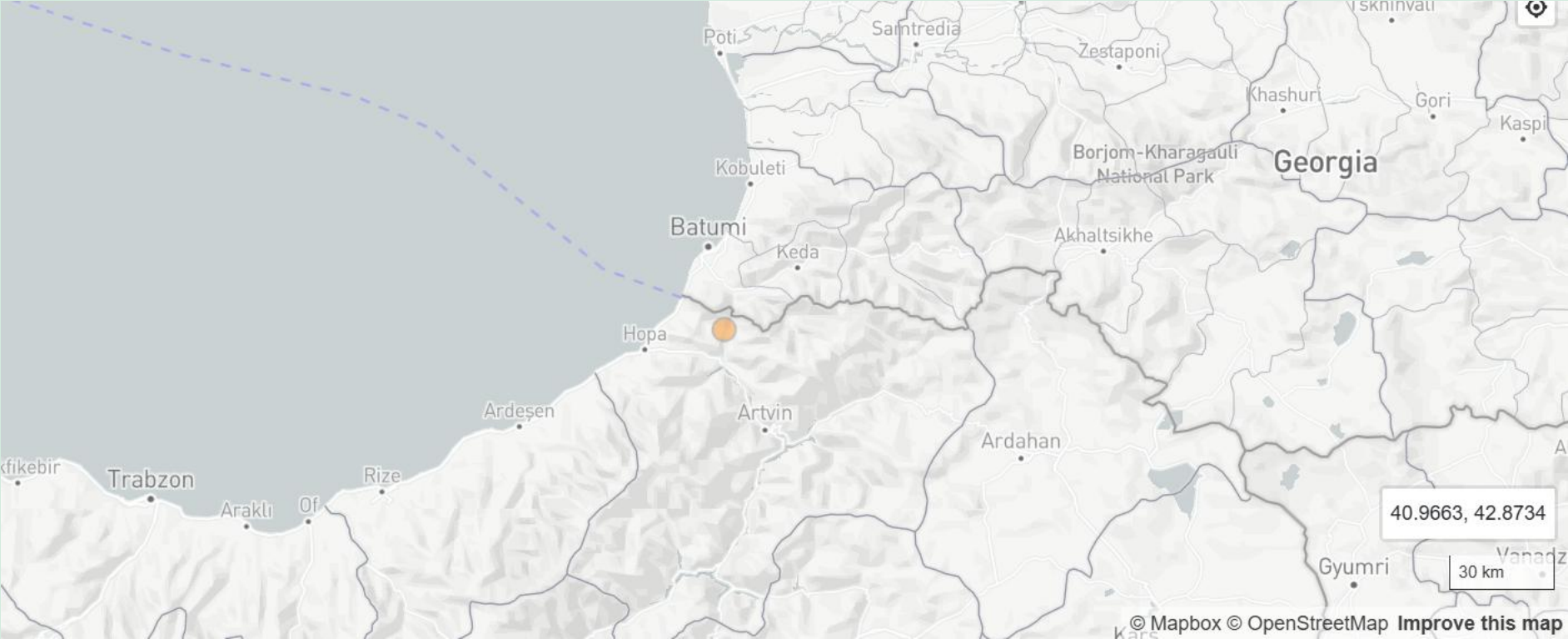


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WOAH Report

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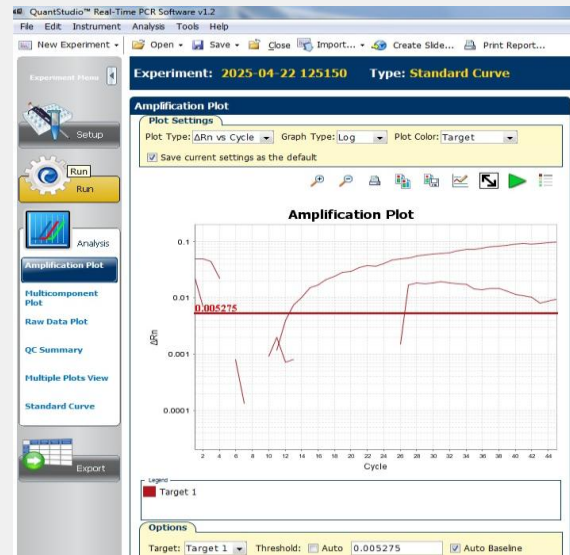
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PCR protocols for *Tropilaelaps* spp. detection in hive debris

BETTER-B Deliverable 6.2 — Task 6.2 Risk assessment and contingency plan for invasive species (Lead IZSLT)

Method details

- Five different Real Time PCR protocols designed «in silico» for the 4 species of *Tropilaelaps* spp.:
- 2 Real Time PCR protocols for *T. mercedesae* (the first with TaqMan probe and the second with MGB probe)
- 1 Real Time PCR protocol for *T. clarae*
- 1 Real Time PCR protocol for *T. koenigerum*
- 1 Real Time PCR protocol for *T. thaii*
- The coding region for cytochrome oxidase (COI) was considered as the target



Fast, easy to apply and feasible when hive inspection is not possible (bad weather, aggressiveness, robbing): a pre-clinical indicator that could be integrated into the official diagnostic procedures and contingency plans.



Art. 4.15.1 detection of incursions of exotic diseases, safe international trade;
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Contingency plan for *Tropilaelaps* spp.

A proactive One Health strategy against a mite the European Union is still free from

Risk assessment

- Entry via honey bee imports, contaminated beekeeping material and other traded goods, migratory beekeeping and illegal movements
- *T. mercedesae* confirmed in Georgia; queen trade towards Türkiye and a climate-driven longer brood-rearing season increase the risk of establishment

Diagnosis and identification

- Morphological identification and on-field detection methods
- Real-time and conventional PCR protocols (Khongphinitbunjong et al. 2015; Anderson & Morgan 2007; Ribani et al. 2020) with sequencing confirmation

Prevention and preparedness

- Training and awareness for beekeepers, veterinarians and competent authorities; hygiene and quarantine measures; continuous monitoring
- Defined responsibilities, resource allocation, plan updates, communication and reporting, legal and regulatory compliance

Surveillance and response

- EU surveillance plans and enhanced border controls at ports and international airports
- Eradication where feasible; when endemic, IPM combining biological control, hard and soft acaricides, physical control, apicultural practices, selection of resistant colonies and post-treatment monitoring

Three interconnected pillars — prevention, training and control — supported by a coordinated and flexible response network with clearly defined roles in each Member State.

Official controls at importation

Honey bee queens



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Art. 4.15.4 collection of diagnostic samples and despatch to a laboratory,
hygiene measures

Official controls on queens imports from Third Countries

IZSLT is performing official controls at queens imports

Diagnosis and identification of exotic pests

- Detailed protocol for detection of *Aethina tumida* and *Tropilaelaps* spp.



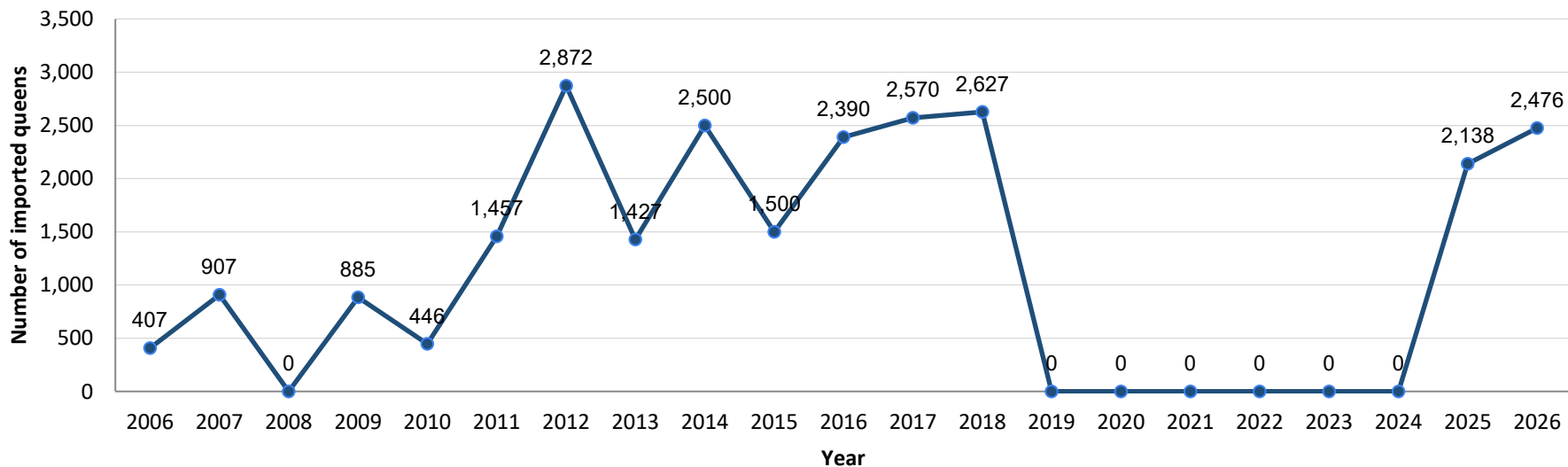


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Official controls on queens imports from Third Countries

IZSLT is performing official controls at queens imports

Official imports of honey bee queens in Central Italy from 2006 to 2026



Managing a SHB outbreak

The Latium region case



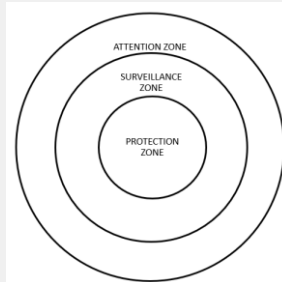
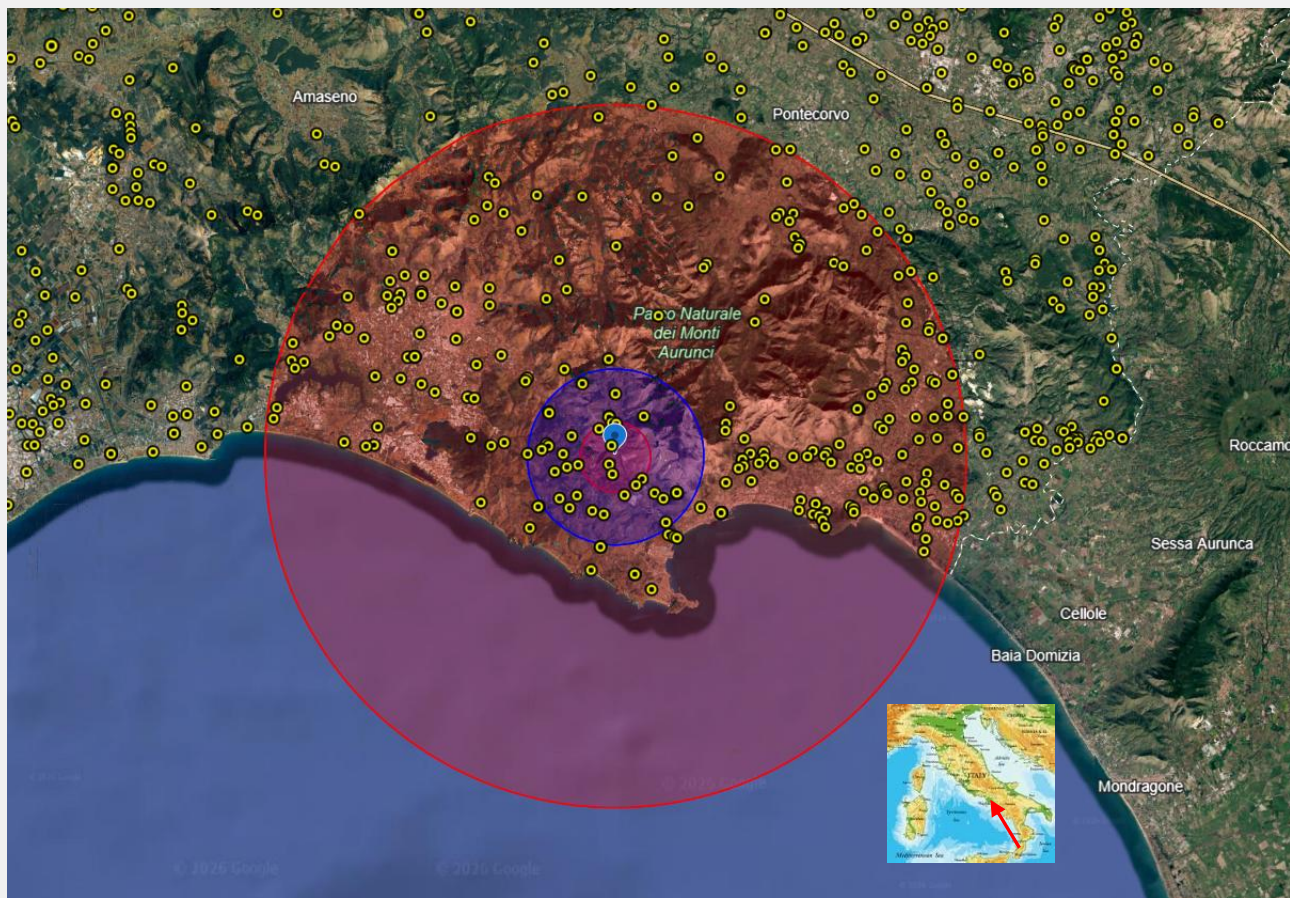
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Art. 4.15.3 official registration of the apiaries in the whole country or region;
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hygiene measures

**First notification
of SHB from 2014
in Central Italy:**

May 2026





Art. 4.15.3 official registration of the apiaries in the whole country or region;
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Managing the outbreak in the Latium region

From an illegal movement of colonies to a full official response — province of Latina, 2026

Detection and confirmation

- 31 May: illegal movement of colonies from Calabria (approx. 700km)
- 2 June: suspicion reported to the Veterinary Services of ASL Latina
- 3–4 June: official sampling and diagnosis at IZSLT
- 5 June: joint inspection and confirmation by the National Reference Centre (IZSve)

Official response

- 9 June: ministerial order — 15 June: regional order with detailed measures
- 16 June: training of 23 official veterinarians — 14 July: training of about 80 beekeepers

Zoning and official controls

- Protection Area: 5 Km ray, zone with 301 registered apiaries. All apiaries and hives are controlled.
- Surveillance Area: 10 km plus. All apiaries are controlled: 5% colonies + traps are placed in all the hives
- Attention Zone: 20 Km ray dispatched sentinel colonies

Three days from the first report of suspicion to official confirmation and seven to the ministerial order: apiary registration and permanent official surveillance are what make a response of this speed possible.

Thank You

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