Guidelines for the surveillance of the small hive beetle (*Aethina tumida*) infestation

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1 Background to outbreaks

The Small Hive Beetle (SHB), *Aethina tumida* Murray 1867 (Coleoptera: Nitidulidae) was detected for the first time in Reggio Calabria, South West Italy on 5th September 2014. Three honey bee colonies were confirmed heavily infested with SHB adults and larvae. Upon discovery, the three nuclei were destroyed and the bees and the boxes immediately deep frozen. A sample of approximately 15 adults and 15 larvae was taken to formally confirm identification at the University of Reggio Calabria. The species *Aethina tumida* was identified on morphological characteristics. On 10th September 2014, additional specimens were sent to the Italian National Reference Laboratory (NRL) for Honey bee health (Istituto Zooprofilattico Sperimentale delle Venezie). The species *Aethina tumida* was also confirmed through morphological identification. In addition SHB adults and larvae were sent to the ANSES European Reference Laboratory in Sophia-Antipolis (France) where the species was also confirmed through morphological identification and through molecular diagnostics on 17th September 2014. On 18th September, confirmation of *Aethina tumida* detection in Italy was notified to the OIE (World Organisation for Animal health).

To date (early March 2015), more than a thousand one hundred apiaries have been inspected in Calabria and approximately 250 apiaries in Sicily. *Aethina tumida* has been confirmed in 61 apiaries located within a 20 km radius area in two provinces of Calabria region (Reggio Calabria and Vibo Valentia) with one exception in Sicily (one apiary in the province of Siracusa, directly linked to a migratory movement back from Reggio di Calabria). Approximately 3,500 honeybee colonies have been destroyed upon discovery of *Aethina tumida* in the apiaries.

SHB originates from Africa where it is not a threat for the local subspecies of honeybees. However, SHB was accidentally introduced in the USA and in Australia over a decade ago as well as into other countries. From these experiences, it has been shown that *Aethina tumida* can be detrimental to western honeybee colonies under certain circumstances. In Portugal *Aethina tumida* had been detected in 2004 during a control on imported queen bees from Texas. All the imported materials were destroyed and stamping out was applied to the apiaries where the imported queens had been introduced. Following the implementation of these measures, no further detection of *Aethina tumida* occurred in Portugal and Europe.

To date the populations of the small hive beetle in Calabria are considered low. The spread of the beetle has been largely documented in other countries (USA particularly). However, to date in Italy, it is not entirely clear if all the tracing of apiary/colony movements have been fully covered, particularly out of the surveillance area. The Italian veterinary services together with the Italian beekeepers have worked and still work very hard to contain *Aethina tumida* in Calabria by killing thousands of colonies to prevent its definite establishment and/or further dissemination in Europe. The contingency management field work in Italy is designed to reduce the risks of this new pest becoming established more widely in Europe and to maintain the small hive beetle populations at a very low level where they might be established. These guidelines aim to provide advice to Member States on apiary surveillance and early detection of the SHB to reduce the risks of further establishment of the beetle across other countries in Europe.

This document provides harmonized guidelines and advice for Member States to detect, monitor and survey for *Aethina tumida* across Europe. The guidelines have been produced to support Member States with implementing a risk based framework. This document outlines what should be recommended as optimum best practice for surveillance, that Member States should endeavour to follow, whilst recognising certain constraints on this such as finance, trained personnel, resources, database and so forth.

It should also be noted that EFSA (European Food Safety Agency) launched a new mandate on a 'Scientific and technical assistance and a scientific opinion concerning the risk of survival, establishment and spread of the small hive beetle (*Aethina tumida*) in the EU' (www.efsa.eu).

2 Biological and epidemiological characteristics

The characteristics of *Aethina tumida* infestation that influence surveillance measures are as follows[1]:

- Biological cycle of the beetle and signs of infestation:
    - The earliest sign is the occurrence of adult small hive beetles on the frames of the hive.
    - A female lays between one thousand and two thousand eggs, in clusters, inside the hive, in wood crevices or directly in the bees' brood cells, but these small eggs (approximately 1.5 x 0.25 mm) are difficult to detect upon visual inspection.
    - The eggs hatch into larvae. Depending on the conditions, particularly the temperature, the larval stage lasts eight to 29 days. The larvae are omnivorous and bore into the combs looking for food.
    - The hive beetle and its larvae eat brood, honey and beebread. In some cases, infestation can destroy the wax combs. Feeding and tunnelling activities of the larvae cause the honey to flow out, creating the fermenting sticky mess and fermentation. Heavy infestations can cause the colony to die or the bees to abscond. All of these are all late signs of late stage infestation.
    - When they reach maturity (known as the wandering phase), the larvae leave the hive and crawl away from the hive, before they dig into the soil, generally at a depth of 1 to 30 cm and close to the colonies (but up to 20 m from the colony), to begin pupation. It is then difficult to detect them but still possible by digging into the soil around the colonies. Soft, moist soil and a temperature of at least 10°C are necessary for the larvae to complete their life cycle. However, the larvae can survive in soil for several weeks (3-4 weeks) at temperatures below 10°C. Adult beetles emerge from the soil after two to 12 weeks, depending on the temperature and the nature of the soil.
    - The adults are sexually mature one to seven days after their emergence. *Aethina tumida* can have several generations per year (one to six) depending on the environmental conditions (primarily the climate and soil composition).

[1] A descriptive leaflet can be found on free access on the EU Reference Laboratory website https://sites.anses.fr/en/minisite/abeilles/eurl-honeybee-health. Upon request, the leaflet is available on different European languages (eurl.bee@anses.fr).

- In total, development from egg to mature female requires between 22 days and two and a half months. The detection of larvae in bee colonies means that the biological (reproductive) cycle has begun. If there are no prior observation data for the same colonies, it is not possible to know how many life cycles have been completed. It should therefore be considered that the soil might contain *Aethina tumida* pupae, meaning that it needs to be treated and the colonies need to be destroyed.

- Spread and potential persistence:
  - The adults are particularly attracted to hive-produced volatiles (bees, pollen, honey).
  - The adults can fly several kilometres to infest new host colonies (2014 OIE Health Code). Their flying activity is most intense two days after their emergence. After this period, their behaviour changes and their flying activity decreases.
  - They can survive nine to 14 days (depending on the source) without water or food, up to 50 days on used empty brood combs and several months on ripe fruits and rotten fruits.
  - Spread of the small hive beetle is enhanced by movements of bees, colonies, swarms, beeswax and beekeeping equipment.
  - The beetle can also spread through the introduction of soil (e.g. through the sale of potted plants), fruits or occasional hosts (e.g. bumblebees, *Bombus* spp.).
  - The occurrence of feral colonies in the environment is a risk of infestation persisting in a contaminated territory that is difficult to control.
  - The infestation of bumblebee colonies (*Bombus* spp.) is possible and has been detected in colonies reared near infested apiaries (Spiewok et al. 2006). *Aethina tumida* is capable of completing a full biological cycle in experimental conditions in *B. impatiens*, but this has never been observed in nature.

- As documented in the USA (Hood 2004), the spread of infestation within a territory is primarily determined by the following factors:
  - the climate and season (the biological cycle of the small hive beetle depends on temperature and humidity conditions - although *Aethina tumida* is able to withstand colder temperatures, the highest impact is usually facilitated by higher temperatures and humidity),
  - the nature of the soil; relatively moist soft, sandy soil is conducive to small hive beetle pupation. Moisture is a limiting factor and there is less impact on colonies if not kept in the shade.
  - the density of colonies in the area (there is greater spread in areas with a high density of apiaries),
  - the structure and organisation of the beekeeping sector (areas and routes of migratory beekeeping, importing apiaries, production of package bees and/or nucleus colonies, trade in beekeeping equipment, management within honey extraction facilities and apiary maintenance buildings, storage of honey, honey houses...).

3 Surveillance objectives

The SHB is a statutory notifiable pest in the European Union (Council Directive 92/65/EEC). There is a legal requirement for any SHB confirmation. There is therefore a legal requirement on beekeepers to notify any suspect findings. Following introduction in Italy, protective measures have been implemented (Commission Implementing Decision 2014/909/EU of 12 December 2014). The dispatch of honey bees, bumblebees, unprocessed apiculture by-products, beekeeping equipment and comb honey intended for human consumption is banned from the infested regions to other areas of the Union. EU legislation prohibits (with the exception of New Zealand) imports of package bees or colonies from Third Countries. It is permitted to import honey bee queens from a very limited number of countries outside the EU (Council Directive 92/65/EEC of 13 July 1992 and Commission Regulation (EC) No 206/2010 of 12 March 2010). The import regulations and protective measures are the main defence against the introduction and the spread of the SHB in Europe. It is therefore crucial that every Competent Authority and indeed beekeeper respect the EU legislation and ensures regular surveillance.

In a country where SHB is considered absent (still an exotic threat), the objectives of the surveillance programme may be to:

- detect any *Aethina tumida* infestation at an early stage in order to eradicate it;
- demonstrate freedom from *Aethina tumida* infestation to maintain the country's infestation-free status. This objective should be specified in relation to the European or national regulations and international standards (OIE), particularly regarding official criteria for recognition of this status.

For an infested country, the objectives are compartmentalization and zoning to:

- demonstrate the absence of *Aethina tumida* infestation for maintaining infestation-free status in certain zones/compartments
- detect any *Aethina tumida* infestation at an early stage in order to eradicate it from infested zones

4 Surveillance methods

Early detection can be ensured by combining extended outbreak surveillance (covering the entire national territory or surveillance zone) with passive surveillance (outbreaks) and active surveillance (programmes) targeting at-risk zones.

For the entire surveillance programme, the epidemiological unit considered is the apiary[2], which can contain one or more colonies. A beekeeper can own more than one apiary. To identify the numbers of colonies to be inspected within each apiary according to the size of the apiary, the expected prevalence and diagnostic sensitivity, please refer to the sample size calculator in Annex 1.

[2] An apiary is a place where one or more bee colonies are kept. A colony (consisting of workers, drones and a queen) lives in a hive. In general, colonies in the same apiary are jointly managed (transhumance, zootechnical and health conditions, etc.).

4.1 Outbreak surveillance

Enhanced passive outbreak surveillance is based on the reporting of suspected cases by beekeepers (or any other stakeholders in the beekeeping sector) to competent veterinary authorities (Directives 82/894/EEC and 92/65/EEC). This surveillance covers all of the apiaries throughout the national territory and must therefore be promoted by the competent veterinary authority in the whole sector using all existing (in-) formal communication channels. Reporting criteria are based on the definition of a suspected case (Refer to paragraph 4.5 Definition of a case). Methods for reporting and investigating suspect outbreaks are set out in the section on Organisation of surveillance (see below).

This outbreak surveillance can be strengthened in at-risk zones meeting the criteria given below. Please see the Flow Chart at Annex 2, which illustrates the type of surveillance (Passive and Active), and the recommended sampling levels.

4.2 Active surveillance

Active surveillance involves the sampling of apiaries in which investigations are being undertaken (Annex 1). This is suitable for infested as well as SHB free countries. This sampling can be designed in various ways, depending on the objective targeted in the Member State in terms of precision and accuracy[3]; and the means dedicated to the surveillance (particularly trained personnel, resources). The proposals given below are ranked from the most robust methodology through to lighter approaches.

- Targeted sampling: selection of at-risk apiaries with a particular risk of being infested (based on beekeeping practices) for the early detection of infestation;
- Representative sampling of all/registered apiaries located in a zone considered at-risk for the early detection of infestation;
- Representative sampling of all/registered apiaries in part or all of the national territory for recognition of the infestation-free zone/country status, if this is the regulatory objective.

4.2.1 Targeted sampling (selection) of at-risk apiaries

The individual criteria for the risk of apiary infestation are as follows:

- Apiaries that have been moved the last 12 months to/from a protection or surveillance zone, or a zone classified as such within 12 months of their migration;
- Apiaries that imported, in the last 12 months, queens / swarms / package bees from a zone recognised as infested or classified as such within 12 months of importation;

These apiaries must be fully recorded and inspected as soon as possible after their identification as described below. Further inspection can be planned according to estimated level of risk and to the

[3] The precision evaluates the dispersal of the measures; the accuracy refers to the systematic errors.

specific situation of the area they moved from, according to events that might shed a different light on risk factors. An event is any new epidemiological information such as outbreaks retrospectively reported in new area, tracing of movements, or field information.

4.2.2 Representative sampling in at-risk zones

The criteria to classify an at-risk zone are as follows:

- A 15-km-radius zone around an international seaport or international airport where at-risk products are imported: bee products (bees, queens, brood, hive products), beekeeping products (beekeeping equipment), other products containing bees in the broad sense (bumblebee colonies or queens, e.g. *Bombus* sp.), ripe fruits or vegetables (e.g. apples and bananas), potted plants. In addition it should be noted that, in many cases, a great deal of freight does not get opened or dealt with at ports but is moved inland into freight depots.

  In the case of a large number of seaports, airport or freight depots, each Member State should be able to evaluate and prioritise the risk points and types e.g. such as tonnage imported or type of goods imported. Member States can prioritise within each category which of the airports or ports or freight depots are highest priority.

  Each Member State will evaluate other risk hotspots as required, for example if imports occur through different pathways than seaports. Illegal imports should also be included as much as possible. By their very nature i.e. illegal they are unlikely to be discovered. Member State might not even know that any had occurred to be able to target. However Member States should always be on the lookout.

- Zones into which susceptible goods are moved e.g., by road or rail from identified surveillance or at-risk areas and protection zones.

Each at-risk zone in the territory must be identified and all the apiaries located in this zone georeferenced.

Three types of actions can be taken in the apiaries in these zones in the following order of priority, considering that, depending on the situation, only one or two actions can be implemented:

- Strengthening passive surveillance: a specific communication strategy is used to inform beekeepers, bee health workers and other beekeeping workers of the risk of *Aethina tumida* infestation and provide them with the knowledge[4] required to detect suspicions of *Aethina tumida* infestation;

[4] [4] A descriptive leaflet can be found on free access on the EU Reference Laboratory website https://sites.anses.fr/en/minisite/abeilles/eurl-honeybee-health. Upon request, the leaflet is available on different European languages (eurl.bee@anses.fr).

In some European Member States, apiaries are to be inspected when hives are moved and/or traded. Therefore in these cases, this information (detection of *Aethina tumida*, number of hives, health status of hives) are already available on a large number of hives and apiaries.

- Quarterly inspections of three large sentinel apiaries (minimum 10 colonies) placed near the maximum risk source (maximum 15 km from seaport, airport, road, rail network). This data are given as an indicative basis (number of apiaries and the number of colonies per sentinel apiary) and should be refined according to further information, eg, modelling tools and epidemiological knowledge.
- Annual inspection (preferably late spring depending on the climate of each Member State) of a random sample of registered apiaries to be able to detect infestation affecting at least 5% of the apiaries with a 95% confidence level of detection. Irrespective of the population size of the apiaries in the defined at-risk zone, the maximum sample size requested is 59 apiaries.

4.2.3 Representative sampling throughout the national territory (according to the regulations)

The following recommendations are given on an indicative basis and have to be refined according to upcoming national or European regulations regarding the recognition of infestation free zones or country.

The national territory can be divided into agro-ecological or geographical zones with a homogeneous risk of infestation (example: beekeeping carried out in mountainous areas will be different from dry prairies or oceanic zones). Each Member state will have full knowledge of the structure of their honeybee sector for hierarchical purposes. Each of these zones should be sampled to be able to detect infestation at minimum prevalence level of 2% of the apiaries with a 95% confidence level. Irrespective of the population size of the apiaries in each agro-ecological zone, the maximum sample size requested is 149 apiaries. The relevance of these sampling criteria should be determined in relation to the national or international regulations establishing prevalence limits for detection. This stratification is to ensure that, in case of large Member states, the entire country is not considered as only one population exposed to the same risk.

However, if further work demonstrates that the sensitivity of the detection process is not 100%, the maximum number for a design prevalence of 2% would be greater than 149 (for example it would be 186 for a technique with a 80% sensitivity).

4.2.4 Practical aspects of apiary inspection

In each selected apiary in the surveillance sample, a certain number of colonies need to be inspected in order to be able to detect infestation affecting at least 5% of the colonies with a 95% confidence level (this means that, irrespective of the size of the apiary, no more than 59 colonies per apiary should be inspected if the sensitivity of the investigation technique is considered 100% effective). For small apiaries (n<20), all colonies must be (see Annex 1).

Data given in this document regarding design prevalence and sample size are based on the mathematical assumption that the investigation technique has 100% sensitivity. It has to be acknowledged that investigation/diagnostic techniques at colony level do not reach 100% and most likely ranges between 90 and 95%. However, at apiary level (the epidemiological unit), considering that in case of an infestation, more than one colony is infested, it can be assumed that the sensitivity of the investigation technique to allintents and purposes 100%.

A colony is inspected as follows:

- Visual observation of frames: small hive beetle detection by observation of frames should take into account the lucifugous[5] nature of the adults. Examination on sunny days (or with any light exposure) is recommended, since the adult beetles will scurry quickly away from the light. The frames should be removed from the hive one by one. Each side of the frame should be quickly observed. The beetles tend to move rapidly along the frame to find a refuge from light and can easily be spotted by an attentive observer. The first frame can be left outside the body or super of the hive to make it easier to handle the other frames. The following frames should be put back into the body or super to prevent robbing in the apiary during the examination.
  Beetles can hide inside the cells of combs. It is also important to examine the lid, the bottom board, the side faces, corners and interstices of the hive. Also debris on the hive floor boards (mesh floors) should be examined using molecular methods (Ward et al. 2007).
- Detection of suspect specimens: If adult insects or larvae are detected during the visual inspection, their characteristics should be compared to the definition of a suspected case (see below) to be able to rule out obvious negative cases (eg wax moth larvae such as *Galleria mellonella* or *Achroia grisella*). If adult insects or larvae correspond to the definition of a case, a sample should be taken and sent to a reference laboratory for identification;
- Collection of suspect specimens: to capture adult beetles, it is preferable to use a mouth aspirator (Annexe 3)[6]. Adult beetles can also be captured between the thumb and index finger. Once they have been captured, it is advisable to promptly kill the individuals in a container such as a sampling tube filled with alcohol (avoid the use of denatured alcohol) to keep them from flying away when the container is opened;

Setting traps: Traps can be placed in colonies and used in combination with the visual observation method to increase the likelihood of detection, or as an alternative to visual observation when climate conditions do not allow colony inspection. However, in this condition traps are much less effective Thus, depending on the zone or surveillance method, it may be decided to use visual observation or traps, but whenever possible the combination is best. In apiaries where inspections are frequently undertaken (sentinel apiaries), surveillance may be more acceptable if traps are particularly used. For single inspections (annual sampling), it may be best to perform visual

[5] Shunning the light.

[6] Mouth aspirators are very simple instruments commonly used in entomology to capture insects. They have a vial fitted with two flexible tubes, one of which is directed to the adult beetle and the other of which is placed in the examiner's mouth. Thin gauze should be placed at the entrance of the tube drawing air (towards the mouth) into the vial so that the beetles are not sucked in by the user.

inspections because of the high detection sensitivity and to avoid a return visit to check the trap. However, attention needs to be paid to the conditions in which traps are used (see below).

The floor traps to be used for the detection of adult small hive beetles are pieces of corrugated plastic with square cells big enough for the beetle to get in, but too small for the bees to enter (approx. 4 x 4 mm). The beetles tend to hide in the flutes of the trap which also suit its thigmotactic[7] behaviour. The traps should preferably be made of a transparent material so that beetles can quickly be detected (Schäfer et al. 2008).

The traps are placed inside the hive through the entrance. The hive does not need to be opened for the operation, which can be done quickly. Only one trap is set per colony. It is important to properly place the trap in contact with the floor of the hive. If not, beetles can seek refuge in the space located between the trap and the floor. For optimum use, traps should be left in hives for a minimum of 48 hours before they are checked. To check the traps, remove them from the hives and then tap them along the edge into a large enough recipient or, if available, a bucket containing soapy water (to prevent beetles from flying away) to dislodge the adults nestled in the flutes. It is also possible to tap them out inside a large plastic bag. The flutes can be visually inspected to see if they are 'blocked' by an individual or not. Using a recipient makes it easier to capture dislodged beetles. It is preferable to use a light-coloured recipient to make it easier to see the collected beetles (which are dark brown).

In cold weather, bees form clusters to maintain warm colony temperatures and preserve heat. Any beetles inside the hive will then tend to seek refuge inside the cluster to benefit from the temperature. They are then less likely to colonise the traps.

There are in addition other types of trap that can be used, eg, sold under such names as “Better Beetle Blaster”, “Beetle Jail” or “AJ’s beetle eater”. These are small containers filled with some oil (cooking oil is suitable, diesel or engine oil should be avoided) and covered by a grid. The trap is placed between the top-bars of two frames. In their attempt to get away from the bees that chase them, *Aethina tumida* adults will enter the trap and drown in the oil. When visiting the colony, traps are withdrawn from in between the frames and examined for the detection of any beetles. If the container is transparent, this observation is easy and straightforward. These traps have been shown to be quiet efficient in North America. They can be used anytime of the year, and particularly during the cold season they should be placed as close to the bees as possible.

Molecular detection of SHB by PCR on hive debris could be an additional diagnostic tool for surveillance (Ward et al 2007, Cepero et al. 2014). The Ward method was developed by spiking debris and has been used in contingency exercises in the UK using dead SHB (various pieces of beetle and life stages). However, this method should also be validated blind in the field, in natural conditions to validate the sensitivity of the method (determination of the detection limit) and to standardise the sampling framework for the purpose of detection. Further experimental and field work is needed to implement these validation steps.

[7] The motion or orientation of an organism in response to a touch stimulus

4.3 Surveillance period

Surveillance by visual observation of beetles inside of hives depends on the temperature conditions. When temperatures are low, the examination of hives can indeed jeopardise the survival of the colony, which will be 'clustered'.

Surveillance using floor trapping method can be undertaken all year long without endangering bee colonies. However, it is necessary to take into account the decreased sensitivity of traps made of corrugated plastic when bee colonies are clustered (see above).

Lastly, it is important to adapt the surveillance period and methods to the expected spread of *Aethina tumida*. The biological cycle of this parasite depends on the temperature and humidity conditions; moreover, movements of live bees and beekeeping equipment are factors in the spread of the beetle (see Section 1). Surveillance should be strengthened from spring to autumn, during the active beekeeping season. It will however be necessarily less intensive in the winter, particularly in colder climates.

4.4 Definition of a case

4.4.1 Suspected case

A suspected case is defined by at least one of the following situations detected upon observation by the beekeeper or someone inspecting the apiary:

- Occurrence in the hive (or beekeeping equipment) of one or more beetles similar *to Aethina tumida*,
- Occurrence in the hive or in the hive's immediate environment (larvae climbing out of the hive to pupate in the soil / 'wandering larvae') of one or more whitish beetle-like larvae similar to *Aethina tumida* (different from the larvae of wax moths),
- Occurrence of at least one beetle in a trap placed inside the hive.

4.4.2 Confirmed case

Confirmation of an initial outbreak located in a zone considered non-infested: (i.e. in the framework of the surveillance plan)

A case of *Aethina tumida* infestation is confirmed based on at least one of the following criteria:

- Identification of an adult small hive beetle (*Aethina tumida*) by the NRL based on morphological criteria, confirmed if needed by molecular identification (e.g. damaged specimen)[8],
- Identification of a small hive beetle (*Aethina tumida*) larva by the NRL based on morphological criteria, systematically confirmed by molecular identification.

[8] The EU RL for honeybee health currently validates a molecular technique to identify *A. tumida* adults and larvae. As soon as the full procedure is ready, it will be made public and freely available to use.

Confirmation of the following cases occurring in protection or surveillance zones established around the confirmed initial outbreak:

A case of *Aethina tumida* infestation is confirmed based on at least one of the following criteria:

- Identification of an adult small hive beetle (*Aethina tumida*) by a the NRL based on morphological criteria, confirmed if needed by molecular identification (e.g. in the case of damaged specimens),
- Identification of a small hive beetle (*Aethina tumida*) larva by the NRL based on morphological criteria.

Note: once an outbreak has been officially recognised, larvae identification can be confirmed based only on morphological criteria in this outbreak, systematic molecular analysis being no longer necessary.

5 Sampling

It is important to sample as many specimens as possible (adults and larvae). Morphological identification is even more reliable if done on undamaged specimens (specimens whose morphological integrity has been preserved, have not been crushed and are in a good state of preservation). This is why the use of a mouth aspirator is recommended for sampling adult beetles and the use of flexible entomological tweezers is recommended for sampling larvae.

All specimens must be killed before being transported. The use of 70% undenatured ethanol is recommended. Place the specimen(s) in a tube containing ethanol and tightly close it. The duly labelled container can thus be sent through the regular or specific post services, in a timely fashion to the laboratory at ambient temperature with the related background data (number of samples, etc.).

It is also recommended to take pictures of suspicious signs observed in colonies, and collect specimens in appropriate sample containers and promptly send them to the NRL so that the alert level may be assessed. Pictures can be emailed directly to the Member State NRL.

The laboratory should be notified by telephone and email of the shipping of samples, so it can be ready to receive and quickly analyse them.

6 Organisation of surveillance

The following chapter is mentioned as an aide memoire for the Member States to specifically address these topics in order to correctly organise their country surveillance systems.

6.1 Central level

Determination of management, scientific and technical support and coordination of surveillance. To be adapted by each country.

6.2 Intermediate level

Determination of the local coordination of stakeholders and the first level of data validation and surveillance organisation. To be adapted by each country.

6.3 Field level

Determination of the people responsible for implementing surveillance (making observations and implementing stages for the verification of suspicions). To be adapted by each country.

7 Data management and use

The following chapter is mentioned as an aide memoire for the Member States to specifically address these topics in order to correctly organise their surveillance systems. .

7.1 Data collection

Apiary inspection form for observations (programmed and in the event of suspicion).

7.2 Transmission and centralisation

Methods for transmitting and entering data from the sheet and laboratory data.

7.3 Validation and processing

Determination of health indicators and their expression.

8 Communication

Decision to invoke and put in action a contingency plan to activate outbreak surveillance and dissemination of surveillance results in particular.

9 Training

In order to organise stakeholder training tailored to the country's situation, the following should be done:

- Prepare a list of participating stakeholders.
- Prepare a list of activities expected from each stakeholder with expected skills and know-how.
- Determine training needs in relation to these guidelines.
- Determine training organisation procedures.
- Make arrangements to check pre-training and post-training skills and know-how and ensure that stakeholders can apply them in practice.

- Make arrangements to reach indirectly as many more stakeholders as possible, via trained stakeholders (training of trainers).

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11 Annex 1

Sample size calculator indicating the number of colonies to be visited in an apiary according to the size of the apiary and to the prevalence targeted.

Total number of units (apiaries) in the zone	50	100	200	300	400	500	600	700	800	900	1000	1500	2000	3000	3500	4000	5000	9000	>35000
To be inspected	48	78	105	117	124	129	132	134	136	137	138	142	143	145	146	146	147	148	149

Number of units to be inspected in order to detect a **prevalence of 2% with a 100% sensitivity technique**

Total number of units (apiaries) in the zone	50	100	200	300	400	500	600	700	800	900	1000	1500	2000	>4500
To be inspected	35	45	51	54	55	56	56	57	57	57	57	58	58	59

Number of units to be inspected in order to detect a **prevalence of 5% with a 100% sensitivity technique**

Total number of colonies within the selected apiary	up to 24	25	30	40	50	60	70	80	100	110	120	140	160	170	200	220	300	400	500
To be inspected	all	24	28	33	37	40	42	44	47	48	49	51	52	53	54	55	56	58	59

Number of colonies to be inspected in order to detect a **prevalence of 5% with a 95% sensitivity technique**

Total number of colonies within the selected apiary	up to 13	14	15	16	18	21	23	26	29	33	38	44	52	62	77	98	134	204	>410
To be inspected	all	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

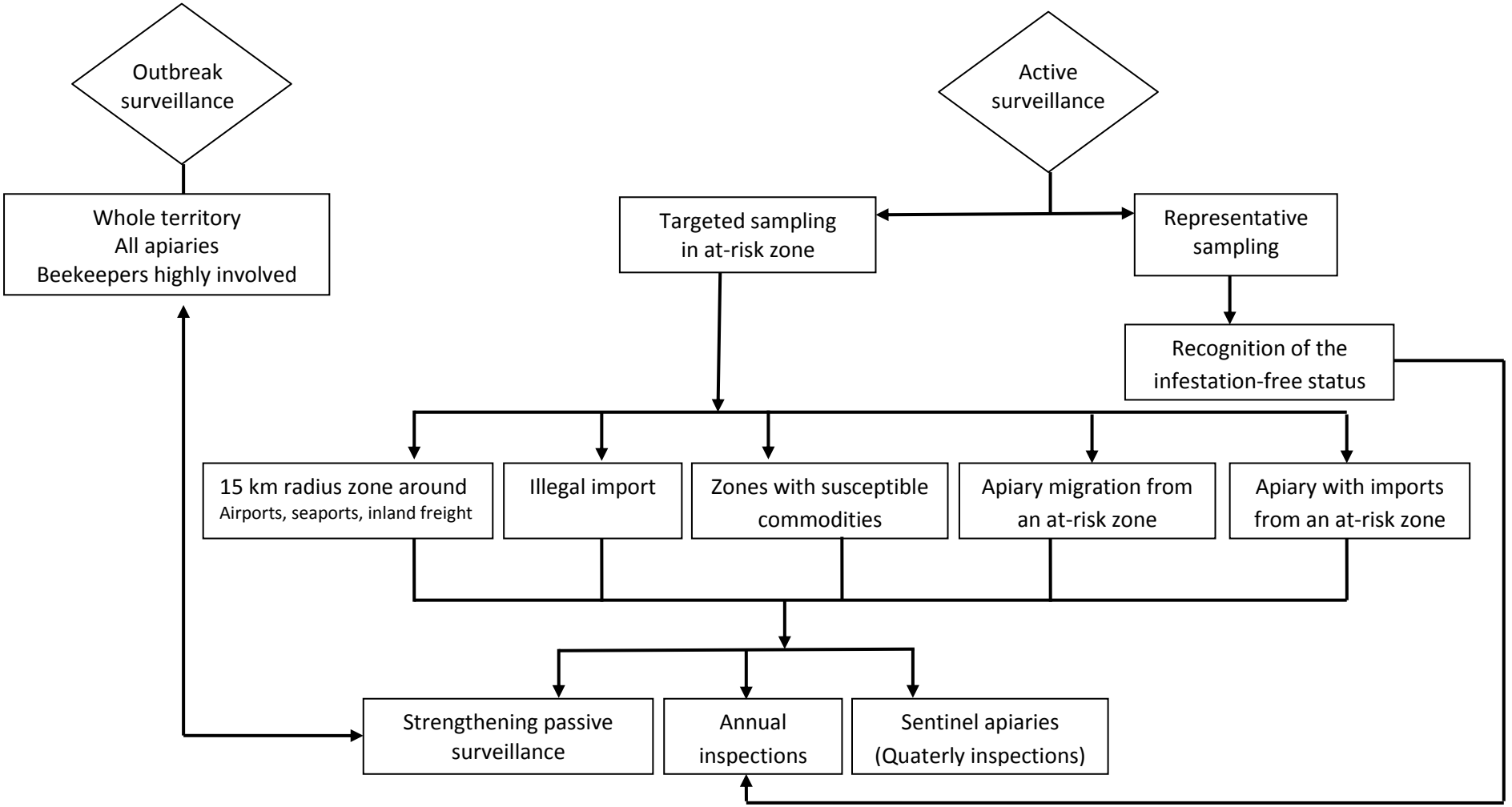
Number of colonies to be inspected in order to detect a **prevalence of 10% with a 95% sensitivity technique**

Total number of colonies within the selected apiary	up to 9	10	11	13	15	18	22	27	35	46	67	115	>345
To be inspected	all	9	10	11	12	13	14	15	16	17	18	19	20

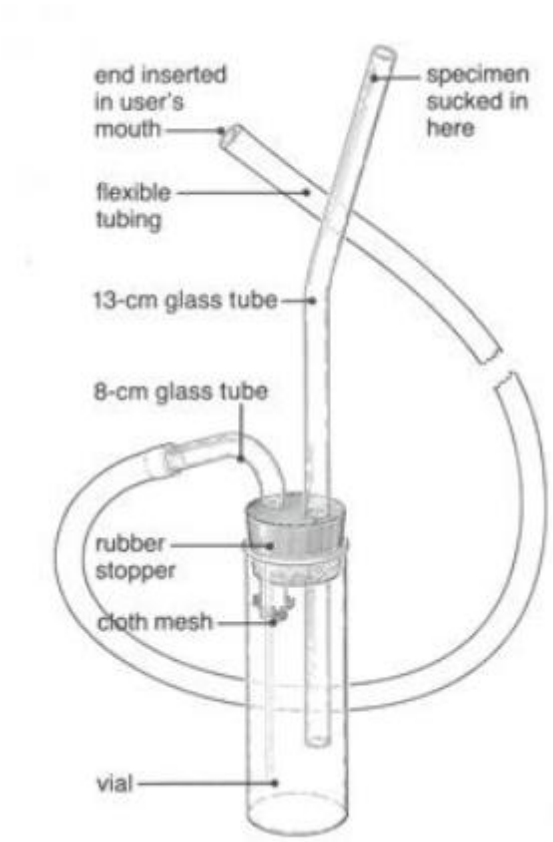
Number of colonies to be inspected in order to detect a **prevalence of 15% with a 95% sensitivity technique**

12 Annex 2

Flow chart with different types of surveillance and sampling level required.



13 Annex 3



Common type of Pooter (Sucking-type specimen aspirator) (www.ars.usda.gov)