Guidelines for a pilot surveillance project on honeybee colony losses

Introduction

Pollinator activity is required for the world's quarter million angiosperm species to reproduce, and as a result for fruits and seeds to be produced. Honeybees (*Apis mellifera* L.) are amongst the most specialised, arguably the most dominant pollinators and are essential for certain agriculturally important crops. There are numerous threats facing honeybee populations world-wide. The recent losses of honeybee colonies in the United States and Canada are alarming (vanEngelsdorp *et al.* 2008, 2011) and European beekeeping provides worrying insights on honeybee hive health disorders with maybe comparable colony losses, although as yet not nearly as clearly established (EFSA report 2009). Recent reports stemming from works of different groups have highlighted a serious lack of standardized surveillance systems needed to allow accurately assess the situation in Europe. A standardized and Europe-wide surveillance programme is required to obtain a reliable and accurate measure of the current status of honey bee health.

The purpose of this document is:

- to review the general principles that constitute the basis for accurate and strategic surveillance

- to draw conclusions on perspectives and requirements of future surveillance of honeybee colony losses

- to propose a surveillance framework to be used by European MS who may be interested in applying for a project grants to allow them to implement a relevant and effective system

- to provide future applicants with useful recommendations and assist in the design and improvement of surveillance systems

This document is **primarily based on conclusions and recommendations** presented in several working groups' meetings and reports (see § 1.3.1, as well as AFSSA report 2008 and EFSA report 2009) and based on the experience of the Member States (MS). The current project to be developed within the framework of the EU RL for honeybee health is mainly based on the conclusions of a 2008-2009 study on colony losses in Europe that was implemented by a consortium of 7 European partners ("EFSA consortium") and financed by EFSA (EFSA report 2009). The surveillance procedures to be implemented will be established by taking into account the objectives, needs, strengths and weaknesses expressed by the countries and the surveillance systems investigated.

In February 2011, the Sophia-Antipolis Laboratory of ANSES, France (French National Agency for Sanitary Safety of Food, Environment and Labor) was designated as the **EU RL in the field of honeybee health** by the European Commission. Its main missions are (1) to coordinate the methods employed in the MSs for diagnosing the relevant honeybee diseases and the training and other information activities throughout the Union, (2) to actively assist in the diagnosis of outbreaks of the relevant diseases in MSs and (3) to develop monitoring and surveillance activities. As such, the EU RL will play the central coordinating role in the establishment of epidemiological surveillance on honeybee pests and diseases.

The **objective** of the pilot surveillance project is to organize co-financed surveillance systems, improving both the effectiveness and the cost-benefits of existing systems at the national and European levels. In this aim, a strict statistically robust formal procedure is proposed here, and as an alternative a lighter touch approach to allow applications from every MS (see § 2.2.4).

1.1 Definitions

Since 2003, there have been reports in Europe and United States of serious losses of bees from beehives. In 2006, the term Colony Collapse Disorder (CCD) was first used to describe a particular phenomenon of sudden colony collapse in the USA (see description in Oldroyd 2007). By contrast, an excess of winter mortality seems to characterize most European colony

losses. However, it is also found in the USA, often mixed together with what is called CCD. Overall, boundaries between CCD and other types of colony losses remain much unclear. In order to encompass all aspects of colony mortality, and avoid excluding any causative or risk factor or any situation, the EFSA consortium decided to exclusively use the term "colony losses" to identify the phenomenon targeted by surveillance systems and literature.

Current consensus within the scientific community is that the origins of colony losses are multifactorial. They likely involve both biotic (e.g. diseases, reduction of resource availability) and/or abiotic factors (e.g. climate changes, phytosanitary and veterinary products). Moreover, some of them seem to be acting as synergists to each another (e.g. *Nosema ceranae* and two pesticide molecules as shown by Vidau *et al.* 2011), making the issue strikingly intricate. Causes and risk factors as well as specific data quantifying and qualifying the problems affecting honeybee colonies still require elucidation. This wide-scale surveillance programme is mainly targeted to descriptive epidemiology, instead of analytical. This must operate - not only on an appropriate geographical scale, but also by taking into account the typological elements.

Whereas the EFSA study needed a generic term ("colony losses") to explore national datasets and assess their respective utility, the Europe-wide surveillance system to be built up in the current project needs to allow more subtle aspects of colony health to be taken into account in future datasets in order to make more thorough typological characterization of European honey bee declines possible. As a result, common strict term usage is required. Set out below are the proposed standard terms and linked definitions to be adopted. The project will distinguish between two main categories, each containing two sub-categories:

Mortality: death of a honeybee colony. A colony is also considered dead if it is clearly unviable; i.e. there are only a few living honeybees remaining present in the hive and no queen is present. For the calculation of the mortality rate, only dead colonies will be counted; weakened colonies are excluded from this calculation. However, the latter will be taken into account for further investigations (see definition below). Additionally, because winter mortality of honeybee colonies is a normal annual seasonal phenomenon in apiaries (AFSSA report 2008), possible correlations between winter mortality and colony losses must strictly rely on relative data (normal winter mortality - usually averaging 10% - *vs.* excess winter mortality). By contrast, mortality during the beekeeping season is relatively abnormal. Consequently, the two following sub-categories are to be distinguished:

(1) **Winter mortality of honeybee colonies:** applies to any honeybee colony found dead at the end of the wintering phase, that was recorded alive in the autumn.

(2) **Honeybee colony mortality during the beekeeping season:** applies to any whole honeybee colony that dies during the beekeeping season. This colony "mortality" is to be strictly distinguished from weakening with depopulation (below).

Weakening: lack of strength of a beehive. Weakening is linked to a decrease in the hive population density over a period of time combined, mostly, with a decrease in hive activity, taking into account the normal seasonal fluctuations of honeybee populations as described in the AFSSA report (2008). Weakening may result in a drastic/abnormal reduction of total brood surface on frames and/or of the number of frames containing brood. Honeybee disorders can be observed, for example behaviour disorders and symptoms associated with diseases. As with mortality during the beekeeping season, a distinction must be drawn between mortality in or around the hive, and depopulation. Therefore, set out below are two different types of weakening that must be taken into account:

(1) **Weakening with apparent honeybee individual mortality:** with an abnormal number of honeybee individuals found recently dead (more than 200 per day) in the close vicinity of the hive(s). In the framework of the present pilot project, the recent mortality of bees just in front of the hive entrance will be taken into account.

(2) **Weakening without apparent adult honeybee individual mortality** but with abnormal symptoms or behavior at the adult or brood level.

Moreover, weakening is combined with a loss of honey production. Because estimate of such a loss is very difficult to clearly establish in a standardized manner, some thinking is needed before deciding whether this parameter will be taken into account.

More specific and detailed criteria for the above definitions (mortality and weakening) need to be clearly established and validated/confirmed taking into account the advice of experts at the beginning of the pilot project during the dedicated workshop.

In some MS (Southern Europe), the course of the season is quite different from the Northern Europe. The hardest time would therefore not be the (mild) winter but the dry and hot season. This has an impact on the timing of weakening during the beekeeping season. The bee mortalities would occur more likely after the honey production and after the increase of bee population. This will have to be taken into account for the management of the three apiary inspection visits (see §2.2.4).

The variety of indicators and definitions that can be applied to colony disorders indicate that all MSs will have to use common case definitions to collect data to estimate the indicators. Simple version of the case definitions will have to be developed for ease of use by field personnel within the surveillance programmes.

1.2 EU Legal framework

The Community legal framework on honeybee diseases and disorders is established by the following[1]:

1.2.1 EU RL designation and missions

- Responsibilities of the EU RLs and NRLs: Article 32 and 33 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls + Annex VII listing the EU RLs
- Commission Regulation (EU) No 87/2011 of 2 February 2011 designating the EU reference laboratory for honeybee health.

1.2.2 Legislation on notification of diseases

Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community (*Aethina tumida* and *Tropilaelaps* mites).

1.2.3 Legislation on intra-EU trade and introduction into the Union of certain species of bees

- Council Directive 92/65/EEC of 13 July 1992 (especially Article 8 and Annex A).
- Regulation (EU) No 206/2010 lays down animal health and certification conditions for introduction into the Union of certain species of bees (*A. mellifera* and *Bombus* spp.) the presence of the American foulbrood, the small hive beetle (*Aethina tumida*) and the *Tropilaelaps* mites (*Tropilaelaps* spp.): subject to compulsory notification throughout the whole territory of the third country or territory concerned.

1.3 Task Force subgroups on honeybee health and scientific input

1.3.1 Scientific input from past and current working groups

The outcomes of the kick-off workshop organized by the EU RL for honeybee health (ANSES, Sophia-Antipolis, France) held in Brussels in June 2011 provided, *inter-alia*, an

[1] All the legal texts can be consulted on the following website: http://eur-lex.europa.eu/

essential input for coordination of the elaboration of a Europe-wide epidemiosurveillance involving the candidate MSs.

Some important outcomes from the working group "Mortality, collapse and weakening of honeybee colonies" of French Food Safety Agency (AFSSA, now named ANSES) (13 meetings in 2007-2008 and one comprehensive report on the situation of honeybee health knowledge and organization of evaluation mainly focusing on France: AFSSA report 2008) and from the EFSA consortium (2 meetings in 2009, one comprehensive report on the surveillance systems in Europe and the utility of contemporary available data: EFSA report 2009, one honeybee health bibliographical database established following strict and consistent rules) also provided well focused input for the design/update of future surveillance strategies. Additionally, the experiences of the "German Bee Monitoring Project", which has been performed continuously since 2004 provided the scientific community with practical information and results on surveillance protocol (Genersch *et al.*, 2010). Finally, the international surveillance network for honeybee colony losses COLOSS (Prevention of COlony LOSSes; COST Action FA0803[2]) has been determining a standardized questionnaire

[2] http://www.coloss.org

to measure the honeybee colony mortality on an International scale. The strategy implemented by the COLOSS network for some years is based on passive surveillance and estimates. The questionnaire is sent by all means possible to ensure a wide distribution. The data collected are analysed and interpreted by the COLOSS network (Working group 1 on monitoring and diagnostic). This strategy is recognised as clearly being unrepresentative considering that beekeepers are sending back their information on a voluntary basis. Nevertheless, the strength of this approach relies on the level of collected data, and this can be quite high in some countries.

1.3.2 Organisational concept

At the European level, the pilot surveillance project will be coordinated by the epidemiology team of the EU RL (who will provide support for the development of the surveillance protocols, implementation of a number of the training activities, follow-up of the programme, data analysis and interpretation at the European level). This team will be supported by a team of experts and field researchers from the various MS, skilled in honeybee diseases and beekeeping, who will contribute to the development of the protocols, data analysis and interpretation.

Each participating MS will designate a coordinating national institution with a coordinator or a coordination team responsible for the national customisation of the pilot surveillance project, its implementation, follow-up and local and national data analysis and treatment. This coordinator will maintain a constant link with the EU RL epidemiology team and will tightly interact with a team of experts and local field honeybee specialists (including a variety of stakeholders within the beekeeping community) to support the surveillance activities (Figure 1).

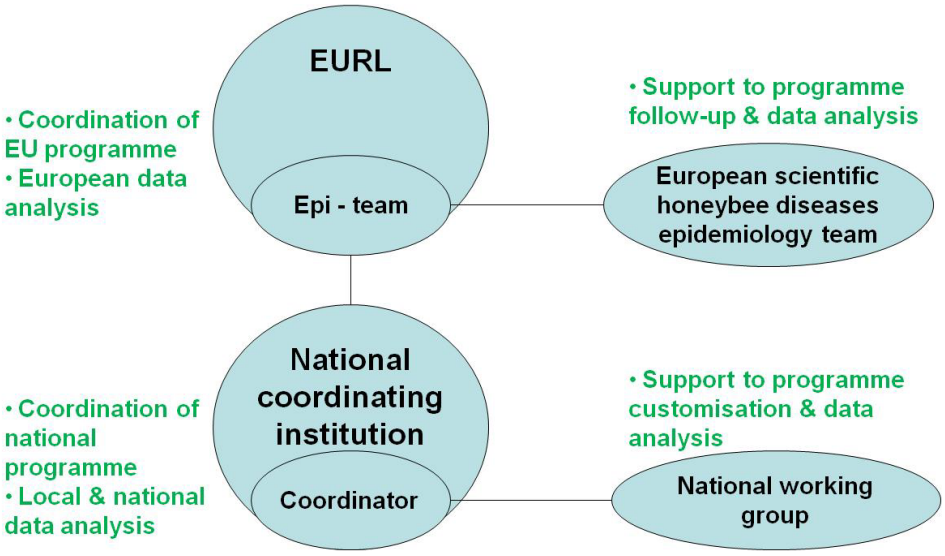

Figure 1: Organization scheme of the pilot surveillance project

1.4 State of the art on honeybee colony losses and surveillance systems in Europe

1.4.1 Main pathogens (diseases) and their distribution in regard to Europe

About thirty major biological pathogen species affecting bees have been identified today: 4 predatory, 4 ectoparasitic and 1 endoparasitic arthropods, 1 endoparasitic protozoan, 4 fungi, 5 bacteria and around 12 viruses (AFSSA report 2008). Some of these viruses as well as some others rarely detected/isolated or recently discovered are present in bees without any clear consequences (e.g. four viruses newly isolated by Runckel *et al.* 2011). Overall, many honeybee viruses have the potential to become pathogenic if the circumstances are right, for example in combination with some other synergistic organisms or circumstances or might represent some weakly pathogenic organisms.

Of these major pathogenic species, the most important in terms of prevalence and importance of known potential damage inflicted on colonies are the following:

- 1 predatory beetle (*Aethina tumida*, the small hive beetle; not present in Europe: see below)
- 3 ectoparasitic mites (*Varroa destructor*, agent of varroosis, and *Tropilaelaps clareae*, as well as *T. Mercedesae* – see below)
- 2 fungi (*Nosema apis* and *N. ceranae*, agents of nosemosis)
- 2 bacteria (*Paenibacillus larvae*, agent of American foulbrood and *Melissococcus plutonius*, agent of European foulbrood)
- 3 viruses known to induce honeybee losses without association needed (Chronic bee paralysis virus CBPV) or in association with *V. destructor* (Acute bee paralysis virus ABPV and Deformed wing virus DWV).

V. destructor requires some particular attention, because it was a primarily exotic mite, which entered Europe in the 1980's. To date, it is endemic in all the European apiaries and was shown strongly linked to winter mortalities in many surveillance studies (Genersch *et al.* 2010, Chauzat *et al.* 2010). Moreover, it is worth noting that *V. destructor* is a vector of some viruses (ABPV and DWV among others). It has been hypothesized that the relatively recent

expansion of this invasive species associated with viruses and with increasing resistance to some acaricides could be a major factor of the currently apparent increase of colony losses.

Among all these majors pests and pathogens, three (two genera) are still absent from Europe: the small hive beetle *A. tumida* and the mite species *T. clareae* and *T. mercedesae* (as well as any other species of the genus *Tropilaelaps* such as *T. koenigerum*). They are listed as notifiable organisms, and measures to prevent or at least reduce their introduction must be established in each country, by the European Commission. Both the beetle and the mites have recently invaded non native areas. *A. tumida* originates from Southern Africa and entered into the USA likely in 1996, where it was proven to be present in 1998. It is now present at least through North Africa, North America, Australia, Mexico, in one of the Carribean islands Jamaica (Thomas 1998, Neumann et Elzen, 2004, Hauser 2004). In 2010, it further extended its range to Hawaii. As for the genus *Tropilaelaps*, it encompasses several species primarily parasitizing non *mellifera* honeybee species of the genus *Apis* in Asia. Among them, at least *T. clareae* and *T. mercedesae*, whose primary host is the Asian native honeybee *A. dorsata* are also parasitizing introduced Western honeybees *A. mellifera* (Laigo and Morse, 1968, Anderson and Morgan 2007). Although their exact geographical range is not fully known, *T. clareae* is thought to be present in North-West Asia, from Iran to New Guinea (Delfinado et Baker, 1961; Burgett et al., 1983). It is now also known to be an economically important pest of *A. mellifera* throughout Asia and is considered an emerging threat to world apiculture. For the time being, no *Tropilaelaps* species has been reported in Europe or in the USA so far. However, with the globalisation of the beekeeping industry there is significant potential for this parasite to be spread worldwide by movement of bees. This represents a serious concern all the more given the much deleterious introduction followed by invasion already observed with the ectoparasitic mite *V. destructor* (see above). Current European regulations impose checks of imported honeybee materials, which are expected to help preventing or at least strongly reducing the risk for the introduction of these two pest genera.

1.4.2 Situation of the surveillance systems of colony losses within Europe

Within the EFSA consortium, a standardized Surveillance Network Assessment Tool (SNAT) was developed to analyse the European colony losses surveillance programmes (EFSA report 2009). Twenty-seven countries were selected to be part of the study. Twenty-five SNATs from 24 countries were completed, received and processed. The SNAT analysis allowed the Countries to be classified into four categories: those with (i) a very good level of compliance

with the standards of a good operating system (1 system), (ii) an upper intermediate level of compliance (4 systems), (iii) a lower intermediate level of compliance (12 systems) or (iv) a low level of compliance (8 systems).

Eighty percent of the surveillance systems were found to comply with less than 50% of the 40 items covered by the questionnaire. This generally low level of compliance reflects a broad margin for improvement in most of the European surveillance systems considered within the project. Concerning surveillance procedures and protocols, of the 18 systems stating that they have in place active surveillance procedures, only 6 can be considered as valid active systems able to produce representative figures of the true colony loss situation for the countries considered.

It was found that colony losses surveillance systems in Europe are characterised by a variety of approaches and operational methodologies. Nevertheless, the majority does share common aspects, in particular the weakness of the systems implemented, and the lack of representative data produced.

1.4.3 Tentative analysis of colony losses data in the EU Member States

Within the same EFSA work, data from surveillance networks were collected and standardised in order to allow analysis at the European level (EFSA report 2009). The only indicator that appeared to be commonly used was the "global colony loss rate" during the over-wintering period. Therefore, all aspects of colony losses (such as summer losses) could not be addressed through this study. Temporal and geographical analyses showed an important variability in colony losses. However, such trends are difficult to interpret considering the wide variation in the quality of the systems that produce these data.

Nevertheless, the project noted (i) a baseline colony loss rate around 10% each year at the European level and (ii) a higher level of colony loss in some countries during the years 2003 and 2008.

This analysis clearly highlights an absence of shared epidemiological indicators, collected following common surveillance procedures and based on comparable populations. Trend analysis and mapping suggest some periods of higher colony loss rates, but these findings should not be over interpreted. They serve to illustrate the fact that existing data collection systems are not robust enough to allow between country comparisons across Europe, or the analysis of trends at the European level.

2 Strategies to enhance the effectiveness of surveillance for honeybee colony losses

The objectives of the pilot surveillance project are to propose harmonised active surveillance procedures that will allow an accurate estimation of colony losses within and throughout participating European countries. Besides, taking advantage of the active procedures to be established, the pilot project will support the implementation of prevalence studies on priority diseases of honeybees in order to estimate incidence following harmonized procedures which use shared epidemiological indicators. In particular, due to the proper characteristics of *V. destructor* (proven association with excess winter colony losses; see above), it is considered crucial to assess the infestation level using consistent protocols throughout European countries in order to get comparable data on populations of this mite before winter.

Note that, for the time being, analyses for detection of chemicals (phytosanitary and veterinary products) are not targeted in the surveillance programme to be built up. Indeed, given the very high costs of such studies and the complexity of colony loss figures, it has been considered that a two-step large-scale survey could be needed in order to be able to specifically target appropriate subsets of cases and a sub-series of chemicals. The first step (present pilot project) is expected to lead to provide typology of the different (and maybe inter-related) types of colony losses. The need for a second step study including possible detection of some chemicals will be further evaluated.

2.1 Issues to be addressed in a general context

2.1.1 General requirements

Harmonisation of surveillance procedures at a European level should lead to the establishment of a consistent and robust set of epidemiological indicators, calculated following the same rules and protocols in all countries, and produced by comparable active surveillance procedures applied across comparable populations according to the climate constraints. This recommendation is essential, as not only will this allow accurate comparisons to be drawn between the statuses of different European countries, and thus facilitate the objective assessment of fluctuating colony losses within Europe. An appropriate tool to monitor colony losses at a European level is important since it will provide national and European decision makers, and also the beekeeping industry, with accurate figures about colony mortality which, in turn could focus control and research activities.

2.1.2 Need for clarification/standardization of case definition

In order to use commonly agreed indicators, the case definition of the events under surveillance will have to be established and validated. Therefore, existing work undertaken by the COLOSS network will have to be valorised. The case definition setting is focused mainly on colony mortality and colony weakening as defined above (see § 1.1). Case definitions will be clearly established at the beginning of the pilot project during the dedicated workshop with the expert consulting group "European scientific honeybee diseases epidemiology team" (see Figure 1).

2.2 Required basis for protocols to be proposed for surveillance of honeybee colony losses

This section discusses main measures needed for honeybee colony losses surveillance to be efficient, which are largely in line with the conclusions and recommendations drawn up from previous analyses (see §1.3 above).

Those measures deal with following issues:

- Estimate of the targeted populations in each participating MS
- Nature of the targeted biological material to sample
- Diseases and syndromes to be targeted
- Surveillance procedure to be implemented
- Data management, data treatment and interpretation
- Training
- Communication
- Assessment and follow-up
- Institutional organization

Basic measures described below must be applied to make any applications eligible. Nevertheless, given the important heterogeneity in currently available surveillance systems between MSs (EFSA report 2009) and in order to make the partnership accessible to every MS, some lighter touch alternatives are proposed for each part of procedure enabling different degrees of participating involvement (see Table 2).

2.2.1 Targeted population

The total honeybee population of the participating countries will have to be included in the surveillance. This means that population census will have to be accessed to serve as sampling

framework or basis on which to establish the population samples (see Annex II). In the absence of an accurate and complete hive census, alternative procedures will have to be proposed and agreed to guarantee a representative sampling of the population in these cases (see Table 2).

2.2.2 Targeted samples

During the apiary inspection visits to be implemented (as described in §2.2.4 below), some biological material will be sampled as follows: one systematic sampling per examined hive during the first visit, additional symptomatic samplings in every hive with symptoms either on adult bees or on brood during visits 1, 2 and 3 (see Tables 1 and 2).

2.2.3 Targeted diseases & syndromes

Note: Some of the below diseases are notifiable diseases in many MS. Hence the question of management of hives with any notifiable disease detected is arising. In these cases, the European and national regulation must be applied. The problem of notification of the diseases found through the pilot programme should be taken into consideration and should be solved because it can interfere with the motivations of the beekeeper to be part of the survey.

The focus will be on the following main honeybee diseases and/or pathogens: varroosis (*V. destructor*), American (*P. larvae*) and European foulbrood (*M. plutonius*), nosemosis (*N. apis, N. ceranae*), Paralysis (CBPV) and the two viruses strongly linked with *V. destructor* (DWV and ABPV). These are known to be present with relatively high prevalence and/or impact in Europe. Additionally, the two following notifiable pathogens will be also searched for: *A. tumida* and *Tropilaelaps* spp. (currently considered absent from Europe).

Clinical records

For all these diseases (pathogens), specific records of clinical symptoms and/or presence of pest arthropods at the colony level will be established using the apiary inspection form. This form has to be systematically filled up by the specific person in charge of apiary's visit (see Annex I).

Laboratory analyses

Additionally, diagnostic and/or quantifying analyses will be carried out at lab as follows:

1) Systematic analyses on systematic samples (see §2.2.2 Targeted samples, §2.2.4 Surveillance procedure and Table 2).

Because of the above described history and characteristics of *V. destructor* and varroosis in Europe, an estimate of the population size of *V. destructor* in every colony under examination will be compulsorily carried out at lab in systematic samples collected during apiary inspection visit 1 (see §2.2.4). For such a purpose, recommended protocol implies house adult honeybee washing. Quantification criteria still need to be established and will be provided in the close future. In the same time as *V. destructor,* the opportunity of honeybee washing must also be taken to search for *Tropilaelaps* mites. No light touch protocol is admitted for these issues.

Finally, the two viruses DWV and ABPV must be systematically searched for. Some light touch protocols may be discussed for this issue (see Table 2).

2) Systematic analyses on symptomatic samples (see §2.2.2 Targeted samples, §2.2.4 Surveillance procedure and Table 2).

Laboratory diagnostic analyses will have to be performed on symptomatic samples collected during apiary inspection visits 1, 2 and 3. Symptoms reported through the apiary inspection forms should serve as the basis for guiding and refining decisions on the laboratory analyses to complete, so that pathogen organisms suspected as being present are searched for and/or quantified. Due to the current lack of harmonisation among diagnostic methods, a tentative table of instructions (see Table 1) established by the EU RL is proposed below in order to allow planning project costs for now. The protocols of specific methods will be discussed with the experts in honeybee pathology involved in the pilot project and the relevant laboratories of the MS participating at this project in order to decide which harmonized approaches to be used and to provide detailed information at the beginning of the programme. The core protocol requires laboratory diagnostic analyses for every of above cited pathogens. Light touch alternatives excluding some of the non *Varroa* analyses are allowed (see Table 2).

Note: For any suspect cases, confirmatory diagnostics can also be carried out by the EU RL.

Targeted disease	Pathogen	Type of sample	Inspection of the colony	Type of laboratory method	Recommended method
Varroasis	*V. destructor*	brood + house adult bees	Symptoms observation, macroscopic observation	Symptoms observation, macroscopic observation and counting	EU-RL recommendations for specific symptoms observation and *Varroa destructor* detection and counting (by washing adult bees)
American foulbrood	*P. larvae*	symptomatic brood	Symptoms observation	bacterisocopic diagnostic completed if needed by molecular diagnostic	OIE recommendations for disease diagnostic by bacterioscopy and identification of the agent by PCR (method validated by the EURL)
European foulbrood	*M. plutonius*	symptomatic brood	Symptoms observation	bacterisocopic diagnostic completed if needed by molecular diagnostic	OIE recommendations for disease diagnostic by bacterioscopy and identification of the agent by PCR (method validated by the EURL)
Nosemosis	*N. apis* and *N. ceranae*	symptomatic adult bees/ dead bees	Symptoms observation	microscopic observation	OIE recommendations
				molecular identification	If microscopy positive, PCR following EU-RL recommendations adapted from OIE recommendations
Paralysis	CBPV	symptomatic adult bees/ dead bees	Symptoms observation	molecular diagnostic detection and quantification	RT-qPCR following EU-RL recommendations
DWV	DWV	house adult bees	Symptoms observation	molecular diagnostic (on systematic samples collected during visit 1)	RT-PCR following EU-RL recommendations
ABPV	ABPV	house adult bees			RT-PCR following EU-RL recommendations
A. tumida	*A. tumida*	symptomatic brood/honey/bee bread cells	Symptoms observation	macroscopic observation	Hive examination following / adapted from OIE recommendations

		adult beetles and their larvae	Detection (per hive)	macroscopic/microscopic observation followed if necessary by molecular diagnostic	If beetles (adult and/or larvae and/or eggs) evoking *A. tumida* have been encountered during hive examination, then morphological identification at lab adapted from OIE recommendations /molecular diagnostic following EU RL recommendations
Tropilaelaps spp.	*Tropilaelaps* spp.	symptomatic brood/honey/bee bread cells	Symptoms observation	macroscopic observation	Hive examination following / adapted from OIE recommendations
		diverse stages of mites (mainly adult females)	Detection during *Varroa* laboratory analysis (counting)	macroscopic/microscopic observation followed if necessary by molecular diagnostic	Any non *Varroa* mite isolated during the house adult honeybee washing on systematic samples must be morphologically studied, and, if *Tropilaelaps* identity may not be clearly excluded, molecular identification should be performed (procedures to be established by EU-RL based on available publications)
			Detection (per hive)	macroscopic/microscopic observation followed if necessary by molecular diagnostic	If any mite suggesting *Tropilaelaps* spp. infestation visible to the naked-eye and different from *V. destructor* have been encountered during hive examination, then morphological identification at lab followed if necessary by molecular identification (procedures to be established by EU-RL based on available publications)

Table 1: Tentative table of instructions for diagnostic analyses

If the incidence/prevalence of any other pest(s) or disease(s) appears more important than previously estimated, their possible inclusion into the list of diseases to be searched for should be discussed by experts and national reference laboratories.

In every apiary inspection, some obligatory **systematic standard sampling** will have to be completed during apiary visits. Collected samples should be stored/preserved in accordance with future instructions (specification planned for next autumn, although a rough draft is available in Table 2). We insistently underline that this is a strict obligation and that it must be done in such a way to allow accurate analyses possible (transport to the respective lab, conservation, identification, traceability).

Possible collaborations with third institutes may be discussed between participants in order to allow the obtained samples to be accordingly identified, preserved and stored.

2.2.4 Surveillance procedure

Given that the aim is to objectively measure the selected indicators in the population, surveillance will have to rely on active procedures implemented by specifically trained personnel. As stated in §2.2, given the large variability in current surveillance systems operated across the MSs and in order to make the partnership accessible to every MS, "recommended procedure" in Table 2 represents the most complete procedure or core protocol, while "possible light touch alternatives" are proposed for each part of procedure enabling different degrees of involvement.

To ensure statistical robustness of the expected data, a **sample of the honeybee population** will be randomly selected according to the level of the expected pest and pathogen prevalence and the relative precision expected for the results (see Annex II). Depending on the general geographical and administrative shape of the participating countries, a population stratification process could be implemented for example using the following criteria: agro-ecological zoning, administrative boundaries, beekeeping farm characteristics (kind of productions, size of exploitation, …).

Important: The colonies in these selected apiaries will be monitored **over the whole duration of the pilot project (exception: see Table 2)**.

Data collection will consist of basic information on beekeeping practices at the farm/apiary/hive under test, clinical observations in hives under test and analyses of samples from hives under test. Practical information, apiary and colony condition and clinical observations as well as biological samples will be collected by specifically trained personnel

using an apiary inspection form to be established within the next few months (see outlines in Annex I). Sample analyses (subset to be agreed based on the detail provided in Table 2) will be carried out directly by the MS relevant laboratory. In addition, a part of samples will be preserved for possible *a posteriori* analyses. Three **apiary inspection visits** will be implemented per year on the selected apiaries. At each visit, colony condition and clinical symptoms will be recorded on the apiary inspection form. The relevant hive samples within each selected apiary (as stipulated in Table 1) will be inspected in order to estimate the prevalence of some diseases/pathogens according to a harmonized protocol and common case definitions. All of these visits will be done in all the selected apiaries within one month (each MS will ensure that an adequate workforce is available in order to complete the work within this one month time frame). The three apiary inspection visits will entail the following:

- Apiary inspection visit 1: A first visit will be performed to collect data relating to beekeepers' practices, location of the beehives and environmental information, record the number of living and healthy colonies and estimate the prevalence of some diseases (pathogens) (at least varroosis (*V. destructor*)). This visit will be implemented at the end of the season, before the wintering period (exact period to be defined in accordance with climatic characteristics of the MS). During this visit, a sample of house adult honeybees will be systematically collected from each colony irrespective of whether any symptoms are observed (i.e. from apparently healthy colonies as well as from any apparently infected/infested colonies). Additionally, specific symptomatic samples will be collected in colonies with any disease symptom (see Table 1).
- Apiary inspection visit 2: A second visit to the same apiaries will take place at the end of the wintering honeybee season (late winter or early spring depending on the specific climate of the MS or geographical unit) in order to objectively record any colony losses that have occurred during the winter. During this visit, only specific symptomatic samples (and/or dead bees) will be collected in colonies with any disease symptom and/or observed troubles (see Table 1). In order to ensure coordination between the COLOSS and the present survey, the beekeeper will be provided with the questionnaire from the COLOSS network during this visit and will be asked filling it up and sending it to COLOSS.
- Apiary inspection visit 3: A third and final visit is planned for the same apiaries during the honey production season in order to objectively estimate the number of lost/ weakened colonies. The period should be selected by the MS depending on its specific climatic characterisitics in such a way that opportunities to observe abnormal mortality

are the most probable. As in visit 2, during this visit, only specific symptomatic samples (and/or dead bees) will be collected in colonies with any disease symptom and/or observed troubles (see Table 1). In order to avoid omission of any season mortality event, the beekeeper will be asked to regularly (e.g. once a month) inspect selected apiaries (external observations of hives) and to alert in case of important mortalities/abnormal behaviours. Additionally, it will be verified during this visit that the questionnaire has actually been filled up and sent to COLOSS.

Search for exotic pest arthropods.

Active search of *A. tumida* and *Tropilaelaps* spp. may be very time-consuming and makes the colony inspection difficult. As a result, it appears more stringent not to provide active search within each selected apiary, but only within the selected apiaries with high risk factor. Because of the life style of *A. tumida* (the last larval stage moults into nymph within the soil), not only bee materials, but also importations of plants and soil material are possible vectors for it to be introduced in a new area. As for *Tropilaelaps* mites, chances for introduction are relatively limited because of the need of brood for the mites to survive.

Some risk factor should be investigated further in order to select apiaries: the selected apiaries having high risk factors might be apiaries homing imported queens (risk for *A. tumida* and *Tropilaelaps* spp.) and apiaries located in the close vicinity of harbours and airports(risk for *A. tumida*).

Overview of data collection:

One apiary inspection form will have to be filled up per visit and per apiary. There will be three different forms to be used according to the position of the visit under consideration (1st, 2nd, 3rd, see Annex I).

- **Visit 1 (main objective = assessement of initial condition)**: general detailed data, clinical observations for each colony under examination, systematic samples (every colony under examination, with or without any symptoms), symptomatic samples (only colonies with symptoms)
- **Visit 2 (main objective = assessement of winter mortality and weakening)**: condition of each colony in each apiary: count of dead and weak colonies, count of non disorder linked increases (bought or split hives)/decreases (sold hives...) in the number of hives and any changes since the first visit, clinical

observations for each colony under examination, symptomatic samples (only colonies with symptoms)

- **Visit 3 (main objective = assessement of mortality and weakening during the beekeeping season)**: detailed description of the losses that have occurred during the season, condition of each colony in the apiary (count of dead and weak colonies, count of non disorder linked increases/decreases) and changes since the second visit, clinical observations for each colony, symptomatic/ dead bees samples (only colonies with symptoms, and/or troubles), alerts by beekeepers

Table 2. Summary of surveillance guidelines for colony mortality, including possible alternatives depending on available means in each MS

	Recommended procedure (core protocol)	Possible light touch alternatives
Targeted population	All apiaries of the targeted zones: this means that professional as well as hobbyist should be included in the sampling frame. If a high proportion of apiaries are usually transferred from one place to another (transhumance), this specific issue should be addressed properly to prevent bias in the sampling.	It is acknowledged that in most countries it is difficult to have exhaustive lists of beekeepers and apiaries. Therefore, the proposed surveillance frame should discuss this issue and estimate the level of uncertainty in the population data set due to the incomplete nature of the census lists. Incomplete information should not prevent implementation of the surveillance programme. Depending on the beekeeping practices and organisation in each MS, it may be appropriate to modify the apiary selection criteria: e.g. apiaries with very small size (less than 5 colonies). For migratory apiaries, MSs should ensure that the location of the colonies under study is known and the apiaries are followed systematically throughout the course of the survey.
Geographical coverage	The sampling should address the whole country. Geographical zoning should be considered as geographical units for the	If it is not possible to take all administrative units as the basis of the sampling in a country, a selection of several administrative units

	sampling strategy. Even if agro-ecological zoning is assumed to be the most relevant to support geographical sampling, administrative zoning may be easier to use. For the purposes of cost-effectiveness, geographical units based on administrative regions (of the size of regions in Spain, Italy, France, UK or a Land in Germany) appears to be the most appropriate.	chosen to represent the variety of the agro-ecological zoning of the country could be done (5 is the minimal number of zones which will be included in the protocol in any case). The representativeness of the selected regions should be discussed in the proposed programme.
Number of apiaries to be included	In each geographical unit, the number of apiaries to be included in the surveillance is determined on the basis of the expected prevalence of the phenomenon to be measured. Considering a prevalence of 15%, an absolute precision of 5% (which means a confidence interval from 10% to 20% in the expected result) leads to a sampling of 193 apiaries per geographical unit (see Annex II-A). If the true prevalence is lower, the relative precision of the result will be lower with the same number of apiaries included. If the prevalence is higher, the relative precision will be better.	According to the practical implementation capabilities of the surveillance in the country, higher or smaller sample sizes could be proposed. Each proposition should be discussed as regards to the expected precision according to the expected prevalence. It has to be taken into account that too small samples will lead to wide confidence intervals that will reduce the power of any statistical comparison between regions or countries (see Annex II-A for more details). If candidate teams do not involve any epidemiologist and need to establish lightened project, we strongly recommend that they ask for advices from some epidemiologist.
Number of colonies to examine	A certain number of colonies will have to be investigated during each visit in order to detect clinical signs of diseases and the presence / absence of some pathogens. The number of colonies to investigate is determined according to the expected prevalence of the diseases inside the apiary. For instance, for an expected prevalence of 20% inside the apiary, the number of colonies to necessarily investigate is up to 15 maximum depending on the total number of hives in an apiary (with a confidence rate of 95%) (see	It is not recommended to reduce the number of colonies to be investigated per apiary as illustrated in the example given column one given that an expected prevalence of 20% is already a high prevalence rate. The number of hives to be examined in each selected apiaries must be determined based on the abacus in Annex II-B.

	Annex II-B). Each investigated colony will have to be clearly and unambiguously identified in order to be able to identify it during the next visit. Standardised labelling system will be proposed and should be adopted by all participating MSs.	
Samples to collect	All sampling protocols must be clearly adhered to. It is vital that sampling recommendations are carefully followed recommendations (and independently of whether all other analyses can be performed immediately – see column 2). Some collaboration with third institute may be established to allow storage and preservation of obtained samples. Visit 1: From each hive (colony) under examination: a systematic sample of house adult honeybees must be taken. The specific receptacle will be stated at the beginning of the programme and will simply have to be filled in with bees (no exact counting). If any beetle or any mite similar to the notifiable exotic pests or any disease symptom is observed, additional sampling must be done following Table 1 after appropriate recording on the apiary inspection form 1 (see Annex I). Specific type of symptomatic samples, amount of biological material to collect, identification and storage conditions will be discussed and decided during the future workshop. A relevant detailed sampling procedure will be further specified and issued.	No alternative procedure proposed. Whether all analyses can be performed immediately or not, each MS should have the capacity to perform diagnostic analyses on a specific subset of samples that will be analyzed *a posteriori* (see below).
Colony observations	A series of clinical observations must be completed and reported on the relevant apiary	No alternative procedure proposed.

to be made	inspection form (see Annex I). As for the two exotic genera, active search during hive inspection must be performed in apiaries with high risk factor (see §2.2.4 p. 21). In apiaries with no particular risk factor, no active search will be required. Nevertheless, suspect mites or beetles must be recorded and sampled.	
Laboratory analyses to be performed	The detection and counting of *V. destructor*, as well as detection of *Tropilaelaps* spp. in house adult bees sampled (systematic samples collected during the apiary inspection visit 1), as well as the identification of any beetle or non *Varroa* mite suggesting *Tropilaelaps* spp. infestation collected within hives, must be systematically done. Investigations for specific diseases and pathogens will be carried out on symptomatic samples (symptomatic samples collected during apiary inspection visits 1, 2 and 3; diagnostic analyses directly orientated by specific symptoms) (see §2.2.3 and Table 1). Detection of DWV and ABPV should be done systematically in systematic samples collected during visit 1.	The counts of *V. destructor* individuals, search for and identifications of unusual mites must be carried out in any case on all systematic samples (Visit 1). Depending on the resources available in the candidate MS, investigations for a proportion of the pathogens listed or for a subset of the total sample may be discussed (see above samples to be collected). In the latter case, the selection of the subsamples to analyze may be performed *a posteriori* on the basis of tendencies revealed by the annual global analyses provided by the EU RL. More specifically, the detection of DWV and ABPV on samples collected during visit 1 could be carried out using the method of case control study (comparing a subset of weakened hives and a subset of hives in good condition at visit2). Specific instruction for preservation will be stated at the beginning of the programme.

2.2.5 Data management, data treatment and interpretation

Management of data collected during the apiary visits (beehive and environment information, direct clinical notations), all the results of *Varroa* counts, apiary assessments, any clinical symptoms observed in colonies and laboratory diagnostic results on the samples collected will require specific IT software which can be used by trans-European surveillance manager

teams. The needs of the development of suitable IT software to manage the honeybee data will be discussed at the EU level and the conditions for an implementation at the EU level needs to be further evaluated to allow to ensure that the resulting datasets are harmonised and usable through a centralized program at the EU RL. Data should be integrated in a database, then sent and centralized at both national and European levels to allow data treatment and analysis at these different levels. The appropriate tools are necessary to integrate and share the data.

A computer security system will be devised in such a way that no nominative data can be accessed by anybody other than the submitter.

Additionally, procedures allowing for feedback to stakeholders on obtained data and results should be clearly integrated into the project at the country level. MS surveillance projects must include formal reporting of the results to all stakeholders (local coordinators, field personnel, participating beekeepers and total beekeepers population – see §2.2.6 below).

2.2.6 Training

The number of persons involved in the surveillance projects will be determined by each participating MS and based on the number of targeted apiary sites to be visited. All personnel will need to follow Standard Operating Procedures not only at national level but also at the European level. In particular, a specific session should focus on basic training for the estimate of introduction risks and the detection of *A. tumida* and *Tropilaelaps* spp.

Therefore, each country will designate two professionals who will act as trainers and training coordinators within their own country. These trainers will attend a dedicated workshop to be organized by the EU RL with the objective of ensuring agreement on standardized operating procedures and ensuring standardized training sessions for personnel involved in each participating country. The trainer workshop will be organized during winter 2011-2012 and national training sessions will have to take place before the beginning of each national programme.

2.2.7 Communication

A report on the centralized global analyses will be provided by the Epidemiological team of EU RL every year to each participating MS. Extension and dissemination of the results of the project work should be an integral part of the proposals at country level and should be conceived as annual reports stemming from this global report. Additionally, a feedback procedure making obtained data and at least some discussions directly available to all

stakeholders and beekeepers involved into the programme should be clearly integrated into the project at the country level

2.2.8 Assessment and follow-up

Key Performance Indicators (KPIs) will be developed in order to monitor the progress of the pilot surveillance programme on national and European levels. To measure and manage progress, KPIs will consist of a number of key variables brought together in a Gantt chart form allowing a continuous estimate/measurement of the achievement of the network's priority activities. These KPIs for the epidemiological monitoring network will be identified as soon as the pilot surveillance project is set up in order to periodically assess its activities.

2.2.9 Institutional organisation

Each MS will designate a national coordinator and a coordinating institution to undertake the pilot surveillance project in his/her country. This coordinator must be supported by a clearly identified working group to help in the customisation of the national pilot surveillance project according to the European guidelines and to assist the coordinator for the data treatment and interpretation at the national level (see Organization concept in § 1.3.2).

The pilot surveillance project will be coordinated by the EU RL, a European scientific honeybee diseases epidemiology team will be established to assist in data treatment and interpretation at European level under the coordination of the EU RL (see Organization concept in § 1.3.2).

3 Conclusions and Recommendations

3.1 Conclusions

An efficient surveillance of honeybee colony losses within Europe is achievable, given the current involvement of many countries and regions within the European Union with this issue. However, important improvements are expected in order to get a usable – consistent and statistically robust – overview of present situation in Europe concerning honeybee colony losses. These improvements mainly rely on strengthening of the statistical robustness of data and standardization and homogenization in order to make trans-European comparisons possible. No single strategy is the optimal applicable to all MSs, due to the great diversity of existing surveillance and of beekeeping practices. Present guidelines define the conditions under which countries can apply for a project grant to carry out surveillance of honeybee colony losses in their country. Despite a rather large flexibility in the present application,

mainly represented by a general scenario allowing a panel of alternatives (see Table 2), some stringency is required, as outlined in this document, in order to obtain stronger datasets in the future and make the most of surveillance actions.

3.2 Recommendations

Improvement of the representativeness of collected data and harmonisation of surveillance procedures at European level are needed in order to make a consistent estimation of colony losses in Europe possible. A specific attention is to be paid to case definition. Here are summarized recommendations arising from above definitions, requirement descriptions and discussions:

a. The projects to be submitted should comply with the core protocol and/or the proposed alternatives (as described in Table 2)
b. The design of surveillance strategy in each country requires a preliminary census or assessment of the total honeybee population in the country (procedures other than an accurate and exhaustive census may be proposed provided that they guarantee a representative sampling of the population)
c. Apiary health surveillance must be based on active standard procedures implemented by specifically trained personnel and involving at least three visits per year per selected apiary
d. Presence of pests, symptoms of diseases and syndromes should be noted through (1) clinical observation directly during apiary inspection visits, (2) identification of specific diseases/pathogens by national reference laboratories.
e. A systematic sampling of biological material must be planned at least during three visits per year and per hive under test. During the first visit, adult bees must be sampled and accordingly marked and preserved. Additionally, during the three visits, if disease symptoms are reported and/or presence of predator and/or non *Varroa* ectoparasite are reported (and recorded noted on apiary inspection forms). In parallel, complementary suspect material must be sampled to confirm presence (or otherwise) through specific laboratory diagnostic analyses.
f. An online and secure computer system should be developed by the consortium of future partners. Data should be managed (keyboarding and dataset

elaboration, statistical analyses, interpretation) through its use, in a strictly standardized manner.

g. An important emphasis within the project must be attributed to training activities of personnel involved in the project. In order to harmonize and standardize effective surveillance activities two coordinator trainers must be designated in each partner country

h. Important emphasis must be attributed to communication: annual reports based on yearly global analyses as well as specific feedback procedure making some data directly available to involved stakeholders should be clearly integrated into the project at the country level

i. Key Performance indicators and project milestones consisting of a limited number of variables brought together in chart form must be established. They must allow a continuous estimate of the achievement of the network's priority activities in order to facilitate its management.

j. Institutional organization relies upon a coordinator / external support group concept, at both the Europe and the country levels. The pilot surveillance project will be coordinated by the EU RL and each surveillance system in each country must designate a coordinator (person or team) and a clearly identified external consulting group.

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Annex I: Outlines of apiary inspection forms to be filled up by the person(s) in charge of apiary visits

Three different apiary inspection forms are to be filled up by the trained person in charge of the visit successively during the three prescribed visits. One apiary inspection form will be filled up per apiary and per visit. Note that each MS must be able to have enough persons in charge of keyboarding the data recorded in apiary inspection forms.

At the top of each apiary inspection form, unique ID numbers specific to the hives under examination must be clearly written. A standardized numbering system will be established and will have to be strictly applied for identification of each beekeeping farm, each apiary and each hive involved in the survey. The way the numbering incrementation will be managed is to be clearly established within each MS in order to strictly avoid any inconvenience such as redundant ID numbers. Note that every hive under examination needs to be clearly identified, in order to allow it to be individually followed during the three visits (Standardised labelling system will be proposed at the beginning of the programme).

Basic information on the beekeeping farm and on the apiary under examination will be collected in apiary inspection form 1. This encompasses beekeeping farm's location (geographical unit, agricultural environment), the kind of activity (professional, hobby, honey/pollen/royal jelly/other productions), bee races used in the beekeeping farm and the total number of apiaries and colonies at the moment when the apiary inspection form will be filled up, location of the selected apiary (including GPS coordinates), bee race used in the apiary under examination and environment characterization of the apiary. Concerning the latter, EU RL is working on suggestions which will be discussed with the consulting group to provide an easy notation in order to get some basic landscape information on the close environment of the apiary under examination (within a radius of 3,000 m around for instance). Such information will be also collected during the 3rd visit (apiary inspection form 3) in order to take some of changes experienced by transhumant apiaries into account (besides questions to the beekeeper about movements undergone by the apiary – see table below).

The following information will be collected in all of the three apiary inspection forms: date, technique and product used for control of *V. destructor* and other diseases, any treatment or

specific manipulations/events (transhumance, supplementary feedings, pollen trap, swarming, queen replacement….) performed on the hives, data for assessing the condition of the apiary under examination and clinical observations following a grid. More specifically, the evolution of the condition of apiary will be assessed taking into account the number of new colonies created from artificial swarm or by any other mean (colony bought and introduced) and the number of fused colonies. Estimation on the number of production colonies, weakened colonies, dead colonies in apiary inspection form 1, 2 and 3 will be assessed through a notation on a specified grid following the defined criteria. Concerning this specific point, criteria of estimation of the bee population/losses of brood and adults etc… will be discussed and decided with the consulting group in order to provide harmonized evaluations to be used by all MSs during the project.

Here is an overview of information and biological samples to be collected during each of the three visits:

Type of expected input	**Detailed input**	**Visit 1**	**Visit 2**	**Visit 3**
ID of targeted sample	Basic information on the beekeeping farm	x		
	Basic information on bees under test (bee race, queens' origin)	x		
	Basic information on the apiary under test	x		
	Any harbor or airport present in the vicinity of the apiary	x		x
	Sampled hives labels	x		
Ecological information associated with the apiary	Environmental characterization (modification observed in spring, transhumance)	x	x	x
Condition of the apiary under test	Estimation on the number of production hives, weakened hives (notation on a specified grid following defined criteria)	x	x	x
Condition of hives under test	Clinical observations	x	x	x
Biological material for laboratory analyses	From each labeled hive : one sample of house adult bees	x		
	From each hive with losses and / or symptoms: samples of adult bees/brood/parasite (mite/ beetles) depending on clinical observations	*	*	*
Undergone experiences from visit 1 to visit 3	Making-up: queen problems, possible storage of honeydew during winter, supplemental food, *Varroa* control, etc.			x
	Date and location of transhumance events between visit 1 and 3			x

x To be systematically collected

* To be collected if any bee or colony losses/symptom/parasite/predator observed

Annex II: Design of the sampling strategy

A. Number of apiaries to select

The number of apiaries to be included in the surveillance is necessarily determined on the basis of the population census on the one hand and of the expected prevalence of the phenomenon to be measured as well as the expected precision on the other hand.

Note that one must settle a prevalence percentage value as a prior, which does not need to stem from concrete studies. If the true prevalence is lower than the assumed one, the relative precision of the result will be lower with the same number of apiaries included. If the prevalence is higher, the relative precision will be better. If some prevalence was assessed through some objective study in some geographical units, one should be aware that the available data are not necessarily representative of the whole country to be explored. Considering a prevalence of 15%, an absolute precision of 5% (which means a confidence interval from 10% to 20% in the expected results) leads to a sampling of 193 apiaries per geographical unit with a census of 10000 apiaries. It has to be taken into account that too small samples will lead to wide confidence intervals that will reduce the power of any statistical comparison between regions or countries. For instance, for a similar census, decreasing sample size to 20 apiaries per geographical unit leads to a confidence interval from 0% to 30%. This means that any phenomenon with no occurrence detected will only be considered having a prevalence < 30%. It is important to keep in mind that decreasing sample size very quickly leads to widening confidence intervals. Besides, the number of apiaries to be investigated does not decrease so much as the census is smaller. For instance, if the estimated population size is 5000 instead of 10000 per unit, 189 apiaries must be examined for an absolute precision of 5% and an estimated prevalence of 15%, if population size is 400, required sample size is 132 apiaries, and so on. As a result, it is very important to evaluate the benefit of punctual money saving when decreasing sample size and to consider the balance between cost and expectable scientific input.

In short, in any case, **proposals for light touch alternatives must contain the following steps**:

1°) A reliable census of the apiary population should be provided (if not exhaustive, please discuss this issue and estimate the level of uncertainty in the population data set due to the incomplete nature of the census lists).

2°) Objectives in term of expected prevalence (default: 15%) and expected precision (default: 5%) must be clearly defined.

3°) The proposed sampling strategy.

Important note: If candidate teams do not involve any epidemiologist and need to establish lightened project on the sample size point of view, we strongly recommend that they ask for advices from some epidemiologist.

B. Number of colonies to examine per selected apiary

Because it is not possible to open all hives within all selected apiaries, it is necessary to determine the minimum number of hives to be inspected to reach an accurate representativeness of the apiary condition. In this aim, it is needed to define a limit expected prevalence rate for targeted diseases/syndroms in order to make the detection of the diseases/syndroms the most likely if present. Depending on the limit expected prevalence rate, an upper limit for the required number of hives to inspect may be assessed (see below tables).

In the pilot project, the default limit expected prevalence rate is set on 5%. Nevertheless, some light touch alternatives with higher limit expected prevalence rates may be discussed.

Numbers of hives to be inspected in order to detect a prevalence of 5%

Total number of hives within the selected apiary	up to 19	20	30	40	50	60	70	80	100	110	120	140	150	170	190	220	300	500
To be inspected	all	19	26	31	35	38	40	42	45	46	47	48	49	50	51	52	54	56

Numbers of hives to be inspected in order to detect a prevalence of 10%

Total number of hives within the selected apiary	up to 11	15	20	30	40	50	60	70	90	110	160	280	900
To be inspected	all	13	16	19	21	22	23	24	25	26	27	28	29

Numbers of hives to be inspected in order to detect a prevalence of 20%

Total number of hives within the selected apiary	up to 6	7	8	10	12	16	22	33	59	190	1000
To be inspected	all	6	7	8	9	10	11	12	13	14	14