



## A pan-European epidemiological study on honeybee colony losses 2012-2013

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## EXECUTIVE SUMMARY

For the first time, an active epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) has been implemented in 17 European member states. Each member state has developed a surveillance protocol based on guidelines produced by the European Reference Laboratory for honeybee health (EURL) to harmonize the surveillance procedures. EPILOBEE project was co-financed by the European Commission (grant of 3 307 803 €) and the member states taking part in the project.

To estimate the mortality of bee colonies (*Apis mellifera* L.) over the winter and during the beekeeping season, three visits were performed by bee inspectors: before winter 2012 (autumn), after winter (spring 2013) and during the beekeeping season (summer 2013). Farming practices and clinical manifestations of the main infectious and parasitic diseases were recorded through a detailed questionnaire resulting in the collection of a very substantial amount of data and samples. In case of disease suspicion, appropriate samples were taken from colonies for subsequent laboratory analyses. Between autumn 2012 and summer 2013, 31 832 colonies located in 3 284 apiaries were fully visited three times by 1 354 bee inspectors. Overall, 8 572 visits of apiaries were implemented in EPILOBEE.

The production of colony mortality rates and disease prevalence on a harmonized basis in all the member states taking part to EPILOBEE demonstrated that the methodology was fully implementable. Winter colony mortality rates ranged from 3.5 % to 33.6 % with a south-north geographical pattern. In Greece, Hungary, Italy, Lithuania, Slovakia and Spain over winter colony losses were below 10%. In Germany, France, Latvia Poland and Portugal mortality rates were between 10 and 15%. In Belgium, Denmark, Estonia, Finland, Sweden and the United Kingdom winter mortality rates were above 20%. Overall rates of seasonal colony mortality (during beekeeping season) were lower than winter mortality and ranged from 0.3% to 13.6%.

The overall prevalence of American foulbrood (AFB) was low in all the member states, ranging from 0 to 11.6%. The prevalence of European foulbrood (EFB) was even lower in the member states. Only five member states observed positive cases of EFB and the clinical prevalence exceeded 2% in only one member state. The varroosis was observed in nearly all the member states. *Varroa destructor* impact on honeybee colonies was also studied through the assessment of the parasitic pressure by sampling all the honeybee colonies before winter. Statistical analysis on this particular epidemiological risk factor will be implemented in the future. The apparent clinical prevalence of nosemosis exceeded 10% in four member states. No clinical case of nosemosis was observed in Denmark, Germany, Finland, Italy and Latvia. Only few clinical cases of paralysis due to the chronic bee paralysis virus were observed in five out of the 17 member states. Although 15 suspect arthropods were collected in seven member states during the first year of EPILOBEE, none of the suspect cases were confirmed for the presence of *Aethina tumida* (Small hive beetles, SHB) or *Tropilaelaps* mites. All these results show that EPILOBEE was a robust system achieving the collection of substantial information.

EPILOBEE project resulted in the compilation of a very substantial amount of data that will be further analysed. The data collected enabled the filling of a web based database. To date, 103 930 laboratory analyses were recorded in the database. Statistical treatments needed various steps of data cleaning in order to include as much information as possible in the analysis. Future data analyses will unquestionably explore the statistical links between the colony mortality and some risk factors including disease prevalence, use of veterinary treatments, the context of beekeeping and other parameters. Several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality. For example it should be remembered that the 2012-2013 winter has been particularly long and cold in many areas of Europe. Indeed EPILOBEE has proven to be a robust European baseline monitoring tool to be used for further work such as applied research, policy development or routine surveillance.

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## Glossary

<b>EPILOBEE</b>	Epidemiological study on honey bee colony losses
<b>EURL</b>	European Union reference laboratory
<b>ANSES</b>	National Agency for food, environmental and occupational health and safety
<b>AFB</b>	American foulbrood
<b>EFB</b>	European foulbrood
<b>CBPV</b>	Chronic bee paralysis virus
<b>SHB</b>	Small hive beetle

## 1. Context

It is known that numerous hazards threaten honeybee populations (*Apis mellifera*) world-wide. Many publications that looked into colony losses from any part of the world reported that several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality (Genersch et al. 2010, Henry et al. 2012, Vanengelsdorp et al. 2013). In the United States and Canada, alarming losses of honeybee colonies were recently reported (Vanengelsdorp et al. 2007, Vanengelsdorp et al. 2012). In Europe, the decrease in honeybee colonies was estimated at 16 % between 1985 and 2005, and the reduction of beekeepers at 31 % (Potts et al. 2010). European beekeeping statements have also provided worrying insights on difficulties in honeybee hive health sometimes accompanied with colony losses (Hendrikx et al. 2010). However, it was also described in recent reports that standardized surveillance systems were needed to accurately assess bee health in Europe (Hendrikx et al. 2010).

In this context, the European Commission asked the European Union Reference Laboratory (EURL) for honeybee health for technical assistance in setting up the first active epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) in Europe. Following the guidelines produced by the EURL<sup>1</sup>, European Member States answered a call launched by the European Commission. The programmes submitted by 17 member states of the European Union complied with the EURL guidelines: Belgium, Denmark, Germany, Estonia, Finland, France, Greece, Hungary, Italy, Latvia, Lithuania, Poland, Portugal, Slovakia, Spain, Sweden and the United Kingdom (European Commission 2012). For the first time, a harmonised active epidemiological surveillance programme on honeybee colony mortality based on randomly selected apiaries and colonies has been implemented.

The objective of the programme was to quantify the mortality of honeybee colonies in each participating member state on a harmonized basis and to estimate the health of the bee population through the assessment of disease prevalence and other information related to beekeeping practices. EPILOBEE also aimed at assisting the member states in undertaking technical and scientific measures for the development of EU veterinary legislation and national systems in the field of bee health, in particular by testing a specifically designed methodology for bee health surveillance and improve their capacity to plan, undertake and complete such surveillance.

This report presents only the results on mortality rates and on disease prevalence produced during the first year of the EPILOBEE programme from September 2012 to September 2013. EPILOBEE project resulted in the collection of a very substantial amount of data that will be further analysed. The data collected enabled the filling of a web based database. Future data analyses will unquestionably explore the statistical links within EPILOBEE data between the colony losses and some risk factors including disease prevalence, use of veterinary treatments, the context of beekeeping and others.

## 2. Protocol of the study

The EPILOBEE surveillance was designed to collect data on a representative sample of apiaries and colonies in each member state by the way of onsite investigations. Representativeness has been reached through a random sampling of apiaries in the entire member state or in some regions of the member state considered as representative of the member state's situation. The sampling frame was based on a two stage random sampling with apiaries as primary units and bee colonies as the secondary units. Beekeepers and apiaries were randomly selected in each member state from a national list of beekeepers as complete as possible (refer to guidelines for more precisions). Within each apiary, a number of colonies was randomly selected in order to be representative of the apiary. This number was designed based on the probability of detection of mortality and bee diseases. The sampling frame was similar for all the member states in order to have a harmonized basis for the

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<sup>1</sup> [http://ec.europa.eu/food/animal/liveanimals/bees/docs/annex\\_i\\_pilot\\_project\\_en.pdf](http://ec.europa.eu/food/animal/liveanimals/bees/docs/annex_i_pilot_project_en.pdf)

calculations. For detailed information on the sampling strategy, please refer to the EU guidelines or contact the EURL.

### **Surveillance protocol**

To estimate the mortality of bee colonies over the winter and during the beekeeping season, three visits were performed by bee inspectors: before winter 2012 (autumn 2012), after winter 2012 (spring 2013) and during the beekeeping season (summer 2013). Farming practices and clinical manifestations of the main infectious and parasitic diseases were recorded through a detailed questionnaire. Each selected colony was fully visited and examined.

The main honeybee diseases clinically investigated in the frame of this programme were the ones regulated (i.e. listed for notification or trade and import rules or for national eradication programmes) at the European level (European Commission 1992). The fungal disease Nosemosis, caused by the fungus *Nosema* spp., which has been described as of importance (Dussaubat et al. 2013, Higes et al. 2013) and covered by the OIE manual (OIE 2013) was also investigated. Spores of *Nosema* spp. ingested by bees induce infection. A heavy infection may deplete the colony and lead to the premature death of bees.

The main parasitic disease of honeybees, varroosis, was recorded based on clinical signs directly observed on colonies in the field. This parasitic infestation induces tremendous losses all over the world (Becher et al. 2013, Genersch et al. 2010) with some rare exceptions (Locke & Fries 2011). The *Varroa destructor* mite is a parasite of adult and brood bees. Subsequent to mite infestation, emerging adult bees show a shortened life span, changes in behaviour and increased disease susceptibility. To achieve the assessment of parasitic pressure on each colony, a systematic evaluation of the parasitic infestation by *V. destructor* mites was implemented by sampling 300 living honeybees during the first visit of the programme (Lee et al. 2010). The bees from each colony were shaken in sealed container. Mites were counted in each sample.

The two main diseases affecting brood, the American foulbrood (AFB) and the European foulbrood (EFB) caused by the bacteria *Paenibacillus larvae* and *Melissococcus plutonius* respectively, were also assessed. AFB is a highly contagious disease regulated worldwide by OIE and by European Union regulation. The spores produced by *P. larvae* induce the disease. Upon infection, the diseased larvae change colour, become ropy and eventually die. This can result in a spotty or mosaic brood pattern of empty cells (dead larvae removed by nurse bees), uncapped cells with remains of diseased larvae and healthy capped cells in the infected colonies. EFB is caused by *M. plutonius* and induces clinical signs on brood: the death of larvae shortly before being sealed in the cells resulting in a mosaic or spotty brood pattern.

A viral disease caused by the chronic bee paralysis virus (CBPV) was also surveyed. Moreover, this surveillance was an opportunity to confirm the absence of two arthropods exotic to Europe affecting honeybee colony elsewhere in the world: the small hive beetle *A. tumida* and the *Tropilaelaps* mites. These mites are parasites of honey bee brood. Feeding on bee larvae and pupae causes brood malformation and death of bees resulting in an irregular brood pattern. The small hive beetle is a parasite and scavenger of honey bee colonies. Adult beetles can cause colonies to abscond. Both adult and larvae beetles feed on honey bee brood, honey and pollen causing brood death, fermentation of honey and comb destruction.

Case definitions were provided by the EURL and discussed amongst the member states during several EPILOBEE workshops. In the protocol, the samples to be collected in case of suspected diseases were precisely described. For all the diseases when a colony exhibited clinical signs of a disease at any visit, affected brood and/or adult bees were sampled for subsequent laboratory analyses to enable disease confirmation. In this report, only the laboratory results obtained on the samples presenting clinical signs from the diseases listed above were reported.

Each member state organized the training of the bee inspectors on the basis of the documents provided by the EURL. Each member state was also in charge of the implementation of the visits in consistent periods of time for comparison purpose. It has to be acknowledged that this key step generated a remarkable work involving a lot of stakeholders belonging to different levels from the ministry to the field.

### ***Data collection and management***

During each visit, the bee inspectors completed a detailed questionnaire recording the farming practices and clinical observations. In addition, laboratory analyses were performed on samples collected during the visit. Data were all collected and stored in a standardized way at European level using an online database via a website developed by the EURL and the French epidemiological surveillance platform for Animal Health. Two member states were currently finishing entering the results of some laboratory analyses in the database at the time of writing. These data will be included in the statistical analysis in the future.

The statistical analyses were performed using a dedicated software (R software, version 3.0.2). Such an important programme recording 8 572 visits and more than 103 930 laboratory analyses induced the evident risk of error in the recorded data. Therefore, a cleaning step of the data to allow the control and the deletion of errors was necessary. Similarly, dedicated R algorithms were used to identify duplicates or nonsense data. The participating member states implemented a great work in correcting the data. Remaining incorrect and missing data were discarded for calculation.

### ***Calculation of the prevalence of the diseases at the apiary level***

The prevalence of the diseases was based on proportion of apiaries affected by a disease. An apiary was considered affected by a disease if at least one of its colonies showed clinical signs of the disease with the confirmation of the disease by a laboratory analysis.

### ***Calculation of the mortality rates at colony level***

The calculation of the mortality rates were reported to the size of the apiaries. This was necessary to have a correct estimation of the mortality at the population level based on the observed mortality on the sampled colonies. Hence the rate of affected honeybee colonies (i.e. colony mortality) was a *weighted average*, by the apiary size, of the affected honeybee colony rate of each apiary.

$$\hat{\theta} = \frac{\sum_{i=1}^n (M_i \cdot \hat{P}_i)}{\sum_{i=1}^n M_i}$$

P<sub>i</sub> was the proportion of colony affected in the apiary (i.e. number of colony affected divided by the number of colonies observed = colonies randomly selected) and M<sub>i</sub> was the size of the apiary (all the colonies of the apiary whether they were randomly selected or not).



### 3. Results

#### 3.1. Population sampled

Table 1: Number of randomly selected apiaries and colonies at the beginning of the programme in the 17 member states taking part to EPILOBEE 2012 – 2013.

	Number of apiaries visited during autumn 2012	Size of the apiaries visited during autumn 2012 (%)			Number of colonies visited during autumn 2012
		<50 colonies	[50-150]	>150 colonies	
<b>Belgium</b>	149	100	0	0	627
<b>Denmark</b>	202	100	0	0	1 394
<b>Germany</b>	223	97.8	2.2	0	1 988
<b>Estonia</b>	197	95.4	4.1	0.5	2 337
<b>Finland</b>	161	100	0	0	787
<b>France</b>	344	95.3	4.7	0	2 477
<b>Greece</b>	161	39.8	43.5	16.8	1 386
<b>Hungary</b>	197	50.8	37.6	11.7	3 934
<b>Italy</b>	184	77.2	19.6	3.3	1 682
<b>Latvia</b>	194	84	14.9	1	1 930
<b>Lithuania</b>	191	39.8	50.8	9.4	2 484
<b>Poland</b>	190	71.6	27.4	1.1	3 207
<b>Portugal</b>	146	96.6	3.4	0	437
<b>Slovakia</b>	190	82.1	15.8	2.1	3 199
<b>Spain</b>	204	40.7	56.4	2.9	2 321
<b>Sweden</b>	151	100	0	0	725
<b>United Kingdom</b>	200	100	0	0	917
<b>Total</b>	3 284	-	-	-	31 832
<b>Mean</b>	-	80.6	16.5	2.9	-

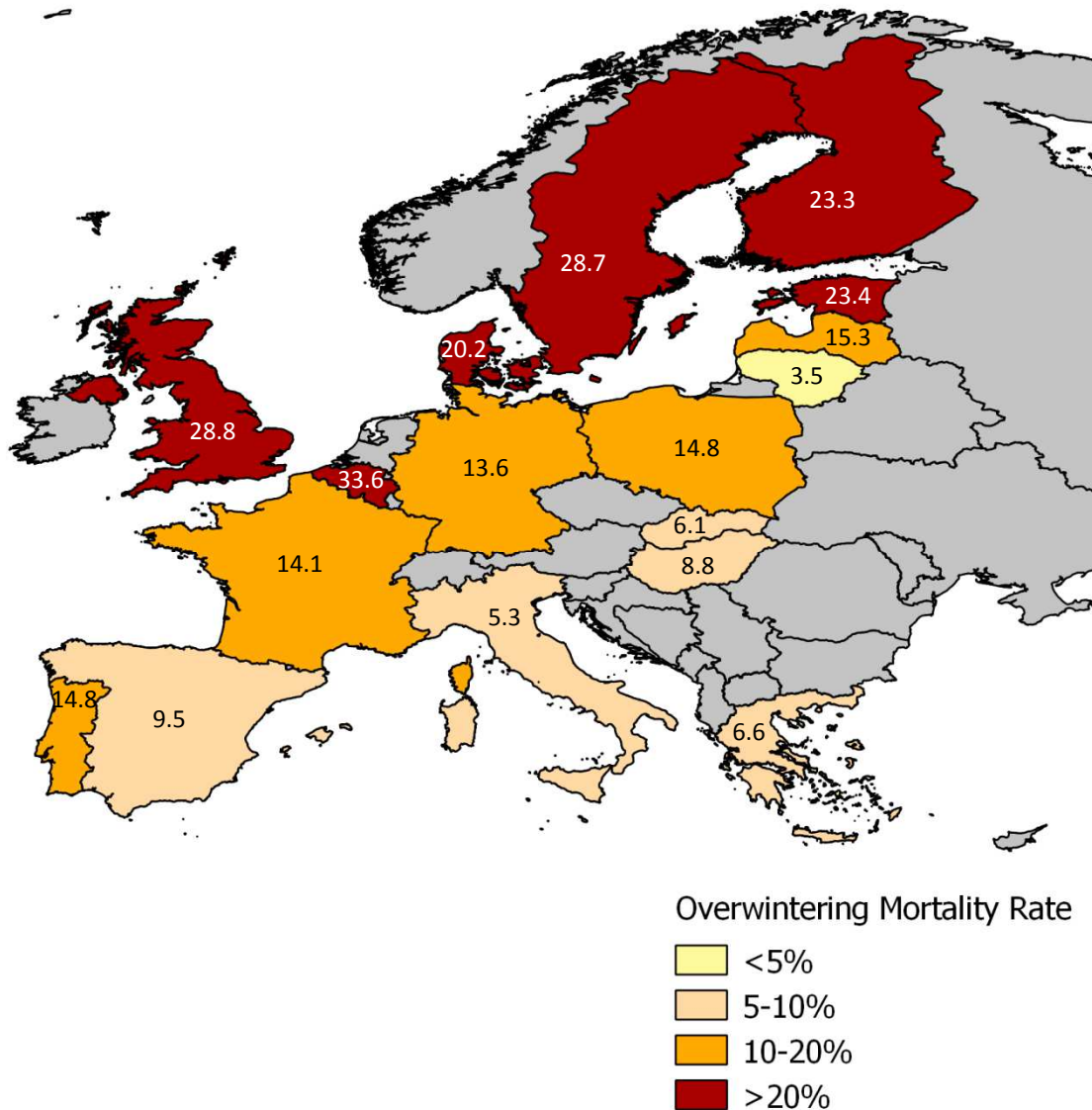
During this first year of EPILOBEE, 31 832 colonies located in 3 284 apiaries were visited before the winter 2012 – 2013. Overall during one year of program, more than 95 000 visits of colonies have been implemented by 1 354 bee inspectors in the 17 member states of the European Union.

Within the 3 284 apiaries randomly selected, the small apiaries (less than 50 colonies) were the most present in 16 member states representing from 39.8 % (Lithuania) to 100 % of the apiaries visited (Belgium, Denmark, Finland, Sweden and the United Kingdom). Large apiaries (more than 150 colonies) were the most present in Greece, and to a lesser extend in Hungary and Lithuania (16.8%, 11.7% and 9.4% of the apiary visited, respectively).

### 3.2. Mortality rates

#### Overwintering mortality (winter 2012 – 2013)

Figure 1: Winter mortality rates in the member states of the European Union recorded by EPILOBEE 2012 – 2013



Rates of colony mortality during winter ranged from 3.5% to 33.6% between the member states (Table 2). In 11 member states, this rate exceeded 10%. Most of the Northern European member states had overwintering mortality rates higher than 10% with the highest rate in Belgium (33.6%). The lowest rate of colony mortalities (3.5%) was recorded in Lithuania.

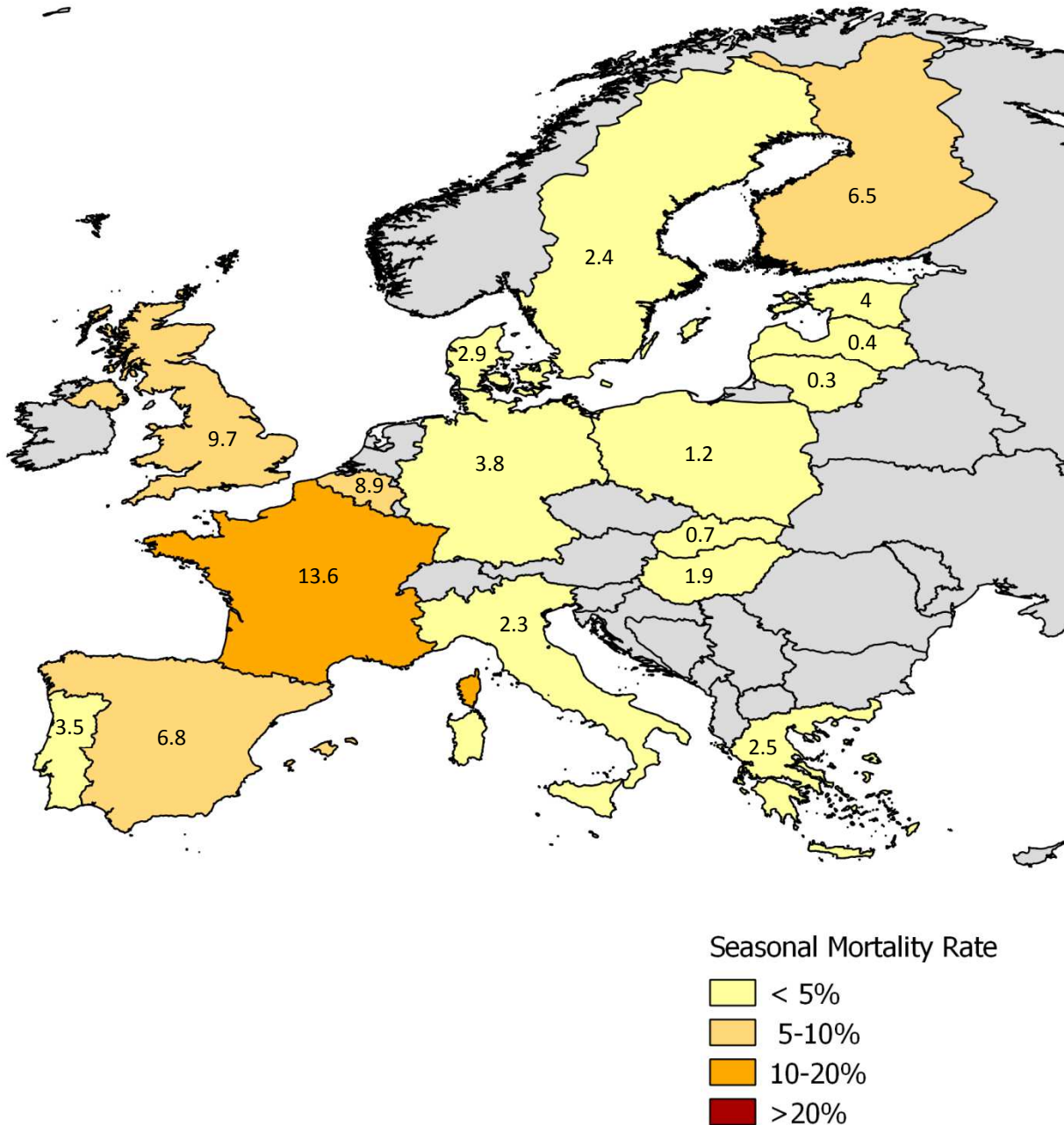
Table 2: Winter mortality rates in the member states of the European Union recorded by EPILOBEE 2012 – 2013;  
95% CI= confidence interval at 95%

	<b>Mortality rate (%)</b>	<b>95% CI inferior limit</b>	<b>95% CI superior limit</b>
<b>Belgium</b>	33.6	26.9	40.4
<b>Denmark</b>	20.2	15.9	24.4
<b>Germany</b>	13.6	10.7	16.5
<b>Estonia</b>	23.4	17.4	29.5
<b>Finland</b>	23.3	19.8	26.7
<b>France</b>	14.1	10.8	17.5
<b>Greece</b>	6.6	4.6	8.7
<b>Hungary</b>	8.8	6.3	11.2
<b>Italy</b>	5.3	3.4	7.1
<b>Latvia</b>	15.3	11.7	18.8
<b>Lithuania</b>	3.5	2.3	4.6
<b>Poland</b>	14.8	11.5	18.1
<b>Portugal</b>	14.8	10	19.6
<b>Slovakia</b>	6.1	3.5	8.8
<b>Spain</b>	9.5	7.2	11.9
<b>Sweden</b>	28.7	25	32.4
<b>United Kingdom</b>	28.8	24.5	33

The colony mortality rate of a member state was an estimation of the *real colony mortality rate* of this given member state. This estimated value was based on a representative sample of the honeybee population of this member state. Therefore, it was necessary to estimate a confidence interval in which the *real colony mortality rate* could be found with 95% probability. The confidence intervals are showed in Table 2.

*Seasonal mortality (spring-summer 2013)*

Figure 2: Seasonal mortality rates (2013) in the member states of the European Union recorded by EPILOBEE 2012 – 2013



Rates of colony mortality during the beekeeping season ranged from 0.3% to 13.6% (Table 3). The seasonal mortality rate was higher than 10% in France only. In 12 out of the 17 member states, the seasonal mortality rates were lower than 5%. Rates were between 5 and 10 % in Belgium, Finland, Spain and the United Kingdom.

Table 3: Seasonal mortality rates (2013) in the member states of the European Union recorded by EPILOBEE 2012 – 2013  
95% CI= confidence interval at 95%

	<b>Mortality rate (%)</b>	<b>95% CI inferior limit</b>	<b>95% CI superior limit</b>
<b>Belgium</b>	8.9	3.7	14.1
<b>Denmark</b>	2.9	1	4.7
<b>Germany</b>	3.8	1	6.6
<b>Estonia</b>	4	1.8	6.2
<b>Finland</b>	6.5	3.4	9.7
<b>France</b>	13.6	9.3	17.9
<b>Greece</b>	2.5	1.1	4
<b>Hungary</b>	1.9	0.5	3.2
<b>Italy</b>	2.3	1.1	3.6
<b>Latvia</b>	0.4	0.1	0.7
<b>Lithuania</b>	0.3	0	1
<b>Poland</b>	1.2	0.1	2.2
<b>Portugal</b>	3.5	0.2	6.9
<b>Slovakia</b>	0.7	0.1	1.3
<b>Spain</b>	6.8	4.9	8.8
<b>Sweden</b>	2.4	0.3	4.5
<b>United Kingdom</b>	9.7	6.8	12.5

### 3.3. Honeybee diseases

#### *Detection of the exotic arthropods SHB and Tropilaelaps mites*

These two arthropods have never been observed in Europe so far. EPILOBEE was a sound opportunity to increase the surveillance and the probability of detection of any signs of their presence in European apiaries. 15 suspect arthropods were collected in seven member states during the first year of EPILOBEE. The subsequent analysis did not identify any of the two exotic arthropods.

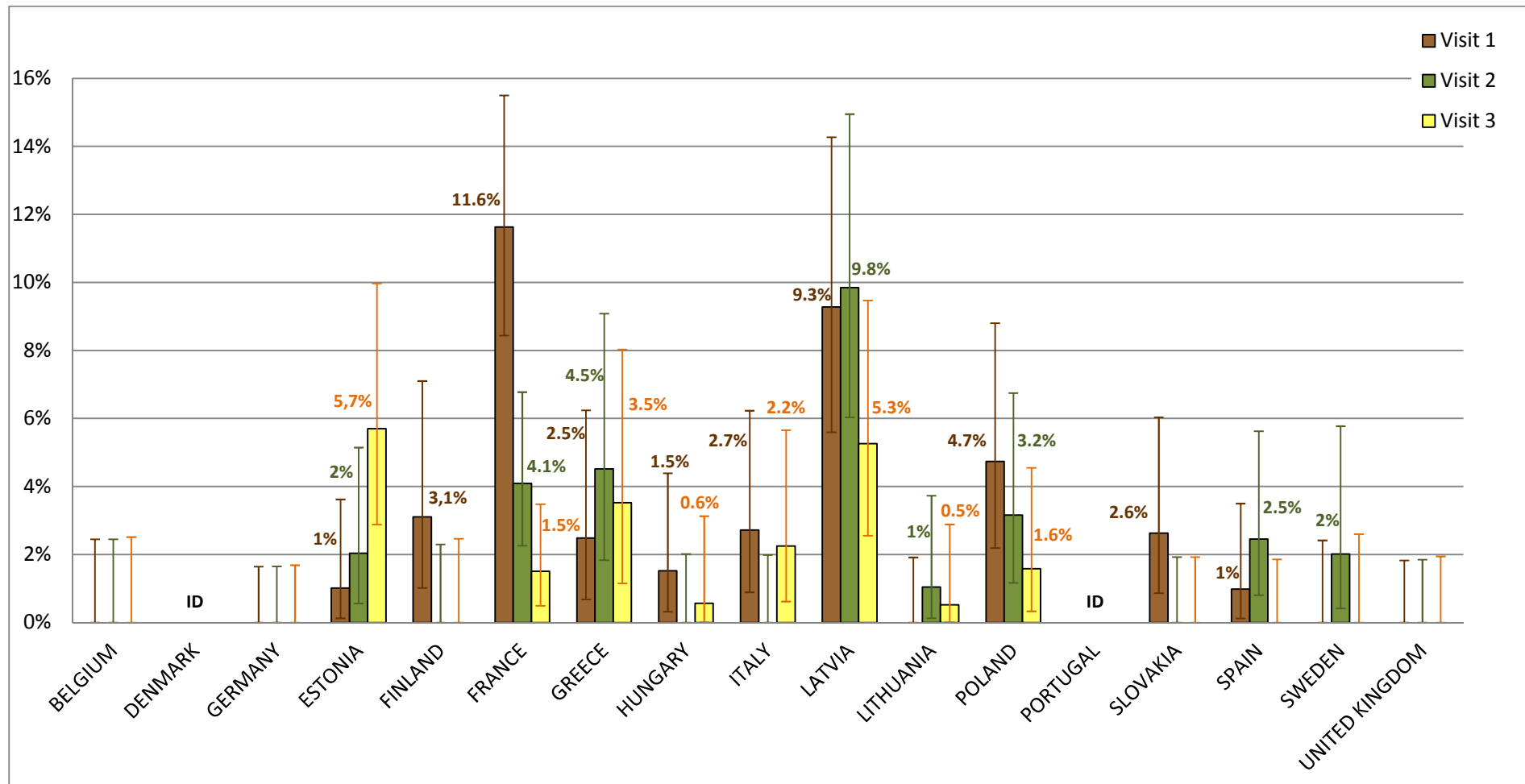
The minimum detectable prevalence of the two arthropods' presence was estimated using the number of apiaries randomly selected in each country (Table 4). The minimum detectable prevalence was very low (below 2%) in all the member states taking part to EPILOBEE. This gave a high level of confidence in the absence of these two arthropods in the member states.

Table 4: Minimum detectable prevalence for the presence of *A. tumida* and *Tropilaelaps* spp mites in the member states taking part to EPILOBEE 2012-2013.

	Number of apiaries visited during autumn 2012	Number of confirmed <i>A. tumida</i> or <i>Tropilaelaps</i> spp infestation	Minimum detectable prevalence in % (design prevalence at 95%)
<b>Belgium</b>	149	0	2,00
<b>Denmark</b>	202	0	1,48
<b>Germany</b>	223	0	1,34
<b>Estonia</b>	197	0	1,51
<b>Finland</b>	161	0	1,85
<b>France</b>	344	0	0,87
<b>Greece</b>	161	0	1,85
<b>Hungary</b>	197	0	1,51
<b>Italy</b>	184	0	1,62
<b>Latvia</b>	194	0	1,53
<b>Lithuania</b>	191	0	1,56
<b>Poland</b>	190	0	1,57
<b>Portugal</b>	146	0	2,04
<b>Slovakia</b>	190	0	1,57
<b>Spain</b>	204	0	1,46
<b>Sweden</b>	151	0	1,97
<b>United Kingdom</b>	200	0	1,49

### American foulbrood

Figure 3: Clinical prevalence of American foulbrood in the apiaries recorded during the three visits.  
ID= incomplete data



The overall prevalence of AFB was low in all the member states. In Belgium, Germany and the United Kingdom no positive case was observed during the three visits. The clinical prevalence exceeded 6 % in only two member states (France at autumn visit and Latvia at autumn and spring visits) out of the 15 member states. Two member states were not included in the analysis because of incomplete data in the database at the time of writing. These data will be included for further analysis in the future. In six member states, the clinical prevalence did not exceed 3 % at any visit (Figure 3 and Table 5 in Annex I).

### *European foulbrood*

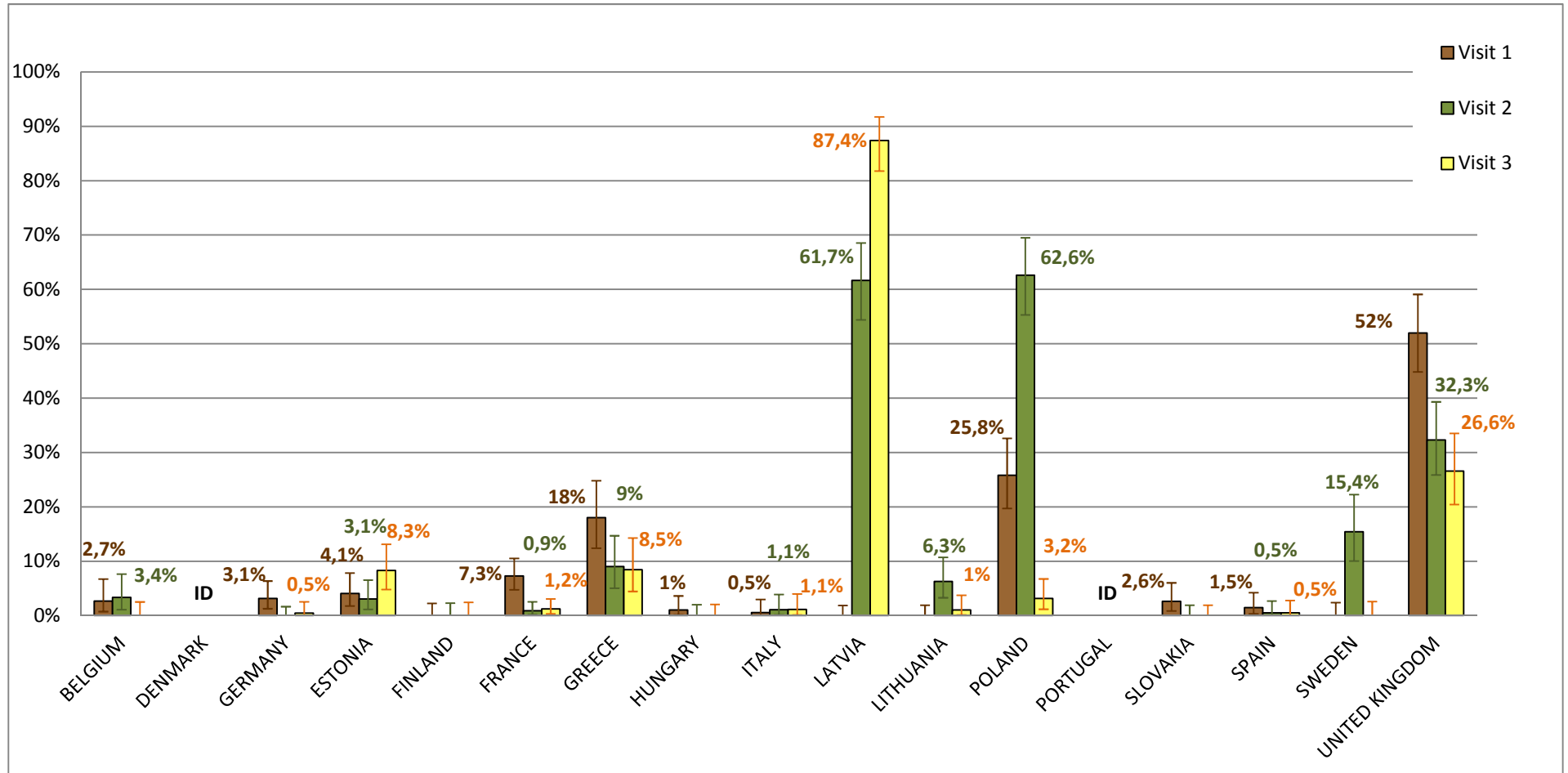
The prevalence of EFB was extremely low in the 15 the member states with complete data. Two member states needed to add some missing data in the database at the time of writing. Only five member states observed positive cases of EFB and the clinical prevalence exceeded 2% at any visit in only one member state (France).

In only two member states (France and the United Kingdom), clinical signs of EFB were observed at all visits. However it is worth to notice that the number of cases decreased with time in these member states (Table 6 and Figure 6 in Annex I and II).



## Varroosis

Figure 4: Clinical prevalence of varroosis in the apiaries recorded during the three visits  
ID= incomplete data

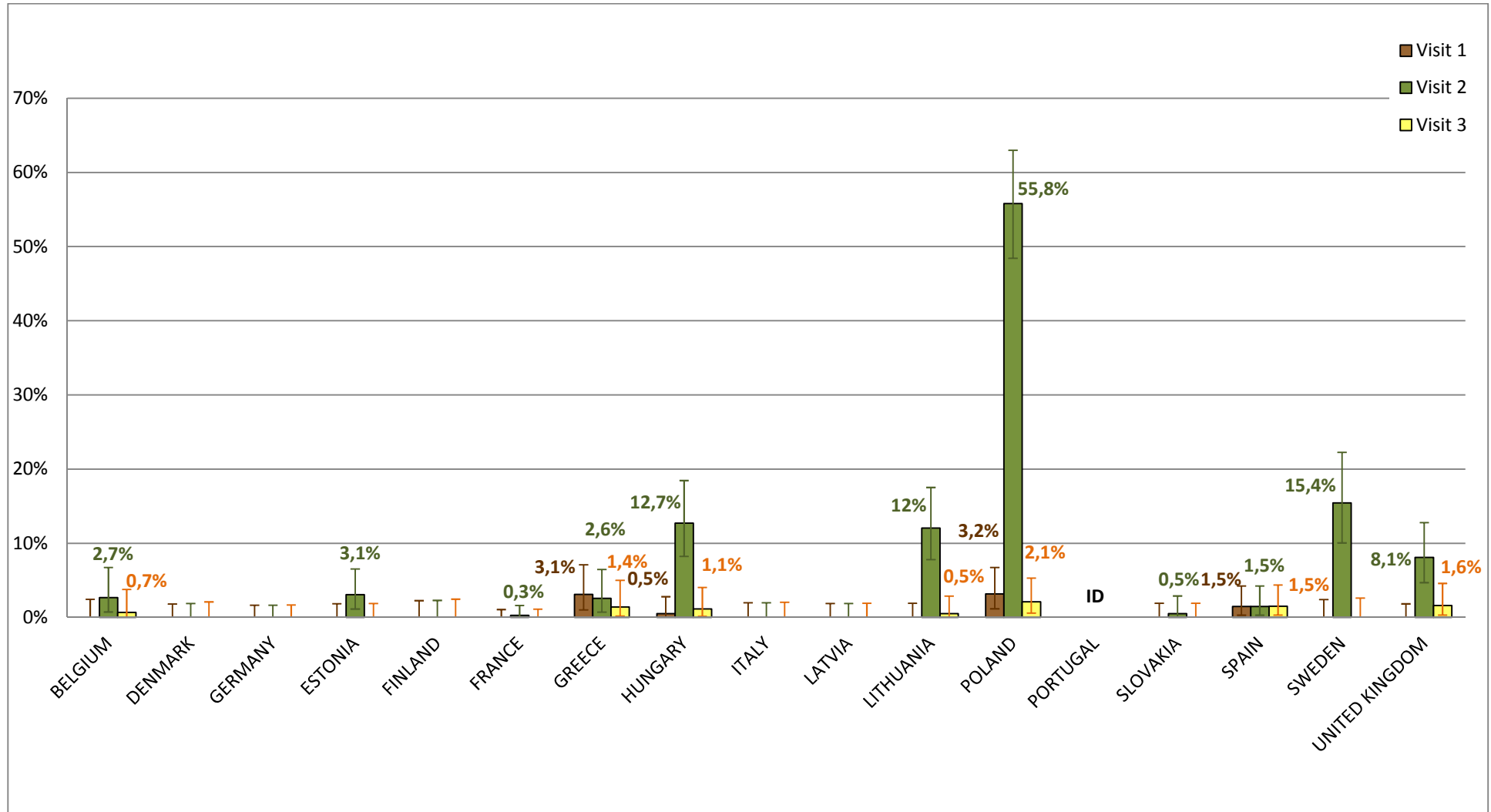


The varroosis was observed in nearly all the member states with complete data (14 out of 15 member states). The data of the two remaining member states will be analysed in the future. In Finland no positive case was observed. In six member states, the prevalence did not exceed 5% at any visit. The varroosis prevalence was higher than 15% in at least one visit in five member states, Greece, Latvia, Poland, Sweden and the United Kingdom (Figure 4 and Table 7 in Annex I).

It should be noted that the assessment of varroosis (the disease) was different from the assessment of the parasitic pressure by *V. destructor* mites. The evaluation of the parasitic pressure was systematically recorded in each colony at the visit performed in autumn 2012 by sampling living bees. Statistical analysis on this particular epidemiological risk factor will be implemented in the future.

## Nosemosis

Figure 5: Clinical prevalence of nosemosis in the apiaries recorded during the three visits  
ID= incomplete data



The prevalence of nosemosis exceeded 10% in four member states out of the 16 with complete data. One member state needed to enter some missing analyses in the database at the time of writing. These data will be analysed in the future. No positive case of nosemosis was observed in Denmark, Germany, Finland, Italy and Latvia. The prevalence increased at visit 2 (after winter 2012-2013) in nine member states reaching 55.8% in Poland (Figure 5 and Table 8 in Annex I). The positive cases reported in Figure 5 were based on analyses performed on living external bees and dead bees collected during the three visits and coming from colonies exhibiting clinical signs of nosemosis according to the case definition detailed in the surveillance protocol.

### **Paralysis**

Some clinical cases of paralysis were observed in five out of the 17 member states. The prevalence of CBPV did not exceed 4% at any visit in these five member states. The prevalence decreased between the visit 1 (before winter) and the visit 3 (during the season) in three member states, France, Italy and the United Kingdom (Table 9 and Figure 7 in Annex I and II).

## **4. Discussion**

Such a project required an enormous effort for the general coordination, the collaboration with the participating beekeepers, the filling and the maintenance of the central data base. It has to be acknowledged that an endless list of people joined their energy to make EPILOBEE a powerful tool for assessing honeybee health. This study was an unprecedented and successfully implemented project delivering scientifically solid representative data on honeybee health in the EU, which is statically comparable among member states.

### **Reliability and robustness of the protocol**

The standardization of the 17 national protocols made possible comparisons and joint statistical analysis of the data. The calculation of mortality rates in EPILOBEE, never used before, was weighted by the size of apiaries, as this could influence the mortality rates (vanderZee et al. 2012, Vanengelsdorp et al. 2008).

It should be remembered that EPILOBEE was a pilot project aiming at monitoring honeybee colony mortality at the European level on a harmonized basis. Even if the national protocols were standardized, the implementation of the protocols in the field was dependent of the particularities of each member states. Results of mortality rates and diseases prevalence of each participating country will be further analysed taking into account the specificities of each member state. Further standardization in the laboratory techniques and the sampling in the field should be required and will be implemented in the future.

### **Winter colony mortality rates**

As previously shown in other studies, the mortality rates varied within a large range (3.5% to 33.6%) (Spleen et al. 2013, vanderZee et al. 2012). These mortality rates should be compared to reference values in order to evaluate if the colony losses recorded in EPILOBEE were above the acceptable levels. It is quite difficult to find published historical values concerning colony losses not only in Europe but elsewhere in the world.

Prior to the introduction of varroa mites into the U.S., beekeepers reported 5–10% winter losses. These losses rose to 15–25% with the introduction of varroa and tracheal mites in the mid 1980s (Vanengelsdorp et al. 2008). In a book edited in 1968 in Belgium, it is stated that normal reported

winter mortality should be inferior to 10 % (Haubruge et al. 2006). In the 2000's, average colony mortality of 10% were considered acceptable for winter in Germany and Switzerland (Charrière & Neumann 2010, Genersch et al. 2010).

Acceptable mortality rates are currently higher when crossing the Atlantic toward USA and Canada. Interestingly, the Bee Informed Partnership program has explicitly explored this question for several years collecting the view of beekeepers (Vanengelsdorp et al. 2008). Acceptable rates of winter colony mortality varied with years (from 2007 to 2013) with a tendency to reduction. The acceptable rate in 2007 was 21.7% (Vanengelsdorp et al. 2008) and 13.7% in 2012 (Spleen et al. 2013). In Canada, 15% was the normal long-term winter mortality in 2010 (Head et al. 2010).

For the purpose of this report, the acceptable level of colony mortality has been considered to be lower than 10%. Under the term acceptable, we understand the level of mortality usually admitted in scientific papers as reasonable by European beekeepers under usual beekeeping conditions (Charrière & Neumann 2010, Genersch et al. 2010, Haubruge et al. 2006, Hendrikx et al. 2010). We acknowledge that this threshold can be discussed because in some areas beekeepers can experience lower or higher mortality rates considered bearable. Furthermore, in other parts of the world (i.e. Northern America) beekeepers and scientists accept higher levels of colony mortality (15%) (Head et al. 2010, Steinhauer et al. 2014).

Therefore to the European references, EPILOBEE winter mortality rates were below the acceptable threshold of 10 % in one third of the member states (Greece, Hungary, Italy, Lithuania, Slovakia and Spain). In all other member states, mortalities were higher than acceptable. In Germany, France, Latvia, Poland and Portugal, mortality rates were between 10 and 15%. In the last third of the member states (6 out of the 17 member states taking part to EPILOBEE – Belgium, Denmark, Estonia, Finland, Sweden and the United Kingdom), mortality rates were above the unacceptable threshold of 20%. If American standards were applied (threshold at 15%) unacceptable winter mortalities would be observed in Belgium, Denmark, Estonia, Finland, Sweden and the United Kingdom.

In order to shed a different light on the statistics, mortality rates were brought back to livestock numbers. Based on figures estimated by the National Reference Laboratories in 2010, provided to the EURL and recently published (Chauzat et al. 2013), member states with winter mortality rates lower than 10% covered about 6 500 000 colonies (ca 47.3% of the total number of colonies in the European Union). The proportion of colonies belonging to the category of member states with winter mortality rates between 10 and 15% represented ca 27.7% of the total number of colonies in EU. Member states with winter mortality rates higher than 20% represented ca 5.0 % of the total EU colonies (a bit more than 684 000 colonies). The member states which did not take part in EPILOBEE represented around 20% of the EU colonies.

When looking at the map, high rates of winter mortality were located in the Northern member states of the European Union suggesting a strong geographical influence probably due to climate. It should be remembered that the 2012-2013 winter has been particularly long and cold in Europe. The effect of long and cold winters on colony survival is well known in cold countries although it has not been accurately documented in scientific papers. Specific statistical analysis will be performed in the future to better study the spatial distribution of colonies losses. The COLOSS network recently published results on colony mortalities recorded through a questionnaire filled in by beekeepers during the winter 2012-2013 in 13 European member states (at most ca 30% of the total number of colonies in Europe). Average losses per country were not provided making the comparison difficult with EPILOBEE data. However in both studies, similar trends were observed in some member states with high losses (Denmark, Estonia, Finland and Sweden for example) or low losses (Lithuania and Slovakia for example) (vanderZee et al. 2014).

## **The sampling frame**

The sampling frame was dependant on the availability of beekeeper lists in the member states. In most member states it was difficult to have exhaustive and updated lists. Although an individual registration system existed for beekeepers in all European member states, this identification was compulsory in only some member states (Chauzat et al. 2013). The random method of selection of the apiaries and of the colonies ensured the representativeness of the data. The representativeness was not dependant on the number of inspected apiaries and colonies.

## **Regulatory context and diseases prevalence**

The production of colony mortality rates and disease prevalence in all the member states taking part to EPILOBEE demonstrated that the methodology was fully implementable.

Disease notification and intra-EU trade rules existed for some diseases at European level e.g. AFB. Eradication policy measures were decided at the national level. This was the case for AFB or EFB. Varroosis was also under national regulation in France, Hungary and Italy. In some cases drug treatments or destruction of affected colonies were required, leading to some biases in the calculation of prevalence (and incidence) and mortality rates. Similarly, beekeepers could have performed medical or technical treatments after a positive diagnosis following a visit. The improvement of health conditions in apiaries participating to a surveillance program has been already shown elsewhere (Chauzat et al. 2010).

For AFB, each national authorities implemented measures as soon as a case was confirmed. Consequently, after the first evidence of the disease, it could be expected that the prevalence of the disease rapidly decreased. Indeed in seven member states, the prevalence of AFB was lower at the last visit in summer 2013 compared to the prevalence at the first visit in autumn 2012. In five member states the prevalence increased during the year (Estonia, Greece, Lithuania, Spain and Sweden). In further statistical analysis, the incidence of the disease (i.e. new cases) will be investigated in detail.

Not surprisingly, cases of varroosis were observed in nearly all the member states. Indeed, varroa mites are present in all honeybee colonies in the EU and worldwide, with some rare exceptions. The wide variation in the prevalence between member states (from the min 0% to the max 87.4%) may come from a difference in the interpretation of the case definition although symptoms were detailed in the surveillance protocol.

The varroosis results were disconnected from the assessment of the parasitic pressure. The presence of mites was systematically recorded in each colony at the visit performed in autumn 2012. The sampling, analysis and result recording in the database caused a lot of work and questions to all the people working from the field to the laboratories. The EU Reference Laboratory acknowledges this massive effort. Future statistical analysis on this particular epidemiological risk factor will link the amount of mites present in the colonies in autumn to their subsequent survival to the winter.

Cases of nosemosis were reported in 11 member states, all located in Northern Europe. Data on nosemosis will be further statistically explored in the future in order to better know the risk posed to honeybee colonies by these pathogens.

## 5. Conclusions

For the first time a European wide program on honeybee health was implemented with epidemiological standardized methods. Mortality rates and disease prevalence were calculated according to a standardized method in all member states. The production of colony losses rates and disease prevalence in all member states taking part to EPILOBEE demonstrated that the methodology was fully feasible and can be implemented and repeated as necessary. EPILOBEE has proven to be a robust European baseline monitoring tool to be used for further work such as applied research, policy development or routine surveillance. It could also be used to cross-check the honeybee data with data received from other sources.

Rates of colony mortality differed widely from one member state to another with significant regional differences. Prevalence of diseases, based on clinical signs observed by bee inspectors, was very low for most of the diseases targeted. American foulbrood and varroosis were recorded in most of the member states. EPILOBEE has generated a very substantial amount of data that will be further statistically investigated. Future data analyses will unquestionably explore the statistical links between the colony losses and some risk factors including disease prevalence, use of veterinary treatments, the context of beekeeping and others parameters.

It should be remembered that the 2012-2013 winter has been particularly long and cold in many areas of Europe. Several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality. The ongoing follow-up of the surveillance on a European scale in 2013-2014 will allow to monitor the evolution of the mortality rates with trends and clusters, disease prevalence and further balance the role of contextual (such as weather conditions) and other risk factors.

## 6. Acknowledgements

This project has been funded by the European Commission, ANSES through the EURL for bee health and each member state participating to EPILOBEE. This program involved thousands of different stakeholders: beekeepers, scientists, laboratories and administrations etc... The EURL for bee health wishes to thank all participants for their substantial involvement in the successful implementation of this programme.

Mike Brown, Franco Mutinelli, Per Kryger and Peter Rosenkranz provided useful remarks and expertise along the EPILOBEE program through the EpiTeam.

## Annex I Tables presenting clinical prevalence of diseases in the apiaries recorded during the three visits

Table 5: Clinical prevalence of AFB in the apiaries recorded during the three visits  
95% CI= confidence interval at 95%; ID= incomplete data

	Visit 1 before winter		Visit 2 after winter		Visit 3 during season	
	%	95% CI	%	95% CI	%	95% CI
<b>Belgium</b>	0	0 – 2.4	0	0 – 2.4	0	0 – 2.5
<b>Denmark</b>	ID	ID	ID	ID	ID	ID
<b>Germany</b>	0	0 – 1.6	0	0 – 1.6	0	0 – 1.7
<b>Estonia</b>	1	0.1 – 3.6	2	0.6 – 5.1	5.7	2.9 – 10
<b>Finland</b>	3.1	1 – 7.1	0	0 – 2.3	0	0 – 2.5
<b>France</b>	11.6	8.4 – 15.5	4.1	2.3 – 6.8	1.5	0.5 – 3.5
<b>Greece</b>	2.5	0.7 – 6.2	4.5	1.8 – 9.1	3.5	1.2 – 8
<b>Hungary</b>	1.5	0.3 – 4.4	0	0 – 2	0.6	0 – 3.1
<b>Italy</b>	2.7	0.9 – 6.2	0	0 – 2	2.2	0.6 – 5.7
<b>Latvia</b>	9.3	5.6 – 14.3	9.8	6 – 14.9	5.3	2.6 – 9.5
<b>Lithuania</b>	0	0 – 1.9	1	0.1 – 3.7	0.5	0 – 2.9
<b>Poland</b>	4.7	2.2 – 8.8	3.2	1.2 – 6.7	1.6	0.3 – 4.5
<b>Portugal</b>	ID	ID	ID	ID	ID	ID
<b>Slovakia</b>	2.6	0.9 – 6	0	0 – 1.9	0	0 – 1.9
<b>Spain</b>	1	0.1 – 3.5	2.5	0.8 – 5.6	0	0 – 1.9
<b>Sweden</b>	0	0 – 2.4	2	0.4 – 5.8	0	0 – 2.6
<b>United Kingdom</b>	0	0 – 1.8	0	0 – 1.8	0	0 – 1.9

Table 6: Clinical prevalence of EFB in the apiaries recorded during the three visits  
95% CI= confidence interval at 95%; ID= incomplete data

	Visit 1 before winter		Visit 2 after winter		Visit 3 during season	
	%	95% CI	%	95% CI	%	95% CI
<b>Belgium</b>	0	0 – 2.4	0	0 – 2.4	0	0 – 2.5
<b>Denmark</b>	ID	ID	ID	ID	ID	ID
<b>Germany</b>	0	0 – 1.6	0	0 – 1.6	0	0 – 1.7
<b>Estonia</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Finland</b>	0	0 – 2.3	0.6	0 – 3.5	0	0 – 2.5
<b>France</b>	7.6	5 – 10.9	6.4	4.1 – 9.6	3.6	1.9 – 6.2
<b>Greece</b>	0	0 – 2.3	0	0 – 2.4	0	0 – 2.6
<b>Hungary</b>	0	0 – 1.9	0	0 – 2	0	0 – 2.1
<b>Italy</b>	0	0 – 2	1.1	0.1 – 3.9	0	0 – 2.1
<b>Latvia</b>	0	0 – 1.9	0.5	0 – 2.9	0	0 – 1.9
<b>Lithuania</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Poland</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Portugal</b>	ID	ID	ID	ID	ID	ID
<b>Slovakia</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Spain</b>	0	0 – 1.8	0	0 – 1.8	0	0 – 1.9
<b>Sweden</b>	0	0 – 2.4	0	0 – 2.4	0	0 – 2.6
<b>United Kingdom</b>	1.5	0.3 – 4.3	1	0.1 – 3.6	0.5	0 – 2.9



Table 7: Clinical prevalence of varroosis in the apiaries recorded during the three visits  
95% CI= confidence interval at 95%; ID= incomplete data

	Visit 1 before winter		Visit 2 after winter		Visit 3 during season	
	%	95% CI	%	95% CI	%	95% CI
<b>Belgium</b>	2.7	0.7 – 6.7	3.4	1.1 – 7.7	0	0 – 2.5
<b>Denmark</b>	ID	ID	ID	ID	ID	ID
<b>Germany</b>	3.1	1.3 – 6.4	0	0 – 1.6	0.5	0 – 2.5
<b>Estonia</b>	4.1	1.8 – 7.8	3.1	1.1 – 6.5	8.3	4.8 – 13.1
<b>Finland</b>	0	0 – 2.3	0	0 – 2.3	0	0 – 2.5
<b>France</b>	7.3	4.8 – 10.5	0.9	0.2 – 2.5	1.2	0.3 – 3.1
<b>Greece</b>	18	12.4 – 24.8	9	5 – 14.7	8.5	4.4 – 14.3
<b>Hungary</b>	1	0.1 – 3.6	0	0 – 2	0	0 – 2.1
<b>Italy</b>	0.5	0 – 3	1.1	0.1 – 3.9	1.1	0.1 – 4
<b>Latvia</b>	0	0 – 1.9	61.7	54.4 – 68.5	87.4	81.8 – 91.7
<b>Lithuania</b>	0	0 – 1.9	6.3	3.3 – 10.7	1	0.1 – 3.7
<b>Poland</b>	25.8	19.7 – 32.6	62.6	55.3 – 69.5	3.2	1.2 – 6.7
<b>Portugal</b>	ID	ID	ID	ID	ID	ID
<b>Slovakia</b>	2.6	0.9 – 6	0	0 – 1.9	0	0 – 1.9
<b>Spain</b>	1.5	0.3 – 4.2	0.5	0 – 2.7	0.5	0 – 2.8
<b>Sweden</b>	0	0 – 2.4	15.4	10 – 22.3	0	0 – 2.6
<b>United Kingdom</b>	52	44.8 – 59.1	32.3	25.9 – 39.3	26.6	20.4 – 33.5

Table 8: Clinical prevalence of nosemosis in the apiaries recorded during the three visits  
95% CI= confidence interval at 95%; ID= incomplete data

	Visit 1 before winter		Visit 2 after winter		Visit 3 during season	
	%	95% CI	%	95% CI	%	95% CI
<b>Belgium</b>	0	0 – 2.4	2.7	0.7 – 6.7	0.7	0 – 3.8
<b>Denmark</b>	0	0 – 1.8	0	0 – 1.9	0	0 – 2.1
<b>Germany</b>	0	0 – 1.6	0	0 – 1.6	0	0 – 1.7
<b>Estonia</b>	0	0 – 1.9	3.1	1.1 – 6.5	0	0 – 1.9
<b>Finland</b>	0	0 – 2.3	0	0 – 2.3	0	0 – 2.5
<b>France</b>	0	0 – 1.1	0.3	0 – 1.6	0	0 – 1.1
<b>Greece</b>	3.1	1 – 7.1	2.6	0.7 – 6.5	1.4	0.2 – 5
<b>Hungary</b>	0.5	0 – 2.8	12.7	8.2 – 18.5	1.1	0.1 – 4
<b>Italy</b>	0	0 – 2	0	0 – 2	0	0 – 2.1
<b>Latvia</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Lithuania</b>	0	0 – 1.9	12	7.8 – 17.5	0.5	0 – 2.9
<b>Poland</b>	3.2	1.2 – 6.7	55.8	48.4 – 63	2.1	0.6 – 5.3
<b>Portugal</b>	ID	ID	ID	ID	ID	ID
<b>Slovakia</b>	0	0 – 1.9	0.5	0 – 2.9	0	0 – 1.9
<b>Spain</b>	1.5	0.3 – 4.2	1.5	0.3 – 4.2	1.5	0.3 – 4.4
<b>Sweden</b>	0	0 – 2.4	15.4	10 – 22.3	0	0 – 2.6
<b>United Kingdom</b>	0	0 – 1.8	8.1	4.7 – 12.8	1.6	0.3 – 4.6

Table 9: Clinical prevalence of CBPV in the apiaries recorded during the three visits  
95% CI= confidence interval at 95%; ID= incomplete data

	Visit 1 before winter		Visit 2 after winter		Visit 3 during season	
	%	95% CI	%	95% CI	%	95% CI
<b>Belgium</b>	0	0 – 2.4	0	0 – 2.4	0	0 – 2.5
<b>Denmark</b>	0	0 – 1.8	0	0 – 1.9	0	0 – 2.1
<b>Germany</b>	0	0 – 1.6	0	0 – 1.6	0	0 – 1.7
<b>Estonia</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Finland</b>	0	0 – 2.3	0	0 – 2.3	0	0 – 2.5
<b>France</b>	2.6	1.2 – 4.9	1.2	0.3 – 3	1.5	0.5 – 3.5
<b>Greece</b>	0	0 – 2.3	0	0 – 2.4	0	0 – 2.6
<b>Hungary</b>	0	0 – 1.9	0	0 – 2	0	0 – 2.1
<b>Italy</b>	1.1	0.1 – 3.9	1.6	0.3 – 4.7	0.6	0 – 3.1
<b>Latvia</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Lithuania</b>	0	0 – 1.9	2.6	0.9 – 6	0	0 – 1.9
<b>Poland</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Portugal</b>	0	0 – 2.5	0	0 – 3.1	0	0 – 2.8
<b>Slovakia</b>	0	0 – 1.9	0	0 – 1.9	0	0 – 1.9
<b>Spain</b>	0.5	0 – 2.7	1	0.1 – 3.5	0.5	0 – 2.8
<b>Sweden</b>	0	0 – 2.4	0	0 – 2.4	0	0 – 2.6
<b>United Kingdom</b>	2.5	0.8 – 5.7	4	1.8 – 7.8	1.6	0.3 – 4.6

**Annex II Figures presenting clinical prevalence of diseases in the apiaries recorded during the three visits**

Figure 6: Clinical prevalence of EFB in the apiaries recorded during the three visits  
ID= incomplete data

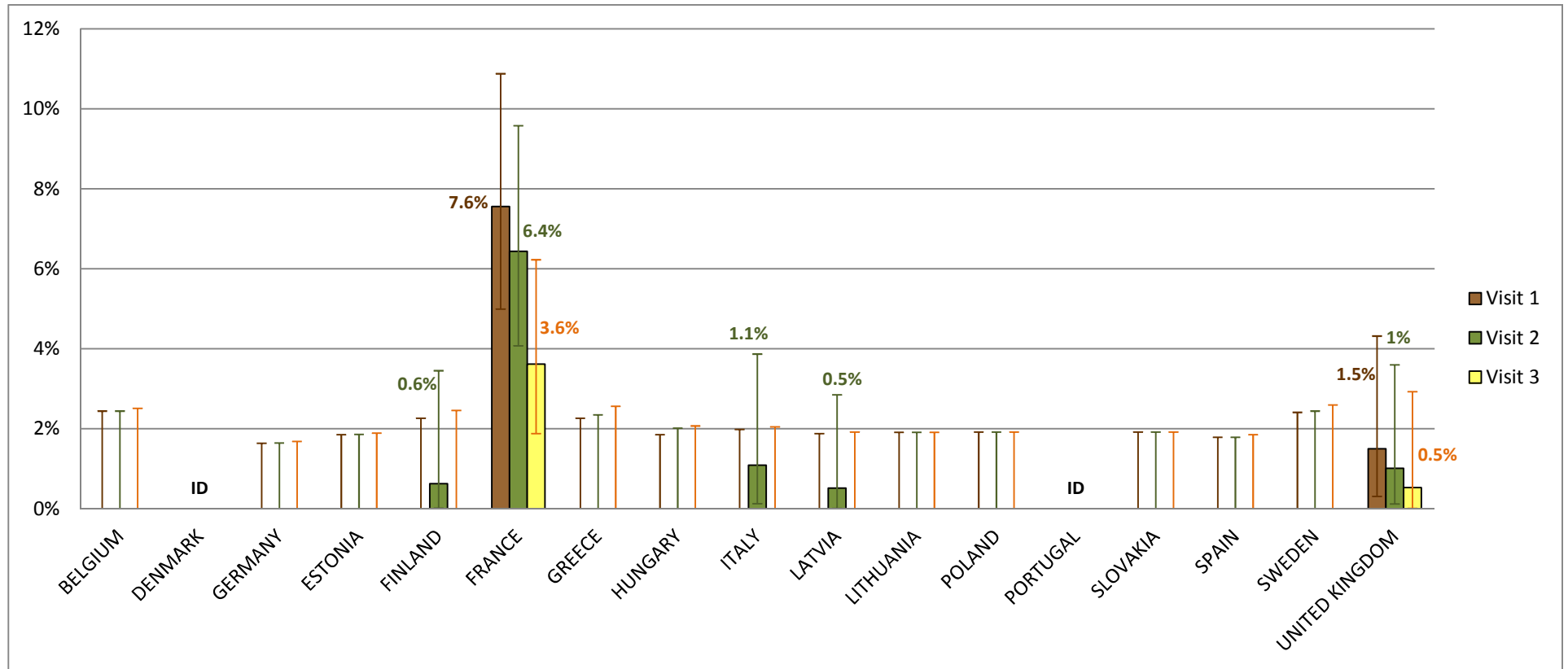
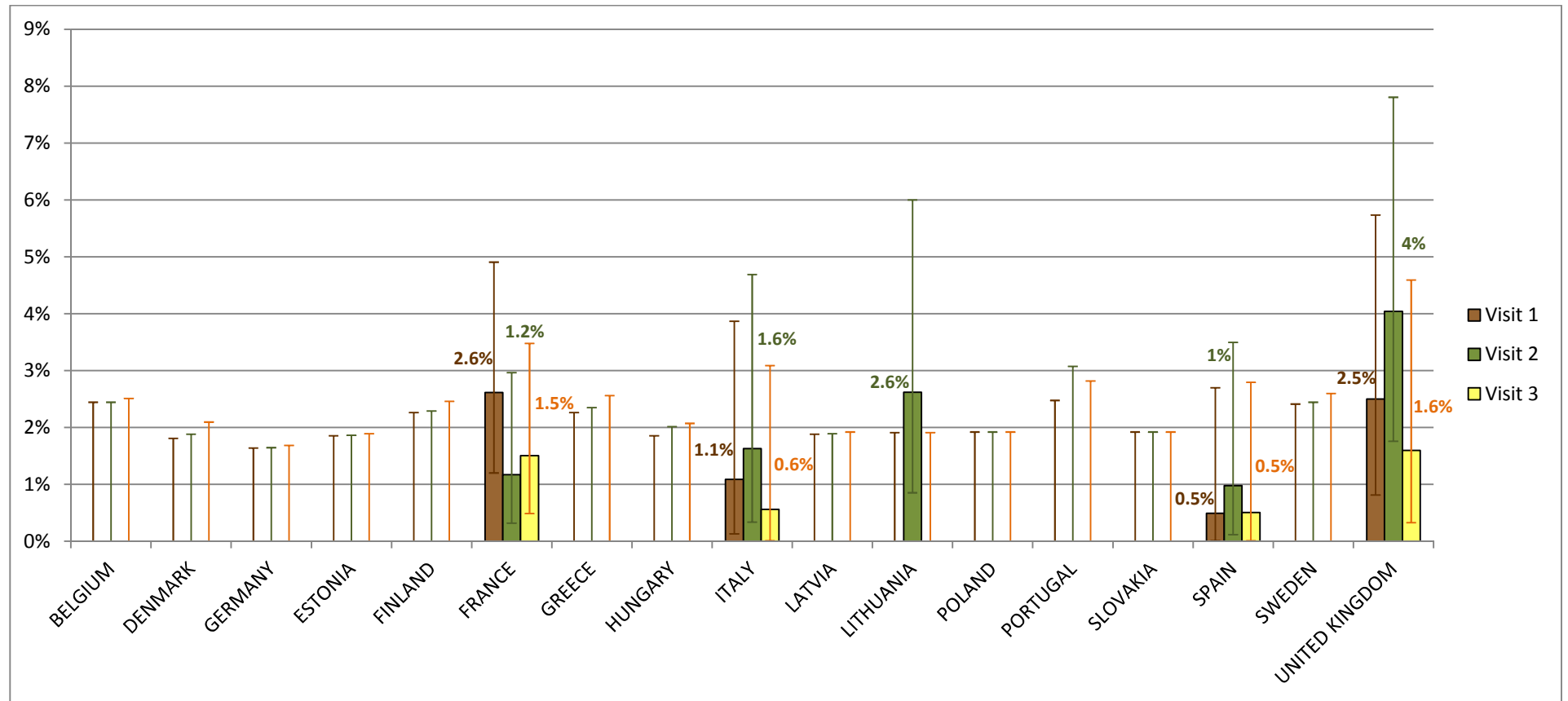


Figure 7: Clinical prevalence of CBPV in the apiaries recorded during the three visits  
 ID= incomplete data



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