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The first pan-European epidemiological study on honeybee colony losses (2012-2014) revealed winter colony losses up to 32.4% and seasonal colony losses up to 11.1%

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Abstract

For the first time, a harmonised active epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) was set up in 17 European Union Member States for two consecutive years. The national protocols were based on guidelines issued by the European Union Reference Laboratory for Honeybee Health (EURL). The objective of the two-year programme was to obtain an overall picture of honeybee colony losses on a harmonised basis in each of the participating Member States.

Winter colony mortality rates ranged from 3.2% to 32.4% and from 2.4% to 15.4% during the first and the second year of the programme. Rates of seasonal colony mortality (2013) ranging from 0.02% to 10.2% did not drastically change during the second year of the programme in 15 of the 16 Member States taking part in EPILOBEE for two years.

This programme was a descriptive epidemiological study enabling the collection of official and comparable data on honeybee health over two years with a methodology that was feasible and repeatable. The outcomes of EPILOBEE are an essential prerequisite to the implementation of future explanatory studies investigating the potential causes of honeybee colony losses such as pesticides and their possible interactions with pathogens or other stress factors.

Keywords

- ★ Epidemiological data
- ★ European Union
- ★ Honey bee
- ★ mortality
- ★ surveillance programme

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** see Table 6.

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Introduction

Over the years, honeybee health has become a major concern. Many publications that have looked into colony losses in any part of the world have reported that several biological and environmental factors acting alone or in combination have the potential to cause 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 reported (Vanengelsdorp *et al.*, 2007, Vanengelsdorp *et al.*, 2009). 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 reports have also provided worrying insights on the difficulties facing honeybee hive health, sometimes accompanied by colony losses (Hendriks *et al.*, 2010). However, it has also been described that standardised surveillance systems are needed to accurately assess bee health in Europe (Hendriks *et al.*, 2010).

To document this phenomenon, a consortium was set up in 2009 following a call launched from EFSA to assess existing surveillance systems and to collate and analyse the data related to honeybee colony mortality across Europe. In the conclusions of the report “Bee mortality and bee surveillance in Europe”, the weakness of the surveillance systems implemented in the European Union was highlighted as well as the lack of comparable data on colony losses. It was concluded that a common operational system to assess honeybee colony mortality at the European level was needed. The recommendations of the report pointed out the need to develop and enhance standardised EU surveillance systems to accurately assess bee health in Europe (Hendriks *et al.*, 2010).

In this context, the European Commission requested harmonised and comparable data at the European level. A call was launched following the guidelines issued by the EURL. The first harmonised active epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) was set up for two years in September 2012 with 17 and 16 European Union Member States participating for the first and second year, respectively. The objective of the two-year programme was to quantify the mortality of honeybee colonies on a harmonised basis in each participating Member State. Simultaneously, the main honeybee infectious and parasitic diseases were investigated based on case definitions and a sampling protocol provided by the EURL to assess honeybee colony health. Information related to beekeeping practices (treatments administered, livestock management), the beekeeper (training, experience in beekeeping), and the environment around the apiaries was also recorded.

Methods

Study design

The EPILOBEE surveillance programme was implemented over two consecutive years (September 2012 to September 2014). It was designed to collect data on a representative sample of apiaries and colonies in each participating Member State through harmonised onsite investigations and a sampling framework. The sampling framework was based on two-stage random sampling with apiaries as primary units and bee colonies as secondary units. Representativeness was reached through a random sampling of apiaries implemented by each Member State either in the entire Member State or in some regions of the Member State considered as representative of the Member State's situation. Beekeepers and apiaries were randomly selected in each Member State from a national list of beekeepers that was as complete as possible. Within each apiary, the number of tested colonies was randomly selected according to the probability of detection of mortality and bee diseases. A total of 17 Member States participated in the programme during the first year, and 16 in the second year (Table 1). About one third of the beekeepers were renewed during the second year, to avoid the population under study being different from the general population. New beekeepers were selected with the same methodology as the one selected during the previous year.



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TABLE 1/ Number of randomly selected apiaries and colonies during the first visits of the two years of the programme in the Member States taking part in EPILOBEE.*England and Wales are reported as one Member State, taking part in the 2012-2013 project only.*

	Number of apiaries visited during		Size of the apiaries visited during autumn 2013 (%) ¹			Number of colonies inspected during ¹	
	Autumn 2012	Autumn 2013	<50 colonies	[50-150]	>150 colonies	Autumn 2012	Autumn 2013
Belgium	149	150	100	0	0	624	644
Denmark	203	212	100	0	0	1,393	1,243
Estonia	197	196	91.3	8.7	0	2,337	1,616
Finland	161	161	100	0	0	787	682
France	343	350	93.7 ²	6.0 ²	0.3 ²	2,265	2,331 ⁶
Germany	223	217	99.1 ³	0.9 ³	0 ³	1,971	1,879
Greece	162	67	40.3	46.3	13.4	2,639	1,060
Hungary	197	185	45.1 ⁴	40.8 ⁴	14.1 ⁴	3,936	3,810
Italy	184	166	79.4 ⁵	17.6 ⁵	3 ⁵	1,969	1,849 ⁷
Latvia	194	190	90	8.4	1.6	1,937	1,918
Lithuania	191	163	51.5	44.8	3.7	2,483	2,061
Poland	190	190	73.2	24.2	2.6	3,207	3,147
Portugal	147	145	95.2	4.8	0	778	865
Slovakia	190	198	88.4	11.1	0.5	3,199	3,036
Spain	204	190	43.7	54.7	1.6	2,325	2,157
Sweden	151	150	100	0	0	730	758
England and Wales	200	-	-	-	-	891	-
Total	3 286	2 930				33 471	29 056
Mean			80.7	16.8	2.5		

1. Unless otherwise stated below, the rates (%) and numbers of colonies inspected were calculated on the number of apiaries visited in autumn 2013

2. The calculation was based on 331 apiaries

3. The calculation was based on 210 apiaries

4. The calculation was based on 184 apiaries

5. The calculation was based on 165 apiaries

6. The calculation was based on 333 apiaries

7. The calculation was based on 163 apiaries

Surveillance protocol

Three visits were performed by bee inspectors each year: before winter (2012 and 2013), after winter (spring 2013 and 2014) and during the beekeeping season (summer 2013 and 2014). Farming practices, description of the environment and clinical signs of the main infectious and parasitic diseases were recorded through a detailed questionnaire. Samples were taken if necessary for further laboratory analyses. Each selected colony was thoroughly inspected and examined.

Each Member State organised the training of the bee inspectors on the basis of the documents provided by the EURL. Each Member State was also in charge of implementation of the visits in consistent periods of time for comparison purposes.

It is important to acknowledge that remarkable work involving many different stakeholders belonging to different levels, from the ministry to the field, was carried out during the two years of

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EPILOBEE, producing an extensive set of data that was as reliable as possible. Particularly, a huge effort was required regarding the data validation (for details see the report produced by Jacques *et al.* 2016).

Data collection and management

The overall information collected can be found in the EPILOBEE reports published on the European Commission website (Laurent *et al.*, 2015). The questionnaire filled in by the bee inspectors was refined and clarified for the second year of EPILOBEE thanks to feedback from the field. Some questions were added for the second year (*e.g.* the record of colony strength) whereas others were removed (*e.g.* location of the migration, name of all the treatments applied in colonies). These modifications improved the forms without compromising the data collected and their comparison throughout the two-year programme. Data were stored in a standardised way in an online European database via a website developed by the EURL and the French Platform for epidemiological surveillance in Animal Health.

The descriptive analyses were performed using R software (version 3.1.0). Due to the size of the database (9,566 apiary visits and 117,269 laboratory analyses the first year, and 8,580 apiary visits and 49,626 laboratory analyses the second year), a data cleaning step was necessary to identify recording errors. Dedicated R algorithms were used to identify duplicates or nonsense data and incorrect or missing data were discarded from the calculation (Chauzat *et al.*, 2016).

Calculation of mortality rates at the colony level

The calculation of mortality rates was related to the size of the apiaries. Hence, the rate of affected honeybee colonies (*i.e.* colony mortality θ) was a weighted average, by the apiary size, of the affected honeybee colony rate of each apiary, and calculated as follows:

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

where P_i was the proportion of colonies affected in the apiary (*i.e.* number of affected colonies divided by the number of observed colonies) and M_i was the size of the apiary (*i.e.* all the colonies of the apiary whether they were randomly selected or not).



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Results

Rates of winter colony mortality from EPILOBEE 2012 – 2013 ranged from 3.2% to 32.4% (Figure 1a and Table 2). In 12 Member States, this rate exceeded 10%. Most of the Northern European Member States had winter mortality rates higher than 10% with the highest rate in Belgium (32.4%). The lowest rate of colony mortalities (3.2%) was recorded in Lithuania.

FIGURE 1 / Winter colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2012 – 2013 (a) and EPILOBEE 2013 – 2014 (b)

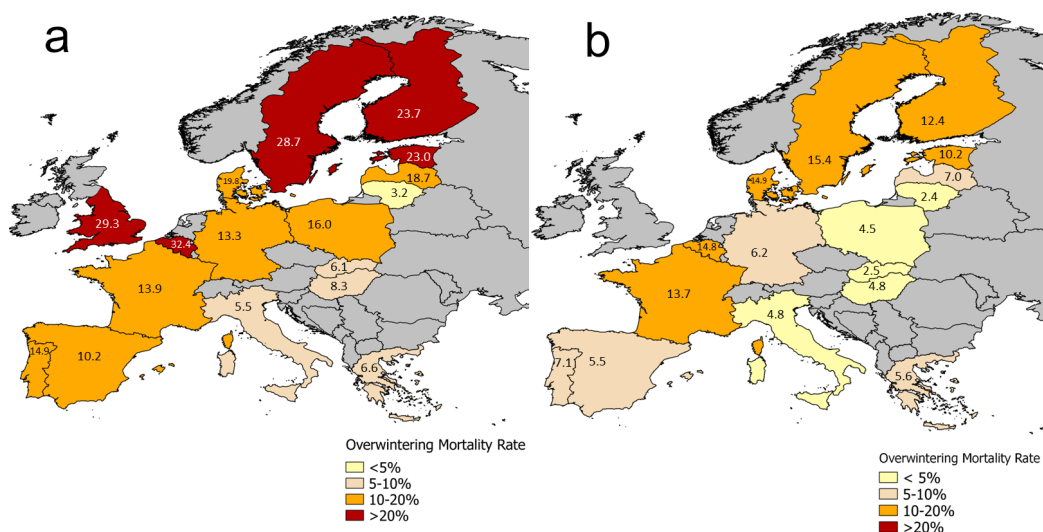


TABLE 2 / Winter mortality rates in the Member States of the European Union recorded in EPILOBEE 2012–2013

	Mortality rate (%)	95% CI ¹ lower limit	95% CI ¹ upper limit
Belgium	32.4	25.4	39.3
Denmark	19.8	15.6	23.9
Estonia	23.0	16.9	29.1
Finland	23.7	19.2	28.1
France	13.9	11.0	16.8
Germany	13.3	10.3	16.4
Greece	6.6	4.5	8.6
Hungary	8.3	5.8	10.8
Italy	5.5	3.6	7.5
Latvia	18.7	14.7	22.7
Lithuania	3.2	1.8	4.7
Poland	16.0	12.4	19.6
Portugal	14.9	10.0	19.7
Slovakia	6.1	3.5	8.8
Spain	10.2	7.8	12.5
Sweden	28.7	24.8	32.6
England & Wales	29.3	24.9	33.7

1. 95% CI = confidence interval at 95%

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Rates of winter colony mortality (2013-2014) ranged between the Member States from 2.4% to 15.4% (Figure 1b and Table 3). The winter colony mortality rates exceeded 10% in six Member States. In five of the 16 Member States, the winter colony mortality rates were lower than 5%. In each Member State, the winter 2013-2014 colony mortality rates were lower than the rates estimated during winter 2012-2013; none of the rates were over 20% (Figure 1).

TABLE 3 / Winter colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2013–2014

	Mortality rate			
	%	Difference between the two years ²	95% CI ¹ lower limit	95% CI ¹ upper limit
Belgium	14.8	↓	11.4	18.3
Denmark	14.9	→	10.9	18.8
Estonia	10.2	↓	7.4	13.0
Finland	12.4	↓	9.3	15.4
France	13.7	→	8.3	19.0
Germany	6.2	↓	3.2	9.1
Greece	5.6	→	0.3	10.9
Hungary	4.8	→	3.4	6.2
Italy	4.8	→	2.3	7.3
Latvia	7.0	↓	5.0	9.0
Lithuania	2.4	→	0.5	4.3
Poland	4.5	↓	2.8	6.1
Portugal	7.1	↓	4.5	9.6
Slovakia	2.5	→	1.4	3.5
Spain	5.5	↓	3.9	7.2
Sweden	15.4	↓	10.7	20.1

1. 95% CI = confidence interval at 95%

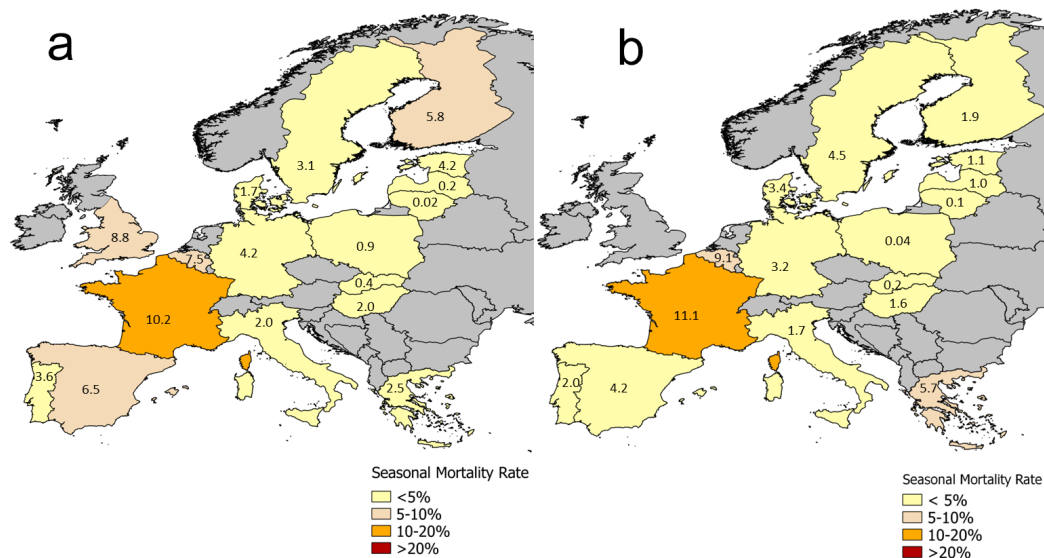
2. ↓: statistical difference between the two years towards a decrease;

→: no statistical difference between the two years

However, it should be noticed that these rates were estimates of the real winter colony mortality rates based on representative samples of the honeybee population in each Member State. The confidence intervals in which the real colony mortality rates could be found with 95% probability were calculated (Table 2 and Table 3). For seven Member States (Denmark, France, Greece, Hungary, Italy, Lithuania and Slovakia), the winter colony mortality rates were not statistically different between the two consecutive years since confidence intervals overlapped. Conversely, winter colony mortality rates decreased statistically during the second year for nine Member States.

Rates of seasonal colony mortality (2013) ranged from 0.02% to 10.2% (Figure 2a and Table 4). The seasonal mortality rate was higher than 10% only in France. The seasonal mortality rates were lower than 5% for 12 of the 17 Member States. Rates were between 5 and 10% in Belgium, Finland, Spain and the United Kingdom (England and Wales).

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FIGURE 2 / Seasonal colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2012 – 2013 (a) and EPILOBEE 2013 – 2014 (b)**TABLE 4 /** Seasonal mortality rates (2013) in the Member States of the European Union recorded in EPILOBEE 2012–2013

	Mortality rate (%)	95% CI ¹ lower limit	95% CI ¹ upper limit
Belgium	7.5	2.5	12.5
Denmark	1.7	0.2	3.1
Estonia	4.2	1.5	6.9
Finland	5.8	2.8	8.9
France	10.2	5.9	14.4
Germany	4.2	0.9	7.4
Greece	2.5	1.0	3.9
Hungary	2.0	0.6	3.5
Italy	2.0	0.5	3.5
Latvia	0.2	0	0.5
Lithuania	0.02	0	0.1
Poland	0.9	0.2	1.6
Portugal	3.6	0.2	7.0
Slovakia	0.4	0.1	0.8
Spain	6.5	4.4	8.5
Sweden	3.1	0.1	6.0
England & Wales	8.8	5.7	11.9

1. 95% CI = confidence interval at 95%

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TABLE 5 / Seasonal mortality rates (2014) in the Member States of the European Union recorded in EPILOBEE 2013–2014

	Mortality rate			
	%	Difference between the two years ²	95% CI ¹ lower limit	95% CI ¹ upper limit
Belgium	9.1	→	4.6	13.6
Denmark	3.4	→	2.1	4.7
Estonia	1.1	→	0.2	1.9
Finland	1.9	→	0.8	3.0
France	11.1	→	4.7	17.6
Germany	3.2	→	1.7	4.7
Greece	5.7	→	0	12.9
Hungary	1.6	→	0.7	2.4
Italy	1.7	→	0.7	2.8
Latvia	1.0	→	0	2.1
Lithuania	0.1	→	0	0.3
Poland	0.04	↓	0	0.1
Portugal	2.0	→	0.9	3.2
Slovakia	0.2	→	0.1	0.4
Spain	4.2	→	2.9	5.5
Sweden	4.5	→	2.1	6.9

1. 95% CI = confidence interval at 95%

2. ↓: statistical difference between the two years towards a decrease;

→: no statistical difference between the two years

Rates of seasonal colony mortality (2014) ranged from 0.04% to 11.1% (Figure 2b and Table 5). Seasonal colony mortality rates were below 5% in 13 Member States. The rate was over 10% only in France. The mortality rate during the 2014 beekeeping season was lower than the rate estimated during the 2013 beekeeping season for nine of the 16 Member States (Figure 2). Conversely, an increase in the seasonal colony mortality rate was observed during the second year for seven Member States (Belgium, Denmark, France, Greece, Latvia, Lithuania and Sweden). The confidence intervals in which the real seasonal colony mortality rates (2014) could be found with 95% probability overlapped with the confidence intervals calculated for the 2013 beekeeping season in 15 of the 16 Member States (Table 4 and Table 5). This means that seasonal colony mortality was statistically different from one year to the other in only one case (Poland), towards a decrease.

Discussion

Reliability and robustness of the protocol

This two-year active surveillance was implemented on a harmonised basis in 17 Member States for the first year and in 16 Member States for the second year, thus allowing comparisons between Member States and joint statistical analyses.

More than 90% of the apiaries randomly selected at the beginning of each year of the pro-

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gramme were monitored throughout each entire year. Given the scale of the programme, this high rate of follow-up shows the great involvement of all the stakeholders in each Member State and emphasises the feasibility and repeatability of EPILOBEE.

Winter colony mortality rates

As discussed previously (Chauzat *et al.*, 2014), no reference values are available for the acceptable level of colony losses during winter. Different winter colony losses have been reported in European countries (Charrière and Neumann 2010, Genersch *et al.*, 2010) and outside Europe (Vanengelsdorp *et al.*, 2008, Head *et al.*, 2010, Spleen *et al.*, 2013, Traynor *et al.*, 2016). For the purpose of the study, honeybee colony mortality of 10% during winter was empirically considered acceptable by the EURL. However, this threshold is debatable, since higher mortality rates can be considered as bearable by beekeepers and scientists.

During the second year of EPILOBEE, winter colony mortality rates were over the acceptable threshold of 10% in one third of the Member States (Belgium, Denmark, Estonia, Finland, France and Sweden). A south-north geographical pattern could be observed. Ten Member States had winter colony mortality rates lower than 10%, which correspond to 64.5% (8,931,600 colonies) of the total estimated number of colonies in the European Union in 2011 (Chauzat *et al.*, 2013). In contrast, Member States with winter colony mortality rates higher than 10% represented 13.2% (1,831,075 colonies) of the total estimated number of colonies in the European Union in 2011. The Member States that did not take part in EPILOBEE represented around 22.3% of the EU colonies (data from 2011).

The mortality rates for winter 2013 – 2014 showed a narrower range (2.4% to 15.4%) than the mortality rates observed during the winter 2012 – 2013. The decrease in winter colony mortality rates over these two years is noticeable. However, this should be interpreted with caution. The confidence intervals in which the real winter honeybee colony mortality rates can be found overlapped for Denmark, France, Greece, Hungary, Italy, Lithuania and Slovakia, meaning that the drop of the winter colony losses for 2013 – 2014 was not statistically significant for these Member States. Conversely, the winter colony mortality rates decreased statistically between the two years for nine Member States (Belgium, Estonia, Finland, Germany, Latvia, Poland, Portugal, Spain and Sweden).

The comparison of the confidence intervals for the seasonal mortality rates did not show any statistical difference between the two years for all Member States, with the exception of Poland for which the seasonal colony mortality rate decreased statistically during the 2014 beekeeping season.

It is known that climate strongly influences winter colony losses but other risk factors may also play a role. Specific statistical analyses have been conducted to explore statistical links between the colony losses and other information collected over the two years (health of the colonies, management of the apiary, use of veterinary treatments, environment) (Chauzat *et al.*, 2016, Jacques *et al.*, 2016). Therefore, there is a need for a holistic assessment of colony health, taking also the environment around the colony into account.

Sustainable outcomes

The first major outcome of this programme was the collection of representative and comparable data on honeybee colony mortality on a harmonised basis in the Member States taking part in EPILOBEE. In addition, this two-year programme enabled enhancement of the general European honeybee colony surveillance structure, methodology and capability of veterinary services, which most probably led, as a consequence, to better management of the European apiculture sector. EPILOBEE allowed the implementation of monitoring tools that did not exist to this extent in Europe prior to the programme. National surveillance systems also benefited from this experience in the field of bee health.



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Furthermore, it has been shown that communication, particularly between beekeepers and veterinary services, increased during EPILOBEE and was a positive outcome of the programme. Some beekeepers participating in the two years of EPILOBEE may have benefited from the successive visits leading to an improvement of management practices and health conditions in the apiaries. The data collected during the two consecutive years for these beekeepers are under study.

Harmonisation of the training of bee inspectors set up in each Member States on sampling, observation and interpretation of clinical signs and detection of exotic arthropods in Europe were key factors to EPILOBEE success. The programme was a good opportunity to increase awareness among beekeepers taking part in EPILOBEE concerning the detection of clinical signs associated with the main parasitic and infectious diseases affecting honeybees.

Perspectives of the EPILOBEE programme

Representative and comparative data on honeybee health were collected over these two years, showing that the methodology implemented in EPILOBEE was feasible and repeatable. However, the methodology was adapted in each Member State taking into account their specificities. The specific diversity in data collection has been included in the statistical analyses. Further harmonisation of national procedures could be implemented at the European level by taking into account the specific characteristics of each Member State highlighted during EPILOBEE. EPILOBEE has shown that harmonisation of sampling protocols and field training is fundamental to collect comparable and robust data. During this programme, a large set of data was collected, requiring significant data management, edition and data mining. Since the programme was originally designed for fewer Member States than finally involved, it might have been necessary to reduce the extent of data collected to better adapt to the size of the project and thus ease overall data management. EPILOBEE was the essential first step for the recording of honeybee mortality and health status at a European scale through a descriptive surveillance programme. However, these two years should be prolonged in order to obtain a significant collection of data on colony mortality that could then be considered a baseline for future studies. For instance, during EPILOBEE, winter 2013-2014 was relatively warmer and shorter than winter 2012-2013, which was particularly long and cold throughout Europe. These two winters were opposite in terms of weather, showing the importance of long-term follow-up.

This descriptive programme, EPILOBEE, was a successful first step that will facilitate future implementation of projects (e.g. explanatory studies) examining other risk factors affecting colony health. For example, the study of potential causes such as pesticides, pathological agents, and food intake either on their own or in combination, could be integrated into future explanatory studies, such as case-control studies, in order to explore their role in honeybee colony mortality. These epidemiological projects require the joint commitment of all stakeholders and planned action strategies.

Acknowledgements

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Mike Brown, Per Kryger, Franco Mutinelli, Marc Schäfer and Sophie Roelandt provided useful remarks and expertise during EPILOBEE through the EpiTeam.

The list of people who took part in this project under the name EPILOBEE Consortium is provided in Table 6.



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TABLE 6 / The EPILOBEE Consortium

Country	Name	Institutional affiliations
Belgium	De Graaf D.	Ghent University, Department of Physiology, Laboratory of Zoophysiology
	Méroc E.	NRL for honeybee diseases CODA-CERVA-VAR
	Nguyen B.K.	Ulg, Faculté Gembloux Agro-Bio Tech
	Roelandt S.	NRL for honeybee diseases CODA-CERVA-VAR
	Roels S.	NRL for honeybee diseases CODA-CERVA-VAR
	Van der Stede Y.	NRL for honeybee diseases CODA-CERVA-VAR
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	Kryger P.	
Estonia	Jaarma K.	
	Kuus M.	Estonian Veterinary and Food Board
	Raie A.	
Finland	Heinikainen S.	
	Pelkonen S.	EVIRA, Veterinary Bacteriology Research Unit, Kuopio
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	Ballis A.	Chambre d'Agriculture du Haut-Rhin
	Barrieu G.	DDPP des Bouches du Rhône
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	Layec Y.	Groupement de Défense Sanitaire Apicole du Finistère
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	Mariau V.	DDPP Indre et Loire
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	Mutel S.	DDCSPP Haut-Rhin
	Oesterle E.	Groupement de Défense Sanitaire Indre et Loire
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	Petit M.	DDPP Finistère
	Pillu P.	DDPP du Cantal
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Schroeder A.	University of Hohenheim, Apicultural State Institute, Stuttgart	



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Country	Name	Institutional affiliations
Greece	Agianiotaki E.	Centre of Veterinary Institutes of Athens
	Arfara S.	Centre of Veterinary Institutes of Athens
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	Hondrou V.	Regional Veterinary Laboratory of Mytilini
	Karipidou S.	Regional Veterinary Laboratory of Kozani
	KatsarosD.	Regional Veterinary Laboratory of Chalkis
	Katzagiannakis A.	Regional Veterinary Laboratory of Heraclio
	Kiriakopoulos A.	Regional Veterinary Laboratory of Mytilini
	Oureilidis K.	Regional Veterinary Laboratory of Kavala
	Panteli A.	Centre of Veterinary Institutes of Athens
	Pantoleon F.	Regional Veterinary Laboratory of Tripoli
	Papagianni Z.	Centre of Veterinary Institutes of Athens
	Papalexiou E.	Centre of Veterinary Institutes of Athens
	Perdikaris S.	Ministry of Rural Development and Food
	Prapas A.	Centre of Veterinary Institutes of Athens
	Siana P.	Regional Veterinary Laboratory of Tripoli
	Skandalakis I.	Regional Veterinary Laboratory of Chania
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