A pan-European epidemiological study on honeybee colony losses 2012-2014

This report has been prepared by

Marion LAURENT, Pascal HENDRIKX, Magali RIBIERE-CHABERT and Marie-Pierre CHAUZAT

on behalf of the EPILOBEE consortium (see next page).

European Union Reference Laboratory for honeybee health (EURL)
Anses
Honeybee pathology Unit
105 route des Chappes – CS 20 111
F-06 902 SOPHIA ANTIPOLIS
France
Tél : 00 33 4 92 94 37 19
Fax : 00 33 4 92 94 37 01
eurl.bee@anses.fr

EPILOBEE consortium

Belgium	De Graaf	Dirk	Ghent University, Department of Physiology, Laboratory of Zoophysiology
	Méroc	Estelle	NRL for honeybee diseases CODA-CERVA-VAR
	Nguyen	Bach Kim	Ulg, Faculté Gembloux Agro-Bio Tech
	Roelandt	Sophie	NRL for honeybee diseases CODA-CERVA-VAR
	Roels	Stefan	NRL for honeybee diseases CODA-CERVA-VAR
	Van der Stede	Yves	NRL for honeybee diseases CODA-CERVA-VAR
Denmark	Tonnersen	Tina	(NRL) Aarhus University
	Kryger	Per	(NRL) Aarhus University
Estonia	Jaarma	Kärt	Estonian Veterinary and Food Board
	Kuus	Merle	Estonian Veterinary and Food Laboratory
	Raie	Arvi	Estonian Veterinary and Food Board
Finland	Heinikainen	Sirpa	Finnish Food Safety Authority EVIRA, Veterinary Bacteriology Research Unit, Kuopio
	Pelkonen	Sinikka	Finnish Food Safety Authority EVIRA, Veterinary Bacteriology Research Unit, Kuopio
	Vähänikkilä	Nella	Finnish Food Safety Authority EVIRA, Veterinary Bacteriology Research Unit, Kuopio
France	Andrieux	Christophe	DDPP du Cantal
	Ballis	Alexis	Chambre d'Agriculture du Haut-Rhin
	Barrieu	Guy	DDPP des Bouches du Rhône
	Bendali	Fatah	Direction Générale de l'Alimentation
	Brugoux	Corinne	Groupement de Défense Sanitaire du Cantal
	Franco	Stéphanie	LNR Abeilles Anses Sophia Antipolis
	Fuentes	Anne-Marie	Groupement de Défense Sanitaire de la Drôme
	Joel	Alain	DDPP Finistère
	Layec	Yves	Groupement de Défense Sanitaire Apicole du Finistère
	Lopez	Jacqueline	DDPP Indre et Loire
	Lozach	André	Groupement de Défense Sanitaire Apicole du Finistère
	Malherbe-Duluc	Laure	Groupement de Défense Sanitaire Indre et Loire
	Mariau	Viviane	DDPP Indre et Loire
	Meziani	Fayçal	Direction Générale de l'Alimentation
	Monod	Denis	Groupement de Défense Sanitaire Apicole des Bouches du Rhône
	Mutel	Sébastien	DDCSPP Haut-Rhin
	Oesterle	Eric	Groupement de Défense Sanitaire Indre et Loire
	Orlowski	Muriel	DDPP de la Drôme
	Petit	Manuel	DDPP Finistère
	Pillu	Patricia	DDPP du Cantal
	Poret	Florence	Groupement de Défense Sanitaire du Cantal
	Viry	Alain	Laboratoire d'Analyses du Jura
Germany	Berg	Stefan	Bavarian State Institute for Viticulture and Horticulture, Bee Research Center, Veitshöchheim
	Büchler	Ralph	LLH Bieneninstitut Kirchhain
	de Craigher	Doris	University of Hohenheim, Apicultural State Institute, Stuttgart
	Genersch	Elke	Institute for Bee Research, Hohen Neuendorf
	Kaatz	Hans-Hinrich	University of Halle-Wittenberg, Zoology Dept., Halle
	Meixner	Marina D.	LLH Bieneninstitut Kirchhain
	von der Ohe	Werner	LAVES Institut für Bienenkunde, Celle
	Otten	Christoph	Dienstleistungszentrum Ländlicher Raum, Fachzentrum Bienen und Imkerei Mayen
	Rosenkranz	Peter	University of Hohenheim, Apicultural State Institute, Stuttgart
	Schäfer	Marc O.	Institute of Infectology, Friedrich-Loeffler-Institut, Greifswald - Insel Riems
	Schroeder	Annette	University of Hohenheim, Apicultural State Institute, Stuttgart
Greece	Agianiotaki	Eirini	Centre of Veterinary Institute of Athens
	Arfara	Stamatia	Centre of Veterinary Institute of Athens
	Boutsini	Sofia	Centre of Veterinary Institute of Athens
	Giannoulopoulou	Marianthi	Regional Veterinary Laboratory of Heraclio
	Hondrou	Varvara	Regional Veterinary Laboratory of Mytilini
	Karipidou	Sonia	Regional Veterinary Laboratory of Kozani
	Katsaros	Dimitrios	Regional Veterinary Laboratory of Chalkis
	Katzagiannakis	Aristomenis	Regional Veterinary Laboratory of Heraclio
	Kiriakopoulos	Antonios	Regional Veterinary Laboratory of Mytilini
	Oureilidis	Konstantinos	Regional Veterinary Laboratory of Kavala
	Panteli	Aspasia	Centre of Veterinary Institute of Athens
	Pantoleon	Fotis	Regional Veterinary Laboratory of Tripoli
	Papagianni	Zoi	Centre of Veterinary Institute of Athens
	Papalexiou	Eleni	Centre of Veterinary Institute of Athens
	Perdikaris	Socrates	Ministry of Rural Development and Food
	Prapas	Athanasios	Centre of Veterinary Institute of Athens
	Siana	Panagiota	Regional Veterinary Laboratory of Tripoli
	Skandalakis	Ioannis	Regional Veterinary Laboratory of Chania
	Stougiou	Despoina	Centre of Veterinary Institute of Athens
	Tomazinakis	Ioannis	Regional Veterinary Laboratory of Chania
	Tsali	Eleftheria	Regional Veterinary Laboratory of Larisa

	Tseliou	Evgenia	Regional Veterinary Laboratory of Kerkyra
	Tsiplakidis	Achillefs	Regional Veterinary Laboratory of Kavala
	Tsompanellis	Efstratios	Regional Veterinary Laboratory of Mytilini
	Vamvakas	Giorgos	Regional Veterinary Laboratory of Kozani
	Varvarouta	Vasiliki	Regional Veterinary Laboratory of Heraclio
	Vourvidis	Dimitris	Ministry of Rural Development and Food
Hungary	Dán	Ádám	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Daróczi	Gyöngyi	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Láng	Mária	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Papp	Melitta	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Paulus	Petra, Deákné	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Pupp	Eszter	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Szaló	Márta	Ministry of Agriculture, Food Chain Control Department
	Tóth	Ádám	National Food Chain Safety Office, Veterinary Diagnostic Directorate (NRL for bee health)
	Zséli	Szilvia	National Food Chain Safety Office, Animal Health and Animal Welfare Directorate
Italy	Bressan	Gianluigi	Ulss22, Bussolengo
	Cerrone	Anna	IZS del Mezzogiorno
	Formato	Giovanni	IZS delle Regioni Lazio e Toscana
	Granato	Anna	IZS delle Venezie
	Lavazza	Antonio	IZS della Lombardia e dell'Emilia Romagna
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	Pintore	Antonio	IZS della Sardegna
	Ricchiuti	Luciano	IZS dell'Abruzzo e del Molise
	Ruocco	Luigi	Ministero della Salute
	Salvaggio	Antonio	IZS della Sicilia
	Troiano	Pasquale	IZS di Puglia e Basilicata
	Voltini	Barbara	Regione Toscana
Latvia	Avsejenko	Jelena	Institute of Food safety, Animal Health and Environment, "BIOR"
	Ciekure	Elīna	Institute of Food safety, Animal Health and Environment, "BIOR"
	Deksne	Gunita	Institute of Food safety, Animal Health and Environment, "BIOR"
	Eglīte	Ineta	Latvian Beekeepers Association
	Granta	Rita	Institute of Food safety, Animal Health and Environment, "BIOR"
	Olševski	Edvīns	Food and Veterinary Service of the Republic of Latvia
	Rodze	Ieva	Institute of Food safety, Animal Health and Environment, "BIOR"
	Stinka	Madara	Food and Veterinary Service of the Republic of Latvia
Lithuania	Sirutkaityte	Rasa	The State Food and Veterinary Service, Animal Health and Welfare department
	Siriukaitis	Sigitas	The State Food and Veterinary Service, Animal Health and Welfare department
Poland	Bober	Andrzej	National Veterinary Research Institute, Pulawy, Poland
	Jażdżewski	Krzysztof	General Veterinary Inspectorate, Warsaw, Poland
	Pohorecka	Krystyna	National Veterinary Research Institute, Pulawy, Poland
	Skubida	Marta	National Veterinary Research Institute, Pulawy, Poland
	Zdańska	Dagmara	National Veterinary Research Institute, Pulawy, Poland
Portugal	Amador	Maria Rita Ramos	Direção Geral de Alimentacão e Veterinaria
	Freitas	Susana	Direção Geral de Alimentacão e Veterinaria
	Quintans	Sofia	Direção Geral de Alimentacão e Veterinaria
	Santos	Patricia Tavares	Direção Geral de Alimentacão e Veterinaria
Slovakia	Březinová	Nicole	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Brtková	Andrea	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Čuvalová	Zuzana	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Filipová	Miriam	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Jurovčiková	Júlia	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Kantíková	Miriam	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Kubicová	Zuzana	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Papierniková	Erika	State Veterinary and Food Administration of the Slovak Republic
	Šulejová	Lucia	State Veterinary and Food Institute, Dolny Kubin,Slovakia
	Toporčák	Juraj	The University of veterinary medicine and pharmacy in Košice, Slovakia
Spain	Ares Cenador	Carmen Maria	Consejería de Agroganadería y Recursos Autóctonos del Principado de Asturias
	Ariza	Javier	Consejería de Agricultura de La Junta de Comunidades de Castilla La Mancha
	Berná Serna	Nieves	GVA, Consejería de la Presidencia de Agricultura, Pesca, Alimentación y Agua
	Cabeza Núñez	Amparo	Consejería de Agricultura y Pesca de la junta de Andalucía
	Casasempere Cascales	Jorge	GVA, Consejería de la Presidencia de Agricultura, Pesca, Alimentación y Agua
	Cid González	Carlos	Subdireción Xeral de Gandería - Consellería do Medio Rural e do Mar - Xunta de Galicia
	Corzán Ripoll	Jose Manuel	Consejería de Agricultura, Ganadería y Medio Ambiente, Diputación General de Aragón

	De Abajo Domingo	Miguel Ángel	Consejería de Agricultura y Ganadería de la Junta de Castilla y León
	Díaz Rey	Roberto	Subdireción Xeral de Gandería - Consellería do Medio Rural e do Mar - Xunta de Galicia
	Esteban Royo	Ángel	Consejería de Agricultura, Ganadería y Medio Ambiente, Diputación General de Aragón
	Fernández Somalo	Pilar	SG de Sanidad, Higiene Animal y Trazabilidad del Mº Agricultura, Alim. y Medio Ambiente
	García Pascualvaca	Alejandra	Consejería de Agricultura y Pesca de la junta de Andalucía
	González Breña	Carlos	Consejería de Agricultura, Desarrollo Rural, Medio Ambiente y Energía , J. de Extremadura
	Mínguez Gonzalez	Olga	Consejería de Agricultura y Ganadería de la Junta de Castilla y León
	Oñate	Maria Luisa	Consejería de Agricultura, Ganadería y Medio Ambiente, Diputación General de Aragón
	Oteiza Orradre	Pedro	Dpto. de Desarrollo Rural, Industria, Empleo y Medio Ambiente, Diputación Foral Navarra
	Pérez Cobo	Iratxe	SG de Sanidad, Higiene Animal y Trazabilidad del Mº Agricultura, Alim. y Medio Ambiente
	Plaza Pérez	Margarita	Consejería de Agricultura y Agua de la Región de Murcia
	Puy Pitarque	D. Juan Ramón	Departamento de Agricultura, Pesca y Alimentación del Gobierno Vasco
	Riol Guinea	Rubén	Consejería de Agricultura y Ganadería de la Junta de Castilla y León
	Romero González	Luis José	SG de Sanidad e Higiene Animal y Trazabilidad del Mº Agricultura, Alim. y Medio Ambiente
	Soldevilla Yanguas	Jose Fernando	Consejería de Agricultura, Ganadería y Medio Ambiente, Comunidad Autónoma de la Rioja
	Soler i Barrasús	Mercè	Dept. Agricultura, Ramaderia, Pesca, Alimentación i Medi Natural, Generalitat de Catalunya
	Soriano González	Mario	Consejería de la Presidencia de Agricultura, Pesca, Alim. y Agua, Generalitat Valenciana
	Vigo López	Virginia	Consejería de Agricultura, Ganadería, Pesca y Aguas del Gobierno de Canarias
	Villarta Rivas	José Luis	Consejería de Agricultura de La Junta de Comunidades de Castilla La Mancha
Sweden	Fabricius-Kristiansen	Lotta	Swedish Board of Agriculture
	Forsgren	Eva	Department of Ecology, Swedish University of Agricultural Sciences
UK	Brown	Mike	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
	Budge	Giles	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
	Grant	Ruth	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
	Marris	Gay	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
	Powell	Michelle	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
	Wattam	Andy	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
	Whiting	Ilex	The National Bee Unit (NBU) - Food and Environment Research Agency (FERA)
EURL staff	Cauquil	Laura	
	Garin	Emmanuel	
	Jacques	Antoine	
	Rivière	Marie-Pierre	
	Saugeon	Cécile	

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EXECUTIVE SUMMARY

Over the years, honeybee health has become a major concern. Sound colony losses were reported by beekeepers and scientists but no official data were available. In 2009 EFSA has launched a project on the description of bee surveillance programmes existing in Europe. The report highlighted the lack of comparable data and common operational systems to assess the mortality of bee colonies.

In this context, the first harmonised active epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) was set up in 17 European Member States for a year, later prolonged for another one (September 2012 – September 2014) following a call launched by the European Commission. The national protocols were based on guidelines issued from the European Union Reference Laboratory for honeybee health (EURL). The objective of the two-year programme was to get a state of play of honeybee colony losses on a harmonized basis in each of the participating Member States. Simultaneously, the main honeybee diseases were investigated based on case definitions and sampling protocols provided by the EURL. This report aims at presenting the main results of the second year of EPILOBEE on colony mortality and on the prevalence of infectious and parasitic diseases, and to compare these results with those obtained during the previous year.

During EPILOBEE, 9 618 apiary visits and 117 342 laboratory analyses have been recorded over the first year while 8 604 apiary visits and 49 042 laboratory analyses were recorded during the second year. Overall 176 769 colonies were visited during the two years of the programme. Winter colony mortality rates ranged from 2.4% to 15.4% during the second year of the programme. In one third of the Member States, the mortality rates were over 10%. Rates of seasonal colony mortality (2014) ranging from 0.04% to 11.1% did not drastically change during the second year from the first year of the programme in 15 out of the 16 Member States taking part in EPILOBEE. However, the overall winter colony losses varied between the two years of the programme in some Member states.

Trends toward decrease were observed for the disease prevalence throughout the two years of the programme. The overall prevalence of AFB was lower than 12% in all the Member States at any visit during the two years of EPILOBEE. The overall prevalence of EFB was even lower (below 8 %) in all the Member States during the two years. Clinical cases of varroosis were observed in nearly all the Member States during the two years of EPILOBEE. Similarly to the first year, the parasitic pressure of *Varroa destructor* was assessed at the visit performed before winter by sampling all the honeybee colonies. Statistical link between the varroa mites' infestation of the colonies and their subsequent survival to the winter is currently understudy. Positive cases of Nosemosis were observed in ten out of the 16 Member States during EPILOBEE 2013 – 2014. The apparent clinical prevalence exceeded 10% in at least one visit in only three Member States. The overall clinical prevalence of chronic paralysis disease did not exceed 2% at any visit in the four Member States with positive cases recorded. *Aethina tumida* and *Tropilaelaps* mites have not been detected in any of the 17 Member States during the two years of EPILOBEE programme. However, it should be remembered that *A. tumida* was detected in Italy in September 2014 outside of and after that EPILOBEE has finished.

The data gathered throughout these two years on various topics (disease prevalence, use of veterinary treatments, the beekeeping context, management...) will be further analysed. Statistical analysis of correlations between the colony losses and potential risk factors is currently understudy. Several factors are known to have an effect on colony losses. For example, the winter 2013 – 2014 has been relatively warmer and more favourable for honeybees than the winter 2012 – 2013 which was long and cold throughout Europe. Climate might have influenced winter colony losses over the two years. Its role in the winter colony mortality should be further balanced with other risk factors that have also certainly played a role.

This programme was a descriptive epidemiological study enabling the collection of official and comparable data on honeybee health during two years with a methodology that was fully feasible and repeatable. The outcomes of EPILOBEE were 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.

TABLE OF CONTENTS

Glossary

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

1. Context

As presented in the first EPILOBEE report[1], worrying honeybee colony losses stressed by the beekeepers and reported by some scientific studies underlined the urgent necessity for epidemiological studies. To document this phenomenon a consortium was set up in 2009[2] 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 standardized EU surveillance systems to accurately assess bee health in Europe (Hendrikx et al. 2010). In this context, the European Commission requested to get harmonized and comparable data at the European level. A call was launched following the guidelines[3] issued by the European Union Reference Laboratory (EURL) for honeybee health. The first harmonised active epidemiological surveillance programme on honeybee colony mortality (EPILOBEE) was set up for two years in September 2012 with 17 Member States participating for the first year and 16 Member States participating for the second year (European Commission 2012 & European Commission 2013). The objective of the two-year programme was to quantify the mortality of honeybee colonies on a harmonized basis in each participating Member State. Simultaneously, the main honeybee infectious and parasitic diseases were investigated based on case definitions and sampling protocol provided by the EURL to estimate the honeybee colonies health. Information related to beekeeping practices was recorded alongside.

EPILOBEE was a descriptive epidemiological study aiming at collecting official and comparable data on the honeybee colony mortality. 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 pilot programme enabled to implement feasible and repeatable methodology for the active surveillance of honeybee colony mortality. This first state of play on honeybee colony mortality in Europe as well as the implementation of an epidemiological active surveillance is a prerequisite to the set-up of further explanatory studies. Future programmes might benefit from the framework established in the Member States thanks to EPILOBEE as well as the resulting data to investigate the potential causes of honeybee colony mortality such as pesticides, pathogens or their possible interactions.

Mortality rates of the first year were revised following corrections brought to the datasets by the Member States coupled with the enhancement of the cleaning steps implemented by the EURL. Results of the first year have shown that in the 17 Member States taking part in EPILOBEE, (Belgium, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Poland, Portugal, Slovakia, Spain, Sweden and England and Wales) the winter colony mortality rates ranged from 3.2% to 32.4%. There are no historical values regarding the acceptable levels of colony losses in Europe, numbers varying according to countries. In the present report, the acceptable level for winter colony mortality was considered lower than 10%, this threshold being open to discussion. In EPILOBEE first year, the overwintering mortality rates exceeded 10% in 12 Member States. A strong geographical variation was observed. The seasonal colony mortality rates ranged from 0.02% to 10.5% depending on Member States and were lower than the overwintering colony mortality rates.

[1] http://ec.europa.eu/food/animals/live_animals/bees/docs/bee-report_en.pdf
[2] http://www.efsa.europa.eu/fr/scdocs/scdoc/27e.htm
[3] http://ec.europa.eu/food/archive/animal/liveanimals/bees/docs/annex_i_pilot_project_en.pdf

The overall clinical prevalence of American foulbrood (AFB) and European foulbrood (EFB) was low in all the Member States during EPILOBEE first year. Clinical cases of varroosis were observed in nearly all the Member States. The apparent clinical prevalence of nosemosis exceeded 10% in at least one visit in four out of the 11 Member States with positive cases of nosemosis. Some clinical cases of paralysis were observed in five out of the 17 Member States. No *Aethina tumida* or *Tropilaelaps* mites have been detected in the 17 Member States during the first year of the programme.

More than 93 383 colonies were visited during 2012 – 2013 by more than 1 573 bee inspectors in the 17 Member States[4].A total of 117 342 laboratory analyses were performed on samples collected during the three visits[4]. This first year of surveillance was implemented with epidemiological standardized methods allowing possible comparisons between Member States.

The present report details the results on mortality rates and on disease prevalence of EPILOBEE programme stretching from September 2013 to September 2014 and compares these results with those obtained last year. The two-year programme resulted in the collection of a very substantial amount of data on different topics (farming practices, use of veterinary treatments, environment...) that lead to current and future data analyses. These analyses will unquestionably explore possible statistical links between the colony losses and some risk factors collected during EPILOBEE.

2. Protocol of the study

The second year of the surveillance took place under the very similar conditions than the first year of surveillance (refer to the first-year report and the guidelines for more precisions). Each Member State prolonged the protocol to the 2013 – 2014 programme with the exception of England and Wales which did not take part in the second year of the programme.

For the second year of EPILOBEE, the total renewal of the beekeepers visited during 2012 and 2013 was recommended for comparison purposes with the data from the first year. Taking into account the difficulty of beekeepers selection and involvement in such detailed protocol, at least one third of the total beekeepers sampled were renewed for the 2013 – 2014 programme compared to the previous year. These beekeepers were selected with the same methodology than the one implemented in the previous year.

Each Member State organised the training of bee inspectors on the basis of documents provided by the EURL, managed the implementation of the visits and stored the data in an online database. At the time of writing the first report, data were not fully registered in the database for two Member States. These data have been included in the statistical analysis since and revised graphs of the first year are shown in this present report. Similarly, improvement of the cleaning steps and corrections brought to the database by the Member States enabled to revise the mortality rates for the first year. Revised maps are shown in this report.

Surveillance protocol

Similarly to the previous year, three visits were performed by bee inspectors: before winter (autumn 2013), after winter (spring 2014) and during the beekeeping season (summer 2014). Farming practices, description of the environment and clinical manifestations 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 fully visited and examined.

[4] Revised figures from 27th January 2015

The fungal disease Nosemosis, the parasitic disease varroosis, the American foulbrood, the European foulbrood and a viral disease caused by the chronic bee paralysis virus (CBPV) were investigated. A systematic evaluation of the parasitic infestation by *V. destructor* mites on each colony was implemented by sampling 300 living honeybees during the autumn visit in 2013.

Clinical prevalence was based on laboratory confirmation conducted on samples collected in colonies exhibiting clinical signs of a disease at any visit. Only the clinical prevalence of the diseases listed above was addressed.

Each Member State organized the prolongation of the surveillance at the national level. It has to be acknowledged that a remarkable work involving a lot of stakeholders belonging to different levels from the ministry to the field was implemented during the two years of EPILOBEE producing a reliable and extended set of data.

Data collection and management

During the field visits, many data were collected through a questionnaire. The overall information collected is listed in Tables 6 and 7 in Annex I and includes information on the beekeeper, information on the selected apiaries, information on the visits set up in each apiary, the general health events observed in the selected apiary prior to the visit, the treatments administered in the apiary prior to the visit, the livestock management implemented in the apiary prior to the visit and information on a randomly selected colonies. The questionnaire filled by the bee inspectors was lightened for the second year of EPILOBEE thanks to the feedbacks from the field. Some questions were rephrased to improve the understanding in the field. Some questions were added for the second year (e.g. the record of colony strength) whereas others were removed from the questionnaire (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 with the data from the first year of EPILOBEE. Data were stored in a standardized way in a European online database via a website developed by the EURL and the French Platform for epidemiological surveillance in Animal Health.

The descriptive analyses were performed using a dedicated software (R software, version 3.1.0). Such a programme recording great quantities of data (9 618 visits and 117 342 laboratory analyses the first year and 8 604 visits and 49 042 laboratory analyses the second year) induces evident risk of errors 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 undertook arduous work in correcting the data. Remaining incorrect and missing data were discarded from the calculation.

Calculation of the prevalence of the diseases at the apiary level

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

Calculation of the mortality rates at colony level

The calculation of the mortality rates was reported to the size of the apiaries. 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.

$$\hat{\theta} = \frac{\sum_{i=1}^{n}(Mi \,.\, \hat{P}i)}{\sum_{i=1}^{n} Mi}$$

Pi 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 Mi was the size of the apiary (all the colonies of the apiary whether they were randomly selected or not).

The *yearly* colony mortality rate represents the colony losses observed throughout the time frame of one year. It was calculated including only the apiaries where the three visits have been implemented. The apiaries where all colonies died between the first and the second visit were also included in the calculation. Such a programme required the involvement of an important number of beekeepers. Over the course of EPILOBEE, all the randomly selected apiaries were not visited three times for several reasons.

These criteria guaranteed to calculate the *yearly* colony mortality rate only on apiaries followed throughout the entire year of EPILOBEE.

3. Results

3.1. Population sampled

Table 1: Number of randomly selected apiaries and colonies at the first visit of the programme in the Member States taking part in EPILOBEE 2012 – 2013 and EPILOBEE 2013 – 2014.

	Number of apiaries visited during		Size of the apiaries visited during autumn 2013 (%)[1] <50 colonies [50-150] >150 colonies			Number of colonies visited during[1]	
	Autumn 2012	Autumn 2013				Autumn 2012	Autumn 2013
Belgium	149	150	100	0	0	627	644
Denmark	202	212	100	0	0	1 394	1243
Estonia	197	196	91.3	8.7	0	2 337	1616
Finland	161	161	100	0	0	787	682
France	344	350	93.7[2]	6.0[2]	0.3[2]	2 477	2331[6]
Germany	223	217	99.1[3]	0.9[3]	0[3]	1 988	1879
Greece	161	67	40.3	46.3	13.4	1 386	1060
Hungary	197	185	45.1[4]	40.8[4]	14.1[4]	3 934	3810
Italy	184	166	79.4[5]	17.6[5]	3[5]	1 682	1849[7]
Latvia	194	190	90	8.4	1.6	1 930	1918
Lithuania	191	163	51.5	44.8	3.7	2 484	2061
Poland	190	190	73.2	24.2	2.6	3 207	3147
Portugal	146	145	95.2	4.8	0	437	865
Slovakia	190	198	88.4	11.1	0.5	3 199	3036
Spain	204	190	43.7	54.7	1.6	2 321	2157
Sweden	151	150	100	0	0	725	758
England and Wales	200	-	-	-	-	917	-
Total	**3 284**	**2 930**				**31 832**	**29 056**
Mean			80.7	16.8	2.5		

[1]Otherwise stated, the rates (%) and numbers of colonies visited have been 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 332 apiaries [7]The calculation was based on 163 apiaries

During the second year of EPILOBEE, 29 056 colonies were visited at autumn 2013 (Table 1). Within the 2 930 apiaries randomly selected at autumn 2013, the small apiaries (less than 50 colonies) represented 80.7% of all the apiaries. Overall more than 83 362 colonies have been visited in 2013-2014 which represents a reduction of 11% compared to the previous year.

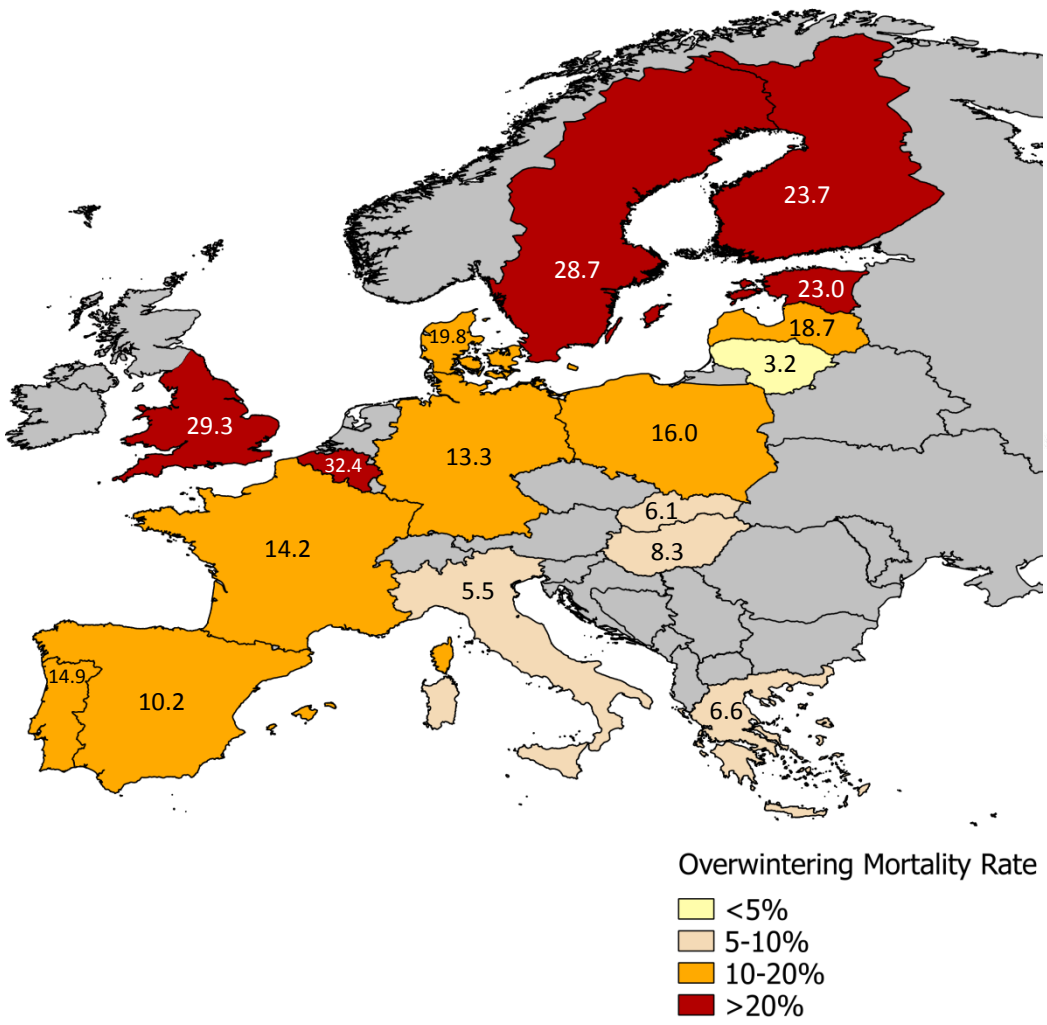
3.2. Mortality rates

The winter and seasonal colony mortality rates for EPILOBEE 2012 – 2013 were revised taking into account complete and updated datasets, and improved cleansing steps. These changes led to revised winter and seasonal colony mortality rates enabling comparisons with data from the second year.

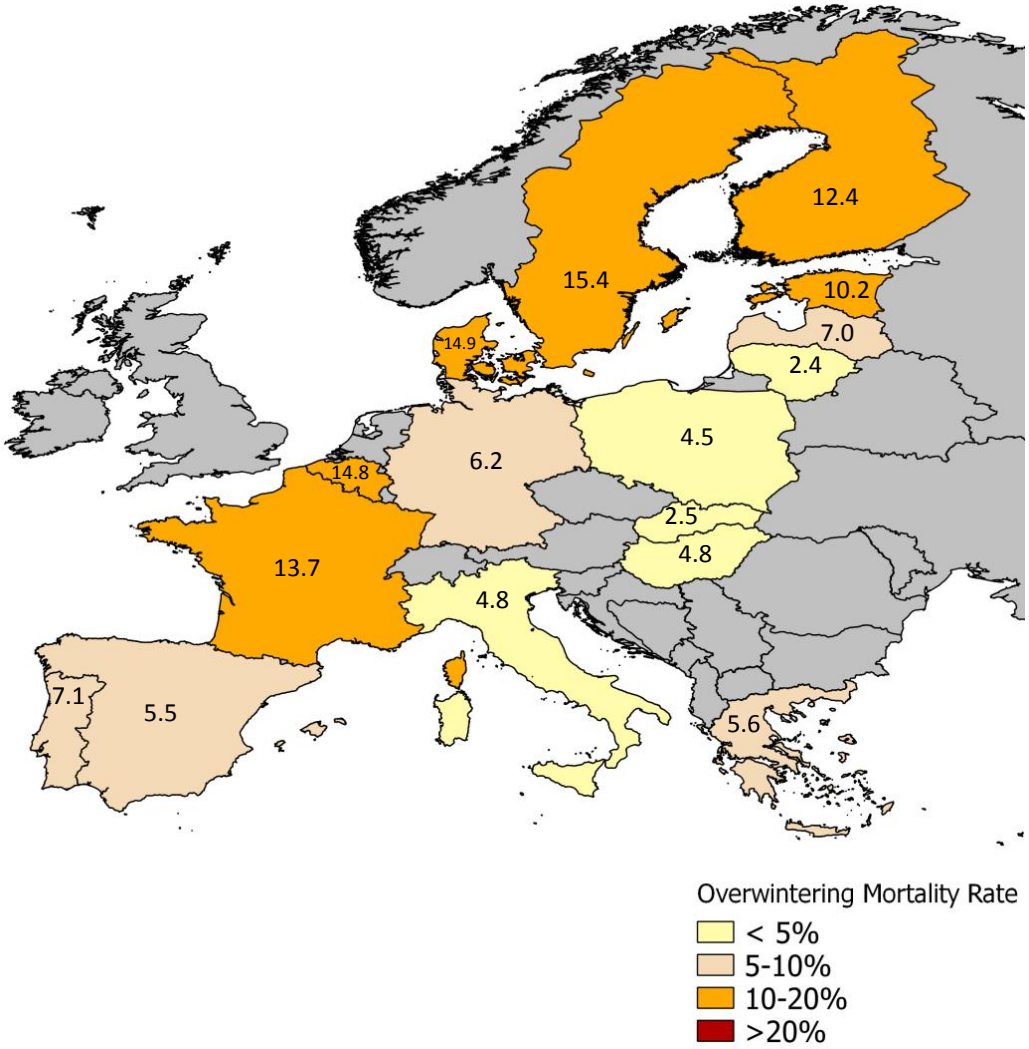
Overwintering colony mortality (winter 2012 – 2013 and winter 2013 – 2014)

Figure 1: Winter colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2012 – 2013 (Revised map) (a) and EPILOBEE 2013 – 2014 (b)

a



b



Revised rates of winter colony mortality from EPILOBEE 2012 – 2013 ranged from 3.2% to 32.4% (3.5% to 33.6% in the report of the first year) (Table 8 in Annex II and Figure 1a). Compared to the rates calculated and released last year, the mortality rates slightly changed (the differences were inferior to 1%) towards an increase or a decrease in 11 Member States. For three Member States, the rates did not change (Greece, Slovakia and Sweden). For Latvia and Poland, the winter colony mortality rates increased (differences were 3.4% and 1.2% respectively) whereas the mortality rate decreased for Belgium (difference was 1.2%). However, each revised rate showed similar confidence intervals to those calculated last year. It should be noted that the revised winter colony mortality rate for Spain exceeded 10% while the revised winter colony mortality rate for Denmark dropped below 20%, leading to colour change in the revised map.

Rates of overwintering colony mortality (2013-2014) ranged between the Member States from 2.4% to 15.4% (Table 2 and Figure 1b). The winter colony mortality rates exceeded 10% in six Member States. In five out 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).

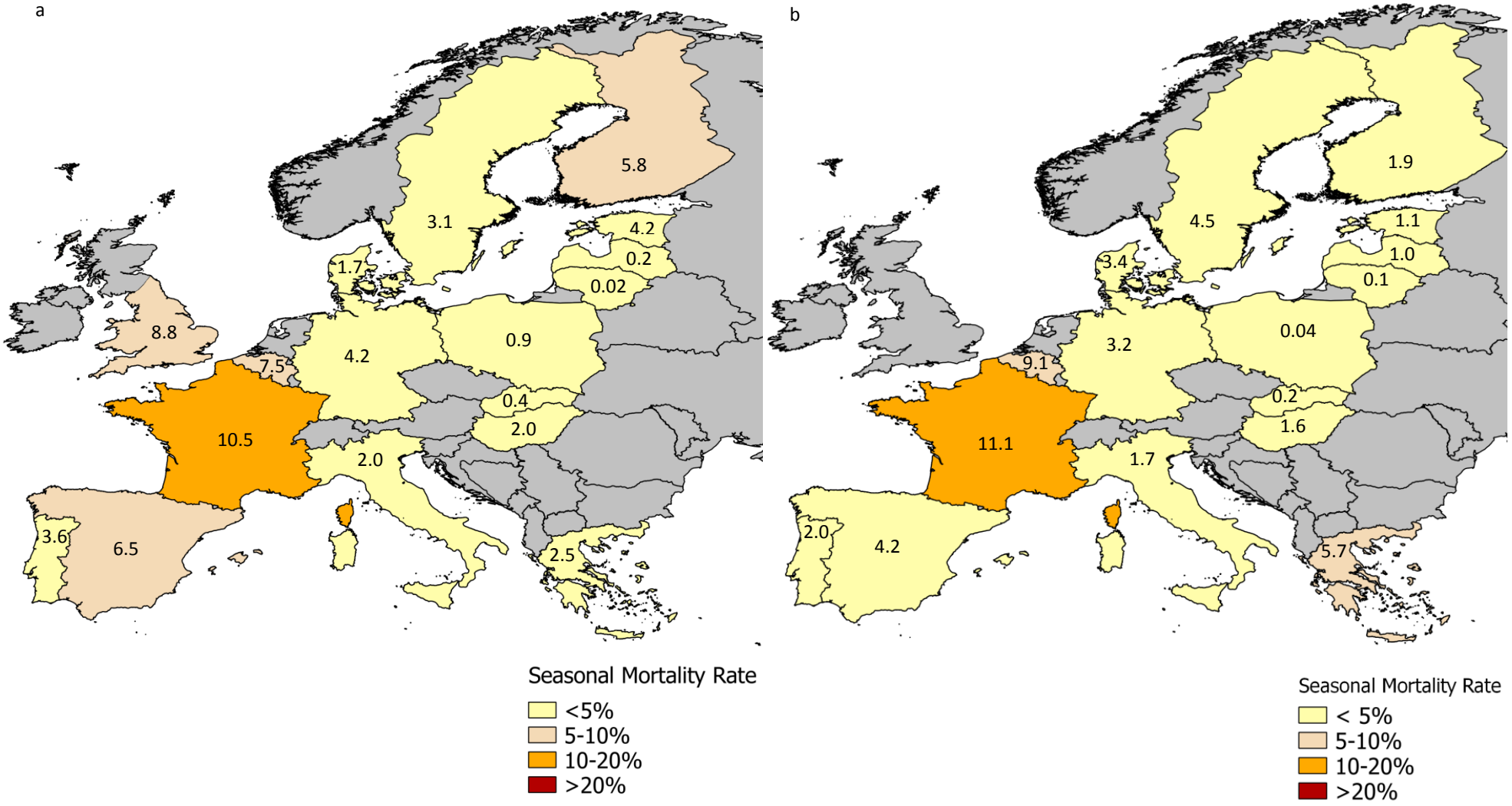
However, it should be remembered that these rates were estimates of the *real winter colony mortality rates* based on representative samples of the honeybee population in each Member State. The confidence intervals in which the *real colony mortality rates* could be found with 95% probability have been calculated (Table 2 and Table 8 in Annex II). For seven Member States, the winter colony mortality confidence intervals from EPILOBEE 2012 – 2013 and EPILOBEE 2013 – 2014 overlapped (Denmark, France, Greece, Hungary, Italy, Lithuania and Slovakia). This means that, for these Member States, there is no statistical difference between the two years for the winter colony mortality rates. Conversely, the winter colony mortality rates statistically decreased during the second year for nine Member States.

Table 2: Winter colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2013 – 2014
95% CI= confidence interval at 95%
↓: statistical difference between the two years towards a decrease; →: no statistical difference between the two years

	Mortality rate (%)		95% CI inferior limit	95% CI superior 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

Seasonal colony mortality (spring – summer 2013 and spring – summer 2014)

Figure 2: Seasonal colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2012 – 2013 (Revised map) (a) and EPILOBEE 2013 – 2014 (b)



Revised rates of seasonal colony mortality (2013) ranged from 0.02% to 10.5% (0.3% to 13.6% in the report of the first year) (Table 9 in Annex II and Figure 2a). Compared to the rates calculated and released last year, the mortality rates slightly changed (the differences were inferior to 1%) towards an increase or a decrease in 13 Member States. For one Member State, the rate did not change (Greece). For three Member States, the rates decreased (the differences were 1.4%, 1.2% and 3.1% for Belgium, Denmark and France respectively). However, each revised rate showed similar confidence intervals to those calculated last year.

Rates of seasonal colony mortality (2014) ranged from 0.04% to 11.1% (Table 3 and Figure 2b). Seasonal colony mortality rates were below 5% in 13 Member States. The rate was over 10% in France only. In nine out of the 16 Member States, the mortality rate during the 2014 beekeeping season was lower than the rate estimated during the 2013 beekeeping season (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).

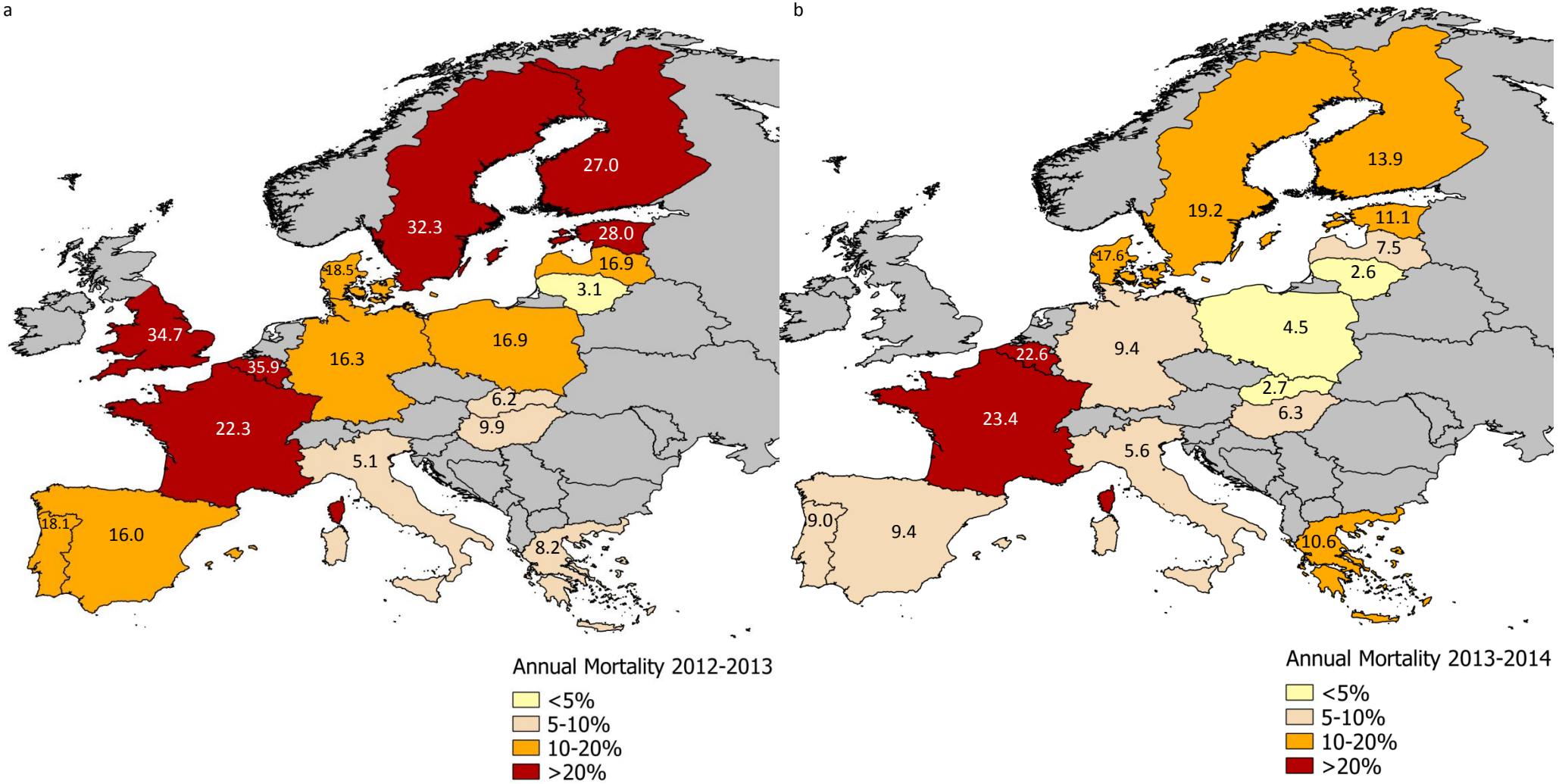
The confidence intervals in which the *real seasonal colony mortality rates* (2014) could be found with 95% probability overlapped with the confidence intervals calculated for the 2013 beekeeping season in 15 out of the 16 Member States (Table 3 and Table 9 in Annex II). This means that the seasonal colony mortality was statistically different from one year to the other in only one case (Poland) towards a decrease.

Table 3: Seasonal mortality rates (2014) in the Member States of the European Union recorded in EPILOBEE 2013 – 2014
95% CI= confidence interval at 95%
↓: statistical difference between the two years towards a decrease; →: no statistical difference between the two years

	Mortality rate (%)		95% CI inferior limit	95% CI superior 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

Yearly colony mortality (2012 – 2013 and 2013 – 2014)

Figure 3: Yearly colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2012 – 2013 (a) and EPILOBEE 2013 – 2014 (b)



The calculation of the yearly colony mortality rates was possible only in apiaries visited three times during one full year of EPILOBEE. This represented 94.9% and 95.1% of the selected apiaries for EPILOBEE 2012 – 2013 and 2013 – 2014 respectively.

The yearly colony mortality rate ranged from 3.1% to 35.9% in the 17 Member States taking part in EPILOBEE 2012 – 2013 (Figure 3a and Table 4). The annual colony mortality rate was lower than 10% in five Member States (Greece, Hungary, Italy, Lithuania and Slovakia). The yearly colony mortality rate throughout the first year was over 20% in one third of the 17 Member States.

For the second year of EPILOBEE, the yearly colony mortality rate ranged from 2.6% to 23.4% in the 16 Member States (Figure 3b and Table 5). The mortality rate throughout the second year was below 10% in nine Member States. The rate was over 20% in two Member States (Belgium and France).

The yearly colony mortality rates for the second year (2013 – 2014) were lower than the rates for the first year (2012 – 2013) with the exception of France, Greece and Italy. For eight out of the 16 Member States, the confidence intervals from the two years of EPILOBEE overlapped, meaning that there is no statistical difference between the two years for these Member States. For the other eight Member States, there was a statistical difference in colony mortality rates between the two years towards a decrease during the second year (Belgium, Estonia, Finland, Latvia, Poland, Portugal, Spain and Sweden) (Tables 4 and 5).

Table 4: Yearly mortality rates in the Member States of the European Union recorded in EPILOBEE 2012 – 2013
95% CI= confidence interval at 95%

	Yearly mortality rate (%)	95% CI inferior limit	95% CI superior limit
Belgium	35.9	28.5	43.4
Denmark	18.5	14.1	23.0
Estonia	28.0	20.4	35.6
Finland	27.0	21.8	32.2
France	22.3	19.8	24.7
Germany	16.3	11.7	21.0
Greece	8.2	5.6	10.8
Hungary	9.9	6.9	12.8
Italy	5.1	2.9	7.4
Latvia	16.9	10.9	22.9
Lithuania	3.1	1.6	4.6
Poland	16.9	13.0	20.8
Portugal	18.1	12.3	23.9
Slovakia	6.2	3.4	9.0
Spain	16.0	13.0	19.0
Sweden	32.3	26.0	38.6
England and Wales	34.7	29.4	40.0

Table 5: Yearly mortality rates in the Member States of the European Union recorded in EPILOBEE 2013 – 2014
95% CI= confidence interval at 95%
↓: statistical difference between the two years towards a decrease; →: no statistical difference between the two years

	Yearly mortality rate (%)		95% CI inferior limit	95% CI superior limit
Belgium	22.6	↓	17.6	27.5
Denmark	17.6	→	13.2	22.0
Estonia	11.1	↓	8.2	14.1
Finland	13.9	↓	10.7	17.2
France	23.4	→	13.3	33.5
Germany	9.4	→	6.1	12.8
Greece	10.6	→	0	22.2
Hungary	6.3	→	4.6	8.0
Italy	5.6	→	2.6	8.5
Latvia	7.5	↓	5.1	10.0
Lithuania	2.6	→	0.6	4.5
Poland	4.5	↓	2.9	6.2
Portugal	9.0	↓	5.9	12.0
Slovakia	2.7	→	1.7	3.8
Spain	9.4	↓	7.1	11.8
Sweden	19.2	↓	14.4	23.9

3.3. Honeybee diseases

The prevalence graphs for the 2012 – 2013 programme were revised taking into account complete and updated datasets. Indeed, data was not fully available for two Member States when writing the first EPILOBEE report.

Detection of the exotic arthropods Aethina tumida and Tropilaelaps mites

These two arthropods have never been observed in the framework of EPILOBEE. During the second year of the programme, nine arthropods suspected to be *Aethina tumida* were collected in three Member States. For these nine suspicions the identification of *A. tumida* was negative.

However, it should be remembered that in September 2014, *A. tumida* was detected for the first time in the Calabria region (southern Italy). No apiaries located in the Calabria region took part in the EPILOBEE 2013 – 2014. Five apiaries were located in the regions surrounding Calabria. The summer visits in these apiaries were performed at the end of July 2014 at the latest.

American foulbrood

Figure 4: Clinical prevalence of American foulbrood in the apiaries recorded during the three visits of EPILOBEE 2012 – 2013 (Revised graph)

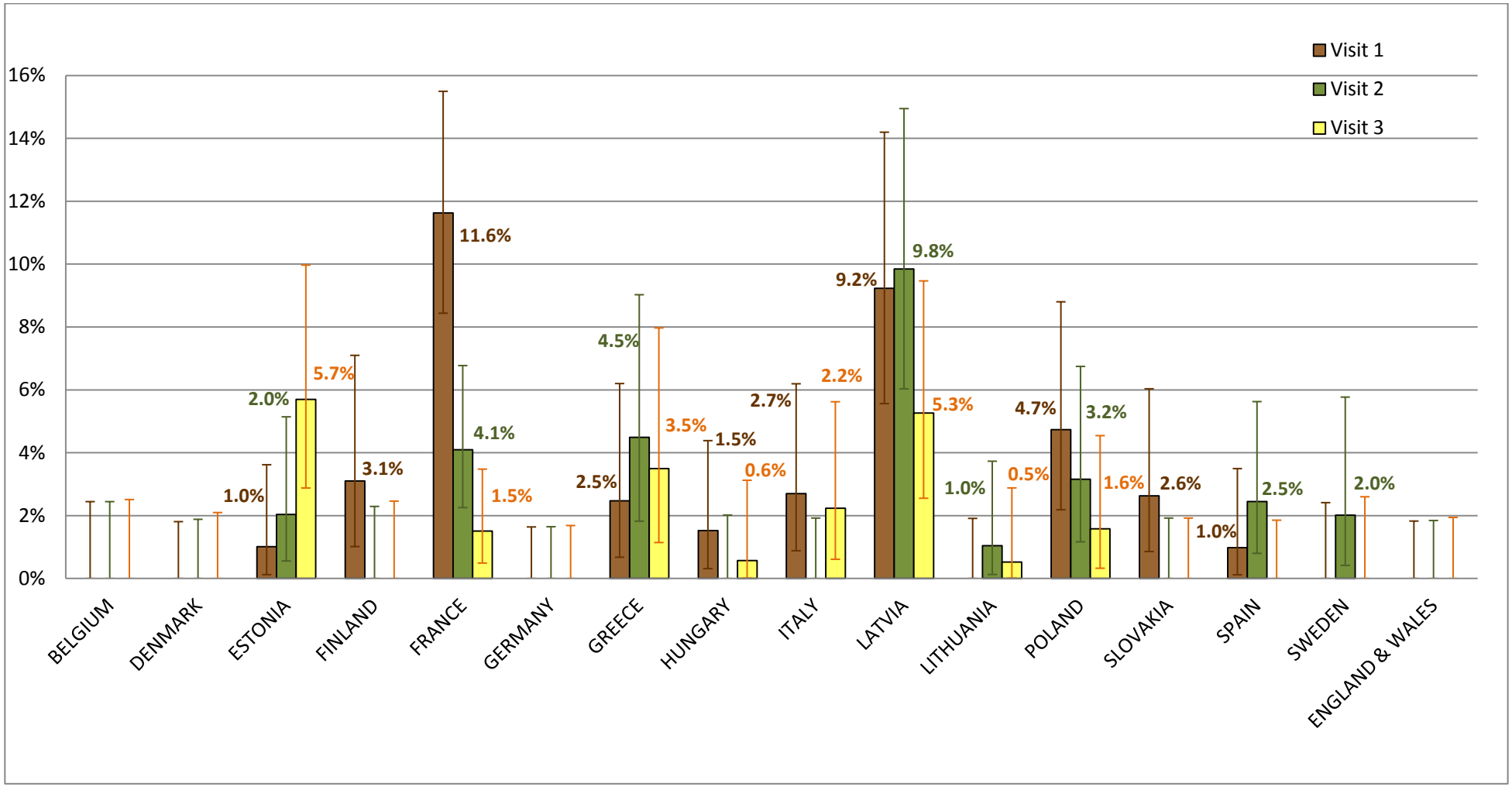
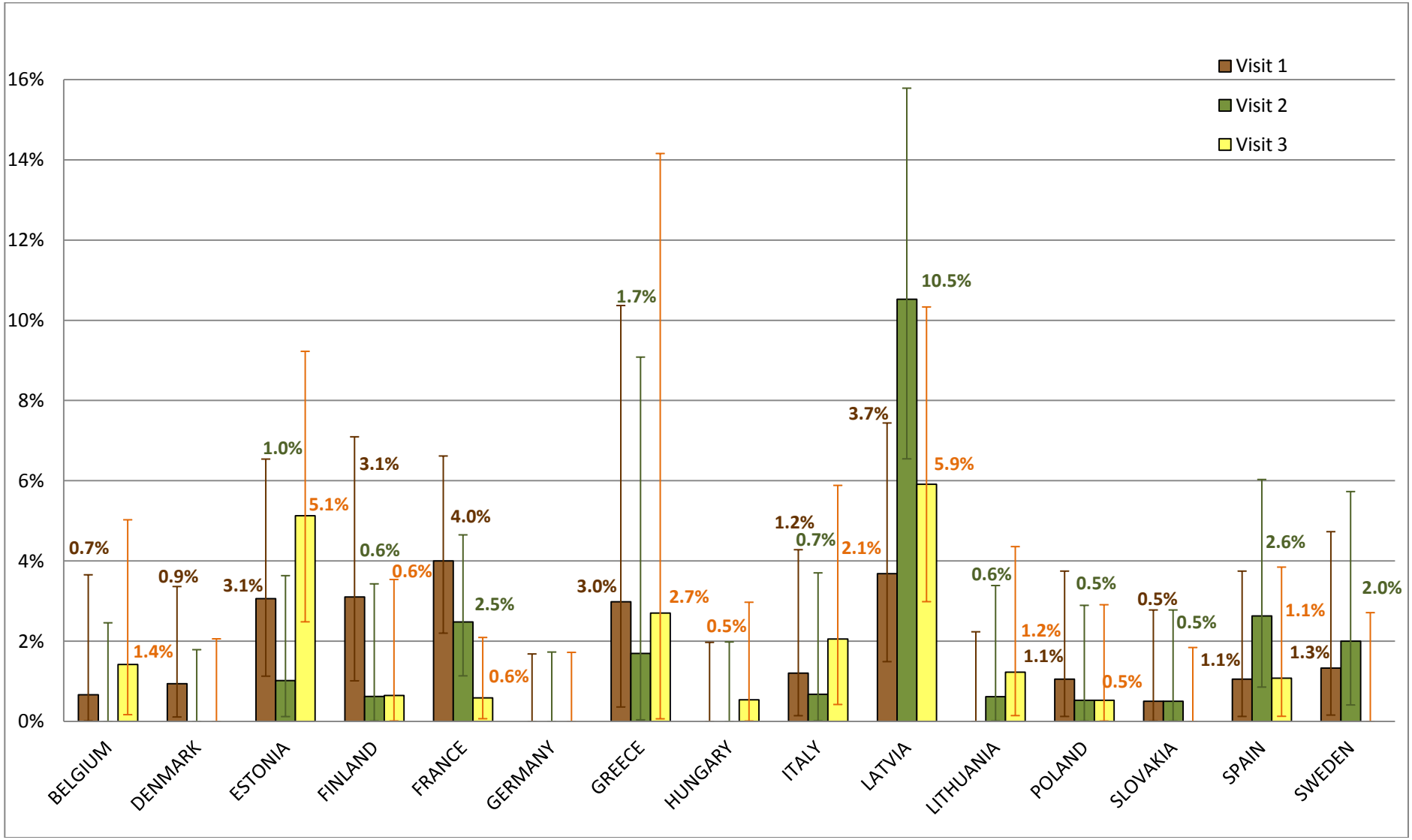


Figure 5: Clinical prevalence of American foulbrood in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014



During the second year of EPILOBEE, the clinical form of AFB was observed in nearly all the Member States (14 out of the 15 Member States with comparable data). In Germany, no positive case was observed. The clinical prevalence exceeded 6% in only one Member State for one visit (Figure 5 and Table 10 in Annex III). The overall clinical prevalence was lower than 12% in all the Member States at any visit during the two years of the programme (Figures 4 and 5).
Similarly to the mortality rates, the clinical prevalence of diseases were estimates of the *real clinical prevalence* in each Member State. The confidence intervals in which the *real clinical prevalence* of AFB could be found with 95% probability were calculated (Table 10 in Annex III). For the 15 Member States with comparable data, the confidence intervals from EPILOBEE 2012 – 2013 and EPILOBEE 2013 – 2014 overlapped at every visit except for France at Visit 1 (autumn 2013). This means that there was no statistical difference between the two years for the clinical prevalence of AFB for these 15 Member States (Table 10 in Annex III and report from the 1st year).

European foulbrood

During the second year of EPILOBEE, the EFB clinical disease was observed in only five out of the 15 Member States with comparable data (Belgium, Finland, France, Italy and Latvia). The overall clinical prevalence did not exceed 5% at any visit (Table 11 in Annex III and Figure 11 in Annex IV). Except for Belgium, all these four Member States had also observed positive cases of EFB during EPILOBEE first year (Figure 10 in Annex IV). The confidence intervals from the two years of EPILOBEE overlapped for the 15 Member States with comparable data at every visit except for France at Visit 1. The overall clinical prevalence of EFB observed during the two years of the programme were not statistically different for the 15 Member States (Table 11 in Annex III and report from the 1st year).

Varroosis

Figure 6: Clinical prevalence of varroosis in the apiaries recorded during the three visits of EPILOBEE 2012 – 2013. (Revised graph)

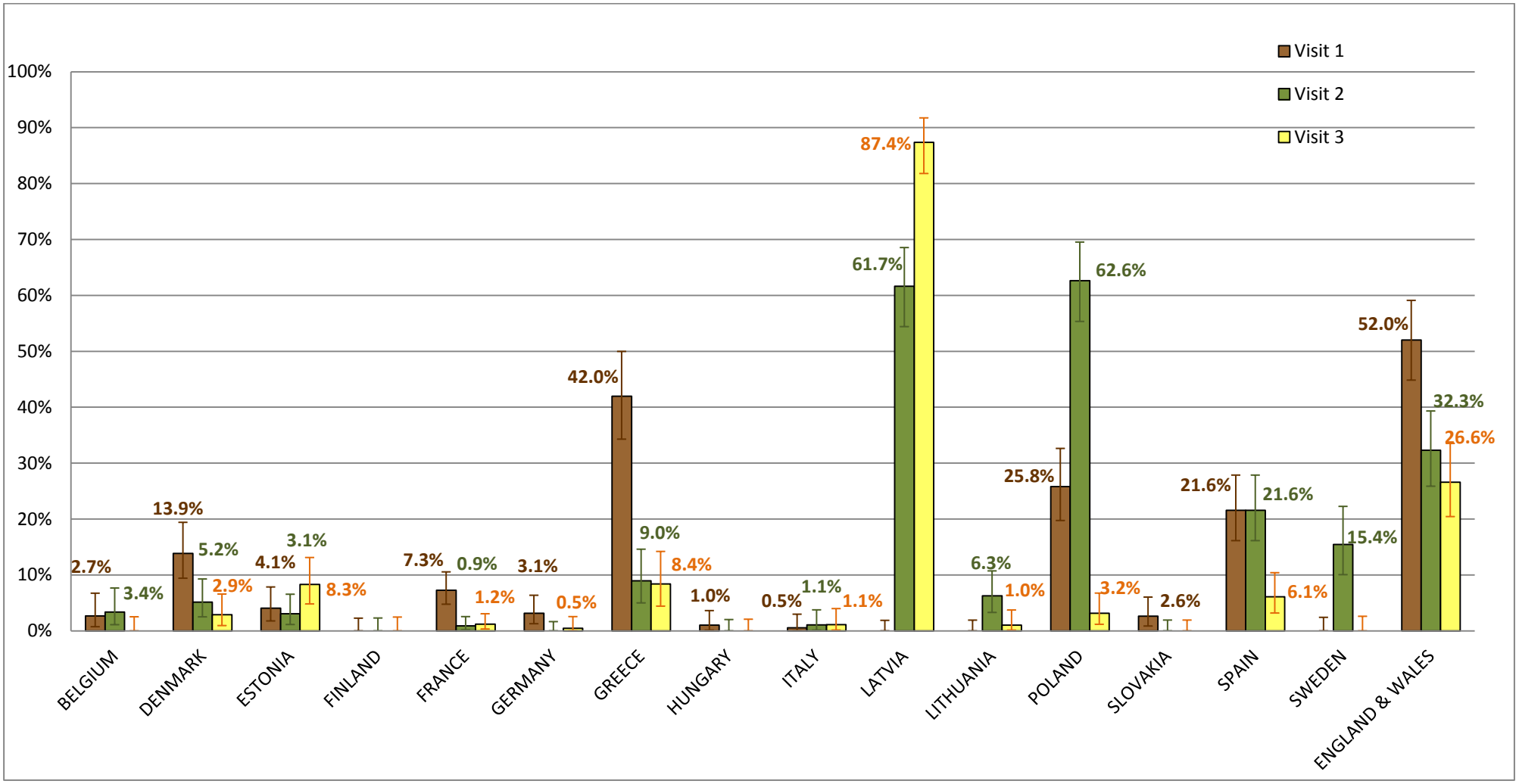
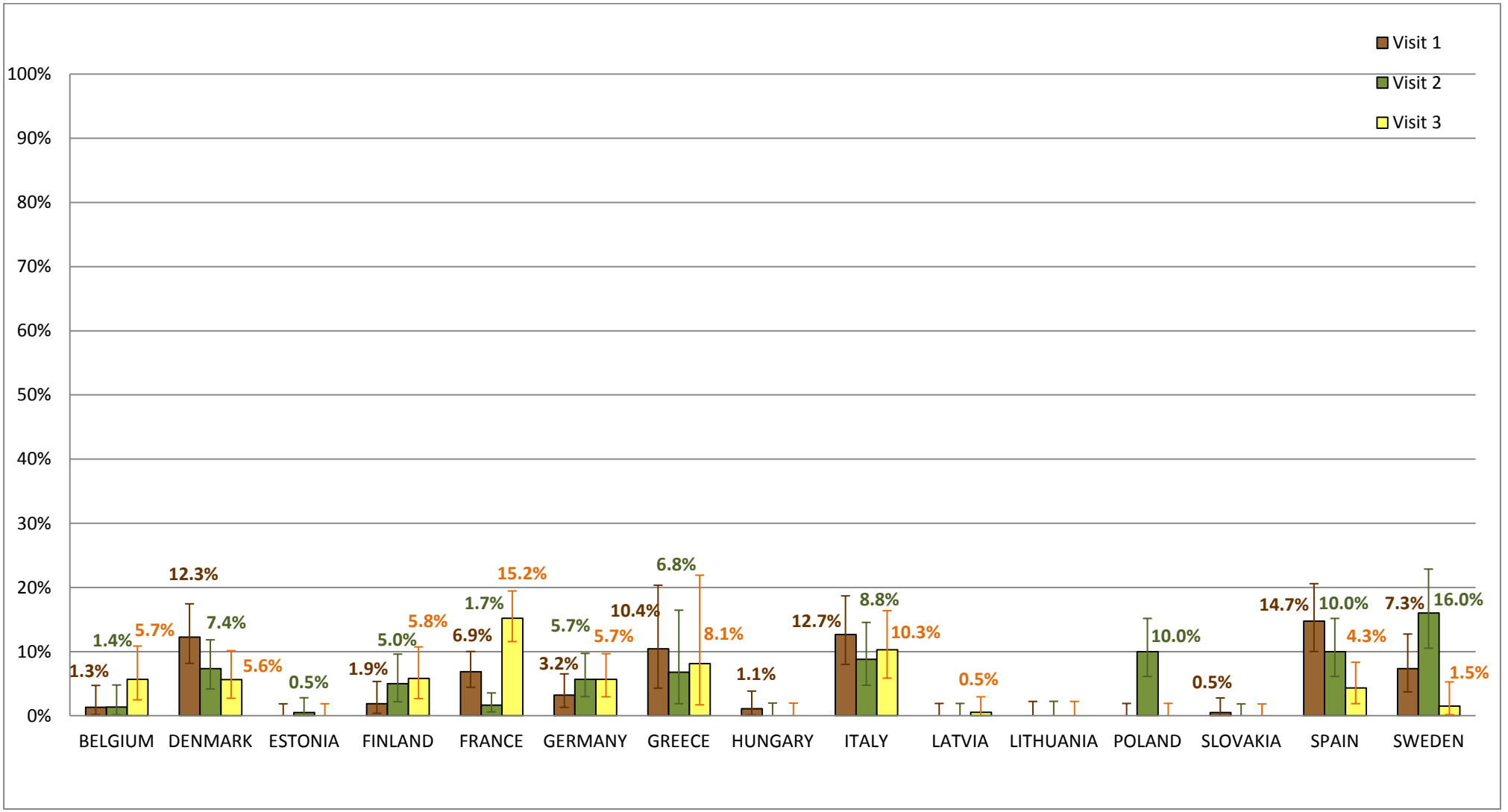


Figure 7: Clinical prevalence of varroosis in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014



During the second year of EPILOBEE, the clinical prevalence of varroosis was observed in nearly all the Member States (14 out of the 15 Member States with comparable data) similarly to the first year of the programme (Figures 6 and 7 and Table 12 in Annex III). In Lithuania, no positive case of varroosis was observed. However, this does not mean that Lithuania was free from *V. destructor*. The varroosis clinical prevalence was higher than 10% in at least one visit in seven Member States with a maximum rate of 16% for Sweden.

The confidence intervals from the two years overlapped at every visit for four out of the 15 Member States with comparable data (Belgium, Denmark, Hungary and Slovakia). For the other Member States, the clinical prevalence of varroosis observed statistically decreased during the second year in at least one visit for six Member States (Estonia, Greece, Latvia, Lithuania, Poland and Spain) and statistically increased during the second year five Member States (Finland, France, Germany, Italy and Sweden) (Table 12 in Annex III and report from the 1st year).

Nosemosis

Figure 8: Clinical prevalence of nosemosis in the apiaries recorded during the three visits of EPILOBEE 2012 – 2013 (Revised graph)

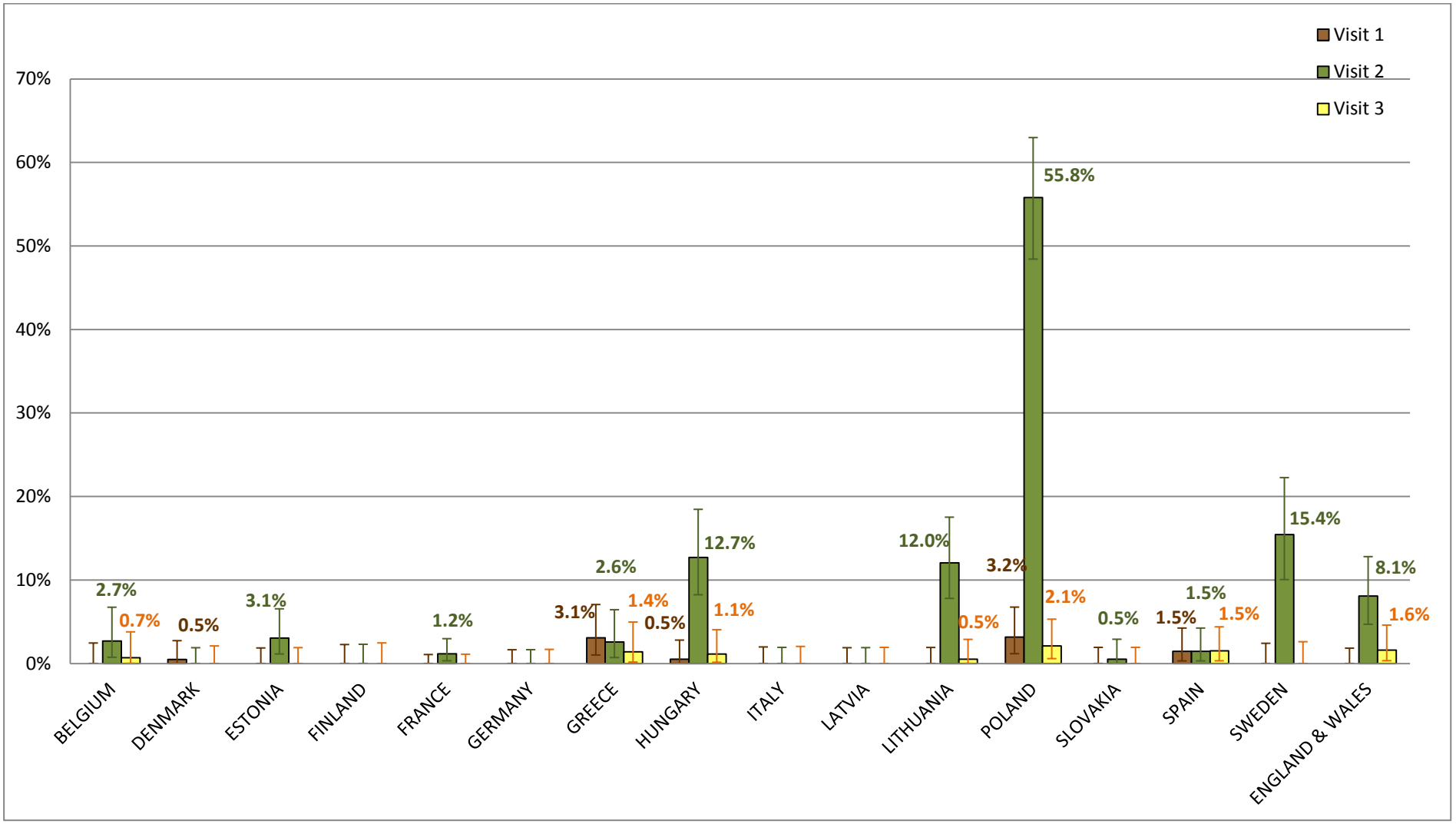
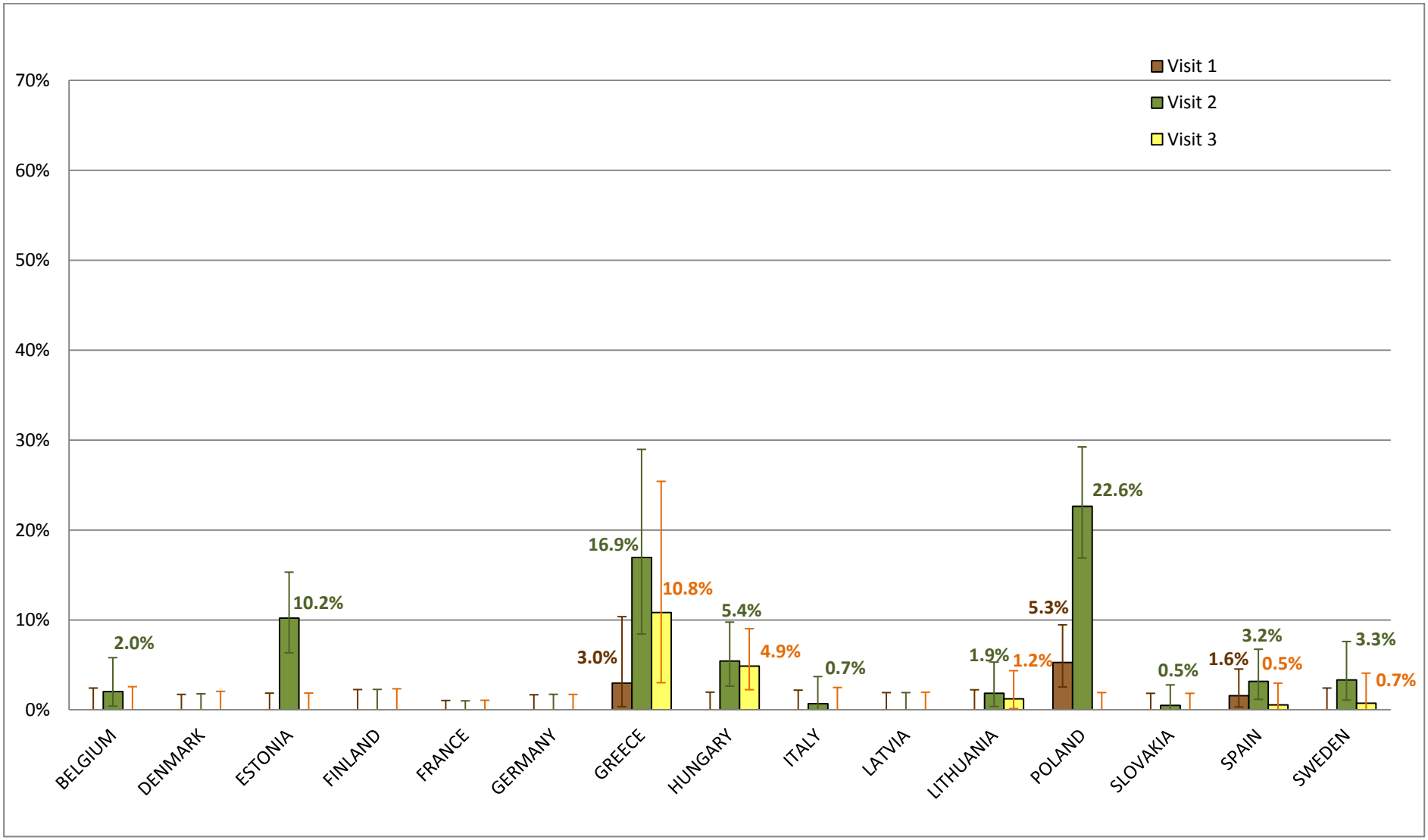


Figure 9: Clinical prevalence of nosemosis in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014



Clinical cases of nosemosis were observed in ten out of the 15 Member States with comparable data. No clinical case of nosemosis was observed in Denmark, Finland, France, Germany and Latvia. The clinical prevalence exceeded 10% in only three Member States in at least one visit (Estonia, Greece and Poland). In all the Member States with positive cases, the prevalence increased at visit 2 compared to visit 1, with a maximum rate of 22.6% in Poland. A similar trend was observed during the first year of the programme (Figures 8 and 9 and Table 13 in Annex III).
As for the first year of the study, the positive cases reported in Figure 9 were based on analyses performed on samples collected in colonies exhibiting clinical signs of nosemosis as detailed in the surveillance protocol.
For 11 out of the 15 Member States with comparable data, the confidence intervals from EPILOBEE first year and EPILOBEE second year overlapped at every visit meaning that the clinical prevalence of nosemosis were not statistically different from one year to the other in these Member States. For the four remaining Member states, a statistical difference in the clinical prevalence of nosemosis was observed between the two years only at visit 2 towards a decrease during the second year for Lithuania, Poland and Sweden and towards an increase during the second year for Greece (Table 13 in Annex III and report from the first year).

Chronic paralysis

Clinical prevalence of chronic paralysis was observed in four Member States (Belgium, France, Poland and Spain). Overall, it did not exceed 2% at any visit in these four Member States. Similarly positive cases of paralysis were noticed in a few Member States during the first year programme (Table 14 in Annex III and Figures 12 and 13 in Annex IV). The positive cases reported in Figures 12 and 13 were based on laboratory analyses performed on samples collected in colonies exhibiting clinical signs of chronic paralysis as detailed in the surveillance protocol. A threshold of 10^8 copies of viral genome per bee was applied to the laboratory results for the calculation of the disease prevalence.
For the 15 Member States with comparable data the confidence intervals from EPILOBEE first year overlapped with the confidence intervals from EPILOBEE second year at any visit meaning that there was no statistical difference between the two years for the clinical prevalence of chronic paralysis (Table14 in Annex III).

4. 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 allowing comparisons between Member States and joint statistical analyses.

More than 90% of the apiaries randomly selected at the beginning of each year of EPILOBEE were monitored throughout each entire year of the programme. Given the importance of the programme, this high rate of follow-up shows the great involvement of all the stakeholders in each Member State and emphasizes the feasibility and repeatability of EPILOBEE.

Winter colony mortality rates

As discussed in the first EPILOBEE report, no reference values exist for the acceptable level of colony losses during winter. Different winter colony losses were reported in European countries (Charrière & Neumann 2010, Genersch *et al.* 2010) and outside Europe (Vanengelsdorp et al. 2008, Spleen et al. 2013, Head *et al.* 2010). For the purpose of the report, the empirical threshold of 10% was considered acceptable by the EURL for European winter honeybee colony mortality; this threshold being open to discussion. Indeed, in some areas of Europe and other parts of the world, higher or lower mortality rates can be considered as bearable by beekeepers and scientists (see report of the first year).

During the second year of EPILOBEE, overwintering 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.

If compared to livestock numbers, the ten Member States with winter colony mortality rates lower than 10% covered about 8 931 600 colonies. This number corresponds to 64.5% of the total estimated number of colonies in the European Union in 2011 (Chauzat et al. 2013). Member States with winter colony mortality rates higher than 10% possessed 1 831 075 colonies which represented 13.2% 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 overwintering colony mortality rates over these two years is noticeable. However, this had to 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 statistically decreased 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 statistically decreased during the 2014 beekeeping season.

The yearly colony mortality rates for 2013 – 2014 were lower than the yearly rates obtained from the first year of EPILOBEE in 13 Member States. However, the confidence intervals of the yearly colony mortality rates from the two years of the programme overlapped for eight Member States (Denmark, France, Germany, Greece, Hungary, Italy, Lithuania and Slovakia) meaning that the difference in the colony losses between the two years was not statistically significant for these Member States. For Belgium, Estonia, Finland, Latvia, Poland, Portugal, Spain and Sweden, the yearly colony mortality rates statistically decreased during the second year of EPILOBEE. Thus, the decrease in colony

losses observed during 2013 – 2014 will be fully analysed, carefully interpreted and put into perspective.

The COLOSS network set up an international study to investigate the honeybee colony losses during winter 2013-2014 in 19 European countries. Similarly to the study conducted during winter 2012-2013, data were gathered through questionnaires voluntarily filled by beekeepers. Even if, the methodology implemented was different from the one set up in EPILOBEE making the comparison difficult, COLOSS preliminary results seem to tend to the similar conclusion of reduced colony losses for winter 2013-2014 (Press release from COLOSS in July 2014).

It is known that climate strongly influenced winter colony losses but other risk factors may also play a role. Specific statistical analyses are currently ongoing 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...).

Diseases prevalence

The clinical prevalence of the two diseases affecting the brood (American foulbrood and European foulbrood) and of chronic paralysis did not drastically change between the two years. The prevalence remained low over the two years of the programme. The confidence intervals in which the *real clinical prevalence* of these three diseases could be found overlapped for the Member States with comparable data meaning that there was no significant difference for this prevalence between the two years of EPILOBEE.

During the second year of the programme there were some variations in the clinical prevalence of varroosis if compared to the data from the first year of EPILOBEE. Indeed, there was a statistical difference in the clinical prevalence of varroosis between the two years in at least one visit in 11 Member States (Estonia, Finland, France, Germany, Greece, Italy, Latvia, Lithuania, Poland, Spain and Sweden). The overall clinical prevalence was lower in the second year compared to the first year. Less variation between Member States in the positive cases might be due to a gain from the observation practice and the interpretation of the varroosis clinical signs by bee inspectors on the field.

As for the first year of EPILOBEE, the clinical prevalence of varroosis should not be confused with the evaluation of varroa mites' infestation. The assessment of the parasitic pressure was evaluated by sampling living bees at the visit performed in autumn 2013 on all the randomly selected colonies. The statistical link between the varroa mites' infestation of the colonies, the subsequent colony survival to the winter and other factors is currently under study.

Sustainable outcomes

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

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

Harmonisation of the trainings 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 for the sensitization of the beekeepers taking part in EPILOBEE to the detection of clinical signs of the main parasitic and infectious diseases affecting honeybees.

Small Hive Beetle introduction in Italy

EPILOBEE was a sound opportunity to sensitize veterinarians, bee inspectors and beekeepers involved in the programme to the detection of *A. tumida* and *Tropilaelaps* mite, two exotic arthropods. *A. tumida* has been detected in some honeybee colonies in the Calabria region on the 5th of September 2014. This outbreak occurred towards the end of the EPILOBEE programme. Late summer visits were implemented in September and October 2014 in 13 Italian apiaries. None of them were located in the regions surrounding the Calabria region or in the Calabria region itself. During the first year of EPILOBEE, the minimum detectable prevalence of *A. tumida* presence was calculated for each Member State taking part in EPILOBEE using the number of apiaries randomly selected for the programme. The minimum detectable prevalence was 1.62% for Italy. No apiary was sampled during the second year of EPILOBEE in the recently infested areas. This was a limit of the programme. To apply the calculated design prevalence to the whole country, the sampling frame should have concerned the entire country homogeneously. Similarly to the dissemination observed in the US (Hood, 2004), *A. tumida* could have been silently present during several months in Italy before official detection.

Perspectives of the EPILOBEE programme

Representative and comparative data on honeybee health were collected during these two years showing that the methodology implemented in EPILOBEE was fully feasible and repeatable. However, the methodology was adapted in each Member State taking into account their specificities. The punctual diversity in the data collection will be included in the statistical analyses. EPILOBEE has shown that harmonisation of sampling protocols and field trainings were fundamental to collect comparable and robust data. Further harmonisation of national procedures could be implemented at European level by taking into account the particularities of each Member State highlighted during EPILOBEE.

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 and thus be considered as a baseline for future studies. For instance, during EPILOBEE, winter 2013 – 2014 has been relatively warmer and more favourable for honeybees than winter 2012 – 2013 which was particularly long and cold throughout Europe. These two winters were opposite in terms of climate showing the importance of long-term follow-up.

Thus, a second step might be to directly investigate the causes of colony losses by conducting specific studies such as case-control studies including analysis on pesticide residues and the recording of the landscape. These factors would be included as potential causes of honeybee losses, alone or with interactions. This type of study needs the set-up of up-front developed actions. Many factors have to be considered including required harmonized analytical techniques. The pilot actions implemented in different Member States as well as in EPILOBEE should be taken as tools in future monitoring systems at European level.

The considerable amount of data collected during EPILOBEE is currently understudy to assess the correlation between the colony bee mortality and some risk factors such as disease prevalence, environment of colonies, farming practices and size of apiaries. This analysis is carried out at the apiary level and at the colony level in order to get epidemiological knowledge on how risk factors affect colony bee mortality.

5. Conclusions

Before the implementation of EPILOBEE, there was no official and comparable data on the colony losses at the European level. The objective of EPILOBEE was to obtain a state of play on the colony mortality in Europe and some knowledge on the health of colonies. This first pan-European descriptive programme allowed the set-up of epidemiological standardized methods in the Member States taking part in EPILOBEE. European and National surveillance systems benefited from this experience in the field of bee health. The active surveillance programmes were adapted in each Member State taking into account the national particularities. Harmonisation in the methodology will be fundamental in further surveillance programme.

Rates of colony mortality differed from one year to another towards a decrease in the second year of EPILOBEE. Significant regional differences in colony losses were also observed. Climate might have influenced winter colony losses over the two years. However its role in the winter colony mortality should be further balanced with other risk factors that also certainly took part. Furthermore, two consecutive years of follow-up are not sufficient to get a trend in colony losses. Long-term collection of data on colony mortality would enable to obtain a representative overview on the colony mortality in Europe. Prevalence of diseases, based on clinical signs observed by bee inspectors, appeared to have similar trends during the second year of the programme. Although American foulbrood and varroosis were recorded in most of the Member States, overall disease prevalence were low for most of the diseases. It should be emphasised that the substantial amount of data gathered throughout these two years on various topics (use of veterinary treatments, the beekeeping context, colony management...) is currently under analysis to get statistical correlations between the colony losses and some risk factors.

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

6. Acknowledgements

This project has been funded by the European Commission, ANSES through the EURL for bee health and each Member State taking part in EPILOBEE. This programme involved thousands of different stakeholders over the two years of the project: beekeepers, field inspectors, scientists, laboratories and administrations. The EURL for bee health wishes to thank all participants for their substantial involvement in the successful implementation of EPILOBEE.

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Annex I Tables presenting the overall information recorded in the database during the visits of EPILOBEE

Table 6: Tables showing the overall information recorded in the database during the visits of EPILOBEE (Part I)

Information on the beekeeper
Age
Type of beekeeping activity
Member of an organisation
Qualification for beekeeping
Apiarist book
Past trainings
Number of colonies belonging to the beekeeper
Number of apiaries belonging to the beekeeper
Information on the randomly selected apiary
Location of the apiary
Number of colonies in the apiary
Honeybee subspecies in the apiary
Targeted production for the apiary
Environment around the apiary
Information on the visits set up in each apiary
Date of the visit
Period of the visit (autumn, spring or summer)
Number of colonies randomly selected at autumn
Number of selected colonies alive at spring
Number of selected colonies dead at spring
Number of selected colonies sold at spring
Number of selected colonies merged at spring
Number of selected colonies used to produce one or several swarms at spring
Number of selected colonies alive at summer
Number of selected colonies dead at summer
Number of selected colonies sold at summer
Number of selected colonies merged at summer
Number of selected colonies used to produce one or several swarms at summer
Location of the apiary since the last visit

Table 7: Table showing the overall information recorded on the apiaries in the database during the visits of EPILOBEE (Part II)

General health events observed in the selected apiary prior to the visit
Events observed prior to the visit
Disease suspected
Laboratory analyses performed
Conclusion of the analysis
Treatments administered in the apiary prior to the visit
Name of the treatment
Date of the treatment
Active ingredient
Dose rate
Duration of the treatment
Frequency
Livestock management implemented in the apiary prior to the visit
Objectives of the actions implemented
Date of these actions
Number of swarms bought by the beekeeper
Number of swarms produced by the beekeeper
Number of queens bought by the beekeeper
Number of queens produced by the beekeeper
Number of colonies divided by the beekeeper
Number of colonies merged by the beekeeper
Number of colonies that have naturally swarmed
Information on a randomly selected colony
Strength of the colony at each visit
Death of the colony
Varroosis diagnosed on the field for this colony
Disease suspected at each visit
Clinical signs observed at each visit
Nature of the samples taken at each visit
Date of the sampling
Laboratory analysis performed on the samples taken at each visit
Laboratory technique used
Result of the laboratory analysis performed on each sample taken
Varroa counting on the systematic samples taken at the autumn visits
Nosema spores counting performed on the samples analyses for nosemosis

Annex II Colony mortality rates in the Member States of the European Union recorded in EPILOBEE 2012-2013

Table 8: Winter mortality rates in the member states of the European Union recorded in EPILOBEE 2012 – 2013 (Revised table)
95% CI= confidence interval at 95%

	Mortality rate (%)	95% CI inferior limit	95% CI superior 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	14.2	11.3	17.2
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

Table 9: Seasonal mortality rates (2013) in the member states of the European Union recorded in EPILOBEE 2012 – 2013 (Revised table)
95% CI= confidence interval at 95%

	Mortality rate (%)	95% CI inferior limit	95% CI superior 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	10.5	6.4	14.6
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

Annex III Tables of the clinical prevalence of diseases in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014

Table 10: Clinical prevalence of AFB in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014; 95% CI= confidence interval at 95%; NA= not applicable
↓: statistical difference between the two years towards a decrease; →: no statistical difference between the two years

	Visit 1 before winter			Visit 2 after winter			Visit 3 during season		
	%	95% CI		%	95% CI		%	95% CI	
Belgium	0.7	0 – 3.7	→	0	0 – 2.5	→	1.4	0.2 – 5.0	→
Denmark	0.9	0.1 – 3.4	→	0	0 – 1.8	→	0	0 – 2.1	→
Estonia	3.1	1.1 – 6.5	→	1.0	0.1 – 3.6	→	5.1	2.5 – 9.2	→
Finland	3.1	1.0 – 7.1	→	0.6	0 – 3.4	→	0.6	0 – 3.5	→
France	4.0	2.2 – 6.6	↓	2.5	1.1 – 4.7	→	0.6	0.1 – 2.1	→
Germany	0	0 – 1.7	→	0	0 – 1.7	→	0	0 – 1.7	→
Greece	3.0	0.4 – 10.4	→	1.7	0 – 9.1	→	2.7	0.1 – 14.2	→
Hungary	0.0	0 – 2.0	→	0	0 – 2	→	0.5	0 – 3.0	→
Italy	1.2	0.1 – 4.3	→	0.7	0 – 3.7	→	2.1	0.4 – 5.9	→
Latvia	3.7	1.5 – 7.4	→	10.5	6.5 – 15.8	→	5.9	3.0 – 10.3	→
Lithuania	0	0 – 2.2	→	0.6	0 – 3.4	→	1.2	0.1 – 4.4	→
Poland	1.1	0.1 – 3.8	→	0.5	0 – 2.9	→	0.5	0 – 2.9	→
Portugal	NA	NA		NA	NA		NA	NA	
Slovakia	0.5	0 – 2.8	→	0.5	0 – 2.8	→	0	0 – 1.8	→
Spain	1.1	0.1 – 3.8	→	2.6	0.9 – 6.0	→	1.1	0.1 – 3.9	→
Sweden	1.3	0.2 – 4.7	→	2.0	0.4 – 5.7	→	0	0 – 2.7	→

Table 11: Clinical prevalence of EFB in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014; 95% CI= confidence interval at 95%; NA= not applicable
↓: statistical difference between the two years towards a decrease; →: no statistical difference between the two years

	Visit 1 before winter			Visit 2 after winter			Visit 3 during season		
	%	95% CI		%	95% CI		%	95% CI	
Belgium	0	0 – 2.4	→	0	0 – 2.5	→	0.7	0 – 3.9	→
Denmark	0	0 – 1.7	→	0	0 – 1.8	→	0	0 – 2.1	→
Estonia	0	0 – 1.9	→	0	0 – 1.9	→	0	0 – 1.9	→
Finland	0.6	0 – 3.4	→	1.3	0.2 – 4.4	→	2.6	0.7 – 6.5	→
France	2.3	1.0 – 4.5	↓	4.4	2.5 – 7.1	→	1.8	0.6 – 3.8	→
Germany	0	0 – 1.7	→	0	0 – 1.7	→	0	0 – 1.7	→
Greece	0	0 – 5.4	→	0	0 – 6.1	→	0	0 – 9.5	→
Hungary	0	0 – 2.0	→	0	0 – 2.0	→	0	0 – 2.0	→
Italy	0	0 – 2.2	→	0.7	0 – 3.7	→	0.7	0 – 3.8	→
Latvia	0.5	0 – 2.9	→	0	0 – 1.9	→	0	0 – 2.0	→
Lithuania	0	0 – 2.2	→	0	0 – 2.3	→	0	0 – 2.2	→
Poland	0	0 – 1.9	→	0	0 – 1.9	→	0	0 – 1.9	→
Portugal	NA	NA		NA	NA		NA	NA	
Slovakia	0	0 – 1.8	→	0	0 – 1.8	→	0	0 – 1.8	→
Spain	0	0 – 1.9	→	0	0 – 1.9	→	0	0 – 2.0	→
Sweden	0	0 – 2.4	→	0	0 – 2.4	→	0	0 – 2.7	→

Table 12: Clinical prevalence of varroosis in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014
95% CI= confidence interval at 95%; NA= not applicable
↓: statistical difference between the two years towards a decrease; ↑: statistical difference between the two years towards an increase; →: no statistical difference between the two years

	Visit 1 before winter			Visit 2 after winter			Visit 3 during season		
	%	95% CI		%	95% CI		%	95% CI	
Belgium	1.3	0.2 – 4.7	→	1.4	0.2 – 4.8	→	5.7	2.5 – 10.9	→
Denmark	12.3	8.2 – 17.5	→	7.4	4.2 – 11.8	→	5.6	2.7 – 10.7	→
Estonia	0	0 – 1.9	→	0.5	0 – 2.8	→	0	0 – 1.9	↓
Finland	1.9	0.4 – 5.3	→	5.0	2.2 – 9.6	→	5.8	2.7 – 10.7	↑
France	6.9	4.4 – 10.0	→	1.7	0.6 – 3.6	→	15.2	11.6 – 19.5	↑
Germany	3.2	1.3 – 6.5	→	5.7	3.0 – 9.7	↑	5.7	3.0 – 9.7	↑
Greece	10.4	4.3 – 20.3	↓	6.8	1.9 – 16.5	→	8.1	1.7 – 21.9	→
Hungary	1.1	0.1 – 3.9	→	0	0 – 2.0	→	0	0 – 0.2	→
Italy	12.7	8.0 – 18.7	↑	8.8	4.8 – 14.6	↑	10.3	5.9 – 16.4	↑
Latvia	0	0 – 1.9	→	0	0 – 1.9	↓	0.5	0 – 3	↓
Lithuania	0	0 – 2.2	→	0	0 – 2.3	↓	0	0 – 2.2	→
Poland	0	0 – 1.9	↓	10.0	6.1 – 15.2	↓	0	0 – 1.9	→
Portugal	NA	NA		NA	NA		NA	NA	
Slovakia	0.5	0 – 2.8	→	0	0 – 1.8	→	0	0 – 1.8	→
Spain	14.7	10.0 – 20.6	→	10.0	6.1 – 15.2	↓	4.3	1.9 – 8.3	→
Sweden	7.3	3.7 – 12.7	↑	16.0	10.5 – 22.9	→	1.5	0.2 – 5.3	→

Table 13: Clinical prevalence of nosemosis in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014;
95% CI= confidence interval at 95%; NA= not applicable
↓: statistical difference between the two years towards a decrease; ↑: statistical difference between the two years towards an increase; →: no statistical difference between the two years

	Visit 1 before winter			Visit 2 after winter			Visit 3 during season		
	%	95% CI		%	95% CI		%	95% CI	
Belgium	0	0 – 2.4	→	2.0	0.4 – 5.8	→	0	0 – 2.6	→
Denmark	0	0 – 1.7	→	0	0 – 1.8	→	0	0 – 2.1	→
Estonia	0	0 – 1.9	→	10.2	6.3 – 15.3	→	0	0 – 1.9	→
Finland	0	0 – 2.3	→	0	0 – 2.3	→	0	0 – 2.4	→
France	0	0 – 1.0	→	0	0 – 1.0	→	0	0 – 1.1	→
Germany	0	0 – 1.7	→	0	0 – 1.7	→	0	0 – 1.7	→
Greece	3.0	0.4 – 10.4	→	16.9	8.4 – 29.0	↑	10.8	3.0 – 25.4	→
Hungary	0	0 – 2.0	→	5.4	2.6 – 9.8	→	4.9	2.2 – 9.0	→
Italy	0	0 – 2.2	→	0.7	0 – 3.7	→	0	0 – 2.5	→
Latvia	0	0 – 1.9	→	0	0 – 1.9	→	0	0 – 2.0	→
Lithuania	0	0 – 2.2	→	1.9	0.4 – 5.3	↓	1.2	0.1 – 4.4	→
Poland	5.3	2.6 – 9.5	→	22.6	16.9 – 29.2	↓	0	0 – 1.9	→
Portugal	NA	NA		NA	NA		NA	NA	
Slovakia	0	0 – 1.8	→	0.5	0 – 2.8	→	0	0 – 1.8	→
Spain	1.6	0.3 – 4.5	→	3.2	1.2 – 6.7	→	0.5	0.0 – 3.0	→
Sweden	0	0 – 2.4	→	3.3	1.1 – 7.6	↓	0.7	0 – 4.1	→

Table 14: Clinical prevalence of CBPV in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014
95% CI= confidence interval at 95%; NA= not applicable
↓: statistical difference between the two years towards a decrease; ↑: statistical difference between the two years towards an increase; →: no statistical difference between the two years

	Visit 1 before winter			Visit 2 after winter			Visit 3 during season		
	%	95% CI		%	95% CI		%	95% CI	
Belgium	0	0 – 2.4	→	0.7	0 – 3.7	→	0	0 – 2.6	→
Denmark	0	0 – 1.7	→	0	0 – 1.8	→	0	0 – 2.1	→
Estonia	0	0 – 1.9	→	0	0 – 1.9	→	0	0 – 1.9	→
Finland	0	0 – 2.3	→	0	0 – 2.3	→	0	0 – 2.4	→
France	0.3	0 – 1.6	→	1.4	0.4 – 3.2	→	1.5	0.5 – 3.4	→
Germany	0	0 – 1.7	→	0	0 – 1.7	→	0	0 – 1.7	→
Greece	0	0 – 5.4	→	0	0 – 6.1	→	0	0 – 9.5	→
Hungary	0	0 – 2.0	→	0	0 – 2.0	→	0	0 – 2.0	→
Italy	0	0 – 2.2	→	0	0 – 2.5	→	0	0 – 2.5	→
Latvia	0	0 – 1.9	→	0	0 – 1.9	→	0	0 – 2.0	→
Lithuania	0	0 – 2.2	→	0	0 – 2.3	→	0	0 – 2.2	→
Poland	0.5	0 – 2.9	→	0.5	0 – 2.9	→	1.1	0.1 – 3.8	→
Portugal	NA	NA		NA	NA		NA	NA	
Slovakia	0	0 – 1.8	→	0	0 – 1.8	→	0	0 – 1.8	→
Spain	0.5	0 – 2.9	→	0.5	0 – 2.9	→	0	0 – 2.0	→
Sweden	0	0 – 2.4	→	0	0 – 2.4	→	0	0 – 2.7	→

Annex IV Figures of the clinical prevalence of diseases in the apiaries recorded during the three visits of EPILOBEE 2012 – 2013 and EPILOBEE 2013 – 2014

Figure 10: Clinical prevalence of EFB in the apiaries recorded during the three visits of EPILOBEE 2012 – 2013 (Revised graph)

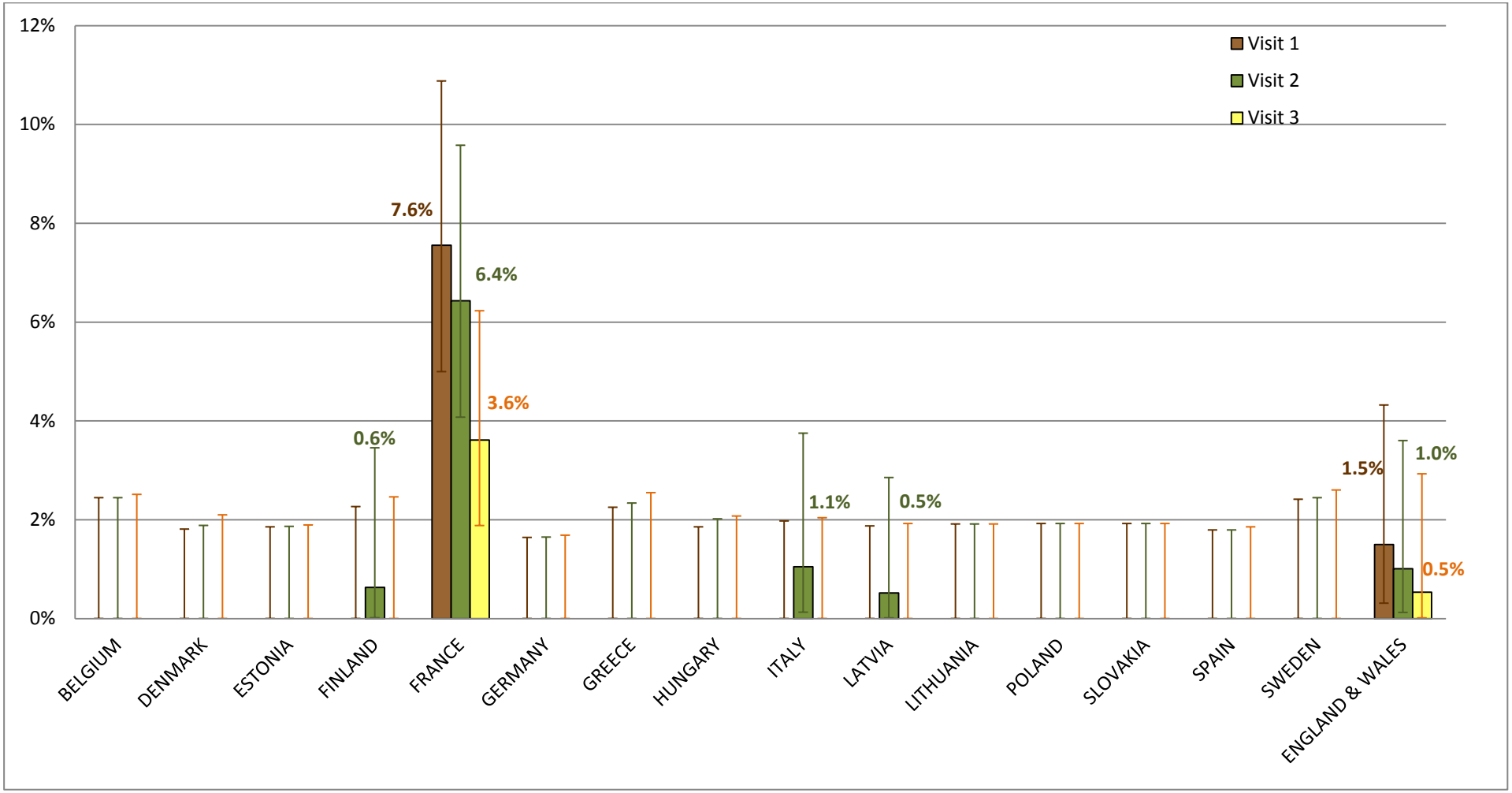


Figure 11: Clinical prevalence of EFB in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014

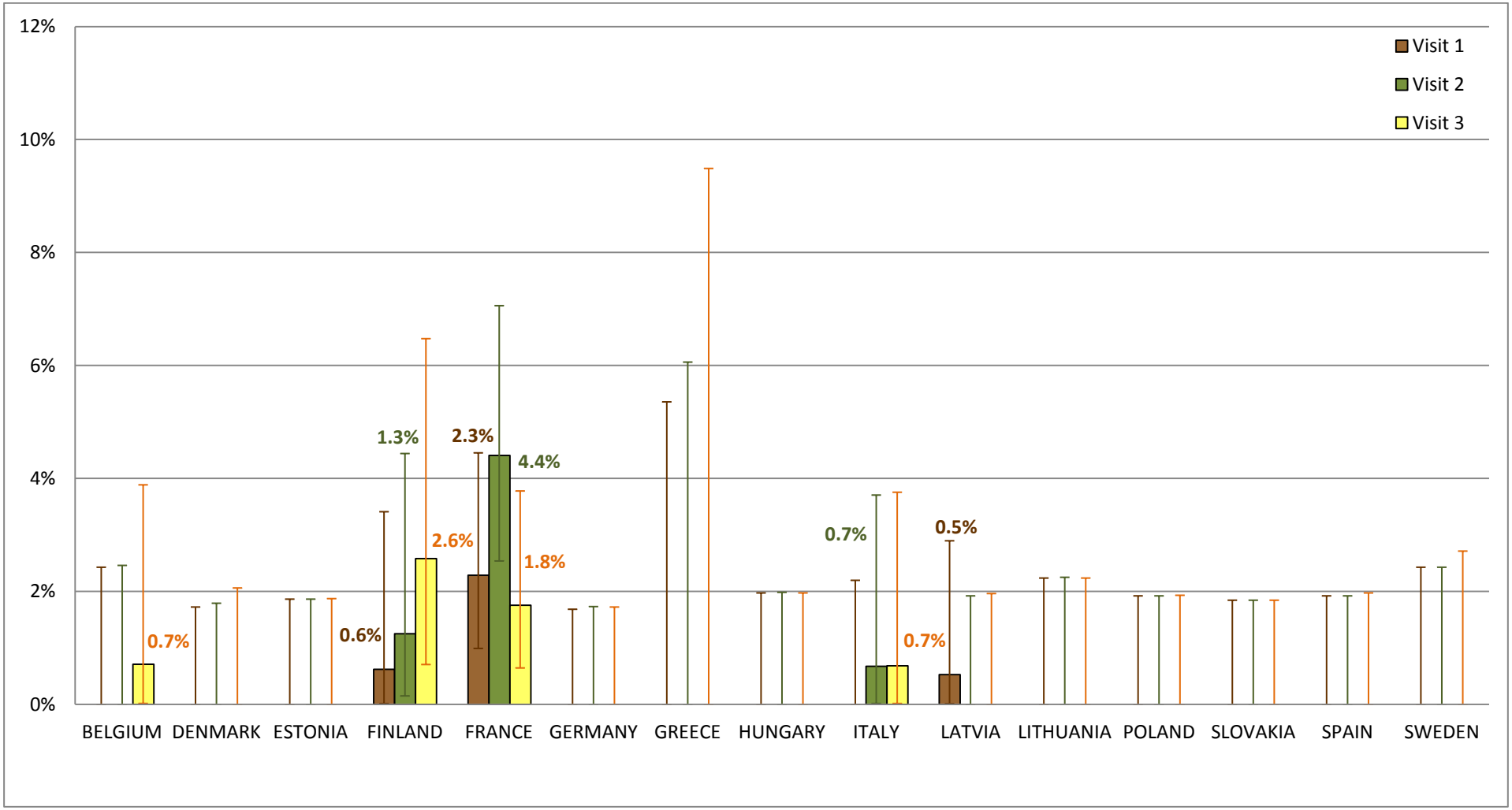


Figure 12: Clinical prevalence of CBPV in the apiaries recorded during the three visits of EPILOBEE 2012 – 2013 (Revised graph)

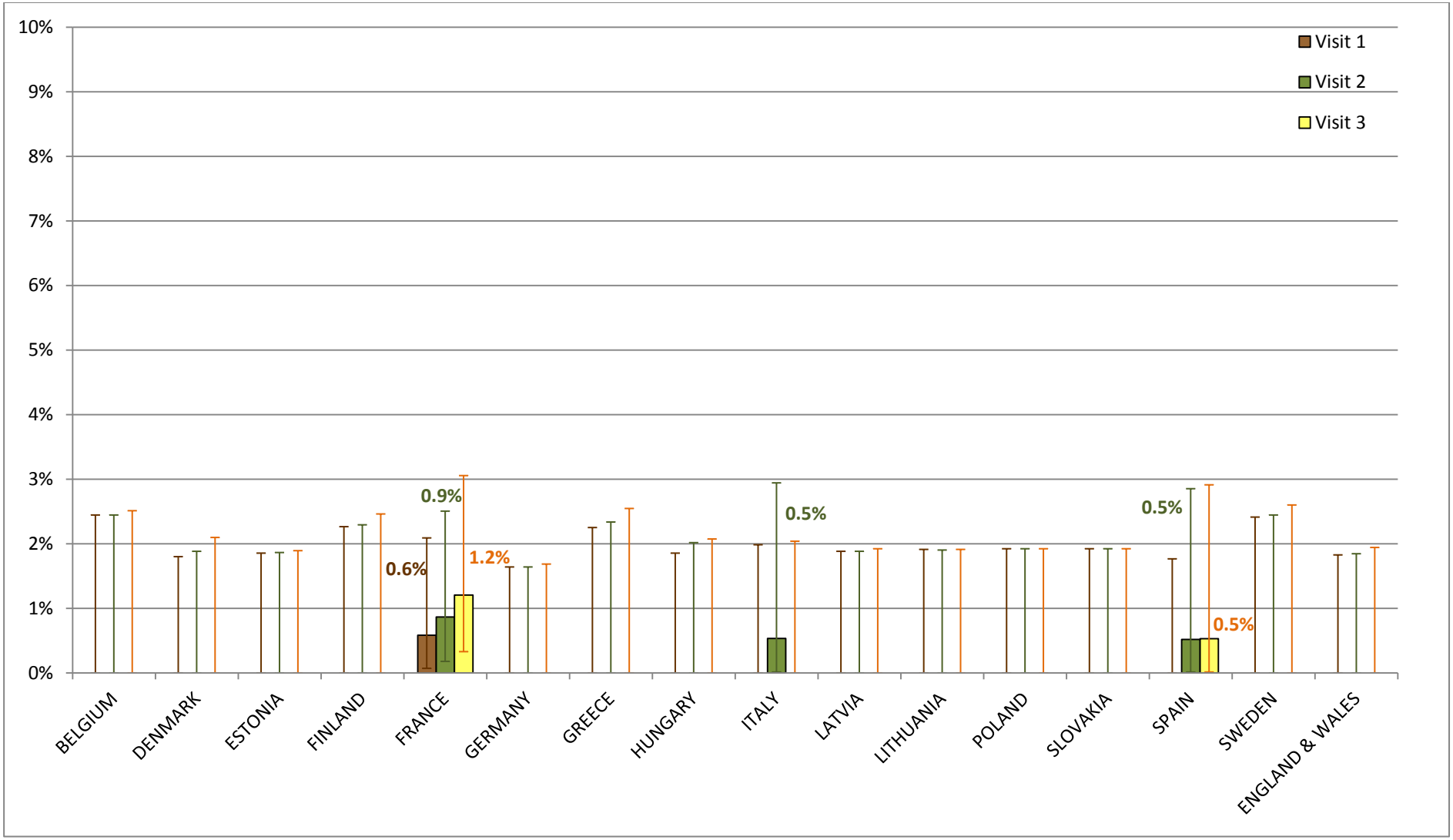
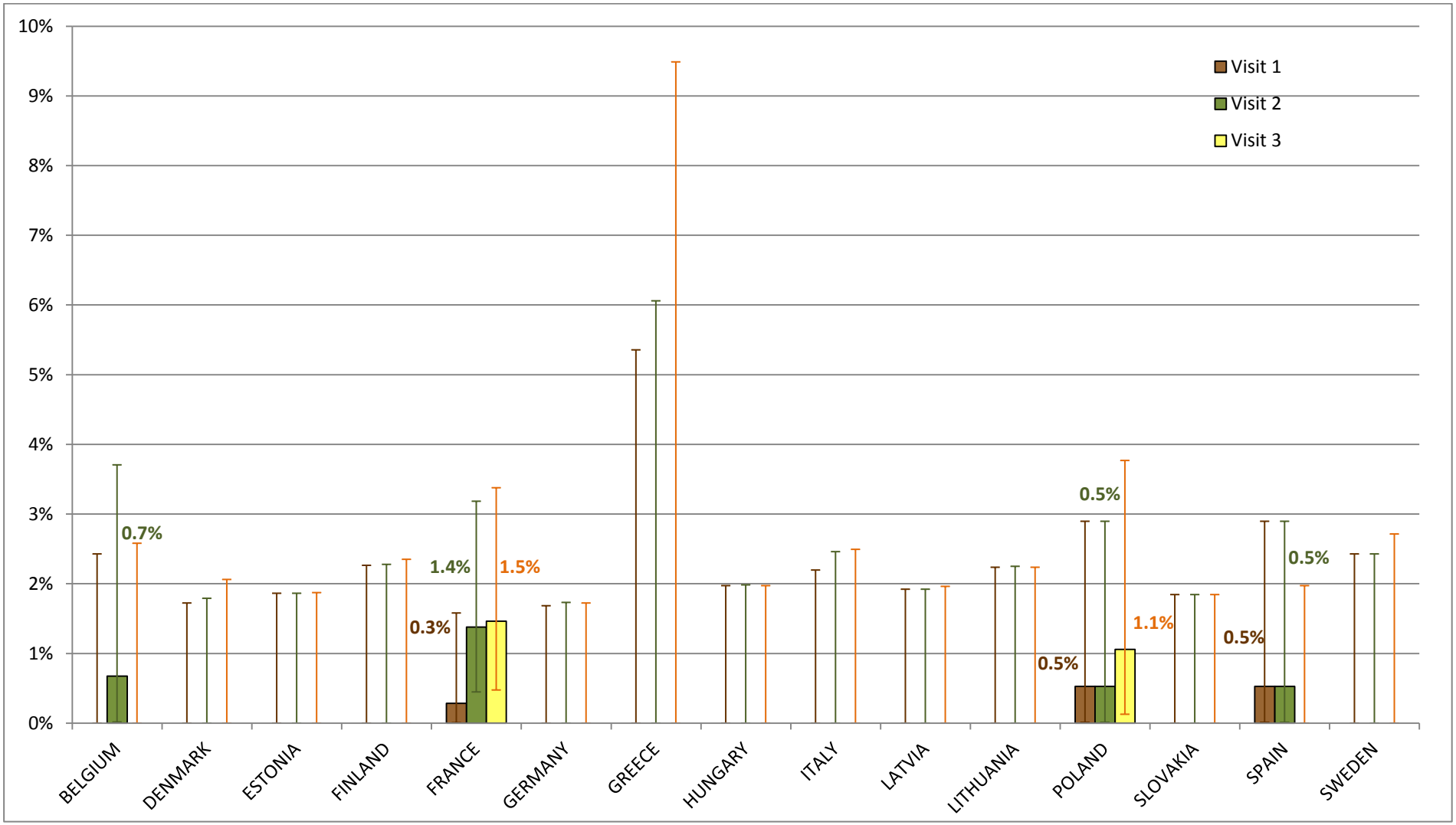


Figure 13: Clinical prevalence of CBPV in the apiaries recorded during the three visits of EPILOBEE 2013 – 2014



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