Risk factors for honey bee (*Apis mellifera* L.) mortality in Estonian apiaries during 2012–2013

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Abstract. In light of the global increase in honey bee colony losses, risk factors regarding beekeeping management practices and honey bee diseases have been studied intensively during the last decade. Some risk factors have been outlined, but the correlation of evidence between relevant factors coinciding with honey bee mortality still needs to be clarified. The current study used the two-year data collected in frames of the European Commission EPILOBEE project. Previously, the data from Estonian apiaries were analysed together with the data from all 17 participating European countries in the consortium. In the current study, data from Estonian apiaries were targeted separately. In total, 196 apiaries containing 2,439 colonies all over Estonia were included in this dataset. The study aimed to clarify the risk factors that would predict colony losses in Estonia. The main factors increasing colony mortality after winter were the size of the apiary, *Varroa destructor* mite count, infestation with *Paenibacillus larvae,* and lack of farmlands around the apiary. No significant risk factors in relation to honey bee summer mortality were detected.

Key words: American foulbrood, Beekeeping management practices, EPILOBEE, honey bee breeds, *Varroa destructor.*

INTRODUCTION

Beekeeping in Estonia is important and widespread but operated rather in small-scale establishments. Estonia is situated in the northern temperate zone that is characterized by the cold winters and summers that tend to have brief warm period. In temperate climates, weather conditions during winter can put substantial pressure on honey bee colony survival (Switanek et al., 2017; Brodschneider et al., 2019). Again, weather conditions during summer can influence the winter survival of honey bees, as shown in a recent study conducted in the northeastern United States (Calovi et al., 2021). Consequently, high colony losses can also occur during the summer following noticeable losses in the winter (Jacques et al., 2017).

However, the climatic conditions *per se* are not responsible for honey bee survival. The mortality of managed honey bee colonies has been explained by a multicausal aetiology. Variable interactions between the causal agents contribute to the presence of honey bee pathogens, chemical pollution, pesticides and factors related to housing,

beekeeping management practices, and the environment surrounding apiaries (Dennis vanEngelsdorp & Meixner, 2010; Clermont et al., 2015; Goulson et al., 2015; Wilfert et al., 2016; Switanek et al., 2017; Bird et al., 2021; El Agrebi et al., 2021). In addition, honey bees should have managed to accumulate an adequate amount of food reserves for surviving the winter (Döke et al., 2015; Lemanski et al., 2020). Honey bee colony losses in Europe exhibit fluctuating patterns depending on the year and several other factors (Jacques et al., 2017; Buendia et al., 2018; Gray et al., 2019; Gray et al., 2020).

The factor often contributing to honey bee survival is the size of the apiary, which reflects the type of beekeeping operation (hobby or commercial) and experience of the beekeepers (Jacques et al., 2017). Most studies find the highest mortality rates in small apiaries operated by hobby beekeepers (Chauzat et al., 2016; Gray et al., 2019).

Although honey bee mortality can vary depending on the year, beekeeping management practices, and the combination of stressors, the most strongly contributing factors are probably pathogens such as varroa mites (*Varroa destructor*) together with associated viruses and the microsporidians *Nosema* spp. (D. vanEngelsdorp et al., 2009; Dennis vanEngelsdorp & Meixner, 2010; Jacques et al., 2017; Thaduri et al., 2019).

Varroa mites not only burden bees mechanically by feeding on their brood and adult honey bees' haemolymph and fat body tissue (Ramsey et al., 2019) but are also effective vectors for many bee viruses, such as deformed wing virus (Wilfert et al., 2016). Nearly 100% of Estonian apiaries are infested with varroa mites (Mõtus et al., 2016).

The current study aimed to analyse risk factors for winter and summer mortality. The data were collected in framesof the pan-European epidemiological project on active surveillance of honey bee colony losses (EPILOBEE 2012–2014) coordinated by the EU Reference Laboratory for Honey Bee Health.

The EPILOBEE project included data from 17 European countries, with overwinter losses from 2% to 32%. Estonia, along with Finland, Latvia, and Sweden, was clustered according to its honey bee mortality rates. It was found to have upper-middle mortality rates in 2012 and 2013. In 2012, Poland was included in the same cluster; Portugal and Denmark were included in 2013 (Jacques et al., 2017). Although the data from Estonian apiaries were analysed within the consolidated dataset, we suspected that the local trends might be overshadowed. In addition, there has been no other broad-based epidemiological study before or after that in Estonian apiaries. Therefore, in this current paper, we only examined the specifics of Estonian colony losses.

MATERIALS AND METHODS

Study population
The used data were collected in frames of a pan-European EPILOBEE project, 2012–2014. The detailed epidemiological study design was described by Chauzat et al. (2016) and Jacques et al. (Jacques et al., 2017).

In total, 196 apiaries containing 2439 colonies throughout Estonia were randomly selected from the beekeeper register managed by The Agricultural Registers and Information Board of Estonia (EARIB). In 2012 the total number of registered apiaries in Estonia was 8,488 (EARIB database, 2012). Within each apiary, the sample size calculator assigned a random and representative number of colonies. The number of honey bee colonies in the selected apiaries ranged from 1 to 170 (median 14; 75% quartile 19). The Estonian Veterinary and Food Board carried out a sampling of the apiaries during

three visits: the first visit in September 2012 (before winter), the second visit in April 2013 (after winter), and the third visit in July 2013, in the middle of the beekeeping season. The sample to detect infectious diseases and parasites consisted of adult honey bees collected from randomly selected colonies within the apiary. Every visit was accompanied by a questionnaire described in detail in the study by (Mõtus et al., 2016).

The colonies sampled for this study were used to estimate the mortality in every apiary. Mortality was calculated as the proportion of colonies the beekeepers reported as lost from all the sampled colonies in an apiary due to any reason excluding predation (brown bear, rodents, woodpecker, etc.). Apiaries were grouped according to the quartiles of the number of colonies in apiary.

Data analysis

To identify factors associated with honey bee winter mortality, a Tobit regression model was used. Winter mortality within apiary (% of colonies lost) was used as a response variable. Tobit regression was used because 35% of apiaries did not have honey bee winter mortality (0%). In the Tobit regression, all cases falling above (or below) a specified threshold value are censored, although these cases remain in the analysis (Tobin, 1958). The outcome variable (winter mortality %) was transformed logarithmically to achieve a normal distribution of variable. The winter mortality minimum was 3% loss of the colonies, maximum was 100%. Univariable analyses were performed, and variables with a P-value < 0.2 were included in the full model. For categorical variables with more than two levels, the Wald test P-value was used. The possible confounding effect of the size of the apiary (categorized into four groups according to quartiles) was individually assessed for every independent variable in separate models. The full model included the size of the apiary, the presence of American foulbrood (yes/no), the average count of varroa mites (categorized into 4 groups according to quartiles), farmlands around the apiary (yes/no), town near the apiary (yes/no), orchards near the apiary (yes/no), type of beekeeping (hobby, part-time or professional), the beekeeper's experience, which was defined by the number of years in beekeeping business, and the bee breed (Buckfast, Carnica, hybrid, Ligustica, and the race Dark European Honey Bee). Backwards stepwise elimination of the variables was used for the final model. Possible interactions and confounders (change of the coefficient over 15% after variable elimination) were controlled.

To identify factors associated with honey bee summer mortality (minimum was 3% loss of the colonies, maximum 50%), a logistic regression model was used. Honey bee summer mortality, as either present (15% of apiaries) or not, was used as a response variable. Model building strategies were the same as in the honey bee winter mortality model (univariable models with apiary size included, followed by backwards stepwise elimination from the full model). The initial full model included four variables: the size of the apiary, farmland around the apiary, town near the apiary, and the average count of varroa mites.

The statistical program STATA 14.2 (StataCorp LP, College Station, TX, USA) was used for the described Tobit regression and logistic regression models.

RESULTS AND DISCUSSION

Winter mortality

The results of the regression analysis to test associations between honey bee winter mortality and the investigated risk factors are presented in Table 1. There was a significant positive association between the size (number of colonies) of the apiary and honey bee winter mortality ($P = 0.001$). The relationship became stronger with the increasing number of colonies in the apiary (Table 1). The group of the largest apiaries contained apiaries with 20–65 colonies but also had one outlier - an apiary with 170 colonies. Removing the outlier from the model did not change the result, so we still consider the results valid (Fig. 1). When included in the consortium, the results of the data analysis of 17 European countries previously reported by Jacques et al. (2017) showed the opposite result as the small hobby beekeepers had a mortality risks twice as high as professional beekeepers. This result could be explained by a lack of experience and professionalism by hobby beekeepers harbouring a smaller number of colonies. The same dataset for all the participants from the first study year showed a negative relationship between winter mortality and apiary size (Chauzat et al., 2016). Like the EPILOBEE consortium study, the four-year survey of beekeepers in China showed that commercial beekeepers operating larger apiaries tended to have a lower rate of colony losses than did hobby beekeepers. However, there was a significant year effect as the mortality trends in different sizes of beekeeping operations in a certain year were not always consistent with the overall trend (Tang et al., 2020). Additionally, a COLOSS survey involving data from 2017–2018 from 36 countries reported significantly higher losses in small apiaries up to 50 colonies (Gray et al., 2020). A study in Austria showed no connection between apiary size and colony losses (Morawetz et al., 2019).

The varroa mite count in colonies significantly affected honey bee winter mortality according to the Tobit regression model, where varroa mite-infected honey bee colonies were grouped using the quartiles of the data ($P < 0.001$). The association became stronger when the average count of varroa mites increased (Table 1).

Guzmán-Novoa et al. (2010) studied the effect of parasite levels, the strength of the colony, and the food reserves on winter mortality in Canada. They found that varroa mite burden could be the main factor explaining the death and reduced populations of honey bee colonies in northern climates.

In case varroa mite infestation is controlled, weather conditions, particularly high temperatures in summer and precipitation in the past year, were the strongest predictors of overwintering survival, as shown in the north-eastern United States while using European honey bee colonies in the study (Calovi et al., 2021). The data on Switzerland's honey bee population support the idea that *V. destructor* is a keyplayer in colony losses, interacting in combination with other pathogens and the effect of the particular year (Dainat et al., 2012).

Apparently, honey bees have also become more sensitive to varroa parasites as evidenced by changes in the *Varroa* infestation rate causing colony loss since 1980, as discussed elsewhere (vanEngelsdorp & Meixner, 2010). VanEngelsdorp & Meixner (2010) reported that a honey bee colony could withstand a couple of thousand mites; currently, 10% infestation indicates imminent colony loss.

Table 1. Results of the Tobit regression model to test associations between honey bee winter mortality (ln percentage) and various parameters of the apiary. The total sample size was 186 apiaries. In the model, 68 of the observations were left-censored (winter mortality 0%) and 118 were uncensored observations (winter mortality 3.2–100%). Apiaries were grouped by quartiles of number of colonies in apiary and the average count of varroa mites per colony data

Variable (n = number of apiaries)	Coefficient (SE)	95% confidence interval (CI)	P-value	Wald test P-value
Size of the apiary				0.001
1–7 colonies (43)	0			
8–13 colonies (48)	1.39 (0.47)	0.46; 2.32	**0.003**	
14–19 colonies (47)	1.14 (0.47)	0.22; 2.07	**0.016**	
≥ 20 colonies (48)	1.95 (0.48)	0.99; 2.91	**< 0.001**	
Average count of varroa mites				**< 0.001**
0–1.09 (47)	0			
1.1–2.99 (45)	1.26 (0.46)	0.36; 2.16	**0.007**	
3.0–8.19 (50)	1.06 (0.45)	0.17; 1.95	**0.019**	
≥ 8.2 (44)	2.15 (0.47)	1.22; 3.07	**< 0.001**	
American foulbrood				
negative (175)	0			
positive (11)	1.67 (0.64)	0.40; 2.95	**0.010**	
Farmlands around the apiary				
no (22)	0			
yes (164)	-1.02 (0.47)	-1.95; -0.09	**0.032**	
Honey bee breed or race				0.142*
Buckfast (20)	0			
Carnica (54)	-0.90 (0.54)	-1.96; 0.16	0.094	
Hybrid (48)	-0.76 (0.56)	-1.88; 0.35	0.176	
Ligustica (46)	-1.14 (0.55)	-2.23; -0.05	**0.041**	
Dark European honey bee (18)	-1.73 (0.72)	-3.15; -0.31	**0.017**	
Constant	0.94 (0.75)	-0.53; 2.42	0.210	

* Variable retained in model as confounder.

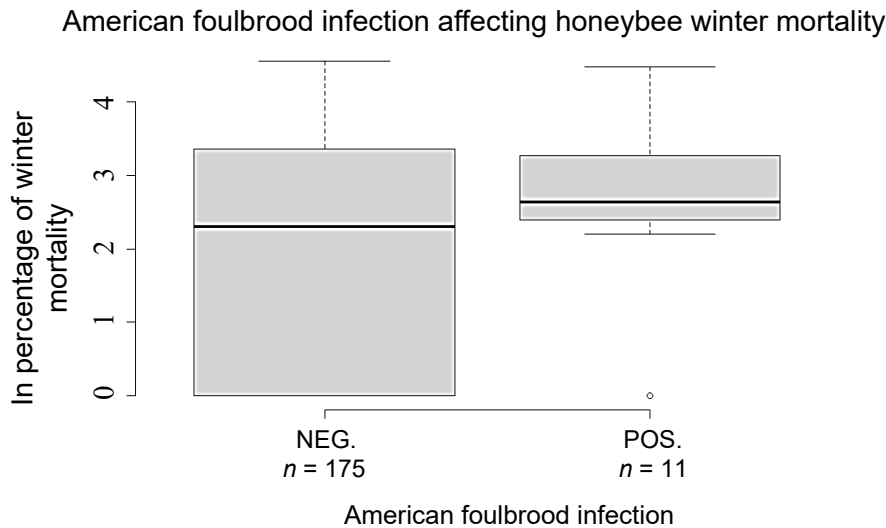Winter mortality of honey bee colonies was significantly higher in colonies infected with American foulbrood ($P = 0.010$) (Fig. 2). American foulbrood, caused by gram+ bacteria *Paenibacillus larvae,* is widespread globally and often results in colony death even in light of sufficient knowledge about this dangerous bee pathogen (Forsgren et al., 2018; Bulson et al., 2021). Lindstrom et al. (Lindstrom et al., 2008) showed that the *P. larvae* spore load in adult honey bee samples was significantly linked to larval mortality in a study conducted in Southern Finland. American foulbrood along with *Nosema* spp. and varroa mite infections were the leading causes of mortality in an Algerian study on seasonal mortality of the cultured honey bee *Apis mellifera intermissa* (Adjlane & Haddad, 2018).

Assessed winter mortality was lower in apiaries located near farmlands (rapeseed, grain, legumes, or grasslands) ($P = 0.032$). It is usually assumed that honey bees are supported by the natural environment around apiaries. Our data reveal the opposite, winter mortality of honey bees was lower when there were farmlands surrounding the apiary.

A statistically significant negative relationship between honey bee breed and winter mortality was found for the honey bees breed Ligustica ($P = 0.041$) and Dark European honey bee ($P = 0.017$) in the model, whereas the overall association between the honey bee breed and winter mortality was not statistically significant ($P = 0.142$).

Size of the apiary affecting honeybee winter mortality
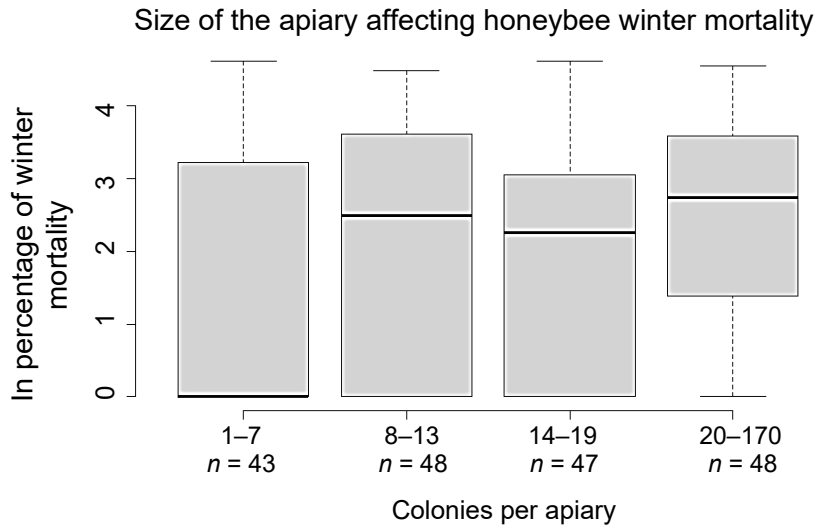
Figure 1. The apiaries were grouped according to the quartiles of the number of colonies in apiary. The last group contains the outlier of apiaries with 170 colonies. Removing the outlier did not change the results, so it was included in the analysis.

American foulbrood infection affecting honeybee winter mortality

Figure 2. American foulbrood infection significantly increased honey bee winter mortality ($P = 0.010$).

Summer mortality

The summer mortality of honey bees was not affected by the size of the apiary ($P = 0.274$) in the multivariate logistic regression model, where crop production around the apiary showed a trend ($P = 0.078$) in reducing summer mortality. However, there

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was a positive trend in the association between summer mortality and large apiaries harbouring over 20 colonies ($P = 0.056$) (Table 2).

Table 2. Results of the multivariate logistic regression model to test associations between honey bee summer mortality, apiary size, and crop production around the apiary ($n = 193$)

Variable (number of apiaries)	OR	95% confidence interval (CI)	P-value	Wald test P-value
Size of the apiary				0.274*
1–7 colonies (49)				
8–13 colonies (49)	1.89	0.51; 7.01	0.339	
14–19 colonies (48)	1.61	0.42; 6.18	0.485	
20–170 colonies (50)	3.32	0.97; 11.42	0.056	
Farmlands around the apiary				
No (23)				
Yes (173)	0.39	0.14; 1.11	0.078	

* Variable retained in model as confounder.

The lack of accordance with consolidated data and overall trends can be explained by the rather modest size of apiaries in general in Estonia (median value of 13.5 colonies per apiary). Nevertheless, our honey bee summer mortality was also not affected by varroa mites. Additionally, the summer mortality tended to be lower in apiaries located near farmlands. This may be an indication that farmlands better support the nutritional needs of honey bees compared to other landscapes and that the previously proven harmful effects of insecticides and pesticides used on farmlands did not have chronic effects on honey bee survival in Estonia. Since no causal relationships were studied, we cannot provide any other speculation for this result.

CONCLUSIONS

The results of this study shed light on the potential risk factors that increase mortality in Estonian apiaries. Honey bee winter mortality was significantly affected by the size of the apiary. Whereby there was only a positive trend in the association between summer mortality and large apiaries harbouring over 20 colonies. Parasitic mite *Varroa destructor* and American foulbrood caused by bacteria *Paenibacillus larvae* significantly affected the honey bee winter mortality. In contrast to the common view that honey bee health is supported by natural environments, there was lower winter mortality detected when there were farmlands around the apiaries. Similarly, the summer mortality tended to be lower in apiaries located near farmlands.

In conclusion, there were more risk factors affecting honey bee winter mortality than summer mortality in 2012–2013. However, the number of apiaries affected by summer mortality in our study was low, meaning that the power of our study was not high enough to be able to identify the significant risk factors. Most probably, the effect of the conditions of the particular year was playing a role.

Studies approaching beekeeping management practices and risk factors for honey bee health in Estonia are rather scarce. Further studies giving us longitudinal data on this field are needed.

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