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## ORIGINAL RESEARCH ARTICLE

### Honey bee pathogens and parasites in Swedish apiaries: a baseline study

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This nation-wide survey including 382 Swedish apiaries is the first to document base-line information of the prevalence and distribution of the ectoparasitic mite *Varroa destructor*, the mite-associated viruses *Deformed wing virus* and *Acute bee paralysis virus*, and the bacterial honey bee pathogens *Paenibacillus larvae* and *Melissococcus plutonius* in the country. *Varroa* and associated viruses were not detected in the northern regions of the country. The bacterium *P. larvae* was detected in 6% of the investigated apiaries and it was absent in more than half of the counties, *M. plutonius* was detected in two apiaries in one county. Other results from our study include questionnaire responses, in which beekeepers reported total winter colony losses of 6.4%. Fifty-three percent of the beekeepers reported to have purchased queens the year preceding this study, and 40.1% moved colonies to the apiary. Queens were imported from European countries and the USA. The movements of bees were one of the factors strongly associated with the prevalence of the disease-causing organisms surveyed and colony losses. The majority of the participating beekeepers were not aware of any disease related signs in their apiaries despite positive laboratory findings. This highlights the importance of further outreach efforts to increase the beekeepers' awareness of diseases and disease management. The results provide a disease baseline for improvements of the surveillance system.

**Keywords:** *Apis mellifera*; surveillance; bee health; winter losses

#### Introduction

Managed honey bees (*Apis mellifera*) perform critical pollination services to several agricultural crops. The economic value of honey bee pollination is estimated to several billion dollars; hence, the health of honey bees is an ongoing concern. Although the numbers of managed honey bee colonies worldwide are steadily increasing (Moritz & Erler, 2016), it is not enough to meet the increasing demand for pollination in agriculture (Aizen & Harder, 2009). Recent large-scale losses of managed honey bee colonies in some parts of the world and the decline of wild pollinators have raised awareness and concern of the lack of pollinators (Burkle et al., 2013). The cause of the reoccurring regional losses in honey bee colony numbers is suggested to be multifactorial; e.g. starvation, climate, pesticides, parasites, and pathogens (Genersch, 2010b; Goulson et al., 2015).

The *Varroa* mite, *Varroa destructor*, is considered to be the main threat to honey bees worldwide (Genersch, 2010b; Le Conte et al., 2010) and an untreated *Varroa* infested honey bee colony is expected to collapse within 2–3 years after the first introduction

of the mite (Amdam et al., 2004). The *Varroa* mite does not only have a direct impact on honey bee health by feeding on fat body tissue (Ramsey et al., 2019), but they also have an indirect impact as a vector for honey bee viruses (Martin, 2001; Mondet et al., 2014). The mite was originally confined to the Eastern honey bee, *Apis cerana*, where a stable host-parasite relationship exists due to a long period of coevolution (Oldroyd, 1999). After a shift from the native host to the Western honey bee, *A. mellifera*, in the last century, the mite dispersed around the globe. Reports of *Varroa* in Europe date back to the 1970s and the first reported Swedish findings were from the island of Gotland in 1987 and on the mainland, in Skåne, in 1991 (Fries, 1987; Fries et al., 1991). The regulations from the Swedish Board of Agriculture (SBA) have since then been aimed at limiting the spread of *Varroa* in the country and had until 2016 not been reported from the most northern parts of Sweden except for close to the Finnish border (Kristiansen, 2002). Two of the viruses associated with *Varroa* infestations (varroosis) are *Acute bee paralysis virus* (ABPV) and *Deformed wing virus*

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(DWV). Symptoms of ABPV infection are paralysis, trembling, inability to fly, gradual darkening and loss of hair from the thorax and abdomen, and premature death of individual bees associated with a sharp decline of adults in the colony (de Miranda et al., 2010). DWV mainly causes benign infections without any signs of disease when transmitted vertically (through drones and queens) or horizontally (through larval food). However, when Varroa mites are feeding and reproducing in the brood cells they transmit the virus to the brood (vectorial transmission) which leads to deformation of the wings, discoloration, short and bloated abdomens, and death. The bees die as pupae or shortly after emergence (de Miranda & Genersch, 2010).

Two of the most economically important honey bee diseases are the bacterial brood diseases American foulbrood (AFB) and European foulbrood (EFB) affecting apiculture worldwide. AFB is caused by the spore-forming bacterium *Paenibacillus larvae* and is not only lethal to individual larvae but to entire honey bee colonies (Genersch, 2010a). Honey bee larvae become infected by ingesting food contaminated with *P. larvae* spores, and the remains of dead infected larvae contain billions of infectious spores that can stay in the environment for decades and serve as sources for new infections (Forsgren et al., 2008; Genersch, 2010a). The disease is epizootic and classified as a statutory notifiable disease in the European Union (Anonymous, 1992). In many European countries, Sweden included, the disease is controlled through the burning of colonies with disease signs combined with beekeeping management techniques to prevent the spread of the infectious agent. Current legislation does not allow European beekeepers to use antibiotics (Anonymous, 2010). Also, the other brood disease EFB, caused by the bacterium *Melissococcus plutonius*, is potentially lethal to honey bee colonies. As with AFB, the honey bee larvae become infected by ingesting contaminated food. The bacteria multiply in the midgut and the infected larvae usually die after four to five days (Forsgren, 2010; Genersch, 2010b).

In Sweden, every beekeeper has the responsibility to prevent the spread of bee diseases and is obliged to register the location of their apiaries. There is no national bee register, but the responsible authority (i.e. the Swedish Board of Agriculture) requires that the number of apiaries and colonies is reported by the beekeepers and recorded by their Country Administrative Board (CAB). The health of honey bees is controlled by local bee inspectors who perform visual inspections of colonies upon disease suspicion or when a beekeeper needs a permit issued by the bee inspector in order to move the bees out of restricted areas (due to AFB outbreaks) defined by the legal authorities. Bee inspectors can send samples to the National Reference Laboratory for Bee Health, NRL, at the Swedish University of Agricultural Sciences, SLU, where the diagnosis of honey bee diseases is performed.

Despite the clear benefits seen from comprehensive disease monitoring surveillance in other animal systems

and the great importance of honey bees, there is little consistency in honey bee surveillance worldwide. Using surveillance studies to establish disease baselines for honey bees is an important first step towards detecting and mitigating emerging biotic threats (Traynor et al., 2016; vanEngelsdorp et al., 2014). The current honey bee disease surveillance mostly consists of apiary inspections and colony population monitoring (vanEngelsdorp et al., 2014). The surveillance of pathogens and parasites of honey bees in Sweden mainly relies on the passive surveillance done through diagnostics related to disease outbreaks as described above. The active surveillance of diseases, since many years reported for other animals, has not included honey bees. This study from 2016 is the first nationwide survey involving multiple honey bee diseases in Swedish apiaries. Our objectives when analyzing the collected data was to investigate (1) the prevalence and distribution of Varroa, two Varroa-associated viruses, ABPV and DWV, and two bacterial pathogens, *P. larvae* and *M. plutonius* in Swedish apiaries; (2) the beekeepers awareness of pathogens and diseases possibly affecting their beekeeping; (3) the potential risk factors for honey bee diseases and winter colony losses.

## Materials and methods

### Study design and selection of apiaries

The study was based on visits to a number of Swedish apiaries, including the sampling of honey bee colonies (*A. mellifera*) and completion of a questionnaire. In Sweden, registration of apiaries is mandatory, and each CAB keeps databases with information about beekeepers' identities and their apiaries. For the purpose of this study, we contacted each CAB and asked for lists of registered apiaries. A random selection of apiaries from these lists was made for each county. The total number of selected apiaries was 385, and this sample size was partly based on sample size calculations for prevalence estimation (to estimate  $50 \pm 5\%$  prevalence with a 95% confidence level) and detection of disease agents (to detect  $<2\%$  prevalence with a 99% confidence level, also with imperfect tests), and on economic constraints. The number of selected apiaries from each county was set to correspond to the proportion of apiaries in the specific county, relative to the total number of apiaries in the country. The CABs were also asked to recommend local bee inspectors that perform their ordinary inspections and that potentially would be willing to perform the apiary visits, given the same financial compensation as for ordinary inspections. The bee inspectors were contacted and, once they agreed to participate, they received written instructions about what apiaries to visit and how to perform the sampling. The bee inspectors contacted the beekeepers to ask for their consent to participate in the study and to set a date for the visit. The visits were performed during the beekeeping season in 2016, from 4 March until 26 November. Every Swedish county ( $N=21$ ) was represented in our study population.

### Sampling of honey bee colonies

Adult honey bees were collected from all apiaries in the study to determine the presence of the Varroa mite, the bacterial pathogens *P. larvae* and *M. plutonius* and the viral pathogens ABPV and DWV.

Five honey bee colonies separately distributed within each apiary were selected based on convenience and sampled by the bee inspector. All colonies were sampled if the apiary had less than five colonies. Approximately 300 adult bees were collected from each selected colony by stroking a small paper box across a brood frame. The samples were kept in a cooling box in the field and stored at  $-20^{\circ}\text{C}$  until analyzed.

### Detection of varroa, *P. larvae*, *M. plutonius*, DWV and ABPV

To detect Varroa, bee samples were stirred for about 1 min in water with a detergent added using an electric household mixer. The bees were washed with a hand shower over a strainer to detect and count the Varroa mites (De Jong et al., 1982; Fries et al., 1991).

The samples were cultured for *P. larvae* according to Lindström and Fries (2005). One hundred bees were crushed in a filter-grinding bag (Bioreba, Switzerland) with 20 mL of sterile water. The fluid produced was poured into a tube and centrifuged at  $4000 \times g$  for 10 min. After centrifugation, the supernatant was removed and the pellet re-suspended in 2 mL sterile 0.9% saline solution. The pellet was incubated in a water bath at  $85^{\circ}\text{C}$  for 10 min to reduce contamination from non-spore forming bacteria before  $10 \mu\text{L}$  was spread onto MYPGP-agar (Mueller-Hinton broth, Yeast extract, Potassium phosphate, Glucose, and Pyruvate) plates. The plates were incubated at  $35^{\circ}\text{C}$  with 5%  $\text{CO}_2$  and after seven days, suspected *P. larvae* colonies were confirmed using real-time PCR. Briefly, a bacterial colony was suspended in nuclease-free water and used as template in the PCR reaction. Real-time PCR using BioRad CFX96 cycler using SsoFast EvaGreen SuperMix (Biorad, US) was performed according to the manufacturer's recommendation and published primers (Martínez et al., 2010)

To determine the presence of *M. plutonius*, 100 adult bees were placed into a filter-grinding bag (Bioreba) with 20 mL of sterile water. The bees were crushed and 1 mL of the fluid produced was immediately subjected to DNA extraction using the QIAamp® genomic DNA isolation mini kit for Gram-positive bacteria (Qiagen, Germany). DNA was eluted with  $100 \mu\text{L}$  elution buffer and stored at  $-20^{\circ}\text{C}$  until processed for molecular diagnostics. Quantitative real-time PCR using BioRad CFX96 cycler using SsoFast EvaGreen SuperMix (Biorad, US) was performed according to the manufacturer's recommendation and using primers previously described (Roetschi et al., 2008).

To determine the quantity of ABPV and DWV, 30 bees were placed in a filter-grinding bag (Bioreba) with 5 mL nuclease-free water and frozen in liquid nitrogen. The frozen bees were crushed and  $100 \mu\text{L}$  of the fluid was used for total RNA extraction using a Qiacube automated extraction robot (Qiagen) and the RNeasy manufacturer's protocol for plant tissue. Eluted RNA was stored at  $-80^{\circ}\text{C}$  until further processed. Reverse transcription-quantitative PCR was run in the BioRad CFX96 cycler using iScript One-Step RT-PCR Kit with SYBR Green according to the manufacturer's recommendation and primers used in Locke et al. (2012).

### Questionnaire

We designed a four-page combined submission form and questionnaire, with 18 questions related to; a) date of sampling, identities of bee inspector and beekeeper, and location of the apiary, b) number of apiaries and honey bee colonies owned by the beekeeper, c) bee health estimations including observations of clinical signs, mortality and potential disease control measures, and d) introduction of bees. The last page of the questionnaire allowed additional free-text comments. The questionnaire was set up as an online form using the Questback software (Questback AS, Oslo, Norway). The questionnaire was filled in by bee inspectors and beekeepers, in conjunction with the sampling, and sent to the laboratory together with the samples. The data from the paper version of the questionnaire was manually entered into the online version. A translated version can be found in the online [supplementary material](#).

### Statistical analysis

The information from the questionnaires and laboratory data were exported to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA). Data handling, descriptive analysis, mapping, and statistical modeling were performed in R statistical software version 3.5.0 (R Core Team, 2018). Model-building strategies followed techniques described by Dohoo et al., 2009 (Dohoo et al., 2009). Descriptive statistics of numeric variables included mean, medians, interquartile ranges, standard deviations, minimum and maximum values. We described categorical variables using frequency distribution tables. The prevalence of Varroa and the honey bee pathogens ABPV, DWV, *P. larvae*, and *M. plutonius* in each Swedish county was calculated by dividing the number of positive apiaries by the total number of apiaries sampled in the county. Prevalence was mapped for visualization.

Apiary level data was analyzed using univariable and multivariable logistic regression models with binomial outcomes. It was not possible to model for ABPV and *M. plutonius* due to the low number of positive findings. We built risk factor models for Varroa infestation, DWV infection level, and *P. larvae* infection. Threshold

values for Varroa were set as infestation rates less than 3% versus an infestation rate equal to or higher than 3% in a honey bee colony. Decreased vigor and increased mortality of bee colonies are usually observed with the higher infestation rate (Barroso-Arévalo et al., 2019; Giacobino et al., 2017). For DWV, the outcome was the number of virus copies per bee in a honey bee colony, i.e. less than  $10^7$  virus copies per bee versus  $10^7$  virus copies or more per bee. A high amount of DWV is associated with disease signs in honey bees (Mockel et al., 2011; Zioni et al., 2011). The *P. larvae* outcome was the absence or presence of the pathogen. Additionally, we modelled risk factors for winter colony losses. The outcome variable for this model was based on the number of colonies lost in an apiary overwinter out of the total number of colonies before winter. The data sources for the explanatory variables of the models came mainly from the questionnaire responses. We obtained data on the number of honey bee colonies in the apiary, number of colonies managed by the beekeeper, observation of disease signs, Varroa treatment, purchase of queens, and colony movement. Due to the biological relevance of Varroa in the transmission of honey bee viruses and colony survival, the mites served as an explanatory variable when modeling for DWV and colony losses.

Regarding the model building process, we assessed the linearity of numeric explanatory variables by visually analyzing scatter plots between each of these variables and the log odds of the outcome. When lack of linearity was identified, we categorized these variables based on cut-points that created categories with an approximate number of observations. The categorical explanatory variables had more than 10% observations in each of its category. Prior to inclusion in multivariable models, univariable models were fit to separately test associations between the explanatory and outcome variables. If  $P < 0.2$  in the likelihood ratio test, the explanatory variable was kept for the multivariable model. Potential collinearity problems were assessed testing associations between pairs of explanatory variables and, if biologically plausible, the Fisher's exact tests were used to check these associations at a level of significance of 0.05. Significant results served as an exclusion criterion for one of the explanatory variables, in which the one that had a weaker univariable association (i.e. a higher  $P$ -value) with the outcome variable was not included the multivariable model. For multivariable modelling, we included the variables that passed the above criteria and possible interactions. The final multivariable model was selected using a backward stepwise elimination procedure and the Akaike information criterion. The variables kept in our models were significant at 5% level, assessed by the likelihood ratio test. Potential confounders in these models were assessed by individually excluding variables from the model and checking a change of at least 20% in the regression coefficients. The model fit

was assessed using Hosmer-Lemeshow goodness of fit tests.

## Results

### Health status, characteristics, and management of apiaries

We received data from 382 out of 385 randomly selected apiaries distributed across all Swedish counties (see supplementary material Figure S1). There was insufficient sampling material from two apiaries to test for ABPV and DWV and for Varroa counts in six apiaries. We excluded questionnaire answers from six study apiaries due to inconsistent data. A summary of the answers from the questionnaire can be found in Table 1. The median number of honey bee colonies in the study apiaries was five, the interquartile range (IQR) = 3–7; the median number of apiaries per beekeeper was one (IQR = 1–2); and the median number of colonies per beekeeper was six (IQR = 3–10).

An overview of the prevalence of Varroa and honey bee pathogens in Swedish apiaries per county is presented in Figure 1. The disease agents with a higher overall prevalence were Varroa (57.4%; 216/376) and DWV (30.2%; 115/380). Varroa was absent in only three counties and the prevalence within apiaries in counties where the parasite was present varied between 16.7 and 77.8%. DWV was not detected in any of the investigated apiaries in the four most northern counties, whereas the prevalence ranged from 10 to 77.8% in apiaries in the other counties. ABPV was only detected in two apiaries in two counties. The bacterium *P. larvae* was present in 6% (23/382) of the investigated apiaries with the highest prevalence of 33.3% and with zero prevalence in more than half of the counties. *M. plutonius* was present in two apiaries from two related beekeepers in the same county.

Fifty-three percent (172/323) of the beekeepers reported to have purchased queens the year preceding this study, and 40.1% (116/289) moved colonies to the apiary. In this period, the purchase of queens and movement of colonies between counties occurred in 19.8% (34/172) and 8.6% (10/116) of the apiaries respectively. One beekeeper unknowingly moved honey bee colonies from a *P. larvae*-infected apiary to an apiary in another county. We received reports of four cases of imports of queens from countries inside and outside Europe (Germany, Slovenia, and the United States) during the five years prior to our study. Most of the beekeepers had been treated against Varroa (88.9%; 330/371). Data on winter losses were reported from 139 apiaries, and the overall proportion of colonies lost over winter in these apiaries was 6.4% (42 out of 655 colonies). We found that the vast majority of beekeepers did not observe any disease related signs although the laboratory assays found the apiaries positive for the disease-causing organisms tested for (Figure 2).

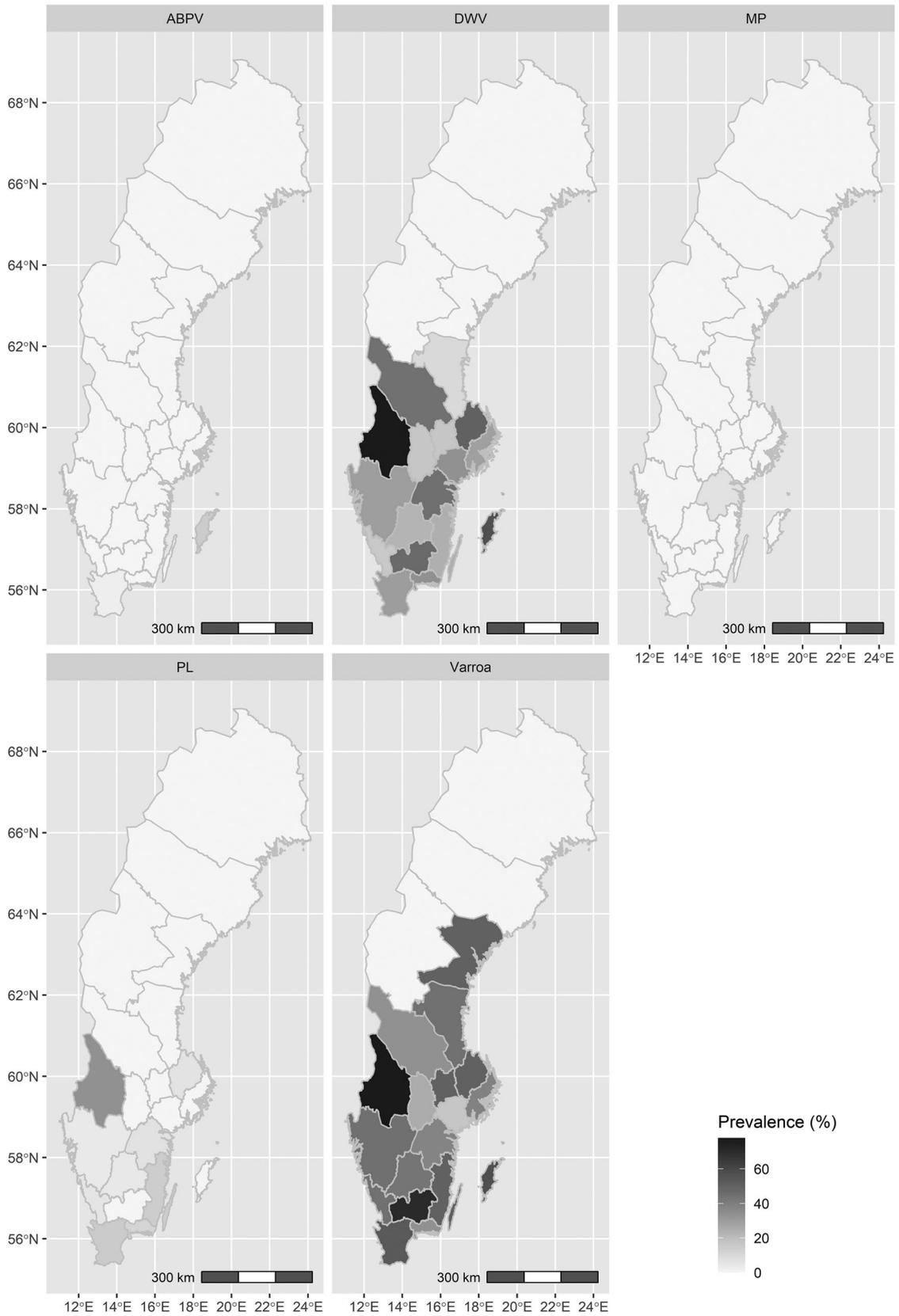


Figure 1. Prevalence of Varroa and honey bee pathogens in Sweden 2016. Acute bee paralysis virus (ABPV), Deformed wing virus (DWV), *Melissococcus plutonius* (MP), *Paenibacillus larvae* (PL), and *Varroa destructor* (Varroa).

Table 1. Summary of questionnaire answers related to at least 2,185 colonies in Swedish apiaries surveyed in 2016. In 8 out of 376 study apiaries there was no information on the number of colonies.

Questions on	No. of apiaries with valid answers	No. of positive answers (%)	No. of colonies <sup>a</sup> (positive answers)
Purchased queens, ≤1 yr	323	172 (53.3)	1089
From within county		135 (78.5)	768
From another county		34 (19.8)	302
From abroad		3 (1.7)	19
Purchased queens, >1 yr	323	151 (46.7)	838
From within county		115 (76.2)	616
From another county		35 (23.2)	215
From abroad		1 (0.7)	7
Moved colonies to the apiary, ≤1 yr	289	116 (40.1)	679
Within county		106 (91.4)	627
From another county		10 (8.6)	52
Moved colonies to the apiary, >1 yr	289	173 (59.9)	999
Within county		157 (90.8)	887
From another county		16 (9.2)	112
Treatment against Varroa	371	330 (88.9)	1964
Winter losses 2015/16	139	29 (20.9)	42 <sup>b</sup>

<sup>a</sup>Not always equal to the total colonies in the apiaries due to missing data.

<sup>b</sup>Out of the 655 colonies in the 139 apiaries.

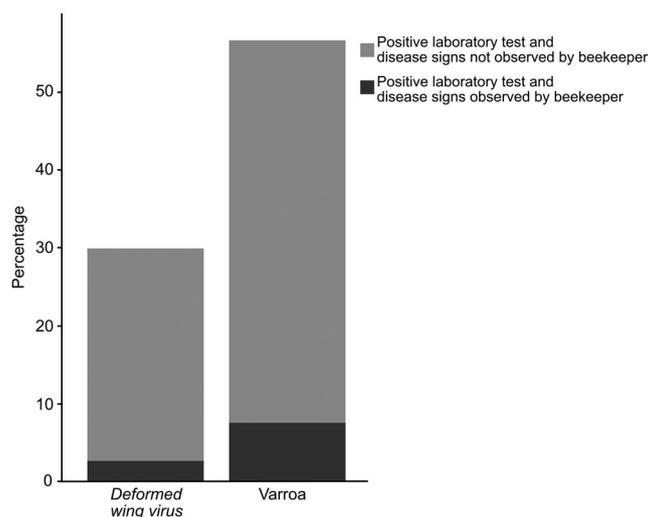


Figure 2. Bar plots comparing the percentage of apiaries tested positive for Varroa and DWV in laboratory assays and the percentage of beekeepers who observed Varroa or any disease signs related to DWV.

### Risk factors for honey bee diseases and colony losses

The results from the risk factor regression models for Varroa, DWV, and *P. larvae* are summarized in Table 2. The odds of an apiary being highly infested by Varroa (i.e.  $\geq 3$  mites per 100 bees per colony) increased by 6% for each unit increase in the number of honey bee colonies. Compared to the apiaries with low levels of Varroa (i.e.  $< 3$  mites per 100 bees per colony) the high-level apiaries were 6 times more likely to contain elevated levels of DWV (i.e.  $\geq 10^7$  virus copies per bee). Apiaries that had received a honey bee colony from another apiary in the previous year had a three times higher chance of being positive for *P. larvae* than apiaries without any colony movement in the previous year.

Considering the factors associated with high winter colony losses (Table 3), low levels of Varroa were the most crucial factor in reducing winter colony losses. The odds for colony losses also dropped if no movement of colonies between apiaries had occurred. With borderline significance, we observed a slight decrease in the odds for colony losses related to a higher number of honey bee colonies kept by beekeepers. There seemed to be confounding effects of the number of honey bee colonies per beekeeper with colony move and Varroa infestation levels in the colony loss model. Excluding this potential confounder from the model had little relevance for the biological interpretation of the associations between the explanatory variables and the outcome. The regression models were well fitted

Table 2. Results from the logistic regression models for risk factors associated with the outcomes high Varroa infestation ( $\geq 3$  mites per 100 bees), high Deformed wing virus (DWV) virus loads ( $\geq 10^7$  virus copies per bee), and *Paenibacillus larvae* infection.

Outcome	Variable	Category	OR <sup>a</sup>	95% CI <sup>b</sup>	P-value
High Varroa infestation	Honey bee colonies in apiary	–	1.06	1.01 – 1.11	0.012
High DWV infection level	Varroa infestation level <sup>c</sup>	Absent or low	1	–	<0.001
		High	5.94	3.64 – 9.82	
<i>Paenibacillus larvae</i> infection	Last colony movement	>1 year	1	–	0.024
		$\leq 1$ year	2.99	1.19 – 8.19	

<sup>a</sup>OR = odds ratio.<sup>b</sup>CI = confidence interval.<sup>c</sup>Varroa infestation levels; Absent or low (<3 mites per 100 bees), High ( $\geq 3$  mites per 100 bees).

Table 3. Results of a logistic regression model for risk factors associated with winter colony losses in Swedish apiaries.

Variable	Category	OR <sup>a</sup>	95% CI <sup>b</sup>	P-value
Colonies per beekeeper	–	0.98	0.96 – 1.0	0.048
Last colony movement	>1 year	1	–	0.023
	$\leq 1$ year	2.39	1.14 – 5.21	
Varroa mite infestation level <sup>c</sup>	Absent or low	1	–	0.002
	High	3.59	1.67 – 8.22	

<sup>a</sup>OR = odds ratio.<sup>b</sup>CI = confidence interval.<sup>c</sup>Varroa infestation levels: Absent or low (<3 mites per 100 bees); High ( $\geq 3$  mites per 100 bees).

according to the Hosmer-Lemeshow statistics ( $0.2 < P \leq 1$ ).

The descriptive results for Varroa, DWV, and *P. larvae*, involving the studied risk factors and the causative agents associated with honey bee diseases are summarized in [supplementary tables S1](#), [S2](#), and [S3](#) respectively. Putative risk factors for winter colony losses are summarized in [Table S4](#).

## Discussion

Sustainable disease surveillance systems are important as they quantify disease levels in different populations and regions and provide data to support the mitigation and prevention of important diseases (Lee et al., 2015). Losses of managed honey bee colonies are of great concern globally, not least in the USA where the organisation Bee Informed Partnership (BIP, [beeinformed.org](#)) has conducted a total of ten winter loss surveys since 2006-2007. They reported total winter colony losses during these years ranging from a low of 22% to a high of 36% and a total annual loss ranging from 34 to 45% (Kulhanek et al., 2017). Another of the many research initiatives to investigate winter losses of honey bee colonies is organized through COLOSS (= Prevention of honey bee COLony LOSSes, currently a non-profit organization). COLOSS use standardized methods for surveys of beekeepers to measure colony loss rates. The COLOSS survey from 2016 reported an overall European loss rate of 12.0% during winter 2015-2016, with marked differences among countries. The estimated overall winter loss rate in Sweden was 15.9% (Brodschneider et al., 2016). A local source of information about winter colony losses in Sweden is the information from the Swedish Beekeepers Association where data on winter colony losses have been recorded

since 1920. The data show an overall winter colony loss rate of 13.1% over the century and 15.9% for winter 2015-2016. (Information from the Swedish Beekeepers Association compiled by the national bee health advisor Preben Kristiansen, pers. comm.). As methodologies, sampling and other factors differ between surveys, direct comparisons between the low overall winter colony loss rates in this study, 6.4%, should be made with caution. An under-estimation of this rate might have occurred due to the low response rate of the related question (139/376, 36%), especially if the non-respondents were beekeepers with large beekeeping operations.

Our data indicate that the odds for high Varroa numbers increase with increasing numbers of colonies in an apiary. This is in line with earlier studies (Frey & Rosenkranz, 2014; Seeley & Smith, 2015) showing that a high density of colonies leads to higher invasion rates and that crowding honey bee colonies in apiaries greatly increases their vulnerability to Varroa. Furthermore, the results reaffirm that low Varroa numbers are the most crucial factor for reducing winter colony losses (Chauzat et al., 2016) and that high numbers of Varroa are correlated to elevated levels of DWV (de Miranda & Genersch, 2010). A risk factor for spreading disease is the exchange and transfer of bees and beekeeping related materials. Half of the responding beekeepers in this study, 53.3%, reported having purchased queens in the previous year and 40.1% had moved colonies between apiaries and regions. One beekeeper moved bees from an apiary later diagnosed with *P. larvae* to another county, which clearly illustrates the risk of unknowingly spreading disease. A few queens were purchased from other European countries and one beekeeper reported illegal imports of queens from the USA.

After the introduction of Varroa in Sweden, the Swedish Board of Agriculture introduced regulations to prevent or at least slow down the spread of the mite in the country. This has not completely prevented the spread, but there are still regions in the north reported free of Varroa and the results from this survey reinforce earlier observations and reports. The Varroa mite acts as a biological vector for viruses like DWV and ABPV, and DWV was detected in all counties except four counties in the far north. The spread of DWV coincides with the presence of Varroa and follows the spread of the mite. The other Varroa associated virus, ABPV, was only detected in one apiary on the Baltic island Gotland and in another apiary in the most southern part of the country (Skåne). The reason for ABPV not being more widely spread may be because the virus is too virulent, *i.e.* kills its host too fast, to spread effectively (Sumpter & Martin, 2004; Traynor et al., 2020). This could explain why the less virulent virus DWV has such a high incidence while ABPV is so sparingly present. It is also worth noting that the counties where ABPV is detected, Gotland and Skåne, are the counties where Varroa was first introduced in the country (Fries, 1987; Fries et al., 1991). At that time (late 80s, early 90s), ABPV was the most dominant Varroa associated virus in Europe before it was replaced by DWV (de Miranda et al., 2010).

The reporting of AFB incidences in Sweden is based on the observation of disease symptoms reported by the bee inspectors to the legal authorities. If one considers the reported number of apiaries with colonies with signs of disease in relation to the estimated total number of apiaries in Sweden, the AFB prevalence in Swedish beekeeping has varied from 0.5% to 1% over the last decade (Data from the Swedish Board of Agriculture compiled by bee health advisor Preben Kristiansen, pers. comm.). There is, however, a reason to suspect an under-reporting of the disease due to factors such as the unrealistic compensation for affected beekeepers. Subclinical levels of *P. larvae* can be detected by microbiological culture or molecular methods from samples of honey, adult bees, and hive debris. In this study, we used microbiological cultivation of *P. larvae* from samples of adult bees, a method proven to be well correlated with signs of disease in the honey bee colony (Forsgren & Laugen, 2014; Nordström et al., 2002), to screen for subclinical levels of the bacterium. Even though only young honey bee larvae develop disease, adult bees are carriers of the infectious agent (Lindström, 2008). We investigated the subclinical presence of the bacterium in a selection of the country's apiaries and in most of the examined apiaries (94%), the bacteria could not be detected. This is an important argument in discussions between beekeepers and regulatory authorities about simplifying the regulations on the management and movement of bee colonies. It is important to highlight that there are many apiaries in

areas free of this pathogen and that status is worth preserving. The causative agent of EFB, *M. plutonius*, was detected in bee samples from only two apiaries. Historically, EFB has been considered less serious than AFB, but reports of more virulent strains of the bacterium and more serious disease outbreaks have become increasingly common in recent years (Grossar et al., 2020). In 2010, Norway had an outbreak of EFB after a long time of no disease that led to extensive investigations and sanitation (Grossar et al., 2020). This supports the relevance of continuous EFB monitoring for the prevention of outbreaks of this disease in Sweden.

The majority of the beekeepers participating in this study were not aware of any disease or any disease related signs in their colonies despite positive laboratory findings, and one beekeeper unknowingly moved colonies from an apiary where *P. larvae* was present. This reaffirms results from other studies and highlights the importance of improved beekeeper training to promote good beekeeping practices (Jacques et al., 2017).

## Conclusions

Active surveillance programs provide superior insight on the prevalence, incidence, and geographic distribution of disease agents and their epidemic potential over passive surveillance based on symptoms and self-reporting. Passive surveillance based on self-reporting of disease by beekeepers is furthermore affected by training, beekeeping practice, social and economic factors leading towards an under-reporting of the true incidence. Improved knowledge of diseases (*i.e.* disease signs, consequences, and regulatory control measures) supplemented by realistic compensation for affected beekeepers might help mitigate the under-reporting of, for example, AFB to the legal authorities. More accurate data on prevalence, incidence, and distribution of diseases at national and international levels, to which this study contributes, would provide the authorities with a superior foundation for improving the legal statute and guidelines for disease prevention and control.

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## Supplementary material

Supplementary Figure 1, Tables S1–S4 and the Questionnaire are available via the ‘Supplementary’ tab on the article’s online page (<http://dx.doi.org/10.1080/00218839.2021.1902679>).

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