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Risk and protective indicators of beekeeping management practices

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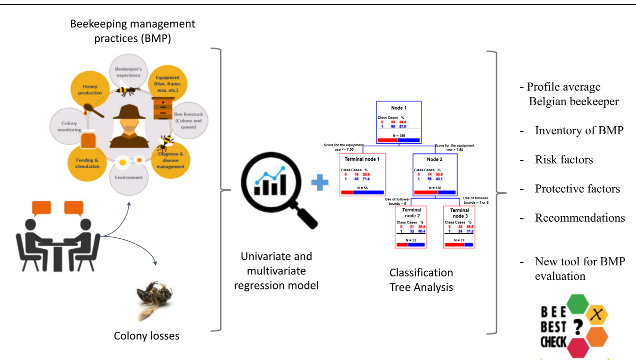
HIGHLIGHTS

- Cross sectional study with face-to-face interviews (n = 186 beekeepers)
- Characterization of beekeeping management practices carried out in Belgium
- Evidences of a relationship between beekeeping management practices and colony losses
- BeeBestCheck online tool freely available on smartphone and computer

GRAPHICAL ABSTRACT



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ABSTRACT

Explaining the reasons for the high honey bee (*Apis mellifera*) colony loss rate in recent years has become a top global research priority in apicultural and agricultural sciences. Although there are indications of the role played by beekeeping management practices on honey bee health, very little information is currently available. Our study aimed to characterize the beekeeping management practices carried out in Belgium, and to determine the relationship between beekeeping management practices and colony losses. Variables obtained from face-to-face questioning of a representative randomized and stratified sample of Belgian beekeepers (n = 186) were integrated into a logistic regression model (univariate and multivariate) and correlated to the declared colony loss rates to identify risk and protective indicators. We used a classification tree analysis to validate the results. We present evidence of a relationship between poor beekeeping management practices and colony losses. The main factors protecting honey bee colonies are the aptitude of the beekeeper to change his management practices, the hive type, the equipment origin and hygiene, wintering in proper conditions (the use of divider boards, i.e. board blocks or space fillers off part of the hive body), the colony strength estimation before wintering, winter monitoring, and last but not least, appropriate integrated pest management. Proper estimation of the *Varroa* infestation level should be performed prior to treatment. The consequences of poor beekeeping practices on honey bee health can be addressed by proper training of beekeepers. An online tool was developed and published for beekeepers allowing them to evaluate the effect of their management practices on colony health.

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1. Introduction

Honey bees (*Apis mellifera* L.) generate a wide range of products for human consumption but more importantly provide irreplaceable pollination services to agricultural and natural ecosystems. The honey bee is a managed eusocial organism. Its health is mainly assessed at the colony level rather than at the individual level (Afssa, 2008). While honey bees enable us to investigate complex health issues affecting social organisms, defining precise risk or protective indicators remains challenging as some stressors are buffered at the colony level (Straub et al., 2015).

Beekeeping management practices (BMP) represent the totality of the actions implemented by a beekeeper to maintain healthy honey bee colonies and to achieve its production objectives (EFSA AHAW panel, 2016; Formato and Smulders, 2011; Ritter and Pongthep, 2006; Rivera-Gomis et al., 2019) (Fig. 1). For example, when facing high pest pressure, beekeepers can reduce hazards through physical or chemical interventions (Giacobino et al., 2016; Jacques et al., 2017). While good management can alleviate stress, poor management can accentuate it. Good management practices must be developed with proper training and experience (Steinhauer et al., 2018). The beekeeper plays thus a key role in maintaining the health status of managed honey bee colonies. However, a clear overview of the main actions carried out by beekeepers and their role in the successful management of honey bees is only partially addressed (Sperandio et al., 2019). Over the last decade, considerable attention has been given to understand stress factors impacting honey bee colony health and losses, but the management practices' impact has often been overlooked. In the literature, very few publications about management practices are available (Sperandio et al., 2019; Steinhauer et al., 2021; Underwood et al., 2019). Nevertheless, national and European monitoring projects such as HealthyBee (Federal Agency for the Safety of the Food Chain, Belgium, 2016-2018), APENET (Porrini et al., 2016) and COLOSS (Gray et al., 2019) highlighted the direct and/or indirect role of the beekeeper in ensuring health and performance of honey bee colonies. Better BMP can be implemented from a short-term perspective by individual beekeepers and may have the potential to reduce colony losses (Clermont et al., 2014). In Belgium, information on the correlation between beekeeping management practices and the registered colony loss rates is still lacking. To date, there is no comprehensive register of beekeeping practices in Belgium. A register with representative and comparable information across the different regions could help target inappropriate BMP.

Honey bee health has been declining since the end of the 1980s in Belgium as well as in the rest of Europe (Ellis et al., 2010; Potts et al., 2010; VanEngelsdorp et al., 2009). Epidemiological standardized methods to collect comparable and robust data were set up with the pan-European surveillance program on honeybee colony losses (Laurent et al., 2015). In 2012–2013, the Belgian winter loss rate was estimated at 34.6%, the highest percentage among 17 participating European countries in the European EPILOBEE study of the same year (loss of 32.8% overall) (Fig. 2). Before the emergence of the *Varroa* mite, no historical data regarding the acceptable (winter) mortality levels of colony losses in Europe were set, and these levels may vary between countries (Chauzat et al., 2016; Steinhauer et al., 2014).

We hypothesize that some implemented BMP can have an impact on honey bee health and consequently on colony losses. Our study aimed to characterize the beekeeping management practices carried out in Belgium, in order to determine the relationship between beekeeping management practices and colony losses.

2. Materials and methods

2.1. The Belgian beekeeping

The monitoring network of the European Honey Programme estimates that 2/3 of the Belgian beekeeping sector is made up of hobbyist beekeepers, who's source of income lay outside beekeeping. They keep bees as a pleasant pastime and for the intrinsic values of beekeeping (El Agrebi et al., 2021). Honey bees are largely maintained in stationary apiaries, for honey production, by hobby beekeepers (1–15 colonies) or experienced hobby beekeepers (16–50 colonies). Apiaries are thus relatively small operations. Beekeepers often have a knowledge based on observation and self-experimentation. One third of the sector is made of semi-professional beekeepers (50–150 colonies) and only seven beekeepers are professional (with more than 150 colonies). The European Union co-finances aid programs for beekeeping. In Belgium, they are developed at regional levels (Flanders and Wallonia), in consultation with representatives of the sector.

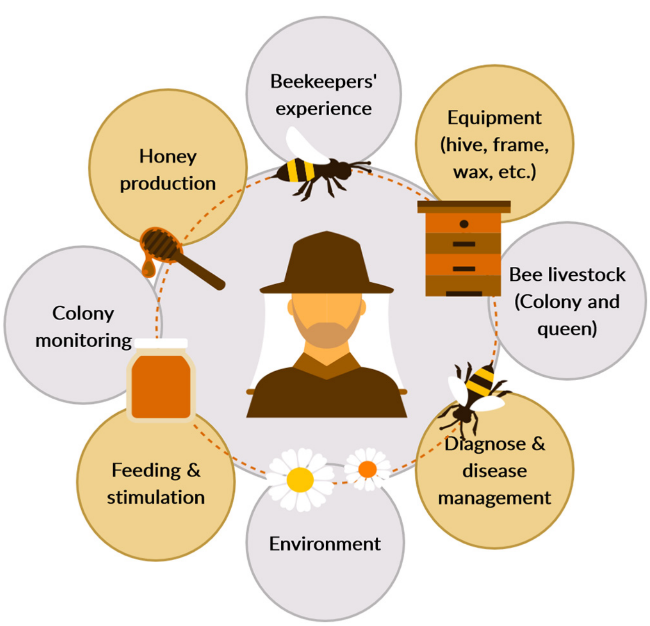

Fig. 1. Beekeeping management practices can affect bee health and survival, alone or combined.

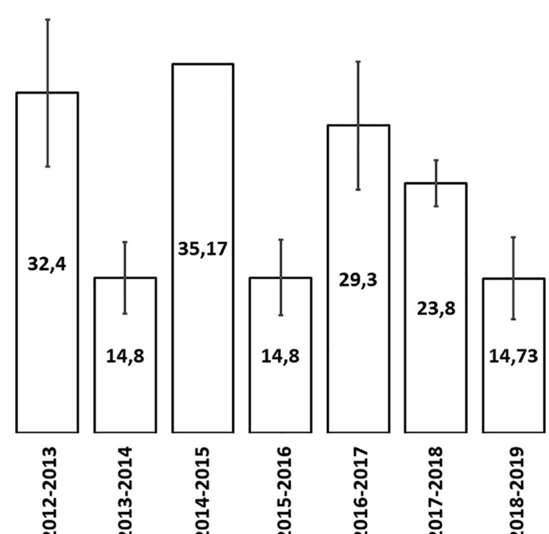

Fig. 2. Winter loss rate in percent from 2012 to 2019 in Belgium.
Legend: Data collected from the EPILOBEE consortium and the Belgian Federal Agency for the Safety of the Food Chain (FASFC) for the years 2012 to 2015, from the Belgian beekeeping federation for the years 2014–2015 (only global data available), from the ULiège-Faculty of veterinary medicine for the years 2015–2016, and from the Belgian institute of health (Sciensano) for the years 2016 to 2019.

2.2. Study design and sample size

A cross-sectional study was carried out from May to November 2016 in Belgium. There is no precise, comprehensive figures for the entire beekeeping sector in Belgium since 2015, as an undefined number of beekeepers are reluctant to the register of competent authority (Federal Agency for the Safety of the Food Chain) or to beekeepers associations. In this study, we started from the list of beekeepers officially registered (FASFC) in 2015 (n = 4949). Out of this list, 20 beekeepers were randomly selected per province (n = 10 provinces) following stratified randomization procedures (computerized random numbers) (Moher et al., 2010).

Potential explanatory variables were obtained from structured face-to-face interviews, with predetermined questions designed in advance and directed towards BMP. To facilitate data processing, most questions were close-ended (N = 140) (dichotomous or multiple choice). Open-ended (N = 3) questions were designed and asked in a simple, neutral, and comprehensive way (van der Zee et al., 2013) and were used to assess beekeepers' concerns. The loss rate of each apiary was assessed. A detailed list of the survey questions and results is available in Appendix 1.

2.3. Data collection

Face-to-face interviews maximize response rate and decrease the risk of bias. The questions were designed in two national languages. For validation, two beekeeping experts and two beekeepers reviewed the survey questions. A pilot test of the survey was carried out with six beekeepers of the intended survey participants. No specific permissions were required for the data collection except for the explicit agreement of the beekeepers. The study was conducted in 2016 to collect data from the management practices of the previous beekeeping season (2015–2016). The mortality recorded in 2016 was thus the result of the beekeeping season 2015–2016. The geographic locations of the beekeepers' apiaries (period 2015–2016) in each province (n = 20), in Flanders (n = 100), and Wallonia (n = 100) are shown in Fig. 3. Surveyed beekeepers' geographic locations in each province (n = 20), in Flanders (n = 100), and Wallonia (n = 100) in 2015–2016. For simplicity, Brussels region was arbitrary grouped with the Flemish Brabant.

2.4. Data on colony losses

The loss rate was based on beekeepers declarations. The overall loss rate is the proportion calculated as the total number of lost colonies (at the end of the winter or end of the season) divided by the total number of colonies before winter. The following definitions are provided to understand the part of methodology carried out in this study. Winter is defined as the period between the end of pre-winter colony preparations and the start of the new foraging season (van der Zee et al., 2013). Seasonal losses occur during the beekeeping season. The year losses are the sum of winter and seasonal losses. The colony loss metric is subject to discussion as BMP vary between regions and between professional and hobby beekeepers. Merging weak colonies into stronger ones decreases the number of colonies in an apiary, but to define those as dead would be inaccurate, so they are considered lost. We have set the acceptable winter mortality level at 10% according to earlier work (Haubruge et al., 2006; El Agrebi et al., 2020; El Agrebi et al., 2021), this rate is generally considered acceptable.

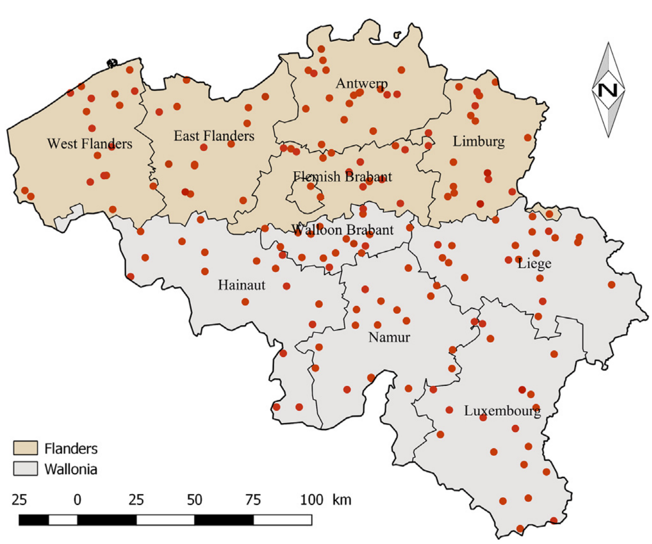

Fig. 3. Surveyed beekeepers' geographic locations in each province (n = 20), in Flanders (n = 100), and Wallonia (n = 100) in 2015–2016.

2.5. Varroa control classification

In Belgium, the strategies used to control *Varroa* are diverse (active substance, formulation, biotechnical control methods, time of treatment, and the treatment frequency), and most beekeepers apply a combination of various *Varroa* control methods. Thus, because statistical methods require a sufficient number of replicates, *Varroa* control methods were classified in models according to most frequent combinations (Table 1). The *Varroa* mite control notice issued yearly by the FASFC (2015–2016) recommended to accurately estimate the *Varroa* mite infestation in the colonies, then to apply two treatments a year: the first after the last honey harvest and the second in winter in the absence of brood when all/nearly all mites are phoretic.

2.6. Equipment scoring

A score was assigned to the origin of the equipment (new, self-made, second hand) and its reuse after colony losses (yes, after disinfection, or no), as well as to the origin of the beeswax (recycled from own beeswax, recycled from commercial beeswax or commercially purchased beeswax). For these three variables, an overall score was calculated for each beekeeper as statistical methods require a sufficient number of replicates and most beekeepers have different combinations of practices regarding the origin of the beeswax, the origin of the equipment and its reuse after colony losses.

2.7. Statistical analysis

2.7.1. Identification of risk and protective indicators using logistic regression

Logistic regression models were performed in Stata SE 14.1® (StataCorp LP, College Station, TX, USA), to evaluate the effect of the selected explanatory variables on the binary outcome loss rate (threshold 10% according to (El Agrebi et al., 2021)). First, a univariate analysis was conducted and odds ratios (OR) with 95% confidence intervals (95% CI) were calculated for each variable. Then, a multivariate logistic

Table 1
Model of the *Varroa* treatments combinations in Belgium.

Model	Treatments combination
A.	No *Varroa* control
B.	Two treatments: thymol after harvest + organic acids (formic, oxalic) in winter
C.	Two treatments: EU authorized veterinary medicinal products (VMP) after harvest (Amitraz and Tau-fluvalinate) + organic acids in winter
D.	One treatment with organic acids (summer or winter)
E.	Two treatments or more; summer and winter with organic acids + other substances (essential oils other than thymol)
F.	One treatment with EU authorized veterinary medicinal products (VMP) (Amitraz and Tau-fluvalinate)

regression analysis was performed using the variables with a p-value < 0.10 in the univariate analysis (in order to be conservative) (Renault et al., 2020). The model was progressively simplified by removing the least significant variable with a p > 0.05. The model was considered complete, either when all variables had a significant p-value (<0.05), or when it could not be further simplified without having a significant difference between the most complex and the simpler model (likelihood ratio test with a p-value < 0.05) (Renault et al., 2020). The goodness of fit was assessed using the Hosmer–Lemeshow goodness-of-fit test (Petrie and Watson, 2014). The limit of statistical significance of the tests performed was defined at p-value ≤ 0.05.

2.7.2. Classification tree analysis

We performed a classification tree analysis (CTA) in an attempt to better understand the relative importance and inter-relations among different risk variables in explaining colony losses using the acceptable level of 10% (El Agrebi et al., 2021). The CTA is a non-linear and non-parametric model that is fitted by binary recursive partitioning of multidimensional covariate space (Breiman et al., 1984). Using Salford Predictive Modeler (SPM) software (Salford Systems, San Diego, CA, USA), the analysis successively splits the data set into increasingly homogeneous subsets until it is stratified to meet specified criteria. The Gini index was used as the splitting method, and 10-fold cross-validation was used to test the predictive capacity of the obtained trees. SPM performs cross validation by growing maximal classification trees on subsets of data then calculating error rates based on unused portions of the data set (Chaber and Saegerman, 2017). To accomplish this, SPM divides the data set into 10 randomly selected and roughly equal parts, with each 'part' containing a similar distribution of data from the populations of interest (i.e. colony strength estimation). SPM then uses the first nine parts of the data, constructs the largest possible tree and uses the remaining 1/10 of the data to obtain initial estimates of the error rate of the selected subtree. The process is repeated using different combinations of the remaining nine subsets of data and a different 1/10 data subset to test the resulting tree. This process is repeated until each 1/10 subset of the data has been used as to test a tree that was grown using a 9/10 data subset. The results of the 10 mini-tests are then combined to calculate error rates for trees of each possible size; these error rates are applied to prune the tree grown using the entire data set. The consequence of this process is a set of fairly reliable estimates of the independent predictive accuracy of the tree, even when some of the data for independent variables are incomplete and/or comparatively small. For each node in a classification generated tree, the 'primary splitter' is the variable that best splits the node, maximizing the purity of the resulting nodes.

3. Results

The completion rate during the face-to-face questionnaire interview was 99.71%. The few absence of answers was due to an alternative BMP (minimal intervention) or a reluctance to talk about the quantity of produced honey.

Beekeepers' age distribution was not normally distributed (Shapiro-Wilk W test for normal data; p-value = 0.0001). Median age was 60 years old (min–max = 20–90, mean = 57, SD = 15, n = 186), 87.2% had followed a beekeeping training, 91.5% were members of a beekeepers association and 59.6% of them used a logbook or took quick notes (23.4%). Beekeepers with 10 years or more of experience represented 54.8% of the subset. The median number of colonies in the apiaries was 8.5 (min–max, 1–60, mean 11.4, SD ± 9.9), i.e. these were exclusively hobby/non-professional beekeepers. The vast majority of the apiaries were located in a rural environment (72.2%) surrounded by agricultural environment and/or gardens with immediate crop proximity (<3000 m) (92%) and estimated abundant vegetation (52.9%). The motivations for beekeeping were various and included interest for honey bees (58.8%), ecological concerns (47.6%), continuing a family activity (21.9%) and honey production (23.5%). The number of colonies and loss rates per season are shown in Table 2.

Table 2
Loss rates for the year 2015–2016 (n = 186 beekeepers).

Year 2015–2016	Average loss rate in percent (95% confidence interval)
Winter	11.8 (9.1–14.5)
Seasonal	3.0 (0.3–5.7)
Yearly	14.8 (11.2–18.3)

The losses due to a lack of hazard prevention, thus a lack of good management practices (GMP) in the colonies, were also assessed. Beekeepers estimated that the lack of GMP and hazards encountered in BMP might have been the cause of 41.7% of the year losses. The most encountered not prevented hazard was colony weakness (Fig. 4).

The Buckfast was the dominant breed of honey bees kept (40.4%), followed by *Apis mellifera carnica* (38.3%) and the dark honey bee (*Apis mellifera mellifera*) (17.7%). Stationary apiaries prevailed; transhumance was practiced by 19.7% of the beekeepers, almost exclusively by Flemish beekeepers (94.4%). The hive type "Dadant Blatt" (10–12 frames) was the most frequent type used in Belgium (46.5%), with 88.5% of use in the Walloon region. In Flanders, the tendency was different, with 53.8% of the beekeepers using the "simplex" hive type.

Queen rearing was practiced by 58.8% of the beekeepers; with a median value of 3 (min–max; 0–72) queens produced per year. Of the self-produced queens, 71% were marked, 56.7% were for personal use. On average, beekeepers bought 1.2 queens a year, 91% of them were reared nationally, and 10.7% came from the EU. In-hive, 51% of the queens were younger than a year, 31.3% were between 1 and 2 years old and 17.7% were older than 2 years. Most new queens were introduced in spring (60%) versus in autumn (40%).

One of the most commonly used reproduction methods was the division of colonies with 48.8%, followed by the introduction of mated queens (42.8%). The average of newly started colonies per year and per beekeeper was 4.6 (min–max; 0–30). About half of these newly started colonies (2.02; min–max: 0–25) were handed off to other beekeepers. The number of introduced swarms was on average 1.28 (min–max: 0–14) per beekeepers a year, 34.2% of these swarms were collected (wild swarms), 33.3% were received from another beekeeper, 25.6% were own swarms recovered, and 9.4 were bought from EU origin.

The most prevalent breeding criterion was honey bee stock gentleness (75.9%), followed equally by the stock productivity and queen laying rate (28.9%). Hygienic behavior and *Varroa* tolerance were only mentioned respectively by 10.7% and 11.8% of the beekeepers.

Winter preparation usually begins after the last honey harvest, starting with an anti-*Varroa* treatment. Adapting the hive space to the colony size by using divider boards[1] was a practice used by 38.5% of beekeepers. Reducing the flight entrance was a common practice (71.1%), as well as the control of the presence/laying activity of the queen (74.3%). Colony strength estimation before wintering was performed by 82.5% of beekeepers, 63.1% of their colonies were estimated as strong, 14.7% as acceptable, and 20.3% as weak. Winter monitoring was implemented by 77.5% of the beekeepers, mostly by controlling the bottom board (68.4%), less than once a month (48.3%). The most practiced airing mode was removing the hive bottom board (60%). A grid was largely used (92%) in the hive as the bottom, and hives were generally 40 cm above the ground.

After winter, beekeepers performed the first hive check-up before April (54.8%). During spring monitoring, 45.3% of the beekeepers used a divider board to reduce the hive space, 88.3% checked the brood

[1] The divider boards are board blocks or space fillers off part of the hive body; so the honey bees are not overwhelmed with space when starting a smaller colony. Yet is easy to move as they grow.

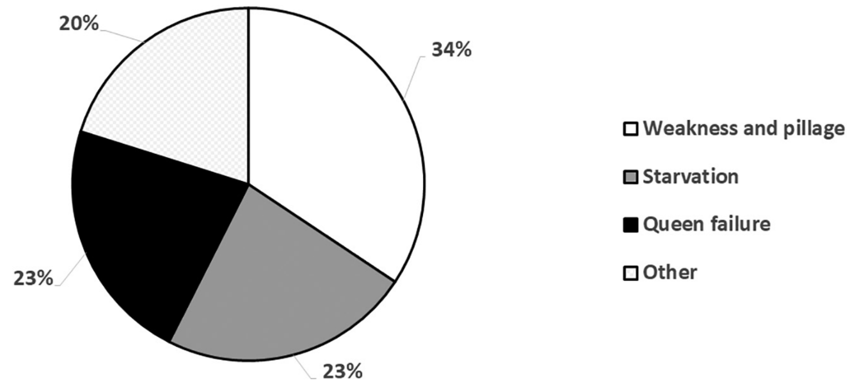

Fig. 4. Distribution of the most encountered (not prevented) hazard leading to colony losses (n = 2175 colonies).

quantity, and the pollen entries (90.7%). After winter, 23.4% of the beekeepers gathered weak colonies with stronger ones.

Swarming control was implemented by 80.6% of beekeepers; the most common control techniques used were royal cell destruction (54.9%), and artificial swarming (33.5%).

In summer, brood quality and uniformity control were done by 85.2% of beekeepers as well as the food quantity, and position (78.7%). Hive pillage by wasps (*Vespula germanica*) was experienced by 36.4% of beekeepers in 2015.

Concerning the equipment and its hygiene, 66.8% of the beekeepers disinfected their equipment after mortality before re-use. The most common disinfection technique used was scraping (53.5%), and using a blowtorch (62%). Reagents as hot water with washing soda or chlorine bleach are also used as disinfectant. Most beekeepers (58.3%) renewed 25 to 50% of the beeswax frames per year. Beeswax was recycled by 32.6% of the beekeepers, or bought by 57.2% (commercial beeswax). Most beekeepers (63.2%) were confronted with the presence of wax moth in the beeswax frames.

Right after harvest, 36% of the beekeepers fed their colonies, 71.6% of them using homemade sugar syrup for this purpose. Winter-feeding was done by 80.2% of beekeepers, the use of commercial products, in this case, was preferred by 63% of the beekeepers, and the average quantity that was given to the bees for feeding was 13.3 ± 4.5 kg. Feeding after winter was done by 44.4% of the beekeepers, and 65.4% of them used a commercial sugar paste.

The actions implemented to control *Varroa* and honey bee diseases in Belgian apiaries are shown in Table 3. The majority of the beekeepers (29.5%) used organic acids (oxalic/formic/lactic) as treatment substances in summer and winter. Thymol in combination with organic acids was applied by 27.3% of beekeepers. Organic acids in a single-use were applied by 10.4% of the beekeepers. Summer treatment with EU authorized veterinary medicinal products combined with organic acids was used by 8.2% of the beekeepers. Only 36.4% of the beekeepers used biotechnical means (drone brood removal, bottom boards screening and powder sugar dusting) in addition to treatments for *Varroa* control. The summer treatment was applied by 62.9% of the beekeepers in August, after the harvest. The winter treatment was mostly applied in December, between Christmas and New Year (57.8% of beekeepers).

Table 3
Implemented actions to control *Varroa* and bee diseases in Belgian apiaries (n = 186).

Category	Sub-category	Variable	Percent
Varroa control	Veterinary advice	For prescription	88
	Varroa management	Automatical treatment without diagnose	80
		Infestation rate determination	19.3
		Counting natural *Varroa* fall	42.2
Diseases	*Varroa* reported infestation rates	Lack of knowledge	82.9
	Biotechnical methods/drone brood removal	Implemented	36.4
	Treatment efficacy check	Implemented	74.3
	Nosema	Detected	6.95
	Deformed wing virus (DWV)	Detected	39.6
		No significant detection in honey bees	80

The honey yield question was not answered by 3.8% of the beekeepers. Of the respondents, 76.9% of the respondents harvested honey twice a year. The average ± standard error production on a yearly base per colony considering all colonies in the apiary was 27.5 ± 16.9 kg per colony per year with a median of 25.0 kg per colony per year (not normally distributed; Shapiro-Wilk W test for normal data, p-value < 0.00001). The average honey yield considering only colonies in full production capacity was 31.03 ± 18.8 kg per colony per year with a median of 27.4 kg per colony per year (not normally distributed; Shapiro-Wilk W test for normal data, p-value < 0.00001). No significant difference was found between Flanders and Wallonia in terms of yearly yield (Wilcoxon rank-sum test) for all colonies in the apiary (p-value = 0.22) and for colonies in full production capacity (p-value = 0.38).

The open-ended questions from the interview allowed the beekeepers to express their concerns about colony losses. Most common concerns were the following: among the colonies that died, a high number had an apparent queen issue on the previous inspection (queenless colonies, drone-laying queens, unfertilized queens), the lack of clear guidelines concerning efficient and alternative varroosis veterinary treatments, trade beeswax quality, and in-hive contaminations.

3.1. Identification of risk and protective indicators of colony losses using logistic regression

3.1.1. Univariate logistic regression analysis

We tested 98 explanatory variables compared to the dependent variable yearly loss rate. We found a significant association between colony losses and the overall global score given to the equipment used (OR = 0.88; 90% CI: 0.79–0.99; p-value = 0.03). The higher the beekeeper scored with the equipment, the more it was considered as a protective indicator (Table 4). The use of divider board(s) also appeared to be a protective indicator, since with beekeepers using a divider board (OR = 0.39; 90% CI: 0.19–0.78; p-value = 0,008) being less likely to have losses. Beekeepers who estimated their colony strength in the fall were also less likely to have losses (OR = 0.37; 90% CI: 0.15–0.89; p-value = 0.03). Beekeepers with the highest number of strong colonies before wintering (76–100%) faced a higher losses risk (OR = 2.33; 90% CI: 1.09–5; p-value = 0.03). The beekeepers who checked their colonies once a month during winter were less at risk for losses (OR = 0.25; 90% CI: 0.12–0.54; p-value < 0,001). Finally checking the efficiency

Table 4
Most relevant explanatory variables evaluated for potential association with yearly colony losses in 186 apiaries, using a univariate logistic regression analysis.

Variable	Variable type	Modalities	Odds ratio	p-Value
Practice improvement	Categorical	Absolutely	Reference	–
		Why not	0.53 (0.24–1.13)	0.10
		No	0.93 (0.42–2.07)	0.87
		Don't know	4.11 (0.19–87.48)	0.37
Hive type	Categorical	Dadant 10–12 frames	Reference	–
		Simplex	1.12 (0.52–2.40)	0.77
		Other hive types	2.05 (0.95–4.41)	0.07
Score given to the equipment origin and hygiene	Continuous	Number	0.88 (0.79–0.99)	0.03
Apis mellifera carnica	Binary	No	Reference	–
		Yes	2.37 (0.93–6.07)	0.07
Use of divider board(s)	Categorical	No	Reference	–
		Yes	0.39 (0.19–0.78)	0.008
		Sometimes	0.34 (0.11–0.06)	0.063
Colony strength estimation	Binary	No	Reference	–
		Yes	0.37 (0.15–0.89)	0.03
Adjust the hive space to the colony size before winter feeding	Categorical	No	Reference	–
		Yes	0.56 (0.29–1.08)	0.08
		Sometimes	0.58 (0.19–1.76)	0.33
Winter monitoring	Categorical	No	Reference	–
		Yes	0.25 (0.12–0.54)	<0.001
Colony strength	Categorical	A	Reference	–
		B	2.38 (0.87–6.50)	0.09
		C	1.11 (0.36–3.48)	0.86
		D	2.33 (1.09–5.00)	0.03
Disease declaration to authorities	Binary	No	Reference	–
		Yes	0.50 (0.24–1.04)	0.06
Infestation rate determination	Binary	No	Reference	–
		Yes	0.50 (0.24–1.04)	0.06
Varroa management with biotechnical methods	Binary	No	Reference	–
		Yes	0.57 (0.31–1.03)	0.06
Treatment efficacy check	Categorical	No	Reference	–
		Yes	0.44 (0.20–0.95)	0.04
		Sometimes	0.88 (0.21–3.59)	0.85
Model of the *Varroa* treatments combinations	Categorical	A	Reference	–
		B	0.52 (0.18–1.50)	0.23
		C	0.47 (0.0–2.39)	0.36
		D	0.63 (0.29–1.35)	0.23
		E	0.38 (0.13–1.15)	0.09
		F	1.87 (0.18–19.73)	0.60

Legend: Statistical significance when p-value < 0.05. Colony strength: A (0–25%), B (26–50%), C (51–75%), and D (76–100%). Treatments model: see Table 1 for definition of models A, B, C, D, E and F.

of the treatment against *Varroa* mite appeared to be a protective indicator (OR = 0.44; 90% CI: 0.20–0.95; p-value = 0.04).

3.1.2. Multivariate logistic regression analysis

The multivariate logistic regression analysis (Table 5) confirmed the significant positive association between colony losses and the equipment score (OR = 0.75; 95% CI: 0.59–0.96; p-value = 0.025) as well as the use of divider boards as protective indicators (OR = 0.094; 95% CI: 0.026–0.32; p-value = 0.00). Supplementary protective indicators of losses were found with the use biotechnical methods to control *Varroa* infestation (OR = 0.22; 95% CI: 0.051–0.96; p-value = 0.04), treatment model E corresponding to two treatments or more; summer and winter with organic acids + other substances (e.g. essential oils other than thymol) (OR = 0.131; 95% CI: 0.017–0.99; p-value = 0.049) compared to model A (no *Varroa* control). The model showed additional risk indicators: beekeepers that were not open to change in their beekeeping practices were at risk of higher colony losses (OR = 8.89; 95% CI: 1.15–68.1; p-value = 0.035) compared to the beekeepers who were willing to improve their BMP, the use of other types of hives other than Dadant-Blatt (OR = 8.62; 95% CI: 1.66–44.61; p-value = 0.01) or combining Dadant-Blatt with another hive types (OR = 8.81; 95% CI: 1.21–55.27; p-value = 0.031) increased the risk of colony losses. Beekeepers who declared overwintering a majority of strong colonies (>75%) were also more at risk of colony losses (OR = 2.24; 95% CI: 0.22–0.88; p-value = 0.437). The Hosmer–Lemeshow test showed that the model fit the data correctly (Ch2 = 6.26, df = 8, p = 0.62).

3.2. Classification tree analysis

The classification tree analysis (CTA) allowed to determine the relative importance and inter-relation among the different risk indicators of colony losses. We conducted the CTA with variables having a p-value < 0.10 from univariate logistic regression analysis. The CTA showed that the score of the equipment (variable importance [VI]: 100) and the use of divider boards (VI: 80.2) were the two predictor variables with the strongest overall discriminating power (Table 6; Fig. 5). Eight additional variables, i.e., variables that did not act as nodes on the selected CTA (Fig. 5), also had significant discriminating power (DP), in decreasing order: the bee breed Carnica (DP: 27.0), tightening colonies before feeding (DP: 13.8), check of treatment efficiency (DP: 11.8), winter check (DP: 11.5), and estimation of the colony strength (DP: 9.9) (Table 6). The root node was first split based on the score of the equipment, clearly indicating that the score of the equipment was the strongest protective indicator. In the first node, when the overall global score of equipment was ≤7.5, 71.4% of the beekeepers (n = 40/56) had mortality rates higher than 10%. In the second node when the overall global score of the equipment was >7.5, 56.9% of beekeepers (n = 130/186) had a mortality rate lower than 10%. For the third node, 31.2% of the beekeepers (n = 77/130) who used one or two divider boards had

Table 5
Results of the final multivariate logistic regression analysis testing the association between the 15 most significant beekeeping management practices out of the univariate model with a p-value < 0.10 and colony losses in n = 186 apiaries.

Variable	Variable type		Odds ratio	p-value
Practice improvement	Categorical	A	Reference	–
		B	0.31 (0.067–1.51)	0.15
		C	8.89 (1.15–68.1)	0.035
Hive type	Categorical	A	Reference	–
		B	4.05 (0.68–24.1)	0.124
		C	8.62 (1.66–44.61)	0.01
		D	8.18 (1.21–55.27)	0.031
		E	0.816 (0.012–51.64)	0.92
Score given to the equipment origin and hygiene	Continuous	Number	0.75 (0.59–0.96)	0.025
Use divider board	Categorical	0	Reference	–
		1	0.094 (0.026–0.32)	<0.001
		2	0.33 (0.04–2.46)	0.028
Varroa management with biotechnical methods/drone brood removal	Binary	No	Reference	–
		Yes	0.22 (0.051–0.96)	0.04
Treatment models	Categorical	A	Reference	–
		B	0.498 (0.086–2.86)	0.436
		C	0.208 (0.010–4.12)	0.303
		D	0.681 (0.18–2.53)	0.568
		E	0.131 (0.017–0.99)	0.049
		F	0.915 (0.038–21.93)	0.956

Legend: Statistical significance when p-value < 0.05. Practice improvement: A (absolutely), B (why not), and C (no). Hive type: A (Dadant 10–12 frames), B (Simplex hive), C (Other types); D (Dadant + other types), and E (Simplex + other types). Treatments model: see Table 1 for definition of models A, B, C, D, E and F.

mortality rates under 10% (Fig. 5). The sensitivity of the tree was 75% (95% CI: 65.1–83.3) and the specificity was 85.6% (95% CI: 76.6–92.1).

4. Discussion

Honey bees (*Apis mellifera* L.) generate a wide range of products for human consumption but more importantly provide irreplaceable pollination services to agricultural and natural ecosystems. To contribute to the maintenance of the population of honey bees, we characterize bee management practices (BMP) carried out in Belgium and present evidence of a relationship between poor beekeeping management practices and colony losses. In general, no significant differences between the two Belgian regions in terms of BMP were found. Our study allowed the identification of risk and protective indicators of BMP and ranked them according to their relative importance and inter-relations among different indicators in explaining colony losses.

According to this study, the winter loss rate reported by the Belgian beekeepers in spring 2016 was 11.8% (±3.6%), which is in line with the winter loss rate of 12.2% published by the COLOSS monitoring group for the same year (Brodschneider et al., 2017). This rate is not particularly alarming given the acceptable losses rate of 10% (El Agrebi et al., 2021).

Varroa control is known to have a tremendous influence on colony losses (Flores et al., 2021; Francis et al., 2013; Noël et al., 2020; van Dooremalen et al., 2012). For *Varroa* control (2015–2016), only four veterinary medicinal products were authorized in Belgium to treat *Varroa*: three based on thymol extract to which *Varroa* have shown resistance for several years (Bonafos et al., 2011) (Thymovar 15 g bee-hive strip, Apiguard 12,5 g gel, and Apilife Var 8 g bee-hive strip (FASFC, 2015)), and one based on flumethrin (PolyVar Yellow 275 mg bee-hive strip) comparable to the fluvalinate molecule, the active ingredient in Apistan (10,3% w/w bee-hive strip), abandoned a few years ago due to *Varroa* resistance (Elzen et al., 2000; Rodríguez-Dehaibes et al., 2005) but still authorized in other EU countries so applicable by cascade[2] in Belgium. A small percentage of beekeepers (8.2%) did not use any *Varroa* control, relying on a *Varroa* resistant honey bee selection or a non-interventionist approach.

Organic acids and thymol are the most widely used control method for *Varroa*. Nevertheless, the beeswax contamination studies related to this same beekeepers sample (n = 186 for multi-residue analysis and n = 124 for flumethrin analysis) (El Agrebi et al., 2020, 2019) revealed the presence of typical residues of beekeeper-applied veterinary medicinal products such as tau-fluvalinate and coumaphos, in 97.3% of the samples, and the presence of flumethrin in 21.8% of the samples. The presence of these veterinary medicinal products is in contradiction with the beekeepers' declaration. These contaminations could come from (e.g.) the recycling of beeswax from varied origins.

Biotechnical methods including drone brood removal (Calderone, 2005), bottom boards screening (Delaplane et al., 2005), powder sugar dusting (Berry et al., 2012) in combination with other *Varroa* control was used by 36.4% of the beekeepers. The use of biotechnical methods to control *Varroa* infestation levels in combination with classical treatments was confirmed to be a protective indicator. This is in line with the study of Giacobino et al., 2015, that showed an increased treatment failure risk when the percentage of *Varroa* infestation prior to treatment was >3% (Giacobino et al., 2015). Sustainable *Varroa* control is a labor-intensive process requiring a combination of different measures, e.g. monitoring of mite fall, drone brood removal trapping (Calderone, 2005; Charriére et al., 2003), and application of miticides in rotation. Such "integrated pest management" needs to consider the population dynamics of *Varroa* as well as the honey bee colony so that measures can be applied at appropriate times of the year (Rosenkranz et al., 2010).

Table 6
Ranking of management predictor variables by overall discriminatory power, using classification regression tree.

Variable	Relative importance
Score given to the equipment origin and hygiene	100
Use of divider boards	80.2
Apis mellifera carnica	27.0
Adjust the space to the colony size	13.8
Treatment efficacy check	11.8
Winter check	11.5
Estimation of colony strength	9.9

[2] The cascade system provides the veterinarian the opportunity to depart from the strict use of registered medicinal products in Belgium. Indeed, it is possible to use a medicinal product for animals of another species or animals of the same species but for another disease. On the other hand, the veterinarian may also prescribe a medicinal product for veterinary use, which is authorized in another Member state of the European Union, a medicinal product for human use and even a magisterial preparation.

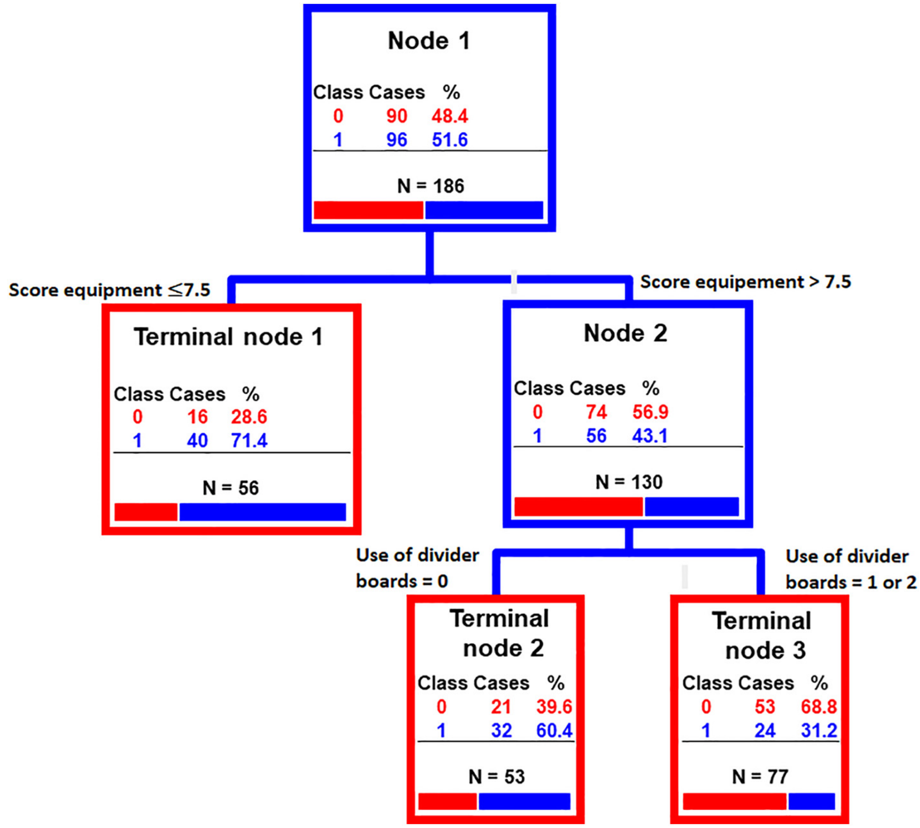

Fig. 5. Classification tree analysis for studying the relative importance and inter-relation among the different risk indicators and the colony losses.
Legend: Class: colony losses above (1) or below 10% (0). The blue-bordered boxes are the nodes that can be further divided into other nodes or terminal nodes. The red-bordered boxes are the terminal nodes that cannot be divided anymore. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In our study, *Varroa* control model in at least two treatments (one in summer and one in winter), one with organic acids and one with alternative substances (mostly essential oils, other than thymol) offered the most protection against colony losses.

Varroa infestation level was rarely estimated prior to treatment. Nevertheless, half of the beekeepers followed up the natural *Varroa* fall (counting *Varroa* natural mortality). Various studies gave contradictory conclusions regarding the accuracy of the natural fall method to determine total infestation rate since natural mite fall is largely determined by the amount of emerging infested brood, but it is in general considered as a good indicator of colony infestation (Branco et al., 2006). The majority of the beekeepers (82.9%) lacked proper knowledge of the *Varroa* infestation rates in their apiaries. These results are worrying as we know that treatment efficiency is highly associated with mite infestation before treatment (Giacobino et al., 2015).

Checking the efficiency of the treatment after its application (applied by 74,3% of the beekeepers) was confirmed to be a protective factor for colony survival. This result is in line with the results of Giacobino et al. (2014) who found that beekeepers who indicated that they did not monitor colonies after mite treatment, were associated with an increased risk of presenting high-intensity infestation and thus colony losses (Giacobino et al., 2014).

The hive type 'Dadant Blatt' used by 46.5% of the beekeepers decreased losses risk compared to all other hive types, the use of other hive types in combination with 'Dadant Blatt' even appeared to increase losses risk. In small apiaries, the use of different types of hives can lead to incompatibility of equipment to remedy problems faced by colonies. The hive type could affect honey bee colony losses by their size, shape, segmentation, building materials, management strategy, or suitability for honey bee parasites (Clermont et al., 2014). The frame of the 'Dadant Blatt' hive is bigger than any other type, this size allows the simultaneous presence of brood and food source in immediate proximity, which might ease colony survival through the winter. A significant relation between loss rate and the global equipment score was found. The overall global score of equipment was calculated as the sum of the scores given to the origin of the equipment (new, self-made, second hand), its re-use after colony losses (yes, after disinfection, or no), and the origin of the beeswax (recycled from own beeswax, recycled from commercial beeswax or commercially purchased beeswax). The higher the beekeeper scored with the equipment global score, the more the factor was protective. Monitoring and keeping the woodenware of hives in good conditions is recommended among best management practices (Heintz et al., 2011), practice good hygiene when dealing with dead colonies (combs, food stores, boxes, etc.) has been ranked and validated as most relevance BMP with a 3.8/4 (Rivera-Gomis et al., 2019). Using own beeswax (preferably capping) is also recommended (El Agrebi et al., 2020; ITSAP, 2017; Vergaert, 2017).

Interestingly, confining the colony to match its need in space and temperature while the colony fluctuates in volume with the use of divider board(s) appeared to be a protective indicator. To date, no other study has looked at this as a potential factor that could influence colony losses. Nevertheless, it has been ranked as a moderately relevant BMP (2.3/4) (Rivera-Gomis et al., 2019).

Beekeepers that estimated colony strength during the beekeeping season and before wintering were less likely to have losses. Moreover, beekeepers that declared the highest number of overwintered strong colonies in fall were those at greater risk of losses. Indeed, the mite population increase is related to colony growth and total incoming and outcoming foragers (DeGrandi-Hoffman et al., 2016). The biggest the colony is, the higher the infestation. Wintering colonies in good conditions and monitoring them through the winter also appeared to be a protective indicator of colony losses. This is rather an indicative of the quality of the BMP that is associated with the success of colony overwintering (Steinhauer et al., 2021).

5. Conclusion

The results of our study indicate that certain BMP are associated to lower colony loss rate. Beekeepers who are not open to improve their BMP are at risk of higher mortality rates. Evolution in management practices is needed as honey bees are exposed to frequent changes in land use, pesticide use, climate, emerging predators, diseases. Adapting BMP to these changes and monitoring the needs of evolving colonies is of crucial importance for their survival. Improving BMP will not prevent all losses, but few behavioural changes including a proper comb management, equipment hygiene, and *Varroa* management, can lead to a non-negligible reduction of the risk of colony losses. We, therefore, recommend the development of a best beekeeping management practices guide, focused on honey bee health rather than on honey production. Having a colony monitoring system in place is also recommended even if it is difficult to conclusively establish the temporal cause and effect relationship. Based on the results of this survey, to improve BMP, an innovative BeeBestCheck tool was designed as inventory to improve BMP and advice beekeepers on their BMP (Appendix 2).

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Abbreviations

BMP	Beekeeping management practices
CTA	Classification tree analysis
FASFC	Federal Agency for the Safety of the Food Chain
ITSAP	Technical and Scientific Institute of Beekeeping and Pollination
Sciensano	Belgian Institute of Health
SPM	Salford Predictive Modeler
ULiège	University of Liège

CRediT authorship contribution statement

Noëmie El Agrebi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Nathalie Steinhauer:** Writing – review & editing. **Simone Tosi:** Writing – review & editing. **Laurent Leinartz:** Software, Writing – review & editing. **Dirk C. de Graaf:** Conceptualization, Writing – review & editing. **Claude Saegerman:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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