A Case-Control Study and Spatial Analysis of Winter Mortality during the Belgian Pilot Program for Honeybee Health Surveillance (EPILOBEE 2012-2013)

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Introduction

Over the last years, an increase in honeybee mortality has been reported in several countries within and outside the EU. In 2011, the EC and EURL set up and co-financed a standardized and Europe-wide voluntary surveillance project in order to obtain reliable and accurate estimates of honeybee colony losses and health as well as information about potential risk factors for these losses (EPILOBEE).

Materials and Methods

The Belgian surveillance program was based on pre-existing studies/data and EU guidelines (EPILOBEE) adapted to a small-scale Belgian beekeeping scenario. The sampling strategy was multi-stage and stratified by province: 150 apiaries were selected from a sampling frame of 3000 registered beekeepers and were visited twice. Questionnaires were completed, collecting info on potential risk factors and mortality. Systematic honeybee samples were screened for *Varroa destructor* infestation by standardized mite counts.
Based on this original prevalence study design, we identified risk factors in a case-control interpretation. Apiary mortality rates were used as a binary dependent variable and questionnaire variables (apiary-beekeeper characteristics, management actions-opinions, landscape diversity, clinical symptoms, acaricide treatment) and *Varroa* infestation were evaluated as covariates in univariate logistic regression (SAS®9.3). Covariates with $p<0.10$ or significant Odds Ratio's were kept and combined in multivariable logistic regression model using backward/forward/stepwise selection protocols (p-stay<0.15 and p-enter<0.1).
A final predictive model was constructed with deviance criterion parameter scaling and after removing outliers. The correlation matrices for the initial dataset (all factors) and final model were checked for pairwise multi-collinearity among variables (rho<0.6 and VIF<10). In ArcGIS®8.0, the predictive risk of mortality was visualized as interpolated background layer. Clusters of high and low mortality were identified in Belgium with SatScan®9.0 (Bernouilli – p<0.05).

Results

Winter Mortality	Colony Loss	95% Conf. Interval		n Apiaries	n Colonies
BELGIUM	**32.71%**	26.54%	39.83%	149	933
Wallonia	**32.84%**	22.93%	42.39%	73	473
Flanders	**32.86%**	25.11%	43.02%	74	453

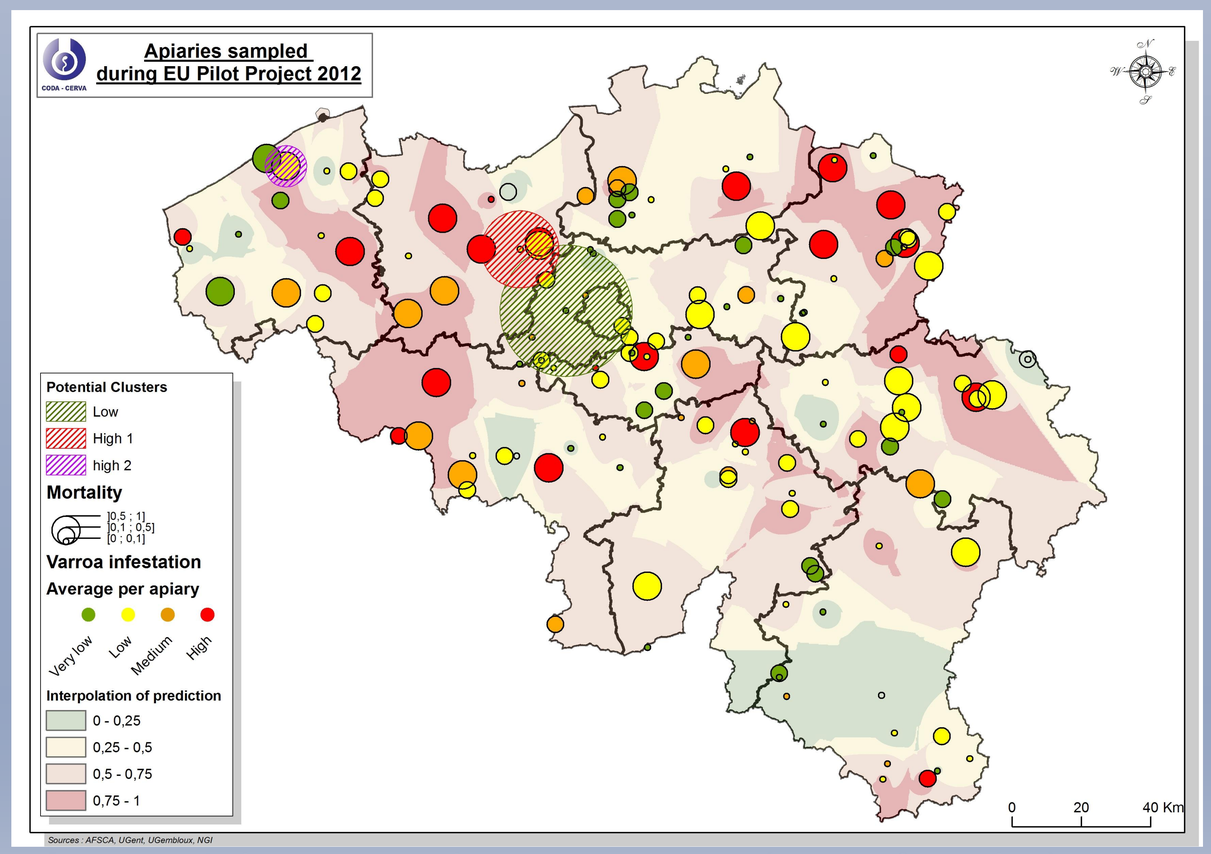

Figure: Colony mortality per apiary (size of circles); most significant potential SatScan clusters (p<0.05 - striped circles); *Varroa* infestation levels (colours); background layer: interpolation of predicted mortality after regression

Covariate	Level of variable	Odds Ratio (95% CI)
Varroa Infestation	Percentage of cols infested with Varroa	4.0 (1.1 - 13.9)
	Any colony high level (>10 mites / 100 bees)	6.6 (2.4 - 18.4)
	High average apiary count (>10 mites / 100 bees)	14.6 (3.0 - 71.8)
Chemical Varroa Treatment	Treat after 1st Sept. or no chemical treatment	3.0 (1.2 - 5.0)
	Treat Thymol before visit 1	0.5 (0.2 - 0.9)
	Treat Other product before visit 1	2.5 (0.98 - 6.2)
	Treat Organic Acid in Winter (between visit 1-2)	0.5 (0.3 - 1.0)
Age of Beekeeper	Beekeeper >65 years old at visit 1	3.1 (1.2 - 8.3)
Beekeeper Opinions	Wanting to keep the apiary at visit 1	0.31 (0.12 - 0.81)
	Wanting to keep the apiary at visit 2	0.30 (0.10 - 0.96)
	Wanting to increase production at visit 2	0.34 (0.14 - 0.82)
Beekeeper observations during winter (between visit 1-2)	Seen swarming in Winter by visit 2	0.35 (0.11 - 1.11)
	Seen colony mortality in Winter by visit 2	115 (26 - 516)
	Seen bee mortality in Winter by visit 2	10.0 (2.9 - 34.7)
Diagnostic test	Requested at visit 1 or visit 2	7.8 (2.2 - 27.2)
	Requested at visit 2	5.4 (1.5 – 19.2)
Landscape diversity	Apiary surrounded by 3-4 types of landscape	3.0 (1.0 - 9.3)

Table: Significant covariates (levels) in the univariable logreg.

Discussion

The final model retained as covariates: age of beekeeper, wanting to continue with beekeeping, surrounding landscapes, average *Varroa* infestation, acaricide treatment <1st Sept. and thymol treatment. It was obvious that this predictive model as well as the SatScan (Bernouilli) model of mortality data do not perfectly predict colony mortality and clusters yet: **AUC=80%** (ROC in Stata®10.0). This is partly due to the highly correlated nature of the questionnaire items, lack of causality for some covariates and due to the multifactorial nature of winter colony mortality. Not all potential risk factors are yet included in the current data layers and models: e.g. virus prevalence data, spatial data on pesticide use, detailed land use data at apiary level.
However, this spatial and risk factor analysis of honeybee winter colony mortality served as a proof of concept for honeybee health surveillance, has generated hypotheses for in depth risk factor and GIS analyses (e.g. use higher resolution landscape data and/or collect data on pesticide use) and highlighted where the case-control study could benefit of an increased future sample size in the prevalence study design. *Varroa* and virus related variables, some management related variables and landscape related variables could be further assessed, e.g. in PCA or CART analysis and subsequent regression modeling.

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