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ORIGINAL RESEARCH ARTICLE

Queen replacement: the key to prevent winter colony losses in Argentina

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Honey bee colony losses during winter are a multi-factorial phenomenon. Environmental conditions, beekeeping practices and different pathogens are all considered as potential causes of honey bee colony losses. However, these factors may be diverse in different regions and there are no regional studies about winter losses in South America. The objective of this study was to identify risks factors associated with winter losses in temperate climate honey bee colonies in Argentina. Parasitic mite infestation level, colony strength measures, and percentage of colonies losses during winter 2013 were evaluated in 62 apiaries distributed in four different regions in east-central Argentina. Data regarding management practices in each apiary were collected by means of a questionnaire. A logistic regression model was constructed to associate management variables with the risk of winter losses higher than 10% of the colonies. Beekeepers who reported replacing less than 50% of the queens in their apiaries showed higher winter losses than apiaries who replaced more than 50% of their queens (OR = 18.15; CI 95%: 1.76–187.43; $p = 0.01$). There were no significant spatial clusters detected in our analysis ($p > 0.05$). Even considering that the winter colony losses can be explained by a complex interaction of factors, requeening appears as one of the most important management practices to reduce this phenomenon in Argentina.

Reemplazo de la Reina: la clave para evitar la pérdida de la colonia de invierno en Argentina

Las pérdidas de colonias durante el invierno son un fenómeno multifactorial. Las condiciones ambientales, las prácticas apícolas y diferentes patógenos son mencionados como potenciales causas de pérdidas de colonias durante el invierno. Sin embargo, estos factores pueden ser diversos en diferentes regiones y no hay estudios regionales sobre la perdida de colonias de invierno en Sudamérica. El objetivo de este estudio fue identificar los factores de riesgo asociados a las pérdidas durante el invierno de colonias de *Apis mellifera* en clima templado de Argentina. El nivel de parasitación con *Varroa destructor*, la fortaleza de las colmenas y el porcentaje de colonias perdidas durante el invierno 2013 fueron evaluados en 62 apiarios distribuidos en 4 regiones en el centro este de Argentina. Los datos de prácticas de manejos aplicadas en cada apiario fueron recolectados mediante una encuesta. Se asociaron las variables de manejo apícola y el nivel de infestación con varroa con el riesgo de presentar perdidas invernales superiores al 10% de las colinas de un apiario mediante una regresión logística. Aquellos apicultores que reportaron reemplazar menos del 50% de las reinas en sus apiarios presentaron mayores pérdidas invernales que aquellos que reemplazan las reinas en más del 50% de las colmenas (OR = 18.15; CI 95%: 1.76–187.43; $p = 0.01$). No se encontraron *clúster* espaciales significativos en nuestro análisis ($p > 0.05$). Aun considerando que las pérdidas de colonias durante el invierno pueden ser explicadas por una compleja interacción de factores, el reemplazo de reinas aparece como una de las prácticas de manejo apícola más importantes para reducir este fenómeno.

Keywords: winter losses; queen replacement; *Varroa destructor*; honey bee

Introduction

High rates of losses during winter have been recently reported in honey bee (*Apis mellifera* L.) colonies in many countries, especially in Europe and North America (Neumann & Carreck, 2010; Pirk, Human, Crewe, & vanEngelsdorp, 2014; Steinhauer et al., 2014; van Der Zee et al., 2014; vanEngelsdorp, Hayes, Underwood, & Pettis, 2008). Although the Latin American beekeeping situation might be different (Vandame & Palacio, 2010), rising winter losses have been registered in recent years. Reasons for high winter mortality are not completely understood. However, the ectoparasitic mite *Varroa destructor*, queen failure and poor nutrition are identified by the beekeepers as main causes for overwintering losses (vanEngelsdorp et al., 2012). High *V. destructor* infestation during the transition to winter bees can cause colony losses due to decreased lifespan of winter bees (van Dooremalen et al., 2012). Additionally, many of the factors ranked as potential causes of mortality are closely linked to management; and management itself was considered an important contributor to winter mortality (vanEngelsdorp, Hayes, Underwood, & Pettis, 2010).

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Apiary management also plays an important role in preparing colonies that survive winter and reach spring time in good conditions (Topolska, Gajda, & Hartwig, 2008). Furthermore, the presence of varroa mites not only cause body weight loss (Duay, Jong, & Engels, 2003), malformation of bees and weakening of colonies (Garedew, Schmolz, & Lamprecht, 2004; Marcangeli, Monetti, & Fernandez, 1992) and reduction of the lifespan of workers (Amdam, Hartfelder, Norberg, Hagen, & Omholt, 2004) but also facilitates the interaction with other pathogens, for example *Nosema* spp. and several viruses (Chen & Siede, 2007; Williams et al., 2010). Varroa has been suggested to be the main cause associated with colony winter mortality (Guzmán-Novoa et al., 2010). Therefore, winter losses could be explained by complex interactions between multiple drivers, headed by varroa mite levels and colony mismanagement.

Beekeeping in Argentina is mainly represented by semi commercial or commercial beekeepers that own fewer than 200 colonies (especially for honey production), distributed among several apiaries (Giacobino et al., 2014). Argentinean honey yield is obtained during summer and most of the production is exported to the USA and European countries (Blengino, 2014). Usually, colonies are treated against *V. destructor* using a commercial acaricide during autumn. Typically, colonies are kept in one place with a few exceptions of colonies that migrate for honey production purposes (Giacobino et al., 2014).

Since environmental conditions, beekeeping practices and both host and pathogens are genetically diverse, the symptoms and causes of honey bee colony losses may be diverse in different regions (Neumann & Carreck, 2010). The objective of this study was to identify risks factors associated with winter losses in a temperate climate region in Argentina.

Materials and methods

Study design and sample size

A total of 62 apiaries (colonies n = 3,735; 95% confidence level; precision <10.5%) were included in the survey, according to the number of apiaries in Santa Fe province (Department of Agriculture from Santa Fe Province, 2008) (Figure 1). Apiaries were randomly chosen following stratified randomization procedures (computerized random numbers) (Moher et al., 2010). Four zones were defined based on the nectar flow period, the eco-region categorization, and agricultural practices (Giacobino et al., 2014): North, Central, Riverside, and South. The apiaries were randomly assigned following stratified randomization procedures (computerized random numbers) to each zone (Moher et al., 2010), according to their proportional distribution.

Autumn survey on management practices: risk exploratory variables

During autumn 2013 we conducted a survey about common management practices performed in the selected apiaries. Information on the potential risk factors was obtained from a checklist questionnaire (available as supplemental material in Giacobino et al., 2014) answered by the beekeepers. We explained to the beekeepers the purpose and the importance of the survey, emphasizing that the answers were anonymous. The questionnaire was divided into three main parts: general items related to the apiary, management practices, and varroa treatment. Table 1 shows a summary of the management practice variables registered in the survey.

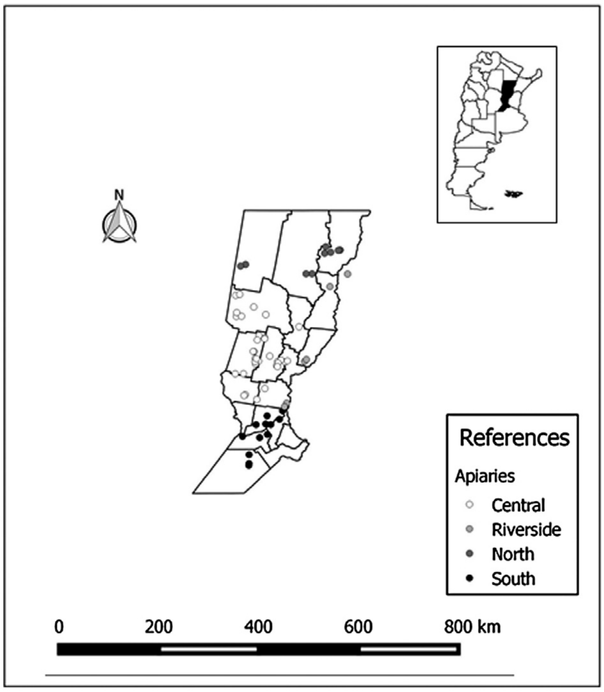

Figure 1. Location of Santa Fe province in Argentina, and apiaries distribution according to zone.

Data collection: colony strength and diagnosis of V. *destructor*

During the autumn, within each apiary a minimum of 6 colonies or 10% of the total number of colonies (in apiaries larger than 60 colonies) were randomly selected to evaluate *V. destructor* infestation level (Lee, Moon, Burkness, Hutchison, & Spivak, 2010). Adult worker bees were examined prior to acaricide treatment, to diagnose the presence of varroa mites using the warm/soap water method (Dietemann et al., 2013). The infestation level was calculated as number of mites per 100 bees (hereafter referred to as percentage of varroa infestation). In addition, the populations of adult bees and brood, as well as pollen and honey reserves were assessed in the colonies by estimating the total area of comb covered by adult bees (CCB), brood sealed (CCBr), honey (CCH) and pollen (CCP) (De Grandi Hoffman et al., 2008). To perform this task, each hive was opened once, each frame was sequentially removed and the percentage of coverage in both sides was estimated.

Table 1. Summary of variables derived from the questionnaire and assessed as potential risk factors for winter losses in apiaries located in central-east Argentina.

General apiary management	Variable description
Region	Geographic location within Santa Fe province: North, South, Central and Riverside
Size of apiary	Number of colonies within each apiary
Average winter mortality	Last three year percentage of colonies lost in the apiary
Average honey yield	Last three year kg of honey harvest per colony
Migratory beekeeping	If colonies are normally moved during winter If yes: to which crops, when and how long
Protein diet	Feed colonies during autumn or spring with natural pollen, supplements or substitutes
Carbohydrate supply	Feed colonies during autumn or spring with sucrose syrup or high fructose corn syrup
Colony multiplication	Percentage of colonies within the apiary that has been used for nuclei production during last spring
Frequency of requeening	How frequently (in years) a queen is replaced in each colony by the beekeepers
Percentage of requeening	The proportion of colonies within each apiary in which queen is replaced by the beekeeper during one season
Annual comb replacement	How many combs per colony are replaced by new ones per year
Wooden ware disinfection	Do you normally disinfect the wooden ware before storage it after harvest season. If disinfection takes place, how?
Autumn treatment against Varroa mites	Active substance that they used Date of treatment Rotation of chemical substance during last 4 treatments
Monitoring varroa infestation level	Checking for the % of varroa infestation level in adult bees, prior to and after treatment
Late winter–Early spring treatment	Active substance that they used Date of treatment

Spring survey on winter mortality

A new questionnaire was distributed to the participant beekeepers during September–October 2013 to assess the following variables: winter treatment against varroa mites (if so, which product was used and application date), whether disinfection of wooden hive parts was performed after honey harvest and before winter and percentage of colony losses over the winter period (since the autumn survey).

Statistical analysis

Varroa level and winter mortality among zones were compared using Kruskall–Wallis and Mann–Whitney U test. Then, apiaries were classified in two groups according to an acceptable percentage of colony loss during winter (Genersch et al., 2010; Le Conte, Ellis, & Ritter, 2010): high (>10%) and low (≤10%) winter mortality losses. Percentage of colonies losses during last winter was considered as the outcome variable.

Potential predictor variables (autumn and spring questionnaire) and winter mortality losses were examined using the Pearson chi square test of independence (χ^2) or Student *T*-test. All variables with a significance value $p < 0.15$ were selected for further analysis in a multivariate logistic model. Collinearity between the selected variables was assessed by a Pearson χ^2 test. When two potential risk factors were associated, only one was used in the multivariable analysis (i.e., the one with the smallest *p*-value in the univariate analysis). A logistic regression model was performed where the explanatory variables were those factors previously selected, whereas the response variable was the winter mortality losses. The estimation method was the maximum likelihood with a convergence criterion of 0.01 to a maximum of 10 iterations. All statistical analysis were carried out using InfoStat software (Universidad Nacional de Córdoba, Argentina).

Spatial analysis

The spatial scan statistic (Kulldorff & Nagarwalla, 1995) cluster-detection method was used to identify and test the significance of specific clusters for a heterogeneous population distribution. The data-set was scanned for clusters with low and high rates of >10% winter losses cases (equivalent to a two-sided statistical test). A likelihood-ratio test statistic was calculated for each cluster and the scanning upper limit was set at 50% of the population at risk. The distribution of winter losses was assumed to be Bernoulli (for instance cases >10% and non-cases ≤10%), the most likely cluster (the cluster that is least likely to have occurred by chance) along with secondary significant clusters (Kulldorff, 2014) were reported. All analyses were performed using SaTScan software version 9.2 (www.satscan.org).

Results

Descriptive analysis

Beekeepers in our study owned from 16 to 90 honey bee colonies in their apiaries (mean ± SD = 42.39 ± 17.67) and the average years of beekeeping experience was of 12.57 ± 8.16. Surveys indicated that beekeeping is usually a complementary economic activity (semi-professional), given that most farmers have other incomes from other activities. More than a half (51.6%) had honey production as a secondary income, 35.9% as their principal income and the rest as a hobby. The colonies were rarely moved (only 4.7% migrated their colonies). Almost seventy percent of the beekeepers (66.12%) did replace their queen. Most apiaries received some carbohydrate supply during autumn (88.5%) and spring (86.8%), independently of the kind of syrup (sucrose or high fructose corn). Pollen substitute was used mostly throughout spring (85.7%) and autumn (54.1%), but only 39% of beekeepers used it during both seasons.

In the autumn, the average percentage of varroa infestation was 5.68 ± 4.19 (mean ± SD), the colonies average strength measures were 8.69 ± 1.16 (CCB), 4.59 ± 1.52 (CCBr), 0.87 ± 0.54 (CCP) and 3.02 ± 1.31 (CCH) (n = 62). The average winter losses was 11.44 ± 8.86% of the colonies per apiary with a minimum of zero and maximum of 50% (n = 46). From a total population of 46 apiaries with winter losses data, 28 were categorized as cases (>10% of colony losses).

Univariate analysis

The variables associated to apiaries with high winter losses (more than 10%) in the univariate analysis were: combs fully covered with brood (CCBr) (p = 0.007), combs fully covered with honey (CCH) (p = 0.023), varroa infestation level (p = 0.026), queen replacement (p = 0.007) and date of treatment (p = 0.005) (Table 2). Apiaries with >10% of winter losses showed less CCBr (4 ± 1.24) and more CCH (3.61 ± 1.24) compared to apiaries <10% of winter losses (5.10 ± 1.27 CCBr and 2.73 ± 1.19 CCH). Moreover, they had more percentage of varroa infestation (7.54 ± 4.12 per colony) than <10% apiaries (4.82 ± 3.44% per colony). Further, 75.8% of the apiaries owned by beekeepers who replaced the queens in their colonies showed <10% of winter losses whereas only 35.3% of the apiaries that did not replace their queen presented <10% of winter losses. All the apiaries that received a late acaricide treatment (after March) presented >10% of winter losses during 2013 (Table 2).

Multivariate analysis

From the associated variables introduced in the forward logistic regression model (only 42 beekeepers wholly answered both questionnaires and were included), we found that only the queen replacement was a significant factor associated with the percentage of winter losses (Table 3). Apiaries whose beekeepers did not replace the queen in their colonies showed an odds ratio (OR) of 18.15 (CI 95% 1.76–187.43; p = 0.01) compared to those who replace their queen regularly. There were not significant spatial clusters detected in the analysis (p > 0.05) and percentage of varroa infestation was alike in all zones (p = 0.22). However, average winter mortality was higher in North and Riverside zones (p = 0.03) (Figure 2).

Discussion

This is the first report of risk factors associated with overwintering colony losses in Argentina. These are similar to losses previously recorded in Germany (Genersch et al., 2010), Croatia (Gajger, Tomljanović, & Petrinec, 2010) and Austria (Brodschneider, Moosbeckhofer, & Crailsheim, 2010) but considerably lower than losses recorded in Canada (Currie, Pernal, & Guzmán-Novoa, 2010), USA (2010; 2012, vanEngelsdorp et al., 2008; Steinhauer et al., 2014), Turkey (Giray, Kence, Oskay, Döke, & Kence, 2010), and other European countries (2014; Nguyen et al., 2010; van der Zee et al., 2012).

Until now, only the Korean haplotype of *V. destructor* has been reported in Argentinean colonies (Anderson & Trueman, 2000; Maggi et al., 2012). Recent studies performed in Santa Fe province (Argentina) have confirmed the same Korean haplotype (Giacobino, 2015).

Table 2. Association between potential risk factors and % honey bee winter colony mortality (univariate analysis).

Potential factors	Winter mortality (%)	n	Mean (SD)	T student p-value
CCBr	>10%	18	4.00 (1.29)	0.007
	<10%	28	5.10 (1.27)	
CCH	>10%	18	3.61 (1.24)	0.021
	<10%	28	2.73 (1.18)	
Varroa infestation level	>10%	18	7.54 (4.11)	0.019
	<10%	28	4.81 (3.44)	

Potential factors		Winter mortality N (%)		χ^2 square p-value
	Variable level	>10%	<10%	
Queen replacement	Yes	7 (24.2)	22 (75.8)	0.007
	No	11 (64.7)	6 (35.3)	
Date of Varroa treatment	Early	5 (29.7)	26 (70.3)	0.002
	Late	5 (100)	0 (0)	

Notes: CCBr: combs with brood; CCH: combs with honey; Early: Feb/March; Late: Apr/May/Jun.

Table 3. Logistic regression model for risk factors associated with honey bee colonies winter losses (2013; *n* = 42).

Predictive variables	Variable level	Odds ratio	95% CI (O.R.)	*p*-value
Constant	–	0.000		0.99
CCBr	–			0.13
CCH	–			0.13
Varroa infestation level	–			0.41
Queen replacement	Yes (Ref.)			–
	No	18.15	1.76–187.43	**0.01***
Date of Varroa treatment	Early (Ref.)			–
	Late			0.99

Notes: Model HL: 0.663; References: CI: confidence interval; CCBr: combs with brood; CCH: combs with honey; Early: Feb/March; Late: Apr/May/Jun.
*Statistical significance < 0.05.

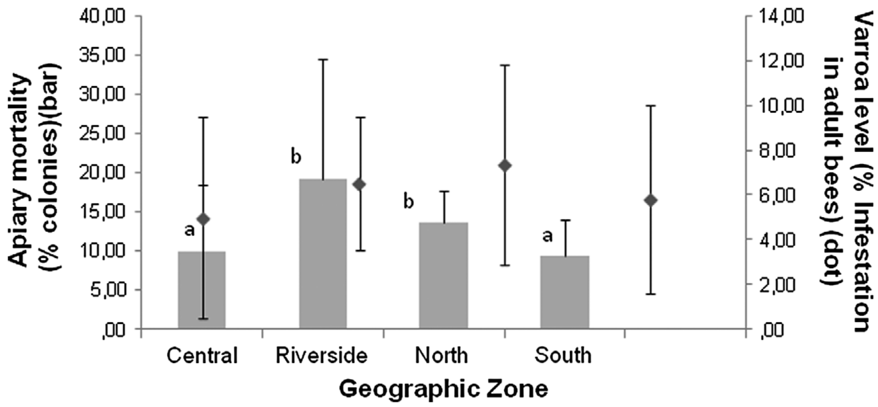

Figure 2. Percentage of varroa infestation during autumn 2013 and winter colony losses (% of colonies per apiary) for all zones in Santa Fe province.
Note: Different letters show statistical significance ($p < 0.05$, Mann–Whitney U test).

When we individually compared all the potential factors associated with winter losses, we found that CCBr, CCH, queen replacement, varroa mites and date of acaricide treatment were significantly associated. This supports the fact that weak colonies during autumn, poor queens, and varroa mites are among the top five most frequent reasons given for colony losses (vanEngelsdorp et al., 2012). High losses occurred when beekeepers reported having observed *V. destructor* in their apiaries during autumn (Nguyen et al., 2010). In addition, when *V. destructor* infestation is reduced in early-treated colonies, before the development of winter bees, this results in higher colony survival after winter (van Dooremalen et al., 2012). Other variables such as carbohydrate and protein supply, migratory beekeeping and the kind of treatment against varroa were not associated with winter mortality in our study, in contrast to some previous studies (Brodschneider et al., 2010; vanEngelsdorp et al., 2008).

Although we evaluated numerous potential risk factors for winter mortality, queen replacement was the most important driven variable explaining colony losses registered during 2013 in Argentina. Beekeepers who did not frequently replace their queen were 18 times more likely to have high winter losses (more than 10% of the colonies per apiary). A similar observation was reported by Gajger et al. (2010) in Croatia where the beekeepers who never requeen their colonies had the highest winter losses. Previous studies reported that queen age (Genersch et al., 2010) or percentage of young queens in the colonies (van Der Zee et al., 2014) were associated, among other factors, with winter losses. Previous results suggest that higher percentages of queen replacement also help to maintain lower mite infestations (Giacobino et al., 2014) since colonies headed by young queens had lower intensity of varroa infestation than those headed by old queens (Akyol, Yeninar, Karatepe, Karatepe, & Özkök, 2007). Also, colonies headed by young queens have a significantly higher chance to survive during winter probably due to significantly higher brood and bee production (Genersch et al., 2010). Finally, since queen replacement improves colony performance and helps maintaining low varroa levels, it seems likely that frequent queen replacement is the main factor significantly reducing honey bee winter colony losses. Therefore, the number of colonies lost can be mitigated by appropriate management practices (vanEngelsdorp et al., 2010).

Losses in Santa Fe province differed between regions, being higher in the Riverside (19.1%) and in the North (13.5%). The observed regional differences in colony winter losses could be explained by environmental factors (Brodschneider et al., 2010). Beekeeping region, overwintering location, and unusual weather patterns seems to be important factors for colony mortality (Giray et al., 2010). However, even when we found that North and Riverside zones had higher winter losses, there were no significant spatial clusters detected in our analysis. Besides the climate differentiation, zones are also quite diverse according to land use. Whereas Riverside is normally used for horticultural crops, North zone is designated mainly for livestock production. Central and South zones are used for milk production and soybean crops, respectively. This might suggest that regardless of differences in weather patterns and landscape features, winter losses are more likely to be affected by management practices than by geographical factors. In this study we only analyzed the presence of *V. destructor*. However, it is possible that other factors such as *Nosema* spp., virus, and pesticide applications, may also contribute to winter mortality. Further studies should be conducted to determine whether there are any other factors (sanitary or environmental) influencing mortality rates in the area.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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