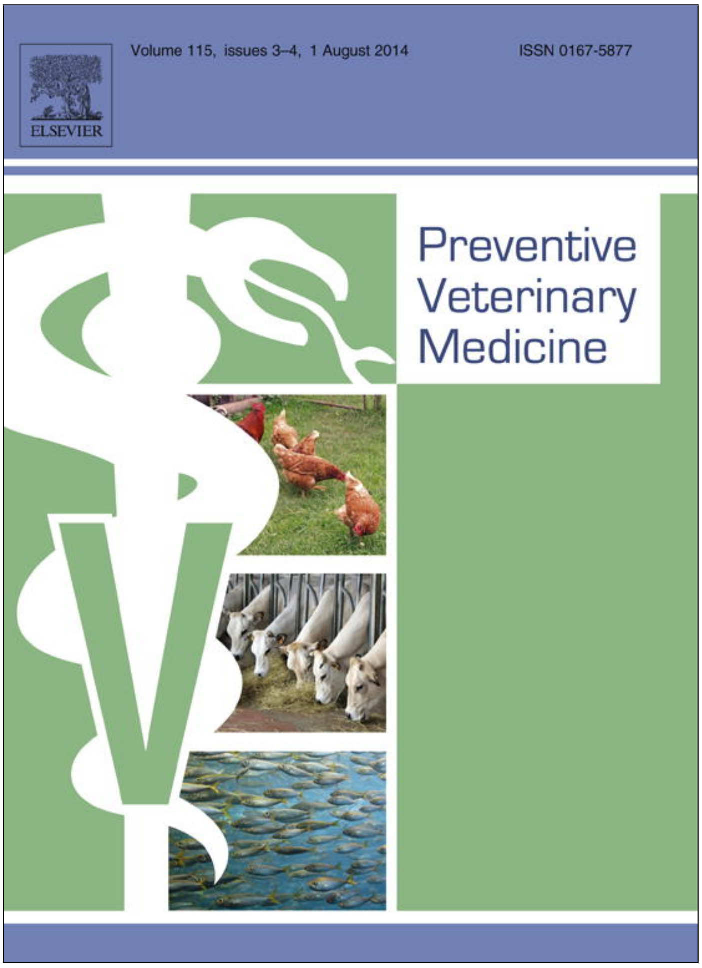
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Risk factors associated with the presence of *Varroa destructor* in honey bee colonies from east-central Argentina

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ABSTRACT

Varroa destructor is considered one of the major threats for worldwide apiculture. Damage caused by varroa mite includes body weight loss, malformation and weakening of the bees. It was also suggested as the main cause associated with colony winter mortality and as an important vector for several honey bee viruses. Little is known about multiple factors and their interaction affecting *V. destructor* prevalence in apiaries from South America. The aim of this study was to identify risk factors associated with *V. destructor* prevalence in east-central Argentina. Parasitic mite infestation level and colony strength measures were evaluated in 63 apiaries distributed in 4 different regions in east-central Argentina in a cross sectional study. Data regarding management practices in each apiary were collected by means of a questionnaire. A mixed-effects logistic regression model was constructed to associate management variables with the risk of achieving mite infestation higher than 3%. Colonies owned by beekeepers who indicated that they did not monitor colonies after mite treatment (OR = 2.305; 95% CI: 0.944–5.629) nor disinfect hives woodenware material (OR = 2.722; 95% CI: 1.380–5.565) were associated with an increased risk of presenting high intensity infestation with *V. destructor* (>3%). On the other hand, beekeepers who reported replacing more than 50% of the queens in their operation (OR = 0.305; 95% CI: 0.107–0.872), feeding colonies protein substitute containing natural pollen (OR = 0.348; 95% CI: 0.129–0.941) and feeding colonies High Fructose Corn Syrup (HFCS) (OR = 0.108; 95% CI: 0.032–0.364), had colonies that were less likely to have *V. destructor* infestations above 3%, than beekeepers who did not report using these management practices. Further research should be conducted considering that certain management practices were associated to mite infestation level in order to improve the sanitary condition in the colonies. Epidemiological studies provide key information to design surveillance programs against one the major threat to worldwide beekeeping.

1. Introduction

Varroa destructor (Anderson and Trueman) (Acari: Mesostigmata) is an obligate ectoparasite of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae). Currently, this parasite is considered almost cosmopolitan (Oldroyd, 1999; Rosenkranz et al., 2010) and one of the main threats to

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worldwide apiculture (Genersch, 2010). During the larval and pupal stages of bees, varroa mites ingest hemolymph, causing body weight loss (Duay et al., 2003), malformation of bees and weakening of colonies (Marcangeli et al., 1992; Garedew et al., 2004) and reduction of the lifespan of workers (Amdam et al., 2004). In addition, *V. destructor* is the vector of several honey bee viruses (Chen and Siede, 2007) and has been suggested to be the main cause associated with colony winter mortality (Guzmán-Novoa et al., 2010).

In Argentina, control of *V. destructor* is especially important, given that Argentina exports about 95% of its national honey production and contributes to 6% of the global honey production (SAGPYA, 2009). Additionally, since numerous economically important crops depend on honey bees for pollination, the loss of honey bee colonies is of ecological concern and is also an economic issue at the global scale.

Alternative strategies to chemical control, including disease prevention and control programs based on epidemiological studies that attempt to identify factors that may explain or contribute to disease outbreak, should be assessed (vanEngelsdorp et al., 2013). Apicultural practices are thought to be responsible for maintaining the virulent forms of pathogens, especially because they contribute to horizontal transmission (Fries and Camazine, 2001). In addition, certain management practices in apiculture, such as having moving colonies or not rotating the acaricides, may indicate that socio-economic factors may tend to artificially improve the performance of certain diseases.

The availability of critical resources for bees depends on the environmental conditions of each geographical zone (Murray et al., 2009). Like in other countries, in Argentina, the amount and quality of forage sources have declined (vanEngelsdorp and Maixner, 2010), especially given that changes in land-use have reduced the diversity of flowering plants (Kremen et al., 2007). When outside food sources for bees become scarce, "robbing" ends up impacting on horizontal transmission of *V. destructor* (Fries and Camazine, 2001).

All this suggests that beekeeping is threatened by multiple and complex drivers involving biological, ecological and socio-economic factors. Neumann and Carreck (2010) reviewed the recent bee colony losses reported in Europe, Japan and the USA and suggested the central role of *V. destructor*, although they also stated that the mite alone cannot explain all the recent losses. Although colony losses have not been recorded in South America, drug resistance and colony losses are some of the most severe problems concerning mite population control at regional scale. In South American apiaries, little is known about risk factors associated with *V. destructor* infestation. Thus, the aim of this study was to identify risk factors associated with autumn *V. destructor* prevalence in east-central Argentina.

2. Materials and methods

2.1. Study design and sample size

A cross-sectional study was carried out from February to May 2013 in Santa Fe province (total surface of 133,007 km^2), east-central Argentina. The percentage of infestation of *V. destructor* was estimated after honey yield and prior to autumn acaricide treatment given that colonies are commonly monitored at this time of the year (Department of Agriculture from Santa Fe province, 2008) and because this is a key practice to guarantee healthy overwintering conditions (Currie and Gatien, 2006). The study was carried out during an extended period (from February to May) because the honey harvest season and treatment time frame vary according to the geographical zone and the beekeeping management practices. During this period, mite loads might be higher because ambient factors such as climate and nectar flow are favorable for mite population growth (Rosenkranz et al., 2010).

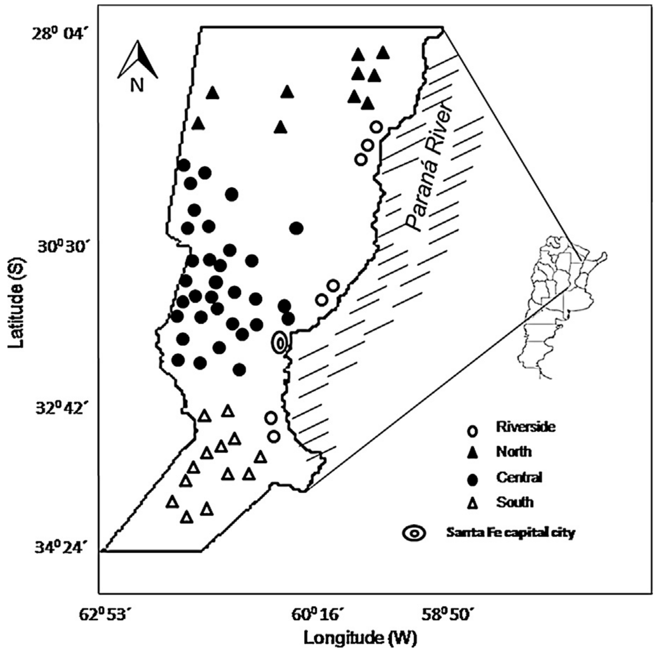

Fig. 1. Apiaries distribution consistent with zone classification of Santa Fe province, in east-central Argentina.

In previous studies, we determined a critical threshold of 3% (mite load above which it is recommended to treat colonies during autumn to avoid severe winter losses) for temperate climate colonies of Argentina. Our results suggested that colonies that go through winter with more than 3% of mite load hardly survive until the following spring (Bulacio Cagnolo, 2011).

A total of 63 apiaries (owned by different beekeepers) were sampled. The sample size was estimated based on the fact that there are 3735 apiaries in Santa Fe province (Department of Agriculture of Santa Fe province, 2008) and 74% of expected prevalence of colonies with >3% (3 mites per 100 bees) of infestation intensity (SENASA, 2007), with 95% confidence level and a precision <10.5%. Four zones were defined based on the nectar flow period and their beekeeping management schedule, the eco-region categorization (Burkart et al., 1999; Arzamendia and Giraudo, 2004) and agricultural practices (Giorgi et al., 2008): North, Central, Riverside, and South (Fig. 1). Apiaries were randomly chosen following stratified randomization procedures (computerized random numbers) and assigned to one of the different regions (Moher et al., 2010), according to their proportional distribution. Spatial stratification is used in large-scale studies to ensure an unbiased number of apiaries. Within each apiary, a minimum of 6 or 10% of the total number of colonies (in apiaries larger than 60 colonies) was randomly selected to estimate *V. destructor* prevalence (Lee et al., 2010). The chosen colonies were managed by the beekeeper like the rest of the colonies in the apiary and according to the usual beekeeping practice. The aim was to guarantee that colonies reflected different management techniques in Santa Fe.

2.2. Data collection: colony strength and diagnosis of V. destructor prevalence

Adult bees were examined to diagnose the presence of varroa mites in bee colonies. Approximately 250 bees were collected from both sides of three unsealed brood combs in a jar containing 50% ethanol. The mites were separated from the bees by pouring the jar content into a sieve with a mesh size of 2 mm (Dietemann et al., 2013). The intensity of mite infestation on adult bees was calculated dividing the number of mites counted by the number of bees in the sample to determine the proportion of infested individuals and multiplying by 100 to obtain the percentage of infestation per colony (Dietemann et al., 2013). In addition, the populations of adult bees and brood, as well as pollen and honey reserves, were measured in colonies by estimating the total area of comb covered (De Grandi Hoffman et al., 2008; vanEngelsdorp et al., 2009) by adult bees, brood, sealed honey and pollen. Once each hive was opened, each frame was sequentially removed and the percentage of coverage in both sides was estimated.

2.3. Survey on management practices: risk exploratory variables

Information on the potential risk factors was obtained from a checklist questionnaire (available as supplemental material) answered by the beekeepers. Beekeepers were explained the purpose and importance of the survey, emphasizing that the answers were anonymous. The questionnaire included 37 questions distributed into three main parts: general items related to the apiary, management practices, and varroosis treatment. Table 1 shows a summary of the variables analyzed. The explanatory variables were the risk factors, whereas the response variable was the prevalence of colonies with more than 3% of *V. destructor* infestation

2.4. Statistical analysis

To establish a relative sanitary condition, previous results obtained in the same region were used to subcategorize the colonies into two levels: high and low, according to their autumn infestation with varroa mites (high: >3%; low: ≤3%) (Bulacio Cagnolo, 2011).

In a first step, a descriptive analysis was performed to identify variables with a large amount of missing observation or a low variability that might be of little value for further investigations. After this validation, the associations between each of the predictor variables and the prevalence of *V. destructor* (>3%) were examined using the

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

Subject	Factors/variables
General data on the apiary	Region: north, south, central and riverside *Size*: number of colonies in the apiary Beekeeping experience: number of years in the activity Scale of beekeeping: hobbyist, semi-professional and professional beekeepers Winter mortality during last 3 years (% of colonies per year) Average honey yield (last 3 years): kg per colony
Management practices	Protein diet: natural pollen, supplements or substitutes Carbohydrate supply: sucrose or high fructose corn syrup Season and purpose when diets are used Colony multiplication: production of nuclei Frequency (in years) of requeening Percentage of requeening colonies by the beekeeper per year[a] Annual comb replacement: how many combs per colony/per year Wooden ware disinfection: yes/no Migratory beekeeping: yes/no Migratory beekeeping: to which crops, when and how long
Varroa treatment	Autumn treatment: how many treatments; active substance Monitoring infestation level; prior to and after treatment: yes/no Late winter–spring treatment: yes/no; active substance: rotation of chemical treatments during last 4 years

[a] This variable refers to the proportion of hives within each apiary in which queen is replaced during one season.

Pearson chi square test of independence (χ^2). All variables with a significance value $P<0.15$ were selected for further analysis in a multivariate logistic model. Colinearity between the selected variables was assessed by a Pearson chi square test of independence (χ^2). 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). Since we collected data on grouped colonies (apiaries) and the unit of analysis was the colony, we adjusted a final mixed-effects logistic-regression with apiary as the random effect. Variables with a $P \le 0.05$, calculated using the Wald test, were maintained in the model. All statistical analyses were carried out using InfoStat software (Universidad Nacional de Córdoba, Argentina).

3. Results

3.1. Descriptive data

Surveys indicated that the Argentine apicultural system is represented by small beekeepers (fewer than 200 colonies per farmer, distributed in several apiaries).

Table 2
Definition and distribution of explanatory variables selected ($P < 0.15$) by univariable analysis, for potential association with high prevalence (>3%) of *V. destructor*.

Definition of variables	Level	No. of hives (%)	% of high prevalence hives	*P*-value
Sampling time	February and the first fortnight of March	228 (59.3)	45.6	<0.0001
	Second fortnight of March and first fortnight of April	114 (29.7)	62.3	
	Second fortnight of April and first fortnight of May	42 (11.0)	81.0	
Protein diet	Natural pollen	24 (6.3)	25.0	0.003
	No or others	354 (93.7)	55.9	
Carbohydrate supply	No-sucrose	318 (84.1)	55.7	0.013
	HFCS	24 (6.4)	25.0	
	Both	36 (9.5)	58.3	
Geographical zone	North	60 (15.6)	65.0	0.007
	Central	204 (53.2)	47.1	
	South	78 (20.3)	56.4	
	Riverside	42 (10.9)	71.4	
Frequency of queen replacement[a]	No	129 (34.1)	58.1	0.121
	Every year	60 (15.9)	60.0	
	Every 2 years	147 (38.9)	46.3	
	More than 2	42 (11.1)	59.5	
% of queen replacement	No or ≤50%	349 (92.3)	55.9	0.010
	>50%	29 (7.7)	31.0	
Hives wooden ware disinfection	No	126 (33.3)	67.5	<0.0001
	Yes	252 (66.7)	47.2	
Monitoring before treatment	No	48 (12.7)	68.8	0.028
	Yes	330 (87.3)	51.8	
Monitoring after treatment	No	84 (22.3)	71.4	<0.0001
	Yes	294 (77.7)	49.0	
Commercial acaricide in 2012	No	18 (5)	72.2	0.099
	Yes	348 (95)	52.3	
Commercial acaricide in 2013	No	30 (8.5)	70.0	0.061
	Yes	324 (91.5)	52.2	

HFCS: high fructose corn syrup.
[a] Excluded of the logistic model because is associated to the variable percentage of queen replacement.

Surveys also indicated that beekeeping is usually a complementary economic activity (semi-professional), given that most farmers have other incomes from other activities. In addition, surveys showed that the mean size of each apiary was 43 ± 18 colonies (mean ± S.D.) and that most beekeepers have been in the activity for more than 10 years (12 ± 8 years). During the last 3 years, average mortality was 15.67 ± 8.50% colonies per year and average honey production was 30.43 ± 11.01 kilograms per colony/year. A total of 209 of the 384 colonies (54.4%) showed an infestation of *V. destructor* higher than 3%. The mean infestation in the colonies prior to treatment was 5.7 ± 6.3%. The mean colony size was 8.7 ± 1.38 combs completely covered with adult bees. We found no significant correlation between colony size and percentage of infestation ($n = 375$; $r = 0.037$; $P = 0.47$) when capped brood ($n = 375$; $r = -0.088$; $P = 0.09$) and pollen ($n = 345$; $r = 0.079$; $P = 0.14$) comb area were analyzed. Although we found a significant correlation between infestation intensity and honey comb area ($n = 345$; $r = 0.188$; $P < 0.0001$), the correlation coefficient was low and the *P* value was influenced by the sample size. Since the sampling time was significantly associated with varroa levels (Pearson X^2, $P < 0.0001$), it was included in the mixed-effects logistic regression model. Three levels were defined for the sampling time variable: February and the first fortnight of March; the second fortnight of March and the first fortnight of April and the second fortnight of April and the first fortnight of May (Table 2).

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. Protein feeding based on natural pollen significantly increased colony size (9.25 ± 0.99 combs covered with adult bees; $P = 0.036$) compared to colonies that were fed with other protein diet or not fed at all (8.66 ± 1.40 combs covered with adult bees).

3.2. Multivariable analyses

Ten out of the 37 potential explanatory variables tested were selected after the univariable analysis (selected variables had a significance value $P < 0.15$; Table 2) to be

Table 3
Mixed-effects logistic regression model for apiary factors associated with *V. destructor* high prevalence (>3%) in hives at the end of the honey harvest season and prior to acaricide treatment (*n* = 384; Santa Fe province, 2013).

Variable	Level	Odds ratio	95% CI (O.R.)	*P*-value
Protein diet	No or others (Ref.)	–	–	–
	Natural pollen	0.348	0.129–0.941	0.037
Carbohydrate supply	No-sucrose (Ref.)	–	–	–
	HFCS	0.108	0.032–0.364	<0.0001
	Both	0.971	0.379–2.568	0.952
% of queen replacement	>50% (Ref.)	–	–	–
	No or ≤50%	3.280	1.208–10.158	0.027
Wooden ware disinfection	Yes (Ref.)	–	–	–
	No	2.722	1.380–5.565	0.005
Monitoring after treatment	Yes (Ref.)	–	–	–
	No	2.305	0.944–5.629	0.067

Intercept = 0.203 (*P* = 0.147); model LR: 71.825; *P* < 0.0001; CI: confidence interval; HFCS: high fructose corn syrup.

included in the mixed-effects logistic regression model. The frequency (*P* = 0.121) and percentage (*P* = 0.027) of queen replacement were associated (Pearson X^2, *P* < 0.0001). Therefore, only the percentage of queen replacement (which refers to the proportion of colonies within each apiary in which the queen is replaced by a new one during one season) was offered to the model. Complete survey data for the potential risk factors included in the model were available for 366 of the 384 colonies sampled (95.3%). The final multivariate model revealed five variables associated with the prevalence of *V. destructor* (Table 3). The apiary random-effect was not significant (*P* = 0.715). The probability of colonies with >3% of varroa infestation decreased when colonies were fed with natural pollen (OR = 0.348; 95% CI: 0.129–0.941; *P* = 0.037), as well as when percentage of queen replacement was more than 50% of total colonies (OR = 0.305; 95% CI: 0.107–0.872; *P* = 0.027).

Colonies owned by beekeepers who indicated that wooden ware was not disinfected after use (question 8, supplemental material) showed a higher prevalence of colonies with infestation >3% (OR = 2.722; 95% CI: 1.380–5.565; *P* ≤ 0.005). Additionally, we found that beekeepers who indicated that they regularly monitored mite levels after varroa treatments (question 12, supplemental material) had lower mite infestation than those who indicated that they did not monitor the colonies after acaricide treatment (OR = 2.305; 95% CI: 0.944–5.629; *P* = 0.067). On the other hand, the prevalence of colonies with infestation >3% decreased when high fructose corn syrup (HFCS) was exclusively supplied to the colonies (OR = 0.108; 95% CI: 0.032–0.364; *P* ≤ 0.0001) (Table 3).

The colonies located in the four geographical zones studied (North = 60; Central = 204; South = 78 and Riverside = 42) were proportionally distributed along with colony abundance. The North (OR = 1.263; 95% CI: 0.354–4.508; *P* = 0.719), Central (OR = 1.450; 95% CI: 0.431–4.881; *P* = 0.549) and South zones (OR = 0.530; 95% CI: 0.152–1.853; *P* = 0.320) were not different from the Riverside zone.

4. Discussion

Honey bee colonies and honey production are threatened at global scale (Genersch, 2010; Higes et al., 2010; Le Conte et al., 2010; vanEngelsdorp et al., 2008, 2009). Identifying and preventing risk factors associated with beekeeping management may help avoid exacerbating this problem. The results of the present study show how certain management practices are correlated with varroa mite prevalence. This is so because most beekeeping management practices are widely common. Nevertheless, given that regional differences may be important for varroa infestation, this sort of monitoring data should be regionally obtained.

The average percentage of infestation on Santa Fe colonies at the end of the harvest season was lower than the critical thresholds for winter survival previously proposed (Liebig, 2001; Fries et al., 2003; Currie and Gatien, 2006; Genersch et al., 2010; Rosenkranz et al., 2010; Bulacio Cagnolo, 2011) given that the impact of *V. destructor* on honey bee colonies varies with climate conditions and in different regions (Currie and Gatien, 2006).

The number of colonies sampled per apiary (6 or 10% of total colonies) was below that recommended for research purposes (Lee et al., 2010) and therefore was a weak point of this study. However, considering the whole objective of this study, we considered that this sample size was adequate.

Five risk factors were found to be associated with a high prevalence (>3%) of *V. destructor*. Firstly, when the protein diet was based on natural pollen supplement, prevalence of colonies with more than 3% of infestation decreased. Natural pollen is more suitable for honey bee colonies because it is consumed more readily than other substitutes (De Grandi Hoffman et al., 2008; Brodscheneider and Crailsheim, 2010). Although colonies supplemented during autumn do not perform better the following spring (Mattila and Otis, 2006), in commercial beekeeping it is thought that pollen availability to overwintering colonies might influence the size and growth of the colony during the following season, and thus extra protein supply during autumn is common. On the other hand, at the beginning of spring, supplementary feeding is a current practice to enhance colony growth when pollen flow is low (Keller et al., 2005; Mattila and Otis, 2006). In any case, the use of protein supplement is a common practice in apicultural management and, as suggested by our results, when natural pollen is used, the prevalence of colonies with high infestation is lower. This is probably because, as reported here, feeding with natural pollen increases colony size and dilutes mite population since this variable is measured as number of mites per bee. Appropriate amount and quality of pollen are helpful against diverse pathogens (Rinderer and Rothenbuhler, 1974; Rinderer and Elliott, 1977; De Grandi Hoffman et al., 2010). Moreover, lower protein storage capacity in *V. destructor*-infested bees may explain the severe impact of the mite on honey bees in temperate zones (Amdam et al., 2004). It is plausible that pollen-supplemented colonies, which are better nourished and larger, may improve their response to any negative impact because susceptibility to disease depends to some extent on nutrition (Field et al., 2002).

A second variable associated with lower prevalence of colonies with infestation >3% was the use of high fructose corn syrup (HFCS) as a carbohydrate supply. Almost all colonies (96.8%) sampled in this study were supplied with some carbohydrate supplement. To provide carbohydrate supplements after honey yield or during periods of death is a common practice (Brodscheneider and Crailsheim, 2010). However, the use of alternative syrups to incorporate carbohydrates might have different effects (Neupane and Thapa, 2005). A higher infestation intensity of *V. destructor* was found when sucrose solution was added to colonies. This can be explained by the fact that bees provided with sucrose syrup produce higher numbers of brood cells (Neupane and Thapa, 2005) and therefore provide a good opportunity for *V. destructor* to reproduce. Also, providing HFCS as carbohydrate supply might decrease the "robbing" behavior among the colonies (Barker and Lehner, 1978) and consequently decrease pathogen horizontal transmission (Fries and Camazine, 2001). Although colonies supplemented with HFCS during autumn have less sealed brood in spring than those supplemented with sucrose, the supplement seems not to affect honey production at the end of the harvest season in temperate climates (Severson and Erickson, 1984). Moreover, if high levels of Hydroxymethylfurfural are avoided (Le Blanc et al., 2009; Ruiz-Matute et al., 2010), HFCS offers some extra advantages such as reduced cost and feeding convenience (Standifer et al., 1977; Somerville, 2000). Since colonies in beekeeping are probably under frequent stress (which affects honey productivity), it is important for beekeepers to consider that nutritional stresses accumulate in commercially managed colonies (Mattila and Otis, 2006).

Another important management practice to highlight is queen replacement. Although queen replacement has not been studied as a potential risk factor associated with *V. destructor*, several studies regarding this issue have been published (Tarpy et al., 2000; Invernizzi et al., 2006; Schneider and De Grandi-Hoffman, 2008; Botías et al., 2012). Requeening is recommended to avoid swarming and to improve hygienic behavior and honey productivity. Similarly to that reported in Uruguay (Invernizzi et al., 2006), in Argentina, it is thought that a queen can maintain a strong colony at least for two seasons. Botías et al. (2012) found that colonies whose queen has been replaced show decreased rates of *Nosema* infections. Our results suggest that higher percentages of queen replacement also help to maintain lower mite infestations since colonies headed by young queens had lower intensity of varroa infestation than those headed by old queens (Akyol et al., 2007). Therefore, the performance of colonies could be improved by replacing the queen periodically in a higher percentage of colonies inside each apiary.

Monitoring and keeping the wooden ware of hives in good conditions is recommended among best management practices (Heintz et al., 2011). Article number 4.14.4 of the Organization for permanent official sanitary surveillance of apiaries in the Terrestrial Animal Health Code (OIE, 2012) establishes that applying hygiene measures, in particular, treating bee colonies and disinfecting the equipment are obligatory tasks to ensure rapid eradication of any outbreak of a disease. While these practices have no biological effect on varroa mite infestation as diets or queen replacement, beekeepers who treat their wooden ware or frequently examine the colonies seem to be more rigorous in their bee management practices. Our results show that beekeepers who indicated that they monitored mite levels after varroa treatments had lower mite levels than those who indicated they did not check for acaricide efficacy. It has been reported that rechecking for treatment efficacy may help to detect acaricide failure or risk of reinvasion problems (Renz and Rosenkranz, 2001). This variable was linked to other suggested practices, such as monitoring previous to treatment (X^2, $P < 0.0001$) and to the use of commercial acaricides during 2012 and 2013 (X^2, $P < 0.0001$ and $P = 0.009$, respectively). This is likely because, generally, beekeepers that adhere to a management program implement several suggested practices that, combined, are expected to improve the productivity of apiaries.

Environmental factors may act indirectly via the host on the parasite status (Rosenkranz et al., 2010). The influence of the geographical zone might indicate an indirect effect of climate and other environmental conditions (e.g. availability of forager crops, use of pesticides, etc.). Likewise, the influence of climate on the growth rate of mites may extend beyond direct effects on mite fertility (Harris et al., 2003). The weather close to the Paraná River (Riverside zone) has "tropical" features (e.g. higher relative humidity). Thus, differences in climate conditions may imply longer pollen flow and consequently longer periods of brood rearing (Mattila and Otis, 2006). The opposite climate effect has been reported in more temperate climates, where apparent adult infestation increases when brood decreases during autumn and winter (Moretto et al., 2001). However, in this study, the more infested colonies did not show significant reduction in the brood area during varroa mite monitoring ($r = 0.037$; $P = 0.476$). Moreover, given that the nectar flow lasts at least until middle autumn (late April), it is plausible that apiaries located close to the Paraná River began their treatments with considerable higher *V. destructor* infestation levels. In addition, we observed that the zone effect was associated with the sampling time period (Pearson X^2, $P < 0.0001$), probably because the sampling time criterion (end of honey harvest season and treatment time frame) varied according to the different zones. However, in this study the prevalence of colonies with infestation ≥3% was not associated with the geographical zone, and it is possible that its influence on the *V. destructor* infestation might be more related with beekeeping management practices conducted in each zone or a high density of apiaries than the environmental factors. For example, Central zone had the largest apiaries (49.22 ± 19.49 colonies per apiary) and they used more frequently natural pollen supplements ($P \le 0.001$), high fructose corn syrup ($P \le 0.001$), and monitored mite levels after varroa treatment than the apiaries located in the other regions ($P \le 0.001$). Additionally, apiaries located in North and Central region replaced the queen periodically in a higher percentage of colonies compared with the other regions ($P = 0.003$). Finally, apiaries located in Central region and in the Riverside zone treated frequently the wooden ware ($P \le 0.001$).

It is possible that Odds ratio calculation overestimates risk magnitude associated to an exposure factor, especially when the disease prevalence is high, as it is the case of Varroa infestation. This condition should be considered when results are analyzed.

5. Conclusions

The prevalence of colonies with *V. destructor* infestation higher than 3% on adult bees seems to be associated with multiple factors, illustrating the complexity of *V. destructor* epidemiology. Feeding natural pollen, using HFCS as a carbohydrate supplement, requeening colonies frequently, monitoring mite levels after treatment and disinfecting the wooden ware of hives are management practices that allow keeping lower *V. destructor* infestations. Thus, regardless of their restrictions, field studies are important because they allow gathering information about colonies under natural conditions and managed by beekeepers. Additional spatial and temporal studies should be conducted to elucidate the complex set of variables that impact on honey bee colonies, especially on *V. destructor* transmission.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2014.04.002.

References

Akyol, E., Yeninar, H., Karatepe, M., karatepe, B., Ozkok, D., 2007. Effects of queen ages on Varroa (*Varroa destructor*) infestation level in honey bee (*Apis mellifera caucasica*) colonies and colony performance. Ital. J. Anim. Sci. 6, 143–149.

Amdam, G.V., Hartfelder, K., Norberg, K., Hagen, A., Omholt, S.W., 2004. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering? J. Econ. Entomol. 97 (3), 741–747.

Arzamendia, V., Giraudo, A.R., 2004. Usando patrones de biodiversidad para la evaluación y diseño de áreas protegidas: las serpientes de la Provincia de Santa Fe (Argentina) como ejemplo. Rev. Chil. Hist. Nat. 77, 335–348.

Barker, R.J., Lehner, Y., 1978. Laboratory comparison of high fructose Corn syrup, grape syrup, honey, and sucrose syrup as maintenance food for caged honey bees. Apidologie 9 (2), 111–116.

Botías, C., Martín-Hernández, R., Días, J., García-Palencia, P., Matabuena, M., Juarranz, A., Barrios, L., Meana, A., Nanetti, A., Higes, M., 2012. The effect of induced queen replacement on *Nosema* spp. infection in honey bee (Apis mellifera iberiensis) colonies. Environ. Microbiol. 14 (4), 845–859.

Brodschneider, R., Crailsheim, K., 2010. Nutrition and health in honey bees. Apidologie 41, 278–294.

Bulacio Cagnolo, (PhD thesis) 2011. Manejo Integrado de *Varroa destructor* (Acari: Varroidae) en colonias de *Apis mellifera* (Hymenoptera: Apidae) en el centro oeste de la provincia de Santa Fe. Facultad de Ciencias Naturales, Universidad Nacional de Mar del Plata, Argentina.

Burkart, R., Bárbaro, N.O., Sánchez, R.O., Gómez, D.A., 1999. Ecoregiones de la Argentina. Administración de Parques Nacionales, Buenos Aires, http://www.sib.gov.ar/archivos/Eco-Regiones_de_la_Argentina.pdf (accessed 10.12.12).

Chen, Y.P., Siede, R., 2007. Honey bee viruses. Adv. Virus Res. 70, http://dx.doi.org/10.1016/S0065-3527(07)70002-7.

Currie, R.W., Gatien, P., 2006. Timing acaricide treatments to prevent *Varroa destructor* (Acari: Varroidae) from causing economic damage to honey bee colonies. Can. Entomol. 138, 238–252.

De Grandi Hoffman, G., Chen, Y., Huang, E., Huang, M.H., 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). J. Insect. Physiol. 56, 1184–1191.

De Grandi Hoffman, G., Wardell, G., Ahumada Segura, F., Rinderer, T., Danka, R., Pettis, J., 2008. Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. J. Apic. Res. 47 (4), 265–270.

Department of Agriculture from Santa Fe province, 2008. Cadena apícola santafecina, http://www.santafe.gov.ar/index.php/web/content/download/66066/320676/file/descargar.pdf (in Spanish; accessed 18.03.13).

Dietemann, V., Nazzi, F., Martin, S.J., Anderson, D.L., Locke, B., Delaplane, K.S., Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P., Ellis, J.D., 2013. Standard methods for varroa research. In: Dietemann, V., Ellis, J.D., Neumann, P. (Eds.), The COLOSS BEEBOOK. Volume II: Standard Methods for *Apis mellifera* Pest and Pathogen Research. J. Apic. Res. 52 (1), http://dx.doi.org/10.3896/IBRA.1.52.1.09.

Duay, P., De Jong, D., Engels, W., 2003. Weight loss in drone pupae (*Apis mellifera*) multiply infested by *Varroa destructor* mites. Apidologie 34, 61–65.

Field, C.J., Johnson, I.R., Schley, P.D., 2002. Nutrients and their role in host resistance to infection. J. Leukoc. Biol. 71, 16–32.

Fries, I., Camazine, S., 2001. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie 32, 199–214.

Fries, I., Hansen, H., Imdorf, A., Rosenkranz, P., 2003. Swarming in honey bees (*Apis mellifera*) and *Varroa destructor* population development in Sweden. Apidologie 34, 389–397.

Garedew, A., Schmolz, E., Lamprecht, I., 2004. The energy and nutritional demand of the parasitic life of the mite *Varroa destructor*. Apidologie 35, 419–430.

Genersch, E., 2010. Honey bee pathology: current threats to honey bees and beekeeping. Appl. Microbiol. Biotechnol. 87, 87–97.

Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., et al., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41, 332–352.

Giorgi, R., Tosolini, R., Sapino, V., Villar, J., León, C., Chiavassa, A., 2008. Zonificación Agroeconómica de la provincia de Santa Fe, vol. 110. INTA, Argentina, pp. 215–224, ISSN 0325-9137.

Guzmán-Novoa, E., Eccles, L., Calvete, Y., Mcgowan, J., Kelly, P.G., Correa Benitez, A., 2010. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. Apidologie 41, 443–450.

Harris, J.W., Harbo, J.R., Villa, J.D., Danka, R.G., 2003. Variable population growth of *Varroa destructor* (Mesostigmata: Varroidae) in colonies of honey bees (Hymenoptera: Apidae) during a 10-year period. Environ. Entomol. 32 (6), 1305–1312.

Heintz, C., Ribotto, M., Ellis, M., Delaplane, K., 2011. Best management practices (BMPs) for beekeepers pollinating California's agricultural crops, Managed Pollinator CAP Coordinated Agriculture Project. Bee Culture 2011, 17–19.

Higes, M., Martín Hernández, R., Martínez Salvador, A., Garrido Ballón, E., González Porto, A.V., Meana, A., Bernal, J.L., del Nozal, M.J., Bernal, J., 2010. A preliminary study of the epidemiological factors related to honey bee colony loss in Spain. Environ. Microbiol. Rep. 2 (2), 243–250, http://dx.doi.org/10.1111/j.1758-2229.2009.00099.x.

Invernizzi, C., Harriet, J., Carvalho, S., 2006. Evaluation of different queen introduction methods in honeybee colonies in Uruguay. APIACTA 41, 1–20.

Keller, I., Fluri, P., Imdorf, A., 2005. Pollen nutrition and colony development in honey bees. Part II. Bee World 86, 27–34.

Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S.G., Roulston, T., Steffan-Dewenter, I., Vázquez, D.P., Winfree, R., Adams, L., Crone, E.E., Greenleaf, S.S., Timothy, H., Keitt, T.H., Klein, A.M., Regetz, J., Rickett, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecol. Lett. 10, 299–314.

Le Blanc, B.W., Eggleston, G., Sammataro, D., Cornett, C., Dufault, R., Deeby, T., Cyr, E.St., 2009. Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee (*Apis mellifera*). J. Agric. Food Chem. 57, 7369–7376.

Le Conte, Y., Ellis, M., Ritter, W., 2010. Varroa mites and honey bee health: can Varroa explain part of the colony losses? Apidologie 41, 353–363.

Lee, K.V., Moon, R.D., Burkness, E.C., Hutchison, W.D., Spivak, M., 2010. Practical sampling plans for *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies and apiaries. J. Econ. Entomol. 103 (4), 1039–1050.

Liebig, G., 2001. How many varroa mites can be tolerated by a honey bee colony? Apidologie 32, 482–484.

Marcangeli, J., Monetti, L., Fernández, N., 1992. Malformations produced by *Varroa jacobsoni* on *Apis mellifera* in the province of Buenos Aires, Argentina. Apidologie 23, 399–402.

Mattila, H.R., Otis, G.W., 2006. Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. J. Econ. Entomol. 99 (3), 604–613.

Moher, D., Hopewell, S., Schulz, K., Montori, V., Gøtzsche, P., Devereaux, P., Elbourne, D., Egger, M., Altman, D., 2010. ConSoRT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 340, c869, http://dx.doi.org/10.1136/bmj.c869.

Moretto, G., Gonçalves, L.S., De Jong, D., Bichuette, M.Z., 2001. The effects of climate and bee race on *Varroa jacobsoni* Oud infestations in Brazil. Apidologie 22, 197–203.

Murray, T.E., Kuhlmann, M., Potts, S.G., 2009. Conservation ecology of bees: populations, species and communities. Apidologie 40, 211–236.

Neumann, P., Carreck, N.L., 2010. Honey bee colony losses. J. Apic. Res. 49, 1–6.

Neupane, K.R., Thapa, R.B., 2005. Alternative to off-season sugar supplement feeding of honeybees. J. Inst. Agric. Anim. Sci. 26, 77–81.

OIE, 2012. Official health control of bee diseases. In: Terrestrial Animal Health Code. Vol. 1: General Provisions. 2012© OIE, http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/en_chapitre_1.4.14.htm (Chapter 4.14; accessed 15.07.13).

Oldroyd, B.P., 1999. Co-evolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. TREE 14 (8), 312–315.

Renz, M., Rosenkranz, P., 2001. Infestation dynamics and reinvasion of *Varroa destructor* mites in honey bee colonies kept isolated and in groups. Apidologie 32, 492–494.

Rinderer, T.E., Rothenbuhler, W.C., 1974. The influence of pollen on the susceptibility of Honey-bee larvae to *Bacillus larvae*. J. Inver. Pathol. 23, 347–350.

Rinderer, T.E., Elliott, K.D., 1977. Worker honey bee response to infection with *Nosema apis*. J. Econ. Entomol. 70, 431–433.

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. J. Inver. Pathol. 103, 96–119.

Ruiz-Matute, A.I., Weiss, M., Sammataro, D., Finely, J., Sanz, M.L., 2010. Carbohydrate composition of high-fructose corn syrups (HFCS) used for bee feeding: effect on honey composition. J. Agric. Food Chem. 58, 7317–7322.

Secretaria de agricultura, ganadería, pesca y alimentos (SAGPYA), 2009. Introducción al Sector apícola Argentino. Agriculture Department, Argentinean Government (in Spanish).

Schneider, S.S., De Grandi-Hoffman, G., 2008. Queen replacement in African and European honey bee colonies with and without afterswarms. Insect. Soc. 55, 79–85.

SENASA (Animal Health National Service), 2007. Situación actual de Varroosis, http://www.senasa.gov.ar/Archivos/File/File3824-varroosis-aituacion-actual-argentina.pdf (accessed 18.03.13).

Severson, D.W., Erickson, E.H., 1984. Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates. J. Econ. Entomol. 77, 1473–1478.

Somerville, D., 2000. Honey bee nutrition and supplementary feeding. Agnote DAI/178. NSW Agriculture, pp. 1034–6848.

Standifer, L.N., Moeller, F.E., Kauffeld, N.M., Herbert Jr., E.W., Shimanuki, H., 1977. Supplemental Feeding of Honey Bee Colonies; USDA Information Bulletin 413. U.S. GPO, Washington, DC, 8 pp., illus.

Tarpy, D.R., Hatch, S., Fletcher, D.J.C., 2000. The influence of queen age and quality during queen replacement in honeybee colonies. Anim. Behav. 59, 97–101.

vanEngelsdorp, D., Maixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Inver. Pathol. 103, 80–95.

vanEngelsdorp, D., Hayes Jr., J., Underwood, R.M., Pettis, J., 2008. A Survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. PLoS ONE 3 (12), e4071, http://dx.doi.org/10.1371/journal.pone.0004071.

vanEngelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., et al., 2009. Colony collapse disorder: a descriptive study. PLoS ONE 4 (8), e6481, http://dx.doi.org/10.1371/journal.pone.0006481.

vanEngelsdorp, D., Lengerich, E., Spleen, A., Dainat, B., Cresswell, J., Nguyen, B.K., Soroker, V., Underwood, R., Human, H., Yves Le Conte, Y., Saegerman, C., 2013. Standard epidemiological methods to understand and improve *Apis mellifera* health. In: Dietemann, V., Ellis, J.D., Neumann, P. (Eds.), The COLOSS BEEBOOK. Volume II: Standard Methods for *Apis mellifera* Pest and Pathogen Research. J. Apic. Res. 52 (1), http://dx.doi.org/10.3896/IBRA.1.52.1.08.