



The Westernmost Mediterranean islands present an endemic varroosis disease pattern

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Abstract

Aim of study: This is the first intensive study of the incidence of *Varroa destructor* (Acari: Varroidae) in the Westernmost Mediterranean islands (Balearics).

Area of study: Mallorca, Menorca, Eivissa and Formentera (Balearic Islands), Spain.

Material and methods: The study was carried out in autumn 2010 and in spring 2011 because the presence of the mite is higher in these seasons. A total of 462 honeybee colonies were analyzed from different apiaries on. One sample of adult bees (worker bees, $n > 200$) and another of bee brood (at all stages of development, $n > 400$) were collected from each colony. All were frozen until analysis. To detect *Varroa* each sample was examined individually basing the study on the guide edited by the World Organisation for Animal Health, with some modifications. The beekeepers involved were interviewed to characterize Balearic beekeeping.

Main results: Regarding the prevalence, results showed that in the Balearic Islands the percentage was higher in spring (34.78%) than in autumn (44.09%) but the infestation rate was lower in both seasons (Autumn: 0.94%, Spring: 0.77%). The focality percentage was also higher in spring (87.10%) than in autumn (82.61%). The data suggests that *Varroa* mites in the archipelago have an endemic rather than epidemic disease distribution pattern.

Research highlights: These results open an interesting scenario to develop a future resistance breeding strategy against *V. destructor* in these small islands.

Additional key words: Balearic Islands; honeybee; anti-varroa treatment; beekeeping; parasitic indexes; field sampling; epidemiology.

Abbreviations used: H (The Kruskal-Wallis H test), %I (infestation rate), %MI (maximum infestation rate), %P (prevalence rate), %F (focality percentage).

Authors' contributions: Conceived and designed the experiments, performed the experiments and analyzed the data: ML. Technical support: MC. Both authors wrote the paper and approved the final manuscript.

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Introduction

In past decades, honey bees (*Apis mellifera*) and their state of health has become an outstanding topic for scientists, beekeepers and society in general, due to the environmental and economic impact caused by the decline of colonies (Potts *et al.*, 2010). Their health status is influenced by nutrition, pesticides and the presence of parasites and pathogens. Currently, one of the key factors worldwide in the loss of colonies is the mite *V. destructor* (Acari: Varroidae); Genersch *et al.*, 2010; Le Conte *et al.*, 2010; Schafer *et al.*, 2010; vanEngelsdorp & Meixner, 2010; Nazzi *et al.*, 2012; McMenamin & Genersch, 2015; Thoms *et al.*, 2019). *Varroa destructor* mite is a viral vector that feeds primarily on the bee fat

body tissue (Ramsey *et al.*, 2019). Deformed wing virus (DWV) is one of the major viruses which *Varroa* may transmit (Gisder *et al.*, 2009). In a research carried out by Jacques *et al.* (2017), apiaries from professional beekeepers showed no signs of diseases, however colonies from amateur beekeepers did show signs of bacterial infection and *V. destructor* infestation, so demonstrating that the adequate formation of beekeepers has an important role on the survival of colonies.

In Spain, *Varroa* was first detected on the border with France in December 1985 (Llorente, 2003). Since then, it has spread over the Iberian Peninsula, Balearic Islands and Canary Islands. The reasons for the expansion of the mite on Balearic Islands are little known. A study carried out by Muñoz *et al.* (2008) confirmed that the Korean

haplotype (K) is that which affects *Apis mellifera iberiensis*, both in the Iberian Peninsula and the two archipelagos.

Here we present the first intensive study of the incidence of *Varroa destructor* (Acari: Varroidae) in the Westernmost Mediterranean islands (Balearics) which is composed of 194 islands and islets divided into two groups (Gymnesian and Pityusic Islands) with different geographic histories. Only the four largest islands (Mallorca, Menorca, Eivissa and Formentera) are inhabited and have honeybee colonies. The specific aims of this research were to: (a) characterize beekeeping on the Balearic Islands through a survey involving beekeepers for the first time and facilitate the improvement of the sector; (b) determine the prevalence and infestation levels of *V. destructor* in the colonies of the Balearics; (c) determine whether it was an endemic disease or an epidemic disease based on the distribution pattern of the parasitized apiaries.

Material and methods

Field sampling and sample processing

A cross-sectional design was executed in two different seasons (autumn 2010 and spring 2011) when the presence of the *Varroa* mite is higher.

The first year (2010) 46 apiaries were selected: 36 in Mallorca, 6 in Eivissa and 4 in Formentera. In the second year (2011), 31 apiaries were selected: 21 in Mallorca and 10 in Menorca (Fig. 1).

At each apiary, 6 bee colonies were chosen randomly making a total of 462 bee colonies (276 in autumn 2010 and 186 in spring 2011). Two samples from each colony were collected: one of adult bees (worker bees, $n > 200$)

and one of bee brood (a section of brood of approx. 20×10 cm was sampled, at all stages of development, $n > 400$). Thus, a total of 924 samples were taken (552 in autumn 2010 and 372 in spring 2011). All samples were kept frozen until analysis (Cepero, 2016).

The analysis of the adult worker bees was based on the guide edited by the World Organisation for Animal Health (OIE, 2012) with some modifications, each being examined individually: first weighed on a precision scale, then a visual examination was carried out to detect any symptoms of disease. The mites were easily detached from the frozen bees and counted against a white background (Garrido, 2013).

In the case of the bee brood analysis, the method described by OIE (2012) was followed with some modifications. In each case, the brood cell capping was removed with a scalpel, larvae were extracted with tweezers and the presence/absence of *Varroa* was determined.

A survey among the beekeepers involved was carried out to characterize Balearic beekeeping. The following information was required: the number and type of the hives from each apiary; the total number of colonies per beekeeper; number, product, dose, application time and dates of administration of the anti-varroa treatment.

Data and statistical analysis

The following indexes were calculated: prevalence rate (%P) = (number of parasitized colonies / total number of sampled colonies) * 100; infestation rate (%I) = (number of mites / bee) * 100; focality index (%F) = (number of parasitized apiaries / total number of sampled apiaries) * 100.

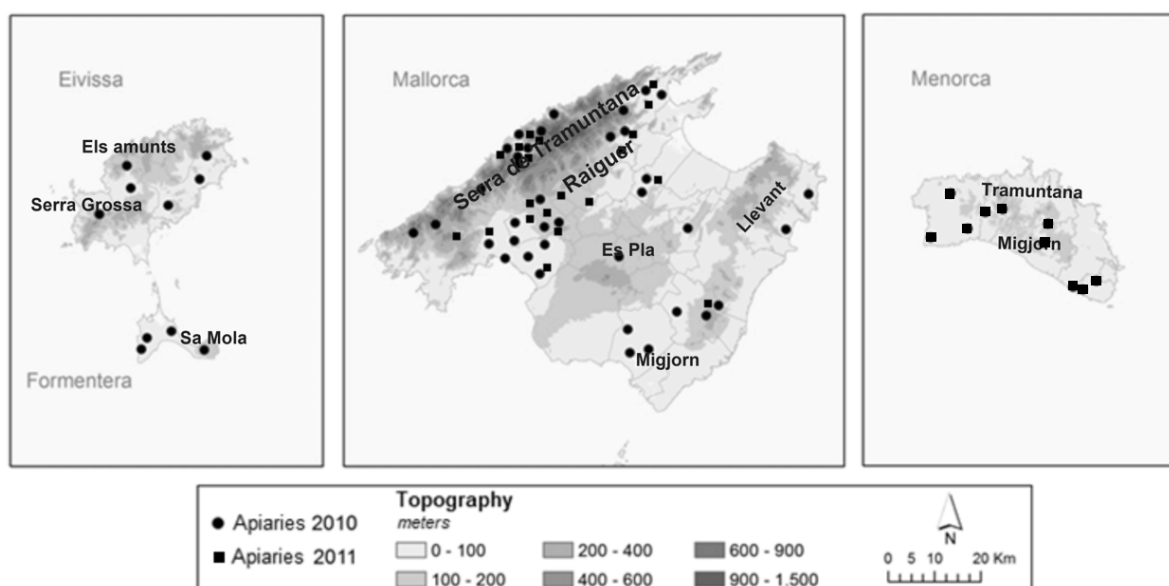


Figure 1. Location of the studied apiaries in autumn 2010 and in spring 2011.

The number of bees was calculated on weight, considering 1 bee = 100 mg (Cepero, 2016). Means and standard deviation were computed for variable. Data were analyzed by Kruskal-Wallis H test analysis of variance (ANOVA) in SPSS v. 20.0 (SPSS Inc., Chicago, IL, USA). A level of $p < 0.05$ was accepted as significant.

The degree of infestation in the colonies were calculated based on the classification developed by Calatayud & Verdu (1995), used by MAGRAMA (2014). The statistical distribution of the number of infested colonies by apiary was compared to a Poisson distribution to determine whether the presence of the mite in an apiary occurs independently and uniformly among the colonies or whether it is influenced by the condition of the other colonies in the apiary as a result of their proximity.

Results

Survey on the characterization of beekeeping in the Balearics

All but 5 owners from each apiary analyzed answered the survey, therefore the detailed information below is extracted from 75 beekeepers in Balearics who, in some cases, did not answer all the questions.

The average size of the apiaries in the Balearic Islands was 22.1 colonies (mean of 22.78, 41, 31 and 9.5 colonies per apiary in Mallorca, Menorca, Eivissa and Formentera, respectively). In accordance to the professional status in the sector and the classification indicated by Spanish legislation (BOE, 2002), most of the beekeepers in the survey had less than 50 colonies (amateurs), 32% had between 51 and 150 colonies (semi-professionals) and 9% were professional (over 150 colonies). In Menorca most of them were professional (62.5%) followed by beekeepers in Eivissa where the proportion was smaller (40%) and those in Mallorca represented 2% of the total.

Regarding to the type of hives, 95.9% of the apiaries were mostly formed by Langstroth and Dadant vertical hives. Only 4% of the apiaries were formed exclusively by Layens hives.

Results of the survey showed that 10% of the apiaries were not treated against mites (6 beekeepers) whereas 57% of apiaries were treated once (33 beekeepers) and 33% twice a year (19 beekeepers). Mallorca is the only island where some beekeepers carried out no treatment. Regarding the first annual treatment, the veterinarian products most frequently used were Apivar (amitraz) 41% of the cases, Apistan (fluvalinate) 25% and Apiguard (thymol) 20%. With reference to the second annual treatment, the products were the same, but in different proportions. The administration of Apiguard in the second treatment increased (38%) in comparison to the first whereas the treatments with Apivar and Apistan decreased (31% and 23% respectively).

The manufacturers of anti-varroa products establish their dosage and duration. Apivar and Apistan recommend using two strips of the product for 6 weeks, whereas Apiguard recommends two doses of the product for 4 weeks or until the evaporation is complete. More than three quarters (76%) of the treatments were properly administered. Apiguard was correctly applied in 95% of the cases compared to Apistan (70%) and Apivar (67.7%).

The comparative analysis between the islands reveals that Formentera had the larger percentage of correct administrations (83.3%), followed by Mallorca (78.8%), Eivissa (60%) and Menorca (57.1%).

No fixed month or season was determined to apply the treatments. This fact increases the possibilities of an inefficient control and/or a development of resistance. This study reveals that 77.8% of the anti-varroa treatments applied fail to comply with Annex II of BOE (2006) whereby treatment for varroosis in September-November is established.

Only three beekeepers in the survey indicated that they had carried out transhumance between municipalities in Mallorca.

Prevalence, infestation and focality levels

The prevalence and infestation rates are shown in Table 1. The mean prevalence rate was higher in spring than in autumn whilst infestation rate was lower. There was no statistically significant difference between the periods of study ($H = 1.453, p = 0.228$).

In a breakdown of the results by island (Table 1), the values of each calculated parameter were higher in Mallorca not only in 2010 but also in 2011 followed by Menorca (where the prevalence in spring was similar to Mallorca in autumn). On the contrary, the lowest values were registered in Formentera. The prevalence of the mite was higher in the Gymnesians (Mallorca and Menorca),

Table 1. Means and standard error of the prevalence (%P) and the infestation (%I) rates in the samples of adult bees in autumn 2010, spring 2011 and for the total samples, and by island. n= number of sampled colonies.

	%P	%I
Autumn 2010 (n= 276)	34.78 ± 4.31	0.94 ± 0.21
Mallorca '10 (n= 216)	39.72 ± 4.91	1.13 ± 0.26
Eivissa '10 (n= 36)	19.44 ± 7.95	0.24 ± 0.12
Formentera '10 (n= 24)	13.00 ± 12.50	0.24 ± 0.24
Spring 2011 (n= 186)	44.09 ± 5.42	0.77 ± 0.15
Mallorca '11 (n= 126)	47.06 ± 6.08	0.61 ± 0.16
Menorca '11 (n= 60)	38.33 ± 10.26	1.11 ± 0.30
Total (n= 462)	38.53 ± 3.35	0.87 ± 0.14

however there was no statistically significant difference between the islands in terms of prevalence and infestation data ($H= 1.185, p= 0.276$; $H= 0.717, p= 0.397$).

With reference to the prevalence of the samples, results obtained have been classified by 7 levels (Table 2). Level 1 presents 1 out of 6 sampled colonies (by apiary) parasitized by *Varroa*. Level 2 presents 2 parasitized colonies and so on; at level 0 none of the colonies was infested. Most of the apiaries analyzed in autumn 2010 (80.4%) showed parasitization in half or less of the colonies (levels between 0 and 3) and approximately 15% more than in spring 2011.

The average value of the infestation rate was very slight (0-1% I) in each period (Table 3). The average value of the MI in autumn is classified as mid-infestation (3-8% I) but the average value of the MI in spring is light (0-3% I).

In autumn 2010, 91.3% of the apiaries showed an infestation rate of average value very slight or slight; the opposite of all the apiaries analyzed in 2011. In autumn of 2010, 3 apiaries in Mallorca presented a serious maximum infestation rate compared to none apiaries in spring 2011. The higher degree of %MI in spring 2011 was moderate in 3 apiaries (1 in Mallorca and 2 in Menorca).

The %F (84.41%) could be calculated from the combined analysis of all adult bees and brood specimens. The %F was higher in spring (87.10%) than in autumn (82.61%). This pattern is repeated in the samples for each period in Mallorca (Table 4). In a breakdown of the results by island (Table 4), the values were higher in Mallorca in 2010 and 2011, followed by Eivissa and Menorca. The lowest values were registered in Formentera.

The statistical distribution of the number of colonies with *Varroa* by apiary was compared with a Poisson distribution to determine whether the presence of the mite in an apiary occurs independently and uniformly between the colonies or on the contrary it is influenced by the condition of other colonies in the apiary as a consequence of close proximity. In the second case a distribution by groups would be observed,

Table 2. Number of the apiaries with all the colonies negative for *Varroa* (level 0), with level 1 (1 colony parasitized by *Varroa* out of the 6 sampled by apiary), level 2 (2 parasitized colonies out of 6), level 3 (3/6), level 4 (4/6), level 5 (5/6) and level 6 (all the sampled colonies parasitized by *Varroa*) in autumn 2010 and in spring 2011.

	Autumn 2010	Spring 2011
Level 0 (0/6)	11	5
Level 1 (1/6)	8	4
Level 2 (2/6)	10	4
Level 3 (3/6)	8	8
Level 4 (4/6)	3	4
Level 5 (5/6)	4	5
Level 6 (6/6)	2	1

Table 3. Number of apiaries where colonies present infestation rate average values (%I) and maximum infestation rate (%MI) very slight (I between 0-1%), slight (I between 1.01-3%), median (I between 3.01-8%), moderate (I between 8.01-15%) or serious (I > 15%) in autumn 2010 and in spring 2011.

	Autumn 2010		Spring 2011	
	%I	%MI	%I	%MI
Very slight	35	17	23	13
Slight	7	14	8	9
Median	4	10	0	6
Moderate	0	2	0	3
Serious	0	3	0	0

that is, a group of apiaries would present a high incidence and the mite would not be present in other groups. The distribution of the number of colonies with *Varroa* by apiary is shown in Fig. 2. If we process the results obtained in Mallorca from the value of the average (2.5 colonies with *Varroa* by apiary); the expected values of the Poisson distribution can be calculated and compared to the observed values (Fig. 2). When adjusting the obtained results to the expected results, it is adjusted to a Poisson distribution; being the correlation coefficient between the expected and observed values 0.88. In the case of Balearics, the number of those colonies parasitized by *Varroa* is not adjusted to a Poisson distribution (the correlation coefficient between the expected and observed values is 0.63). In this distribution the spike of non-infested colonies is notable. Eleven out of sixteen non-infested apiaries are located on the smallest islands (Menorca, Eivissa and Formentera) and in Sóller (Mallorca).

Discussion

The results of the survey on the characterization of beekeeping in the Balearics show the low presence of professional beekeepers (only 9% of the surveyed beekeepers are professional, with over 150 hives) characterized by a high percentage of amateurs, small or

Table 4. Focality percentage (%F) in the samples of adult bees and brood in autumn 2010 and in spring 2011 by island. n= number of apiaries analyzed.

	%F
Mallorca '10 (n= 36)	86.11
Eivissa '10 (n= 6)	83.33
Formentera '10 (n= 4)	50.00
Mallorca '11 (n= 21)	95.24
Menorca '11 (n= 10)	70.00

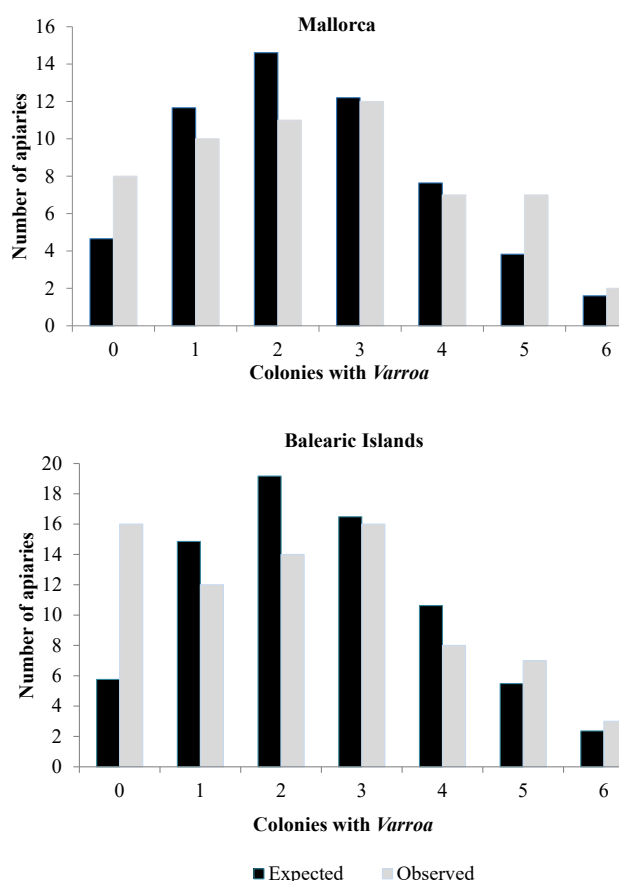


Figure 2. Number of colonies with *Varroa* by apiary in the samples from Mallorca and Balearic Islands. Grey bars are observed values while black bars are expected values based on a Poisson distribution with mean of 2.5.

producing for self-consumption (59% of the surveyed, with fewer than 50 hives). This contrasts with the rest of Spain that presents 22.51% professionals and it is the second country in the EU, with more professionals dedicated to the sector (MAGRAMA, 2014). Furthermore, the low proportion of beekeepers who move their colonies is notable (only 4% of the surveyed) compared to the usual methods in the centre and south/southeast of Spain (MAGRAMA, 2014). The fact that anti-varroa treatments do not conform to the legal indications is notable, which is probably a reflection of the low professional level of the sector. Only 8% of the surveyed beekeepers did not carry out anti-varroa treatment and between those who did it, 24% were incorrectly administered (regarding the dosage or duration of the treatment). More than 30% Apivar and Apistán treatments were incorrectly administered, which is a fact to be considered concerning the possible appearance of resistance to these products (Giacobino *et al.*, 2017). More than three quarters (77.8%) of the treatments were administered out of the period indicated in BOE (2006) which establish and regulate the national program to fight and control honeybee diseases.

From the results obtained in prevalence and infestation rates, we conclude that the prevalence was higher in spring than in autumn, but without statistically significant difference between the periods of study. Infestation rates were lower because bee population increased in spring (decreasing the ratio mite/bee which is the %I). Our data coincides with Marcangeli *et al.* (1992) whose studies showed that in autumn the number of mites decreases but the infestation rate increases. The prevalence observed coincide with the results obtained by Higes *et al.* (2010) in the Iberian Peninsula which registered a prevalence of 32.70% in autumn (34.78% in Balearics). Nonetheless, the results are lower than those observed in colder climates such as in Polonia (Pohorecka *et al.*, 2011), Canada (Guzman-Novoa *et al.*, 2010) and Finlandia (Varis *et al.*, 1992). These results confirm the studies of Moretto *et al.* (1991) that showed that infestation rates are higher in colder regions. Additionally, the infestation rate of the mite obtained in Balearics is higher than those found in tropical regions, which coincides with the studies showing that the population growth is lower in these regions (Moretto *et al.*, 1991; Eguaras *et al.*, 1994; Rosenkranz *et al.*, 2010).

When adjusting the results obtained of the distribution in the number of colonies with *Varroa* by apiary in Mallorca with the expected results, it is adjusted to a Poisson distribution. Nonetheless, in Balearics, the number of those colonies parasitized by *Varroa* is not adjusted to a Poisson distribution (the correlation coefficient between the expected and observed values is 0.63). In this distribution the spike of apiaries with non-infested colonies is notable. Eleven out of sixteen apiaries which are not parasitized by the mite are located on the smallest islands (Menorca, Eivissa and Formentera) and in Sóller (Mallorca). The results obtained in Balearics coincide with those obtained in apiaries in Turkey by Cakmak *et al.* (2003), who concluded that this distribution pattern is that expected of a typical endemic disease caused by a generalist host instead of a typical epidemic disease of a highly contagious parasite which was described previously (Finley *et al.*, 1996). Factors that may contribute to the development of an endemic epidemiological situation are the isolation of the groups (most noted on the smallest islands), the low number of hives per beekeeper (60% of the surveyed beekeepers owns less than 20 hives) and the fact that most of them increase the number of colonies adding natural colonies (instead of obtaining them from other places). This facts together with climatic factors (moisture and temperature) (Le Conte & Arnold, 1987, 1988; Patzold & Ritter, 1989; Moretto *et al.*, 1991; Rosenkranz & Engels, 1994; Kraus & Velthuis, 1997; Harris *et al.*, 2003) could contribute to the low incidence of the mite in Balearic Islands and explaining the spike of apiaries where the mite is not found and the high percentage of non-parasitized colonies (65.22% in autumn and 55.91 in spring).

More studies are needed to better understand this scenario in the Archipelago. It may be interesting to use these islands, applying the methodology to evaluate the desirability (Stear *et al.*, 2001) of resistance breeding against *V. destructor* used in the study made by Guichard *et al.* (2019). The Balearic Islands could prove to be an adequate place to develop breeding programs in future strategies, involving beekeepers and research institutes.

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