Reliability of the main field diagnostic methods of *Varroa* in honey bee colonies

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Additional keywords

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SUMMARY

Varroa destructor is the main health problem in Western beekeeping. The quantification of this mite in beehives is an important factor in veterinary inspections and for beekeepers to apply treatments, monitor their success, or assess varroa mite control efforts. In this paper were evaluate different diagnostic methods to quantify mite population levels in beehives: i) mites dislodged with sugar powder and with ethyl alcohol from adult bees samples brushed from honey combs and from brood combs, ii) mites removed from worker brood cells and iii) mite fallen on a screening bottom board for a period of four days. Recording the number of mites that fell naturally onto the screened bottom board was the only method that showed a significant correlation with the total number of varroa in beehives. Quantification in adult bee samples or bee brood samples can only be used for indicative diagnoses. Sugar powder only dislodge a third of the mites from adult bees.

Fiabilidad de los principales métodos de diagnóstico de *Varroa destructor* en colonias de abejas

Palabras clave adicionales

Apis mellifera.
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RESUMEN

El ácaro *Varroa destructor* es el principal problema de sanidad animal al que se enfrenta nuestra apicultura. La cuantificación de la población de este ácaro es un factor de gran importancia para la inspección veterinaria y los apicultores, además determina la necesidad de realizar o no realizar tratamientos. En este trabajo se evalúan diferentes métodos de diagnóstico: i) ácaros desprendidos con azúcar en polvo o etanol del cuerpo de abejas adultas barridas de cuadros de alimento y cría, ii) ácaros obtenidos de celdillas de cría de abejas obreras y iii) ácaros caídos a los fondos de las colmenas en un período de 4 días. El número de ácaros recogidos de los fondos de las colonias presenta una correlación positiva con el número total de ácaros presentes en las colonias. La cuantificación del número de ácaros presentes en las abejas adultas o en la cría de obrera solo puede ser utilizada de forma indicativa para el diagnóstico. El uso del azúcar en polvo solo desprende un tercio de los ácaros presentes en el cuerpo de las obreras adultas.

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INTRODUCTION

Varroa destructor (Anderson and Trueman, 2000) is the main health problem in Western beekeeping. *Varroa* mites feed on bee hemolymph, transmit other diseases that are mainly viral, and may be responsible for a large number of cases of colony losses. *Varroa* treatments can be the source of other beekeeping problems, such as the accumulation of residues in the wax or the lack of effective treatments due to the resistance of mites to products used for *Varroa* control (Orantes *et al.*, 2010; Guzman-Novoa *et al.*, 2010; Rosenkranz *et al.*, 2010).

Varroa infest both adult bees and bee brood, but only reproduce inside cells with capped brood. This lifecycle permits the use of diagnostic methods in adult bees or capped brood (Rosenkranz *et al.*, 2010).

The OIE Manual (2008; updated 2012) recommends three methods for diagnosing *Varroa*: examination of the hive debris, examination of adult bee samples, and examination of the bee brood. However, some of the diagnostic methods described in the manual are imprecise. These methods have been revised and extended upon in the BEEBOOK (Dietemann *et al.*, 2013). Although they are principally used for research purposes, some of these methods may also be used by beekeepers for controlling *Varroa* populations in beehives. Knowledge of *Varroa* is an important factor in making informed decisions, such as when to apply treatments, how to assess the effectiveness of the treatments, or when evaluating other control methods that can be used in the fight against *Varroa*.

Table I. Number of *Varroa* recorded (mean ± SD) in adult bee samples from brood combs and honeycombs with powdered sugar and ethyl alcohol. Colonies= 7 (Número de ácaros *Varroa* registrados (media ± SD) en las muestras de abejas adultas procedentes de cuadros de cría y alimento, obtenidas mediante el uso de azúcar polvo y alcohol etílico. Se tomaron muestras de 7 colmenas diferentes).

Varroa in adult bee samples from honey combs dislodged with powdered sugar 6.57 ± 5.94	*Varroa* in adult bee samples from brood combs dislodged with powdered sugar 7 ± 4.55	Total *Varroa* in adult bee samples from honey combs and brood combs dislodged with powdered sugar 13.57 ± 9.59 34.29 %
Varroa in adult bee samples from honey combs dislodged with ethyl alcohol 10.43 ± 4.86	*Varroa* in adult bee samples from brood combs dislodged with ethyl alcohol 15.57 ± 8.38	Total *Varroa* in adult bee samples from honey combs and brood combs dislodged with ethyl alcohol 26.00 ± 8.81 65.71 %
Total *Varroa* in adult bee samples from honey combs 17.00 ± 6.61 42.96 %	Total *Varroa* in adult bee samples from brood combs 22.57 ± 9.45 57.04 %	TOTAL *VARROA* 39.57 ± 13.92 100 %
Total number of adult bees in samples from honey combs 201.57 ± 62.63	Total number of adult bees in samples from brood combs 209.57 ± 44.31	Total number of adult bees in samples from honey combs and brood combs 411.14 ± 99.88 Infestation rate of adult bees 10.47 ± 4.92

Although different diagnostic methods have been evaluated in several studies (Calatayud and Verdú, 1993; Flores *et al.*, 2002; Branco *et al.*, 2006; Lee *et al.*, 2010), some major issues remain to be resolved, such as the development of rapid, simple and non-destructive methods that can be applied by beekeepers to productive beehives. Moreover, some methods have not been adequately assessed, while others, such as the evaluation of natural mite fall on beehive bottom boards need to be standardized.

The aim of our research is to assess the different methods commonly used by veterinary inspectors and beekeepers to diagnose *Varroa* in the field. These methods are compared with the actual mite population in beehives to determine their reliability.

MATERIAL AND METHODS

The tests were performed in the experimental apiary at the University of Córdoba, Spain (41° 44' 30.519" N, 6° 16' 41.3322" W) from November 2011 to January 2012.

The research was carried out in seven Apis mellifera iberiensis colonies in Langstroth hives fitted with a screened bottom board (4 mm mesh) and a lower tray. In a first step (November/24/2011), each of the colonies was inspected. The number of combs covered with adult bees and bee brood area was recorded by visual estimation (Burgett and Burikam, 1985; Delaplane *et al.*, 2013).

On November/29/2011 several diagnoses were then performed in each colony as follows:

1. A sample was taken of adult bees brushed from the central brood comb. Ten grams of powdered sugar was then added to each sample, after which the bee samples were shaken and sieved for 5 minutes on a tray to collect fallen mites. Fifty ml of ethyl alcohol (50 %) was added to the sample and the bees were shaken and sieved again under a stream of pressurized water. The remaining mites were collected and counted. Finally, the total number of adult bees in each sample was counted.

2. Another sample was taken of adult bees brushed from a honeycomb of each colony. Mite infestation in the bees was then determined using powdered sugar and ethyl alcohol following the same procedure as described above.

3. The central capped bee brood comb was removed from each colony. The capped brood cells were then opened as a model of cross from the center to the ends of the combs. Each cell was classified by the presence or absence of infested bee brood.

4. From November/25 a sheet of cardboard covered with petroleum jelly was placed in the bottom of the hive for a period of four days to collect the mites that fell on the sheet. The number of mites was then recorded.

5. To determine the total *Varroa* population, the colonies were treated with Apivar® (amitraz 1gr/colony) (November/29) and a sheet covered with petroleum jelly was placed in the lower tray of the beehive. The number of mites that fell on the sheet was counted every 4 days for a period of 42 days.

Statistical analyses were carried out using the SPSS for windows 8.0 program (SPSS Inc., Chicago, IL, USA).

RESULTS

Table I shows the number of *Varroa* recorded in the samples of adult bees taken from the brood combs and honey combs and screened with powdered sugar and ethyl alcohol. As can be seen in the table, only 34.29 % of the mites were dislodged from the adult bees when shaking with powdered sugar. Moreover, the number of *Varroa* was higher in the samples of adult bees from

Table II. Estimation of the amount of adult bees and bee brood in the colonies (early control of the colonies), ratio of infested bee brood, natural mite fall over four days on screening bottom board, and actual *Varroa* population subjected to chemical treatment (Estimación de la cantidad de abejas adultas y cría presentes en las colonias (control inicial), porcentaje de cría infestada, parásitos caídos de forma natural en un período de 4 días y población de *Varroa* censada después de realizar un tratamiento químico).

Colonies	Number of combs covered by adult bees	Number of brood combs	Number of inspected capped brood cells	Number of infested capped brood cells	Percentage of infested cells	Natural mite that fell on the bottom board over four days	*Varroa* subjected to chemical treatment
1	6	4	118	15	12.71	260	3300
2	5	2	152	45	29.61	133	983
3	5.5	2.5	150	51	34.00	224	1855
4	4.5	2	124	21	16.94	102	1379
5	4.5	3	159	6	3.77	76	1410
6	6.5	3	137	14	10.22	82	988
7	5	4	139	17	12.23	142	1791
mean±s.d.	5.29±0.76	2.93±0.84	139.86±15.03	24.14±16.99	17.07±10.88	145.47±70.94	1672.29±795.31

the brood combs than in the bee samples from the honey combs [22.57±9.45 vs. 17.00±6.61(n= 7)]. However, no significant differences were observed between the two samples (Mann-Whitney nonparametric test: p= 0.749).

Table II shows i) The initial inspection of the beehives (amount of bee brood and adult bees) ii) the percentage of cells infested with *Varroa* in each beehive; iii) the number of *Varroa* mites that fell onto the sticky sheet placed on the bottom board; and iv) the total number of mites that fell with the chemical treatment. The statistical analysis of the data (Pearson's correlation coefficient; see **table III**) shows that only the diagnosis made with the number of mites that fell naturally on the screened bottom board was significantly correlated with the total number of *Varroa* in the beehives subjected to chemical treatment. Moreover, the total natural mite fall showed a significant linear regression for the actual amount of *Varroa* in the beehives (p= 0.019).

Total *Varroa*= 306,247 + 9,38 ∗ natural mite fall over a four-day period.

DISCUSSION

Estimating the mite population in beehives in a reliable manner is an important factor in *Varroa* control. It is useful for detecting the infestation threshold for treatments against the mite, performing diagnoses after treatments to evaluate their effectiveness, or determining the success of methods used by beekeepers to control *Varroa* infestations.

Fries *et al.* (1991) used natural mite fall and infestation in adult bee samples and bee brood samples to detect *Varroa* in beehives with low infestation levels. Their results indicated that natural mite fall is the most effective method for estimating mite populations, followed by brood samples and adult bee samples. However, the aim of their study was to detect mites, but not to evaluate the accuracy of the method for determining the actual mite population in beehives. The aim of our study was to assess the reliability of *Varroa* diagnostic methods used by veterinary inspectors and beekeepers under field conditions, especially in au-

Table III. Correlation (Pearson's correlation test) between different diagnostic tests and *Varroa* population recorded in the beehives with chemical treatment (Coeficientes de correlación de Pearson entre las diferentes pruebas diagnóstico y población total de *Varroa* estimada después de haber realizado un tratamiento químico).

Samples	Pearson's correlation test (r_p)	Two-sided t-test of significance (p-value)
A. Number of mites in samples of adult bees from honey combs dislodged with powdered sugar.	-0.357	0.432
B. Number of mites in samples of adult bees from brood combs dislodged with powdered sugar.	0.017	0.971
C. Number of mites in samples of adult bees from honeycombs dislodged with ethyl alcohol.	-0.128	0.785
D. Number of mites in samples of adult bees from brood combs dislodged with ethyl alcohol.	0.110	0.814
E. Number of mites in samples of adult bees from brood combs (A+C).	-0.363	0.423
F. Number of mites in samples of adult bees from brood combs (B+D).	0.177	0.705
G. Total *Varroa* in adult bee samples (A+B+C+D).	-0.140	0.764
H. Percentage of infested brood cells.	-0.096	0.837
I. Mite fall on bottom boards over four days.	0.837	0.019

tumn season, when the research was carried out and when the colonies need healthy bees for overwinter. Moreover, the apiary was in a temperate zone, where bee brood is present in hives throughout the year (the study was conducted from November 2011 and found that the mean number of brood combs was 2.93 ± 0.84. N= 7; see **table II**). For this reason, it is important to know mite populations in the fall when treatments are applied to the hives or to determine infestation levels prior to overwintering.

The results showed a significant correlation only between the mites that fell on the screened bottom board and the total number of *Varroa* mites subjected to the chemical treatment. This result is in line with previous results which found a significant and positive relationship between fallen mites and total number of *Varroa* in beehives (Calatayud and Verdú, 1993; Flores *et al.*, 2002). This method can therefore be considered a reliable diagnostic method for assessing mite populations in apiaries.

Moreover, the data permitted establishing a significant linear regression model between mites collected on the bottom board over a four-day period and the total number of mites in the hives. These models can be used to determine when the *Varroa* population reaches the appropriate threshold for treatment, or the lack of effectiveness of the treatments due to either the development of resistance to treatments or mishandling. However, the test was carried out at a specific location and a time of the year, so further research would be needed to confirm the results throughout the year.

Four-day periods were chosen to record natural mite fall based on previous results and to seek a balance between the effectiveness of the method and the workload. Maintaining the sticky board for more than four days increases the accuracy of the technique, but the accumulated debris make it more difficult to quantify the mites (Flores *et al.*, 2002). This balanced relationship between the recording period and the reliability of the method allows to count the mites directly without having to use more sophisticated methods with solvents for the debris (Dietemann *et al.*, 2013).

The main disadvantage to this diagnostic method is that it must be performed in hives fitted with a screened bottom board (with grids). However, many beekeepers do not have this type of hives, and must therefore resort to other diagnostic methods using adult bees or sealed brood cells.

The assessment of infestation in samples of adult bees and bee brood is a widely used technique. Dietemann *et al.* (2013) reviewed several methods for quantifying mites and estimating the total mite population in beehives. Their review, however, is mainly directed at research. Although some of these methods are commonly used by beekeepers to assess mite populations, several authors have raised serious concerns about the routine use of these methods by professional beekeepers due to their lack of reliability or destructiveness (Fuchs, 1985; Branco *et al.*, 2006, Lee *et al.*, 2010). Moreover, the existence of bee brood in the fall and winter in several temperate climates hinder the assessment of *Varroa* infestations.

Fuchs (1985) found a significant correlation between the amount of mite in samples of adult bees (shaken in detergent water) versus total mite population in beehives. However, the results showed a high variability, raising doubts about the reliability of the method for determining the total *Varroa* population. In order to improve the accuracy of the method , other authors have recommended a sample size ranging from 175 to 300 bees, preferably taken from different frames (brood combs and honey combs) (Delaplane, 1997; Strange and Sheppard, 2001; Lee *et al.*, 2010; Dietemann *et al.*, 2013). Unlike these authors, our results did not show a significant correlation between the infestation rate of the samples of adult bees and the total mite population in the beehives, although the samples from six of the seven hives exceeded the recommended number of bees and were taken from brood combs and honeycombs **(table I)**.

In line with other authors (Fuchs, 1985; Calderone and Turcotte, 1998; Branco *et al.*, 2006; Lee *et al.*, 2010; Dietemann *et al.*, 2013), we found a higher number of mites in adult bees from brood combs than honey combs, although the differences were not significant **(table I)**. This suggests that the error could be larger if the sample is taken only from honeycombs or only from brood combs.

Macedo and Ellis (2002) proposed the use of inert dusts to dislodge mites from adult bees. In our work we have used two methods to assess mite infestation in adult bees. The first method consisted of powdered sugar, which was followed by washing with alcohol. Both methods were performed in the same sample of bees. The results showed that the powdered sugar method dislodged only 34.29 % of the mites in the sample, while the remaining 61.75 % were dislodged by washing with alcohol **(table I)**. Our results differ from those of Macedo and Ellis (2002) and Dietemann *et al.* (2013), who found both treatment methods to be equally effective.

Given our results, we think that *Varroa* diagnoses in adult bee samples could only be used for indicative purposes. We therefore suggest sampling adult bees from brood combs. If no mites are recorded, the colony would have a low infestation rate; however, if a larger number of mites is recorded, it would not be a reliable indicator of the actual *Varroa* population in beehives.

Measuring the infestation rate of sealed bee brood is another diagnostic method (Dietemann *et al.*, 2013). Beekeepers often use this method to estimate *Varroa* in capped drone brood cells, since the mites show a higher preference for drone brood cells than worker brood cells (Fuchs, 1990). Drone brood is present in the beehive in spring and summer. If the drone brood is not infested, the *Varroa* population is likely to be low in the beehive, and will not pose a risk to the colony. In the fall and winter, however, the drone brood is not present in the colonies and estimations can only be made in worker brood cells (Dietemann *et al.*, 2013). Moreover, it is important to determine mite infestation rates in the colonies in the fall and winter as this is the time of year when bees are usually treated against *Varroa*. Since our research was carried out from Nov-

ember to January, drones were not present and worker brood was used. The results did not show a significant correlation between percentage of infested brood and the actual mite population in the colonies subjected to chemical treatment. A significant correlation was not found either between the number of infested cells and the infestation rate in adult bees. Similar to adult bees, sampling bee brood could be an indicative technique, but is not a reliable method for assessing the actual mite population in the beehives.

CONCLUSIONS

In conclusion, our results showed that only the number of *Varroa* that fell over a 4-day period was significantly correlated with the actual mite population in beehives. The other methods we have evaluated could be useful for indicative purposes, but are not reliable for determining the total *Varroa* mite population in beehives.

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