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Article

Prevalence and intensity of varroosis and nosemosis of honey bees (*Apis mellifera*) in six regions of the state of Jalisco, Mexico

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Abstract:

Jalisco is one of the main honey bee producing states in Mexico. However, information on the parasitoses that affect the productivity of honey bee (*Apis mellifera*) colonies in the state is limited and addresses only a few regions. The objective of this study was to determine the prevalence and intensity of two parasitic diseases of *Apis mellifera* —varroosis (*Varroa destructor*) and nosemosis (*Vairimorpha* spp.)— in six regions of Jalisco. Bees from 365 colonies collected during the spring were analyzed. Varroosis was the most frequent parasitosis (90 %), and nosemosis was the least frequent (15 %). The infestation or infection levels of these parasitoses were generally low: <5 % (mites per 100 bees) for varroosis, and <310,000 spores/bee for nosemosis. The regions with the highest prevalence and intensity of *V. destructor* were the Highlands, the Center, and the South, while infections by *Vairimorpha ceranae* —the only species of the fungus found— were significantly higher in the Southeastern and Southern regions. It is advisable to carry out epidemiological studies at other times of the year in order to detect possible seasonal effects of parasitoses for the purpose of designing strategies for their control.

Keywords: *Apis mellifera*, *Varroa destructor*, *Vairimorpha ceranae*, Jalisco, Mexico.

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Introduction

Beekeeping in Mexico is an activity with a high ecological, social and economic impact. Western honey bees (*Apis mellifera*) provide an important environmental service by pollinating native flora, which helps maintain ecosystems, and pollinating economically important crops[(1)]. In addition, beekeeping is an important source of employment and income for rural areas and of foreign currency for the country through the export of honey[(2)]. Mexico is one of the leaders in the production and export of honey in the world, and Jalisco is one of the main honey producing states in the country. Jalisco reached the third place in honey production in 2021 with 6,073 t, with an inventory of over 145 thousand hives [(3)].

Honey bee diseases cause losses estimated at approximately US$6 million per year in Mexico[(4)]. It is therefore important to know their prevalence and distribution in order to control them. Among the diseases and parasitoses affecting honey bees, varroosis caused by the ecto-parasitic mite *Varroa destructor* is the most common. This parasite is considered to be the health issue that causes most damage to the beekeeping industry worldwide[(5-7)]. The mite feeds on the hemolymph and fat tissue of the bee; it inhibits its immune system, shortens

its life, and is a vector of viruses[7,8-11]. *Varroa destructor* was first reported in 1992 in Veracruz, Mexico[12], and is currently distributed across the country[13]. Another important parasitosis is nosemosis, which is caused by two species of microsporidia fungi: *Vairimorpha apis* and *V. ceranae*. These fungi infect the epithelial cells of the bee ventricle, causing digestive disorders that weaken and shorten the life of infected insects and reduce honey production[14,15]. *Vairimorpha apis* has existed for many years in Mexico, but *V. ceranae* was detected in 2010[16], although evidence of its presence since 1995 was later obtained in the state of Mexico[17].

Regarding the sanitary status of honey bee colonies in the state of Jalisco, the prevalence and degree of infestation of the mite *V. destructor* were recently reported in two regions of the state[18]. The average prevalence was 88 % and the infestation level was 5.2 % (number of mites in 100 bees). In addition to the above, in Jalisco there is information on the presence and intensity of infections by *Vairimorpha* spp. fungi in municipalities in the south and southeast of the state, but not in other regions. 83.7 % of the positive samples showed light infections[19].

For beekeeping purposes, the state of Jalisco has been divided into six different regions that vary in topography and climate, and include the Highlands, Central, Northern, Sierra Amula, Southern and Southeastern regions. More than half of the state's producers and hives are located in the South and Southeast regions[20].

Since the existing information on the presence of parasitoses affecting honey bees in the state of Jalisco is partial and available only for some regions, it was considered relevant to generate updated information on the level of infestation or infection of two of the main parasitoses affecting honey bees in Mexico[13] and on whether there is any relationship between them and the different regions of the state. Therefore, the objective of this study was to determine the prevalence and level of varroosis and nosemosis infestation or infection in honey bee samples from six regions of the state of Jalisco, Mexico.

Material and methods

Sampling

Brood and adult bee samples were collected from colonies located in 30 municipalities within the six beekeeping regions of the state of Jalisco (Table 1) at the beginning of spring, and during March, April and May 2018. Two or three apiaries were visited in each municipality, and five colonies were randomly selected from each apiary. A total of 365 colonies were sampled, from each of which the following samples were collected: 1) a sample of approximately 300 adult bees collected from the brood nest in a 250 ml pet container with

70% ethanol for diagnosis and determination of *V. destructor* infestation levels in adult bees; 2) a comb sample (10x10 cm) containing capped brood with pigmented-eye pupae, which was transported in a cooler with refrigerants for diagnosis and determination of *V. destructor* infestation levels in the brood; 3) a sample of approximately 70-80 adult bees in a 250 ml pet container with 70% ethanol, obtained from the entrance of each hive, for the diagnosis and determination of *Vairimorpha* spp. infection levels.

Table 1: Regions, municipalities, and number of honey bee samples collected

Region	Municipality	No. Samples
Higlands	Atotonilco	11
	Lagos de Moreno	10
	Tepatitlán	15
	Zapotlanejo	15
Center	Cocula	15
	Jamay	13
	Tlajomulco	15
	Tonalá	15
Sierra Amula	Autlán	10
	Cuautitlán	10
	La Huerta	10
	Mascota	15
	Tonaya	10
North	Colotlán	5
	Encarnación de Díaz	15
	Huejúcar	5
	Santa María	5
	Teocaltiche	15
	Yahualica	15
South	Gómez Farias	8
	San Gabriel	10
	Sayula	15
	Tapalpa	16
	Tolimán	5
	Zapotiltic	15
	Zapotlán el Grande	12
Southwest	Concepción de Buenos Aires	15
	Pihuamo	15
	Tamazula	15
	Tecalitlán	15

Sample processing

Tests for varroosis in brood, varroosis in adult bees, and nosemosis were performed at the Bee Research Center (Centro de Investigaciones en Abejas, CIABE) located at the Southern University Center (Centro Universitario del Sur, CUSur) of the University of Guadalajara, in Zapotlan el Grande, Jalisco, Mexico. DNA analysis of spores from *Vairimorpha* spp. positive samples was also performed in order to differentiate between *V. apis* and *V. ceranae*. These analyses were performed at the Honeybee Research Centre of the School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada.

Diagnosis and quantification of *Varroa destructor* in adult bees and brood

For adult bees, the ethanol wash technique was used[21]. The container of each sample was shaken for 3 min to separate the mites from the bees, and the contents were poured into a strainer with 8-frame/inch wire mesh. A plastic container covered with a white cotton cloth was placed under the strainer. The bees were retained on the wire mesh, and the mites, on the white cloth. Subsequently, the mites and bees were counted to determine the percentage of adult infestation (number of mites in every 100 bees). In order to determine mite infestation in the brood, the procedure was performed by direct observation under a stereoscopic microscope. In each comb sample, 200 cells were uncapped to look for the presence of *V. destructor* in order to count the number of cells with mites present and thus determine the percentage of infested cells.

Diagnosis and quantification of *Vairimorpha* spp.

Infection by *Vairimorpha* spp. was diagnosed and quantified by observation and counting of parasite spores[22]. Briefly, the abdomens of 60 bees per sample were macerated with 60 ml of H_2O in a mortar, and a drop of the macerate was placed on a slide to observe the parasite spores under an optical microscope (Olympus CX31; CDMX, Mexico) at 400 X. In positive samples, the intensity of the infection was determined by counting the spores in a Neubauer chamber.

Nosemosis-positive samples were also subjected to molecular diagnosis in order to differentiate between *V. apis* and *V. ceranae*. Spore DNA extraction and PCR procedures were performed according to a standard protocol[23]. For PCR amplification, a set of three specific primers was used in a triplex PCR consisting of co-amplification of the 16S rRNA gene from *V. apis* and *V. ceranae*, with the honey bee ribosomal protein S5 (RpS5) gene as a control for the reaction.

PCR reactions were performed on an Arktik thermocycler (Thermo Scientific; Missisauga, ON, Canada). Each reaction contained 1.5 µL of 10x pH PCR buffer (New England BioLabs; Pickering, ON Canada), 0.5 µL of 10 nM dNTPs (Bio Basic Inc; Markham, ON Canada), 1 µl of each 10 µM oligonucleotide, 2 µL of DNA, 0.2 µL of Taq polymerase 5 U/µl (Applied Biological Materials Inc.), and 8.8 µL of nuclease-free H_2O (Invitrogen; Burlington, ON, Canada). The primer sequences used, as well as the amplification cycles were those described by Hamiduzzaman *et al*[23].

PCR products were separated by electrophoresis on 1.1% agarose gels and stained with ethidium bromide. The amplified bands were captured with a digital camera inside a UV Transilluminator (Benchtop-ItM Imaging System; Upland, CA, EUA).

Statistical analyses

To determine whether there were differences between regions for the prevalence of parasitoses in the studied colonies, the data were analyzed using tests of equality of proportions and the Benjamini-Hochberg correction. Before analyzing and comparing such continuous variables as the intensity of infestations or infections, the data were subjected to Shapiro-Wilk and Bartlett tests for the purpose of analyzing the assumptions of normality and homoscedasticity, respectively. The data did not have a normal distribution and were not homoscedastic; therefore, they were analyzed with nonparametric statistical tests. In order to compare the intensity of varroosis and nosemosis between regions, the data were subjected to Kruskal-Wallis tests. When the differences were significant, pairwise comparisons of treatments were made using Dunn's test and the Benjamini-Hochberg correction. All statistical analyses were performed with the R 3.3.1 software (Foundation for Statistical Computing, Vienna, Austria).

Results

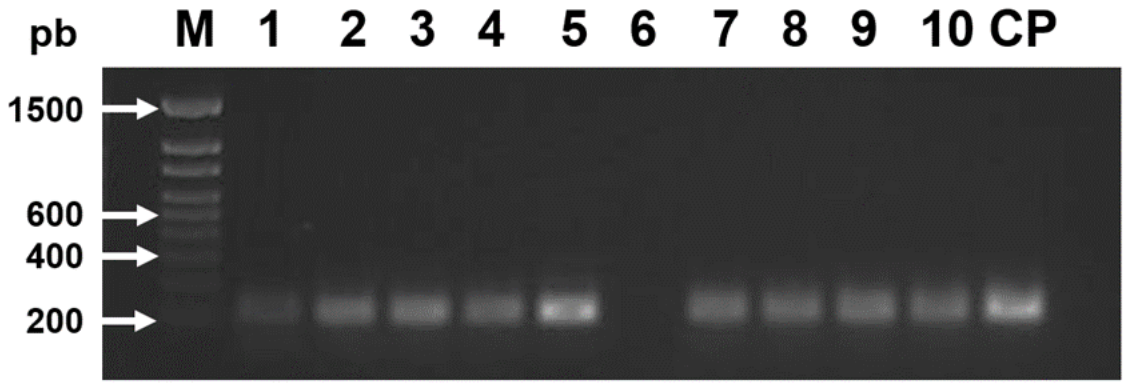
The most prevalent parasitosis in Jalisco was varroosis, which was detected in 90 % of the sampled colonies, while nosemosis was detected only in 15 % of them (Table 2). Of the two *Vairimorpha* species infecting honey bees, only *V. ceranae* was detected (Figure 1), while *V. apis* was not. Table 2 shows the intensity of the diagnosed etiological agents.

Table 2: Prevalence and average intensity of parasitosis (infestation or infection) from parasites affecting honey bee colonies in the state of Jalisco, Mexico

Parasites	N	Prevalence (%)	Intensity ± SE
Varroa destructor/brood	365	90.1	4.50 ± 0.34[1]
Varroa destructor/adults	365	90.1	4.15 ± 0.19[1]
Vairimorpha ceranae	365	14.5	161,046 ± 38,055[2]

SE= standard error.
[1]Number of mites in 100 brood cells or in 100 adult bees.
[2]Number of spores per bee.

Figure 1: Photograph of an agarose gel showing bands of 218 base pairs of a *Vairimorpha ceranae* ribosomal RNA gene fragment in columns 1 to 5 and 7 to 10. A positive control (PC) is used in the RT-PCR reaction



In the results by region, the prevalence of *V. destructor* in both brood and adult bees was significantly higher in the Highlands, Center, and South than in the North ($P< 0.05$; Tables 3 and 4). In addition, the intensity of *V. destructor* infestations in brood also varied among regions. The most intense parasitism of the mite in the brood was found in colonies in the Southern and Highlands regions with 7.1 ± 1.0 % and 5.6 ± 0.8 %, respectively. These infestation levels were significantly higher than those found in the colonies of the other regions, except for the Central region ($\chi^2= 43.0$, sd= 5, $P<0.01$; Table 3). In adult bees, there were also differences between regions. The most intense parasitism by *V. destructor* was again found in colonies of the Southern and Highlands regions, with 4.6 ± 0.4 % and 5.9 ± 0.5 %, respectively. The mite infestation intensities of adult bees in colonies in these two regions and the Central region were significantly higher than those of bees from the Northern

region, at only 2.7 ± 0.4 %, but did not differ from the infestation intensities found in colonies of the other regions (χ^2= 34.3, sd= 5, P<0.01; Table 4).

Table 3: Prevalence and average intensity of *Varroa destructor* parasitosis in the brood of honey bee colonies in different regions of the state of Jalisco, Mexico

Region	**N**	**Prevalence (%)**	**Intensity ± SE**[1]
Highlands	51	88.2[a]	5.60 ± 0.83[a]
Center	58	81.0[a]	4.15 ± 0.53[ab]
Sierra Amula	55	56.4[b]	2.31 ± 0.61[c]
North	60	65.0[b]	3.84 ± 0.81[bc]
South	81	86.4[a]	7.07 ± 1.01[a]
Southeast	60	66.7[b]	3.09 ± 0.60[c]

SE= standard error.

[1]Number of mites in 100 brood cells.

[abc] Different letters indicate significant differences (P<0.05).

Table 4: Prevalence and average intensity of *Varroa destructor* parasitosis in adult workers of honey bee colonies in different regions of the state of Jalisco, Mexico

Region	**N**	**Prevalence (%)**	**Intensity ± SE**[1]
Highlands	51	98.0[a]	5.89 ± 0.55[a]
Center	58	98.3[a]	4.50 ± 0.49[ab]
Sierra Amula	55	90.9[a]	3.60 ± 0.42[bc]
North	60	75.0[b]	2.70 ± 0.43[c]
South	81	92.6[a]	4.61 ± 0.42[ab]
Southeast	60	86.7[ab]	3.72 ± 0.45[bc]

SE= standard error.

[1]Number of mites in 100 adult bees.

[abc] Different letters indicate significant differences (P< 0.05).

For nosemosis, the prevalence of parasitosis caused by *V. ceranae* was relatively low, ranging from 7 to 18 %, with no significant differences between regions (P>0.05, Table 5). The intensity of infection caused by this parasite was relatively low and ranged between 39,375 ± 10,625 to 309,091 ± 166,960 spores/bee in the positive colonies of the different regions studied, among which there were significant differences for the level of infection (χ^2= 11.1, df= 5, P< 0.05).

Table 5: Prevalence and mean intensity of nosemosis (*Vairimorpha ceranae*) in adult workers of honey bee colonies in different regions of the state of Jalisco, Mexico

Region	N	Prevalence (%)	Intensity ± SE[1]
Highlands	51	17.6	66,667 ± 11,024[bc]
Center	58	12.1	107,143 ± 22,961[ab]
Sierra Amula	55	18.2	130,000 ± 21,016[a]
North	60	6.7	39,375 ± 10,625[c]
South	81	17.3	189,286 ± 63,800[a]
Southeast	60	18.3	309,091 ± 166,960[a]

SE= standard error.
[1]Number of spores per bee of the samples that tested positive.
[abc] Different letters indicate significant differences (P<0.05).

Discussion

Varroosis was the most prevalent parasitosis, as it was diagnosed in 90 % of the colonies sampled in Jalisco; however, the average levels of infestation by *V. destructor* were low, with less than 5 % parasitism in both brood and adult bees. These results are in agreement with a previous study carried out in the state, which found a prevalence of 88 % and an infestation level of 5 % of the parasite in colonies located in the southern and southeastern regions of Jalisco[18]. This study, however, was broader, as it included regions of central and northern Jalisco that were not studied in the aforementioned work.

In other regions of Mexico, results similar to those of this study have been observed. For example, in the north of the country, in the state of Zacatecas, a varroosis prevalence of 88 % and an infestation level of 5 % in autumn and of 3.5 % in spring were reported[24]. Although the level of infestation was reported in different seasons, the results are not far from those found in this study. In the central region of the country, in the State of Mexico, a 100%

prevalence of varroosis was found in five municipalities in the eastern zone, with the highest infestation rate of 7.9 %, and the lowest, of 3.5 %[25]. Alternatively, in southeastern Mexico, specifically in the state of Yucatán, a varroosis prevalence of 62.9 % was found in honey bee colonies with an infestation level of only 1.7 %[26]. Both percentages are lower than the ones reported in this study and in other Mexican states, possibly due to the fact that the aforementioned studies were carried out in the central and northern states of the country where the climate is temperate to cold, as opposed to Yucatan, where the climate is tropical. The environment, together with the Africanization of honey bee colonies, is known to be one of the most influential factors in *V. destructor* infestations[27]. In Yucatan, the degree of Africanization of bees is significantly higher than in other regions of the country[28]. In general, honey bee colonies located in temperate climates tend to be more susceptible to the mite because they tend to have bees with predominantly European ancestry, in addition to the stress caused by the winter weather conditions that affect the survival of colonies, because during part of the winter, queen bees stop laying eggs or drastically reduce their laying rate and, consequently, the adult bee population[29]. In contrast, in tropical regions, honey bee colonies are less affected by varroa mite infestations, largely because these regions are dominated by African ancestry, which is strongly associated with characteristics that confer honey bees a higher degree of resistance to the parasite compared to predominantly European bees[24,30-32].

Although the prevalence of varroosis is high in Jalisco and other regions of the country, this is an expected result because the behavior of the bees and their current management favor the dispersion of *V. destructor* between colonies. Bees (worker bees and drones) frequently enter other colonies, carrying mites with them[33]. Also, the robbing behavior (robbing honey from other colonies) of the bees favors the dispersion of the mite[7]. In addition, the short distance between hives in the apiaries favors the mite dispersion[34].

The average level of *V. destructor* infestation found in the colonies in Jalisco was generally low, less than 5 %, as recommended by the Mexican standard for varroosis control[35]. However, in some regions, such as the South and Highlands, infestation levels were higher than 5 %, while in the North they were lower than 4 %. This could be partly explained by the density of bee hives in the various regions, as in the south, the largest number of bee hives in the state is concentrated in a relatively small territorial extension compared to the other regions, while in the north, there is a lower concentration of bee hives in a larger territorial extension[20].

Among the implications of these results, in certain regions such as the South and Highlands, the increased parasitism by *V. destructor* may affect colony development and honey production, as demonstrated by Medina-Flores *et al*[36] and Emsen *et al*[37], who found that colonies with more than 5 % infestation by *V. destructor* produced significantly less honey than colonies with lower levels of parasitism. Arechavaleta-Velasco and Guzman-Novoa[38]

also found that colonies with 2 % infestation by *V. destructor* produced 65 % more honey when treated, compared to colonies with 7 % infestation that were not treated. Therefore, it is recommended that beekeepers in regions where high levels of varroosis (>5%) were found monitor and use mite control measures in their colonies more frequently, keeping the number of treatments to a minimum in order not to promote parasite resistance to acaricides.

Nosemosis was the least prevalent honey bee disease in the state of Jalisco, as it was diagnosed in only 15% of the sampled colonies; in all positive samples only *V. ceranae* was detected, and in no case *V. apis*. It is possible that *V. ceranae* has displaced *V. apis*, as seems to have occurred in other countries[39,40]; but it is also possible that *V. ceranae* has historically been the only species of *Vairimorpha* present in Jalisco, which is difficult to prove, since this is the first study that has identified the species of *Vairimorpha* infecting bees in Jalisco. Further studies are necessary to support these hypotheses. The intensity of the infection with *V. ceranae* was relatively low, as all positive samples were classified as having very light levels of infection (< 310,000 spores/bee)[41].

The present study detected no significant differences in the prevalence of *V. ceranae* between regions; however, there were significant differences in the intensity of infections, as colonies in the Southeast, South and Sierra Amula regions exhibited higher levels of infection than colonies in the rest of the regions. These results can be explained, at least partially, by the prevailing type of climate, as these regions are more humid, which favors the presence and development of microsporidia[14].

The prevalence and intensity of the nosemosis infections observed in this study were lower than those previously found in Yucatan, with a frequency of 74 % and an infection intensity of 1’480,000 spores/bee[26]. In Nayarit, the prevalence of nosemosis was also higher than in this study (55.4 % in winter and 33 % in summer), but the intensity of the infection was lower, with an average of 145,000 spores/bee in winter and 47,000 spores/bee in summer[42]. The low prevalence of nosemosis found in this study coincides with that of a study carried out in Zacatecas, as the presence of *Vairimorpha* spp. was detected in only 4.7 % of the sampled colonies[43]. This could be due to the fact that the climate of the areas of Zacatecas where the samples were taken is similar to that of the highlands of the state of Jalisco, where most of the samples of this study were collected.

In the only previous study that tested for nosemosis in bees in southern and southeastern Jalisco, nosemosis-positive colonies were found to have light or less than light levels of infection[19], similarly to the results presented herein. This coincidence between these two studies may be due to the fact that in most regions of the state of Jalisco there are no environmental conditions that favor the multiplication of the etiological agents of the disease, at least in spring, when the samples of both studies were collected. The intensity of the nosemosis infection of honey bees is usually seasonal. In countries with temperate and cold

climates and at latitudes above 30º, the intensity of *V. apis* or *V. ceranae* infections peaks in the spring and early summer, but decreases in other seasons[14,15]. In contrast, in the Mexican highlands, *V. ceranae* infections are more intense in summer and autumn, and less intense in winter and spring[17]. Therefore, colonies should be sampled in the summer and fall in order to determine if the intensity of *V. ceranae* infection is higher than in spring. These studies would allow to determine whether nosemosis is a serious problem for the beekeeping industry in the state of Jalisco and, if it is, during what seasons of the year it constitutes a serious issue.

Conclusions and implications

The most prevalent honey bee parasitosis in the state of Jalisco was varroosis, which was detected in 90 % of the colonies, while the least prevalent parasitosis was nosemosis, detected in 15 % of the sampled colonies. In addition, of the two *Vairimorpha* species analyzed in the samples, only *V. ceranae* was detected. The levels of infestation or infection in the case of these parasitoses were generally low: the level of varroosis was <5%, while nosemosis infections were classified as very light infections (<310,000 spores/bee). The regions with the highest prevalence and intensity of *V. destructor* infestations in both brood and adult bees were the highlands and the central and southern regions. For the prevalence of nosemosis, no significant differences were found between colonies of different regions, but the levels of infection were highest in the Southeast, South and Sierra Amula regions. Further studies are recommended, with samplings in different seasons of the year and for several years, to determine under what conditions and during which seasons the studied parasitoses may be more harmful to the beekeeping industry, as well as to design adequate control strategies.

Acknowledgments and conflict of interest

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