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## Variation in the prevalence of *Varroa*, *Nosema* and *Acarapis* in two regions of Campeche State, Mexico

Variación de la prevalencia de *Varroa*, *Nosema* y *Acarapis* en dos regiones del estado de Campeche, México



Domínguez-Rebolledo Álvaro<sup>1</sup><sup>ID</sup>, Lemus-Flores Clemente<sup>2</sup><sup>ID</sup>, Salgado-Moreno Socorro<sup>2</sup><sup>ID</sup>, Dzib-Cauich Dany<sup>3</sup><sup>ID</sup>, Chi-Maas Daniel<sup>4</sup><sup>ID</sup>, Loeza-Concha Henry<sup>5\*</sup><sup>ID</sup>

<sup>1</sup>Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias - Mocochá, Yucatán, México,

<sup>2</sup>Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nayarit, Compostela, Nayarit, México. <sup>3</sup>Tecnológico Nacional de México/Instituto Tecnológico Superior de Calkiní. <sup>4</sup>Instituto

Tecnológico Superior de Hopelchén. Hopelchén, Campeche México. <sup>5</sup>Colegio de Postgraduados, Campus

Campeche, Sihochac, Champotón, Campeche, México. \*Author for correspondence: Henry Jesús Loeza

Concha, Programa de Maestría en Ciencias Bioprospección y Sustentabilidad Agrícola en el Trópico,

Colegio de Postgraduados Campus Campeche, Carretera, Haltunchén-Edzná; C.P. 24450; Champotón,

Campeche, México. E-mail: alvaroedr@gmail.com, drclemus@yahoo.com.mx,

coco\_salgado@hotmail.com, dadzib@itescam.edu.mx, dani\_franc.chi@outlook.es,

loeza.jesus@colpos.mx

### Abstract

Bees are susceptible to diseases, which cause significant economic losses in beekeeping. The objective was to evaluate the prevalence of *Varroa* sp, *Nosema* sp and *Acarapis* in apiaries located in two regions in Campeche, Mexico. 116 bee samples from 10 apiaries were evaluated. The prevalence of *Varroa* was lower in Hopelchén compared to Sihochac (98.5 and 100 %), the prevalence of *Nosema* was lower in Hopelchén compared to Sihochac (85.9 and 88.0 %). The *Varroa* infestation level was higher in Hopelchén compared to Sihochac (7.32 and 3.73 mites in 100 bees), however, the *Nosema* infestation level was lower in Hopelchén compared to Sihochac ( $78 \times 10^5$  and  $23 \times 10^6$  spores per bee), and not the presence of *Acarapis* was found in none of the evaluated regions. In both regions a positive correlation was found between the presence of *Nosema* and *Varroa*. There is a 1.08 higher probability of finding *Nosema* in the presence of *Varroa*. We conclude that in both regions there is a high prevalence of *Varroa* and *Nosema* and the presence of *Acarapis* was not detected.

**Keywords:** pathogens, bees, *Apis mellifera*, mite, infestation.

### Resumen

Las abejas son susceptibles a enfermedades, las cuales causan importantes pérdidas económicas en la actividad apícola. El objetivo fue evaluar la prevalencia de *Varroa* sp, *Nosema* sp y *Acarapis* en apiarios ubicados en dos regiones en Campeche, México. Se evaluaron 116 muestras de abejas de 10 apiarios. La prevalencia de *Varroa* fue menor en Hopelchén comparada a Sihochac (98.5 % y 100 %), la prevalencia de *Nosema* fue menor en Hopelchén en comparación a Sihochac (85.9 % y 88.0 %). El nivel de infestación de *Varroa* fue mayor en Hopelchén en comparación a Sihochac (7.32 y 3.73 ácaros en 100 abejas) sin embargo, el nivel de infestación de *Nosema* fue menor en Hopelchén en comparación a Sihochac ( $78 \times 10^5$  y  $23 \times 10^6$  esporas por abeja), no se encontró la presencia de *Acarapis* en ninguna de las regiones



evaluadas. En ambas regiones se encontró una correlación positiva entre la presencia de *Nosema* y *Varroa*. Existe una probabilidad de 1.08 más alta de encontrar *Nosema* ante la presencia de *Varroa*. Concluimos que en ambas regiones existe una alta prevalencia de *Varroa* y *Nosema* y no se detectó la presencia de *Acarapis*.

**Palabras clave:** patógenos, abejas, *Apis mellifera*, ácaro, infestación.

## INTRODUCTION

Beekeeping is one of the main economic activities in the Yucatan Peninsula in Mexico, comprising Yucatan, Campeche, and Quintana Roo states, whose honey is preferred in both domestic and international markets due to its organoleptic characteristics associated with the unique flowering type in the Peninsula. However, this activity is threatened by the presence of diseases such as varroasis, nosemosis and acariosis ([Loeza et al., 2020](#)). Varroasis is caused by the *Varroa destructor* mite, nosemosis by the microsporidia *Nosema apis* and *Nosema ceranae* and acariosis is caused by the tracheal mite *Acarapis woodi*. These three diseases are of sanitary and economic importance for beekeeping ([Arechavaleta et al., 2021](#)) because their presence can reduce honey production or even cause the partial or total loss of hives ([Martínez & Medina et al., 2011a](#)).

*Apis mellifera* bees are susceptible to *Varroa*, *Nosema* and *Acarapis*, which have a detrimental effect on the development and productivity of colonies ([Guzmán et al., 2010](#)); this is because *Varroa* is an ectoparasite, which feeds on the fat bodies of bees affecting the phoretic development of larvae, which reduces their lifespan and reproductive capacity of queen bees and drones, in this sense varroasis is considered the main threat to beekeeping in the world ([Ramsey et al., 2019](#); [Genersch et al., 2010](#)). *Nosema* is a microsporidian fungus that spreads through fecal matter infecting the epithelial cells of the midgut of younger bees, affecting digestive functions, leading to malnutrition, physiological aging, reduction of hypopharyngeal glands and premature death of bees ([Forsgren & Fries, 2010](#)), and *Acarapis* is an endoparasitic mite of the adult bee that affects the respiratory system, the alterations it causes in the respiratory system depends on the number of parasites in the trachea causing mechanical damage such as obstruction of the air passages preventing their normal functioning ([Delannoy & Gislainne, 2006](#)).

Even though the damage caused by these pathogens is well documented ([Otis & Scott-dupree, 1992](#); [Çakmak et al., 2003](#); [Higes et al., 2008](#); [Le Conte et al., 2010](#)), in many countries there is a lack of diagnostics, so there is a lack of knowledge about the health status of bee colonies, or of pathogens that coexist with bees which can lead to the inappropriate use of chemicals for their control thus causing natural resistance of pathogens ([Rodríguez-Dehaibes et al., 2005](#); [Branco et al., 2006](#); [Martínez et al., 2011b](#)), in this sense, maintaining good bee health has become a challenge faced by beekeepers.



The objective of this research was to determine the variation in the prevalence of *Varroa*, *Nosema* and *Acarapis* in two regions of Campeche State, Mexico.

## MATERIALS AND METHODS

### Sampling localities

The research was carried out in the apiaries of the Higher Technological Institute of Hopelchén, Campeche located at 19°76'41" north and 89°86'68" west at 100 m a.s.l. with a minimum temperature of 23 °C and maximum of 376 °C, a precipitation 140. 7 mm and a humidity of 74 %, and in the apiaries adjacent to the Postgraduate School campus Campeche located Sihochac locality, Champotón, Campeche at 19.50°13'89" north and 90.58°61'11" west at 20 m a.s.l. with a minimum temperature of 23 °C and maximum of 35 °C, a precipitation of 163 mm and a relative humidity of 98.5 %. Both regions are dominated by warm sub-humid (awo) climate (w), with summer precipitation of less than 5.0 mm, mean annual precipitation is 1,050 mm, with rainfall from May to October the annual temperature varies between 19.5 and 32.5 °C, considering a mean is 26 °C ([WeatherSpark 2021](#)).

### Sample size

We randomly sampled 64 hives from 5 apiaries in the Hopelchén town and 52 hives from 5 apiaries in the Sihochac town during June to August 2020 and 2021.

### Sample collection

Samples were taken to diagnose the presence and level of infestation of *Varroa*, *Nosema* and *Acarapis*; approved by the animal welfare committee of the Postgraduate School of Mexico, so approximately 300 bees were collected from each hive, between the third and fourth frame of the brood chamber, and preserved in a solution of absolute ethanol until they were analyzed. The methodology used in this research was carried out according to the Mexican Official Standard [NOM -001-ZOO-1994](#), National Campaign against varroasis of bees.

### Varroa diagnosis

This diagnosis was performed using the methodology described by [De Jong et al. \(1982\)](#) with modifications ([Loeza-Concha et al., 2020](#)) so the procedure consisted of shaking the jars with the bees at 60 rpm for 10 min, after which the contents were placed in a conical container containing a 3 mm mesh, this container was filled with absolute alcohol until the bees were completely covered, Afterwards, the samples were shaken with a glass rod in a circular motion to detach the mites from the bees, and by gravity these were deposited at the bottom of the cone, finally, the solution was decanted through a white cloth and the



number of mites was recorded. Varroa infestation level was determined by dividing the number of mites found by the number of bees observed and the result multiplied by 100 ([De Jong et al., 1982](#)).

### **Nosema diagnosis**

This diagnosis was made using the Cantwell technique described by [Loeza et al., \(2021\)](#), so this procedure consisted in the maceration of 25 abdomens of adult bees, then the macerate was filtered in a 0.2 mm sieve and placed in a sieve where a drop was taken to analyze in the optical microscope at 400X; the positive samples were evaluated again in a Neubauer chamber to determine the number of spores present, the level of infestation was obtained by dividing the number of spores observed by 80 and multiplying the result by 4 million.

### **Acarapis diagnosis**

The diagnosis was made with the methodology described by [\(Bailey, 1985\)](#), using 20 adult bees which were fixed with entomological pins on a dissection table, then with a watchmaker's forceps and scalpel heads and the first pair of legs of each of bees were removed, then a transverse cut was made through the mesothorax, between the first and second pair of legs to expose the thoracic ring. This was placed on a slide in a cranial-caudal position and a drop of 10 % lactic acid solution was added for 24 hours in order to clarify the tissues and facilitate the revision, these samples were observed under an optical microscope at 400X to determine the presence or absence of the mites.

### **Prevalence of diseases**

This diagnosis was determined by multiplying the number of hives with the presence of the parasite (Varroa, Nosema or Acarapis) by 100 and divided by the total number of hives evaluated per period ([Loeza et al., 2021](#)).

### **Statistical analysis**

Data were analyzed using a one-effect model, where effects evaluated were Hopelchén and Sihochac localities, under a completely randomized design. To determine differences between prevalences of both diseases, a Chi-Square test was performed, and to determine differences between different levels of Varroa and Nosema infestation in the Hopelchén and Sihochac regions, a comparison of means was performed using the t-Student test. To relate the presence of Varroa, Nosema with the study region, a Pearson correlation test was used. Finally, to determine if the presence of Nosema has an effect on the presence of Varroa, an odds ratio (OR) analysis was used using the Statistical Package for the Social Sciences (SPSS) version 20.0 software [IBM, 2011](#)).



## RESULTS

Varroa prevalence was lower the Hopelchén region compared to Sihochac region, 98.5 and 100 %, respectively, however, no statistical differences were found ( $p=0.365$ ), likewise, Nosema prevalence was higher for Hopelchén region compared to Sihochac region, 85.9 %, 88.0 %, respectively, the statistical test indicated that there were differences between the prevalences by region ( $p=0.023$ ). Varroa infestation level was higher in Hopelchén region compared to Sihochac region, 7.32 and 3.73 mites per 100 bees, respectively, finding statistical differences between both regions ( $p= 0.048$ ), Varroa infestation level was higher in the Hopelchén region compared to Sihochac region, 7.32 and 3.73 mites per 100 bees, respectively, finding statistical differences between both regions ( $p= 0.048$ ). Nosema infestation level was lower for Hopelchén region compared to Sihochac region,  $78 \times 10^5$  and  $23 \times 10^6$  spores per bee, respectively, presenting differences between both regions ( $p=0.022$ ), as shown in Table 1. The presence of *A. woodi* was not found in any of the regions evaluated. A positive correlation ( $p=0.000$ ) between the presence of Nosema and Varroa was found in both regions. The odds ratio (OR) indicated that there is a 1.08 higher probability of finding Nosema in the presence of Varroa, however, this probability is not statistically significant.

**Table 1. Variation in the prevalence and infestation levels of Varroa and Nosema in two regions of Campeche State**

Region evaluated	TH	Prevalence	VILH	Prevalence	NILH
Hopelchén	64	98.5 % <sup>a</sup>	7.32 <sup>a</sup>	85.9 % <sup>a</sup>	$78 \times 10^5$ <sup>a</sup>
Sihochac	52	100.0 % <sup>a</sup>	3.73 <sup>b</sup>	88.0 % <sup>b</sup>	$23 \times 10^6$ <sup>b</sup>

TH= Total hives, VILH= Varroa infestation level per hive (mite/100 bees); NILH= Nosema infestation level per hive (spores/bee), <sup>a,b</sup> different literals indicate significant statistical difference ( $p < 0.05$ )

## DISCUSSION

Nosemosis as well as varroasis represent the main sanitary problems for beekeeping worldwide, due to the harmful effects they cause in bees by the loss of hemolymph and fat bodies which reduces their useful life and productive capacity, (Ramsey *et al.*, 2019; Genersch *et al.*, 2010; Branchiccela, 2014) in this sense the results found in these two regions of Campeche State suggest that the simultaneous infestation by Varroa and Nosema in the colonies, represents an important sanitary problem, which indicates that in the region there is a low level of technification or total or partial ignorance of the presence and control of Varroa and Nosema.



In this sense, [Guzmán-Novoa et al. \(2010\)](#) indicate that the Varroa mite is the main cause of death of bee colonies, since it is 85 % associated with the loss of hives. Likewise, [Higes et al. \(2008\)](#) indicate that the presence of *Nosema* in colonies can cause the sudden collapse of bee colonies, establishing a direct correlation between *Nosema* and the death of bee colonies in field conditions, in this sense, the presence of Varroa and *Nosema* in the territory of Campeche state represents a danger and the possible loss of colonies.

According to the above mentioned in both regions evaluated (Hopelchén-Sihochac) it was found that 92.25 % of the evaluated colonies presented simultaneous infestation by Varroa and *Nosema*, 6.89 % presented Varroa in absence of *Nosema* and 0.86 % presented *Nosema* in absence of Varroa, highlighting that all the evaluated hives had some infestation.

Varroa prevalence found in the study regions (Hopelchén and Sihochac) was 98.5 % and 100 %, respectively, with infestation levels of 7.32 and 3.73 mites per 100 bees, respectively, the results can be compared with other states of the republic such as Yucatán, where 63.6 and 97 % prevalence and an infestation level of 2.89 and 0.2 mites per 100 bees have been reported ([Martínez et al., 2011b](#); [Martínez & Medina, 2011a](#)), likewise, the prevalence and infestation level of Varroa in Zacatecas State was 62 % and 1.70 mites per 100 bees ([Medina-Flores et al., 2014b](#)), in Mexico State a prevalence of 100 % and an infestation level of 0.5 to 22.1 mites per 100 bees has been reported respectively [Martínez-Cesáreo et al., 2016](#)), in Nayarit a prevalence of 65.9 % and 79.1 % with an infestation level of 1.31 to 2.55 mites per 100 bees has been reported ([Loeza et al., 2020](#)).

Likewise, Varroa presence has been reported in hives around the world, as in Colombia where [Salamanca et al. \(2012\)](#) found 45 % of hives with presence of the mite, similarly, [Calderón & Sánchez \(2011\)](#) found a 42 % prevalence in Costa Rica, [Soroker et al. \(2011\)](#) found 21 % prevalence in Israel, [Torres & Barreto \(2013\)](#) found 11.13 % prevalence in Brazil, [Moretto & Leonidas \(2003\)](#) reported an infestation from 2.33 and 24.69 mites per 100 bees and [Guzmán-Novoa et al. \(2010\)](#) reported an infestation level of 3.1 to 5.1 mites per 100 bees in Ontario Canada. According to these results, it is suggested that Varroa presence in the country represents an alarming problem due to the high prevalence rates reported.

Similarly, *Nosema* prevalence in the study regions (Hopelchén and Sihochac) was 85.9 and 88.0 %, respectively, indicating that the presence of this pathogen is considered high, therefore the results found in this research are higher than those found in other states of the republic as Nayarit State where the prevalence was from 33.0 to 55.4 % in hives forming for fertilization nuclei ([Loeza et al., 2020](#)), likewise, in Yucatan Peninsula [Martínez & Medina \(2011a\)](#) found a prevalence of 81.8 %, in the Jalisco State [Tapia-González et](#)



*al. (2017)* reported a prevalence of 100 %, similarly in a study conducted in the northwest of the Mexican Republic *González et al. (2020)* reported the prevalence of *Nosema apis* in Mexicali at 56.58 %, Tijuana with 58 % and San Luis Río Colorado with 7.89 %; and as for the presence of *Nosema ceranae* it was reported in the Mexicali Valley with a prevalence of 15.79 %, Ensenada with 2.63 %, Tijuana with 1.32 % and San Luis Río Colorado with 1.32 %.

Likewise, the presence of *Nosema* has been reported in different parts of the world such as Spain where they found a prevalence of 55.17 %, in Costa Rica 67.5 %, in Chile 78.26 %, in Argentina 91.5 % (*Calderón & Sánchez, 2011; Hinojosa & Gonzalez, 2004; Tiranti et al., 2017; Pacini et al., 2016; Medina-Flores et al., 2014a*), according to the above mentioned it is possible that *Nosema* is found worldwide possibly due to the resistance of the microsporidium to low and high temperatures, which has allowed it to thrive in different regions of the world (*Forsgren & Fries, 2010*).

The present investigation reports the absence of *A. woodi* in the study regions (Hopelchén- Sihochac), therefore, the results coincide with those obtained by (*Martínez & Medina, 2011a; Martínez-Cesáreo et al., 2016; Loeza et al., 2020*) where they indicate the absence of the tracheal mite in Yucatan, State of Mexico and Nayarit, in this sense, we agree with *Loeza et al. (2020)* who indicate that the absence of the mite can be attributed to its unadaptability to the different tropical regions of Mexico, however, this pattern cannot be clarified because last reports on infestation levels date from 1985 to 1986 in northeastern Mexico (*Eischen, 1987*).

## CONCLUSION

The present study confirms the presence of Varroa in 98.5 and 100 %, and *Nosema* in 85.9 and 88.0 %, and a simultaneous Varroa and *Nosema* infestation of 92.2 %. The presence of *A. woodi* was not found in any of the regions evaluated, the present study gives an overview of the lack of technification, as well as little or no knowledge on the proper use of treatments for the control of bee diseases, in this sense, a research door is opened in the field of bee health in Campeche state, Mexico.



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#### Errata Erratum

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