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Article

Occurrence of varroasis and nosemosis in *Apis mellifera* hives in Tomalá municipality, Honduras

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Abstract: Beekeeping in Honduras represents a significant activity within the livestock sector, facing threats from pests and diseases that hinder its development and productivity. **Objective:** To determine the parasitic load of *Varroa destructor* and *Vairimorpha* (*Nosema*) spp. in *Apis mellifera* hives in Tomalá municipality, Lempira department, Honduras, and its association with hygienic behavior, productivity, and the presence of other pests. **Materials and methods:** The study analyzed 57 hive samples collected from 19 apiaries. The infestation rate of *V. destructor* was determined. The average infection rate of *Vairimorpha* spp. was assessed using the Cantwell method. Associations between parasitic loads and productivity, hygienic behavior, and other pests were evaluated through stratified analysis based on the application (or not) of treatment against *Varroa* spp. Associations were determined using the non-parametric Mann-Whitney U and Kruskal-Wallis tests. **Results:** Infestation rates for *V. destructor* were 3.48% in adult bees and 6.82% in larval stages. The use of chemical treatment showed no association with the *V. destructor* infestation rate in either larvae or adults ($p \geq 0.05$). However, the presence of the small hive beetle (SHB) was associated with higher *V. destructor* infestation in larvae ($p < 0.007$) within untreated hives. The *Vairimorpha* spp. infection level was classified as very light, with an average of 5×10^4 spores per sample. The *Vairimorpha* spp. load showed no association with the variables studied ($p \geq 0.05$). **Conclusion:** The results indicate that varroosis is an important disease in Tomalá municipality, Lempira department, Honduras, associated with hygienic behavior and the presence of SHB in hives. Nosemosis exhibited a very light level of infection.

Keywords: infestation; Africanized bee; animal health; mite; Central America

1. Introduction

Beekeeping is an important economic activity worldwide, since honey and bee-derived byproducts are traded. Additionally, the demand for food grows along with the human population, leading to an increase in plant production, and beekeeping plays a fundamental role through pollination [1]. This economic activity has minimal environmental impact. In Honduras, during the period 2016–2019, the value of honey exports grew by 14.1%, and in terms of volume, a global increase of 43.4% was recorded, rising from 0.47 t in 2016 to 1.39 t in 2019 [2].

Varroasis is an important parasitic disease in beekeeping caused by the ectoparasite *Varroa destructor* [3], which is classified into two haplotypes: Korean (K) and Japanese (J). Of these, the K haplotype has been identified in the region in a study conducted in Nicaragua [4]. This ectoparasite can lead to colony collapse by reproducing in brood cells, where it feeds on larval hemolymph, or during the phoretic phase, where it feeds on the bees' fat bodies [5]. Infestation by *V. destructor* and associated viral coinfections are among the main reasons for honeybee population decline [6]. *V. destructor* infestation shows a negative correlation with honey production, with potential losses exceeding 50% [7].

In this study, we determine the association between *V. destructor* infestation rates and hive hygienic behavior, as this has been shown to be a factor of genetic resistance in bee breeding programs by reflecting social immunity against the parasite [8]. Another factor that may also be related to *V. destructor* infestation rates is the presence of other pests such as the small hive beetle (SHB) [9] or ants [10], which may weaken the hive or modify social behavior.

Nosemosis is among the most common pathologies affecting adult honeybees, caused by the microsporidia *Vairimorpha* (*Nosema*) *apis* and *V.* (*Nosema*) *ceranae* [11,12]. Nosemosis may be asymptomatic or can cause significant damage [13]. *V. ceranae* infects worker bees, induces premature maturation of nurse bees, and creates colony imbalance [14]. When the queen can produce sufficient offspring to compensate for worker loss, the infection may remain asymptomatic. However, when over 80% of bees are infected with more than 10 million spores, colony collapse occurs as the queen cannot lay enough eggs, reducing the number of nurse and forager bees [13].

Honduras has previously experienced varroosis outbreaks. In 2019, apiaries in Tomalá municipality exhibited high bee mortality at hive entrances, prompting field visits for sample collection and tau-fluvalinate treatment in some local apiaries. However, periodic sampling remains necessary to ensure timely surveillance. Therefore, this study aimed to determine the parasitic loads of *V. destructor* and *Vairimorpha* (*Nosema*) spp. associated with hygienic behavior, productivity, and presence of other pests in *Apis mellifera* colonies in Tomalá municipality, Lempira department, Honduras.

2. Materials and methods

2.1. Study site

The research was conducted in *A. mellifera* apiaries managed by producers in Tomalá municipality, Lempira department, located in Western Honduras. The area features an altitude ranging between 600 m and 1800 m above sea level with irregular terrain [15]. The climate presents two annual seasons: a dry season from November to May, and a rainy season from June to October [16].

2.2. Population and sample

Samples were collected in 2021 between July and September, corresponding to the post-harvest period characterized by reduced flowering and increased parasitosis. In Tomalá Municipality, a total of 311 Africanized bee colonies were documented, distributed across nineteen apiaries managed by an equal number of beekeepers. For sample size determination, three random colonies were selected from each of the nineteen apiaries, totaling 57 colonies (18.32% of Tomalá's colonies). This sampling approach aligns with recommendations from the Manual of Honey Bee Diseases published by the Inter-American Institute for Cooperation on Agriculture [17], which suggests examining 15% to 20% of colonies for representative *Varroa* spp. studies.

Data collection forms were completed during sampling through direct observation and beekeeper interviews. The evaluated variables included annual honey production (kg/colony), presence of other pests (ants and SHB), artificial feeding, and application of chemical treatments (Tau-fluvalinate) for *V. destructor* control.

2.3. Determination of *Varroa destructor* infestation rate in bee larvae

To determine the *V. destructor* infestation rate in bee larvae, a brood frame with the highest percentage of sealed brood was selected from each colony. Using forceps, larvae were extracted from cells to quantify affected and unaffected individuals. The larval infestation rate was calculated using Equation (1) [18].

$$\text{Infestation in larvae} = \frac{\#\ \text{Number of affected cells}}{\#\ \text{Total number of cells counted}} \times 100 \tag{1}$$

2.4. Determination of *Varroa destructor* infestation rate in adult bees

The protocol described by De Jong et al. [19] was used to determine the *V. destructor* infestation rate in adult bees. For this purpose, a frame with a high number of adult bees on both sides was selected and inspected to ensure the absence of the queen bee. Bees were collected by sweeping a pre-labeled empty jar vertically across each comb surface to allow bee entry. The jar was then closed, shaken, and the process repeated until 200–300 bees were obtained. Samples were stored at room temperature and analyzed within 2 h [20]. After removal from the apiary, the counting process was performed using two 4-inch PVC adapters (one with external threading and one with internal threading) and a fine mesh fabric placed between the threaded connectors. Liquid detergent was added, and groups of bees were washed over the fabric. Subsequently, both the bees and *V. destructor* mites found in the sample were counted to calculate the infestation rate using Equation (2), according to Valladares et al. [21].

$$\text{Infestation in adults} = \frac{\#\ \text{Number of Varroa mites}}{\#\ \text{Number of bees}} \times 100 \tag{2}$$

2.5. Determination of *Vairimorpha* (*Nosema*) spp.

The determination of *V.* (*Nosema*) spp. was carried out according to the procedure recommended by the World Organisation for Animal Health (WOAH), as per Duquesne et al. [22], to detect and evaluate the mean spore infection rate based on microscopy. The abdomens of 60 bees were crushed with a mortar in ultrapure water at a ratio of 1 mL per bee. The suspension was filtered through two layers of muslin and centrifuged for six minutes to remove large debris and purify the spores. Subsequently, the pellets were resuspended in a homogeneous suspension to restore the initial dilution of 1 mL per bee. The samples were placed in a calibrated hemocytometer (Malassez counting chamber) and microscopic examination was performed to count the spores [22].

2.6. Determination of hygienic behavior (HB)

For the determination of hygienic behavior (HB), the pin-kill test (P) was applied. Using an entomological pin, 100 pupae were punctured and sacrificed in either horizontally aligned cells or in a ten-by-ten pupae grid on selected combs from each colony. For measuring the degree of HB, a colony was considered hygienic when it removed 80% or more of dead brood within 24 h. The percentage of hygienic behavior was determined using Equation (3), as described by Newton and Ostasiewski [23].

$$\text{Hygienic behavior} = \frac{\#\ \text{Number of pupae removed}}{\#\ \text{Total punctured treated cells}} \times 100 \qquad (3)$$

2.7. Statistical analysis

A descriptive analysis of the data was performed, including measures of central tendency and the Shapiro-Wilk test. Since normality was not observed, the median was used for both the *V. destructor* infestation rate and *Vairimorpha* spp. parasitic load. To determine their association with hygienic behavior and honey production, Spearman's correlation analysis was applied. For assessing associations with categorical independent variables, non-parametric tests (Mann-Whitney U or Kruskal-Wallis) were employed. All statistical tests for association determination were conducted independently for hives with and without prior treatment history. Data were stored and analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.

3. Results

The infestation rates for *V. destructor* in the dispersal and larval phases were 3.48% and 6.82%, respectively. Previous application of chemical treatment showed no association with *V. destructor* infestation rates in either larvae or adults ($p \geq 0.05$). The median *V. destructor* infestation in the larval phase was 7.06% in hives that had not received treatment against *V. destructor*, a value similar ($p \geq 0.05$) to the 6.52% infestation observed in treated hives. For the dispersal phase, infestation was 2.94% in untreated hives, similar to the 4.88% observed in treated hives ($p \geq 0.05$) (**Table 1**). The median value obtained for *Vairimorpha* spp. was 50,000 (95% CI: 10,000–260,000) spores per sample.

Table 1. Infestation rate for *Varroa destructor* in *Apis mellifera* from apiaries in Tomalá municipality, Honduras. 2021.

Stratification by previous treatment application	Statistic	% *V. destructor* infestation in larvae	% *V. destructor* infestation in adults
No treatment	*n*	36	36
	Median	7.06	2.94
	Interquartile range (IQR)	2.83–9.04	1.23–4.91
With treatment	*n*	21	21
	Median	6.52	4.88
	Interquartile range (IQR)	3.10–8.00	1.70–6.72
Comparison	Asymptotic significance (2-tailed)	0.697	0.208

* According to the Mann-Whitney U test.

The Shapiro-Wilk significance values for *Vairimorpha* spp. spore load, and *V. destructor* infestation in larval and dispersal phases were all <0.05, indicating non-parametric distributions. The Kruskal Wallis test revealed that in hives treated against *V. destructor*, the presence of pests such as ants and SHB was not associated with *V. destructor* infestation in adult bees or with *Vairimorpha* spp. spore counts ($p \geq 0.05$). However, in untreated hives with PEC presence, the median *V. destructor* infestation in larvae was 9.80%, significantly higher than the 7.23% observed in hives with ants and the 2.04% in pest-free hives ($p < 0.05$). This association was not found in hives that had received chemical treatment against *V. destructor* (**Figure 1**).

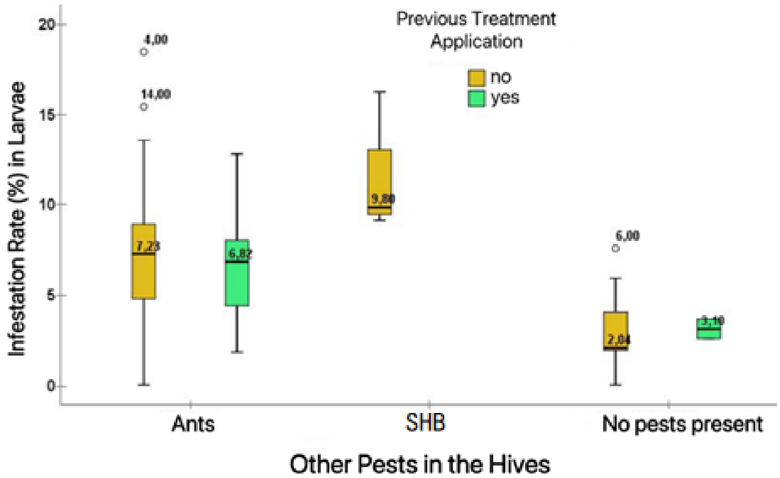

Figure 1. Infestation rate of *Varroa destructor* in *Apis mellifera* larvae in Tomalá hives according to pest presence, Honduras. 2021.
SHB: The small hive beetle.

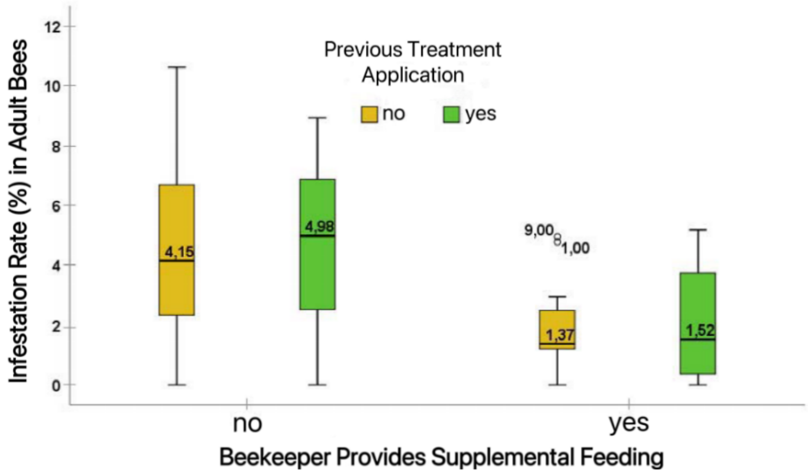

Figure 2. *Varroa destructor* infestation rate in *Apis mellifera* adults according to the practice of artificial feeding in hives in Tomalá, Honduras. 2021.

No association was found between the feeding variable and the infestation rate of *V. destructor* in larvae, nor with the *Vairimorpha* spp. load ($p \geq 0.05$), regardless of previous treatment application. However, within hives that had not received treatment, the *V. destructor* infestation rate in adult bees that did not receive feeding was 4.15%, a value higher than the 1.37% observed in hives receiving artificial feeding ($p = 0.010$). This association was not observed in hives that had received previous treatment against *V. destructor* (**Figure 2**).

Honey production showed no association ($p \geq 0.05$) with infestation rates in either larvae or adult bees, regardless of previous treatment application (**Table 2**). Additionally, no association was observed between *Vairimorpha* spp. parasitic load and honey production ($p \geq 0.05$).

Table 2. Spearman correlation between the infestation rate of *Varroa destructor* in *Apis mellifera* and honey production in apiaries in Tomalá municipality, Honduras. 2021.

Variables	Statistics	Honey production hive/year (kg)	% *V. destructor* infestation in larvae	% *V. destructor* infestation in adults
Honey production hive/year (kg)	Correlation coefficient	1	−0.147	−0.099
	Significance (2-tailed)*	.	0.394	0.564
	N	21	36	36
% *V. destructor* infestation in Larvae	Correlation coefficient	0.033	1	−0.041
	Significance (2-tailed)*	0.889	.	0.811
	N	21	21	36
% *V. destructor* infestation in Adults	Correlation coefficient	−0.176	0.172	1
	Significance (2-tailed)*	0.446	0.455	.
	N	21	21	21

*According to the Spearman's Rho test.
Dark gray cells correspond to hives with treatment, and light gray cells to hives without treatment.

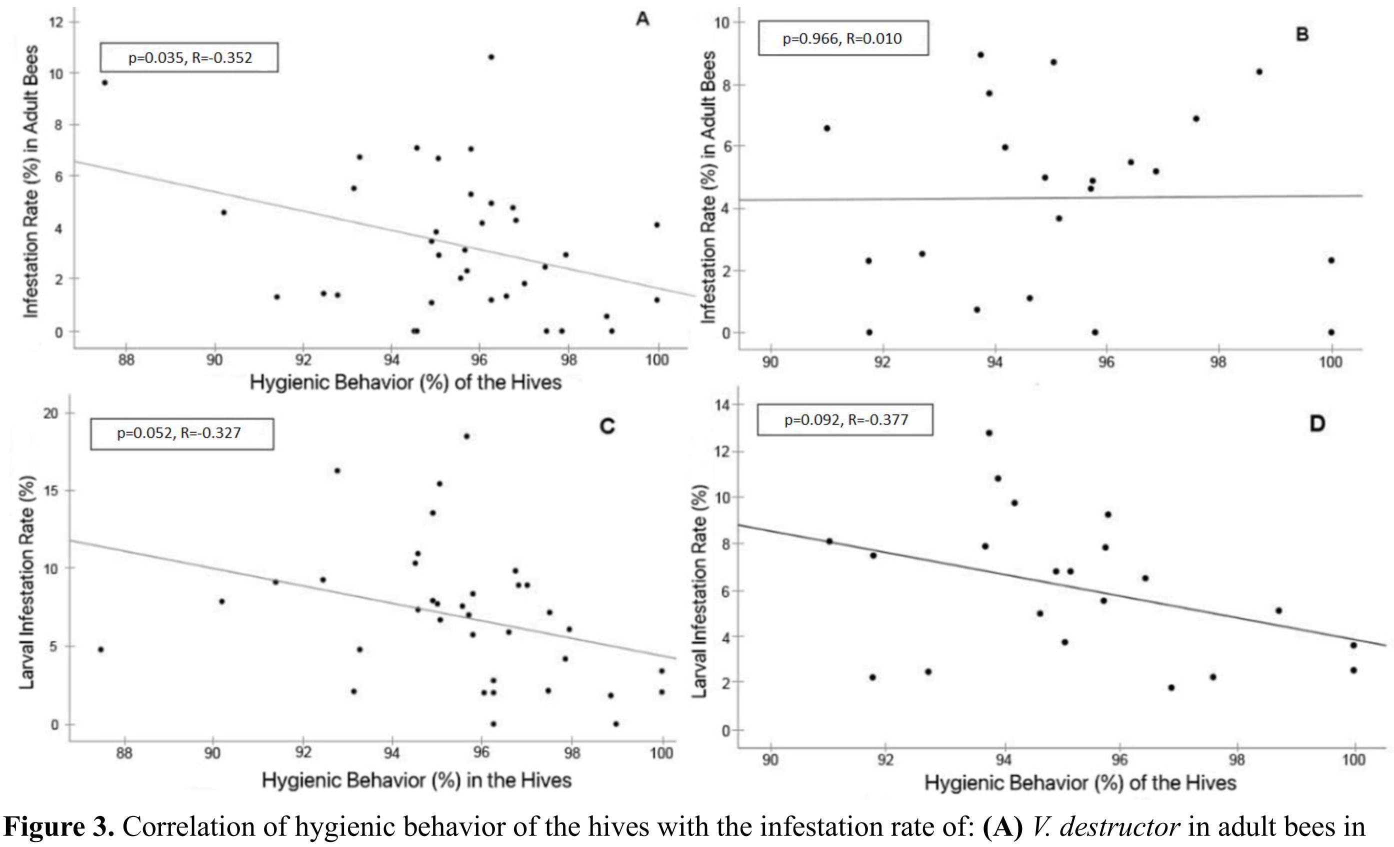

Figure 3. Correlation of hygienic behavior of the hives with the infestation rate of: **(A)** *V. destructor* in adult bees in untreated hives; **(B)** the infestation rate of *V. destructor* in adult bees in treated hives; **(C)** the rate of *V. destructor* infestation in larvae from untreated hives; **(D)** the infestation rate of *V. destructor* in larvae from treated hives. Tomala, Honduras. 2021.

In hives that had received treatment, hygienic behavior showed a weak but significant negative correlation with *V. destructor* infestation rates in adult bees ($R = -0.352$, $p = 0.035$). In contrast, hives that had not received treatment showed no correlation between hygienic behavior and adult bee infestation rates ($R = 0.010$, $p = 0.966$). For larval infestation rates, a weak correlation was found between hygienic behavior and *V. destructor* infestation in both treated and untreated hives, though this was not statistically significant ($p \geq 0.05$) (**Figure 3**).

4. Discussion

The infestation rate of *V. destructor* in adult bees was greater than 5% but less than 10%, classifying it within a moderate infestation range that requires treatment for mite control [24]. However, no significant difference was observed between *V. destructor* infestation rates in adults and larvae of hives treated and untreated with Tau-fluvalinate, suggesting either mite resistance or an inadequate treatment protocol. Tau-fluvalinate was previously considered effective against *V. destructor*, but indiscriminate use has led to resistance development, including cross-resistance among pyrethroid-based products. This is associated with a leucine-to-valine substitution mutation at position 925 (L925V) in the voltage-gated sodium channel [25].

In Tomalá municipality in 2019, high honeybee mortality was reported; however, varroosis in Honduras has never caused a serious health problem, and it is likely associated with the absence of viral diseases. The infestation rate found in adult bees in this study is higher than that observed in neighboring countries of Honduras, such as Nicaragua, where infestation rates close to 3% were reported for the period from 2009 to 2015 [26]. Meanwhile, another study in the same country reports a national infestation of 4.3% for the period from 2012 to 2016 [4]. The elevated values in the *V. destructor* infestation rate in the hives are indicative of deficiencies in apiary management. As reflected in a study conducted in Cuba, where they found a medium infestation rate (5.01%–10.00%) in hives that had not received any treatment against the mite [24].

In this study, it was found that in hives that had not received treatment, the *V. destructor* infestation rate in larvae was higher when SHB was present compared to those without pests (**Figure 1**). Other studies have shown that *V. destructor* infestation can facilitate the entry of other parasites, as found in a study in Madagascar, where it was observed that SHB was only present in hives with *V. destructor*. This may be because the mites could reduce the innate immune system of the bees, making mite-infested colonies more vulnerable to other pathogens. However, it is also possible that the presence of SHB facilitates *V. destructor* infestation [27].

Hive management is essential to maintain low infestation of *V. destructor*; a common practice is providing supplemental feeding during periods of low floral availability [28]. In this study, it was found that in untreated hives, the *V. destructor* infestation rate in adult bees was higher in colonies that did not receive artificial feeding. The primary objective of artificial feeding is to prevent colony decline due to lack of nectar and pollen sources, enabling them to maintain immunity against various pathogens [29]. Additionally, it has been reported that sugar feeding mixed with plant materials, such as mint, cinnamon, and chamomile, can help control bee pathogens, including Varroa mites [29]. Furthermore, it has been demonstrated that protein feeding can increase the lifespan of bees parasitized by *V. destructor* [30].

The correlation results between the *V. destructor* infestation rate in adult bees and the hygienic behavior of untreated colonies showed a negative correlation. It has been observed that a high degree of Africanization in the region's bees is associated with their excellent hygienic behavior [31]. In honeybees, this behavior reflects social immunity against parasites and diseases. Therefore, it is considered one of the main genetic resistance factors in selective bee breeding programs, as it is one of the most promising ways to reduce *Varroa* mite infestation [8].

In this study, low intestinal loads of *Vairimorpha* spp. were found, corresponding to a very light classification (1×10^4–1×10^6 spores/bee), similar to results from a study conducted in Costa Rica that reported 26% of hives with very light loads (and 18.0% with light loads (1×10^6–5×10^6 spores/bee)) [32]. Although low values were found in the parasitic load of *Vairimorpha* spp.

It is advisable to maintain periodic sampling for proper monitoring and identification of circulating species, as nosemosis can be caused by *N. apis* which is considered to be of low virulence, but can also be caused by *N. ceranae* (Type C nosemosis) with higher virulence, which has displaced *N. apis* as the main cause of nosemosis in honeybees, potentially endangering the survival of *A. mellifera* in Honduras [33,34].

5. Conclusions

Varroosis in the apiaries of Tomalá municipality showed an infestation rate in adult *A. mellifera* bees within the medium range (5.01%–10.00%), indicating the need for control measures such as the selection and breeding of hives with high hygienic behavior, which was associated with lower infestation rates. Additionally, artificial feeding, as well as the absence of other pests, was associated with reduced infestation. The infestation level for *Vairimorpha* spp. was in the "very light" range (less than 100,000 spores per sample). The parasitic load showed no association with the study variables; however, species identification in Tomalá's apiaries remains necessary.

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