



Fluctuation of *Nosema* spp. infection levels in honey bees under tropical conditions in Mérida, Yucatán



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Abstract:

Nosemosis is a disease of adult bees, caused by the microsporidium *Nosema* spp., which affects the midgut of bees. High levels of infection in a colony can lead to population reduction, low honey yield, and colony loss. To identify the period with the highest level of *Nosema* spp. infection in Africanized bee colonies and determine its fluctuation, a total of 361 samples of bees were collected from 19 Africanized bee colonies between September and May. The data on the number of spores in adult bees were correlated with temperature, relative humidity, and rainfall. The number of spores per bee varied during the study period. The highest number was recorded in September with $857,781 \pm 2.23$ spores per bee, followed by October and November with $542,188 \pm 1.03$ and $217,813 \pm 0.32$ spores per bee, respectively. A correlation was observed between the number of spores per bee, relative

humidity, and rainfall, indicating that climatic conditions may influence the disease; therefore, more studies are required over the years to monitor its behavior.

Keywords: *Nosema* spp., *Apis mellifera*, infection, climatic factors.

Received: 12/05/2023

Accepted: 11/09/2025

Beekeeping, like any primary activity, is threatened by various diseases and parasitoses that affect the development of bee colonies. Nosemosis is caused by the microsporidium *Nosema* spp., which was recently reclassified as *Vairimorpha*⁽¹⁾; however, this reclassification was not widely recognized by other scientists in the beekeeping area worldwide⁽²⁾; therefore, while the controversy is resolved, in this paper, it will continue to be maintained as the genus *Nosema*. The microsporidium infects the epithelial cells of the midgut of different bee castes, altering the digestion and absorption of nutrients in workers, drones, and queens, which causes malnutrition in bees due to the inability to assimilate nutrients⁽³⁾. The damage it can cause is severe if the level of infection is high, such as population reduction, low honey yield, and colony loss. It is currently proven that the disease is negatively affecting beekeeping activity worldwide^(4,5).

For honey bees, it has been described that *Nosema apis* and *Nosema ceranae* are two species of microsporidia that cause nosemosis. Previously, *N. apis* was considered to be the only causative agent of nosemosis in Western *Apis mellifera* bees, that is, bees from the American continent⁽⁶⁾. Nevertheless, in 2006, it was identified that *N. ceranae* could also infect populations of *A. mellifera* from all over the world, not just the Asian bee, its original host⁽⁷⁾. *N. apis* is characterized by moderate virulence, to the extent that bee colonies can sometimes recover on their own under favorable environmental conditions⁽⁶⁾. In contrast, *N. ceranae* is more virulent; it has been associated with low honey production, as well as the weakness and mortality of bees in colonies, with no visible signs of the disease⁽⁸⁾.

Studies conducted in Europe showed that the prevalence and intensity of *Nosema* spp. infection in *A. mellifera* are not constant throughout the year, and they fluctuate between seasons and geographical regions, with high levels of infection in spring and autumn⁽⁹⁾. Likewise, in Serbia⁽¹⁰⁾ and in Switzerland and Turkey⁽¹¹⁾, the prevalence of *Nosema* spp. is not constant, and temperature can influence the incidence of nosemosis.

In North Asia⁽¹²⁾, *Nosema* spp. is present in areas with subarctic climates or warm summers, with no significant differences in the incidence of infection between the two climates. In

North America, high levels of *N. ceranae* infection were found in spring and summer, being detrimental to honey bees and colony productivity as long as infections exceeded one million spores per bee⁽⁵⁾. Similarly, in the United States of America, it was shown that the intensity of *Nosema* spp. infections in honey bee colonies varies according to the season of the year, with high levels of infection in spring and low levels in autumn⁽¹³⁾.

On the other hand, studies carried out in Costa Rica with Africanized bees found the presence of *N. ceranae* with a prevalence of 70.7 % and a range of 1 to 25 million spores per bee⁽¹⁴⁾; also, in the same country, it was found that the fluctuation of nosemosis is variable, with the highest number of spores per bee in July⁽¹⁵⁾.

In the case of Mexico, the fluctuation over the months has not been studied; research has been conducted regarding the intensity of the infection in certain areas and in different climates, but there has not been constant monitoring of fluctuations⁽¹⁶⁾. Under the tropical conditions of Yucatán, Mexico, it was observed that the infection caused by *N. ceranae* negatively affects the onset time and duration of foraging activity in bees of Africanized origin, as well as the longevity of the workers⁽¹⁷⁾.

Due to the above, the importance of nosemosis, and the few studies on the seasonal behavior of *Nosema* spp. in tropical climates and Africanized honey bees, the objective was to identify the period with the highest level of *Nosema* spp. infection in Africanized bee colonies, as well as to determine its fluctuation over nine months of the year (September-May).

The present study was conducted in an apiary of the Faculty of Veterinary Medicine and Zootechnics of the Universidad Autónoma de Yucatán, located in the town of Xmatkuil (20° 51' 51" N, 89° 36' 45" W), 15.5 km from the city of Mérida, Yucatán. The region has a warm sub-humid climate with rainfall in summer (A_{w0}), an average annual rainfall of 1,127 mm and 94 mm per month for the city of Mérida, an average annual temperature of 26.8 °C, and an average annual relative humidity of 78 %⁽¹⁸⁾.

Before starting the sampling, a preliminary diagnosis was made to determine the level of infection in all bee colonies, all of which were positive. Subsequently, bee samples were collected from 19 colonies with two bodies (a brood chamber and a super) housed in Langstroth hives, with naturally fertilized Africanized queens. Sampling was conducted in the morning every 14 d, from September to May, yielding a total of 361 bee samples. To determine the level of infection of each colony, between 100 and 150 adult bees were collected from the upper part of the second body or super. From each sample, 60 bees were taken to determine the presence of *Nosema* spp. and quantify the severity of the infection (number of spores/bee), counting the spores present in the digestive tract of the workers. According to Cantwell's technique, the analysis consisted of removing the abdomen of the 60 adult bees, which were placed in a mortar to which 60 mL of distilled water was added⁽¹⁹⁾.

The abdomens were macerated until a homogeneous mixture was obtained, which was then filtered through a sieve to remove impurities. To count the number of spores in the bee sample, a drop of the macerated solution was deposited in both grids of a Neubauer chamber and then observed under a compound microscope at 400x magnification. The results of the analysis were used to calculate the average level of infection per bee.

During the sampling period, monthly climatological data on relative humidity, rainfall, and environmental temperature were obtained from the Meteorological Observatory of the city of Mérida, Yucatán, provided by the National Water Commission (CONAGUA, by its Spanish acronym).

Data for the variables of average number of spores per bee, relative humidity, rainfall, and environmental temperature were correlated through a multivariate analysis using Pearson's correlation and a regression analysis via a linear model from the statistical package Statgraphics Plus 5.1[®] (2001).

According to the results of this study, it was observed that the level of disease infection was variable during the nine months of the study. It was observed that September was the month with the highest infestation record, with an average of $857,781 \pm 2.23$ spores per bee.

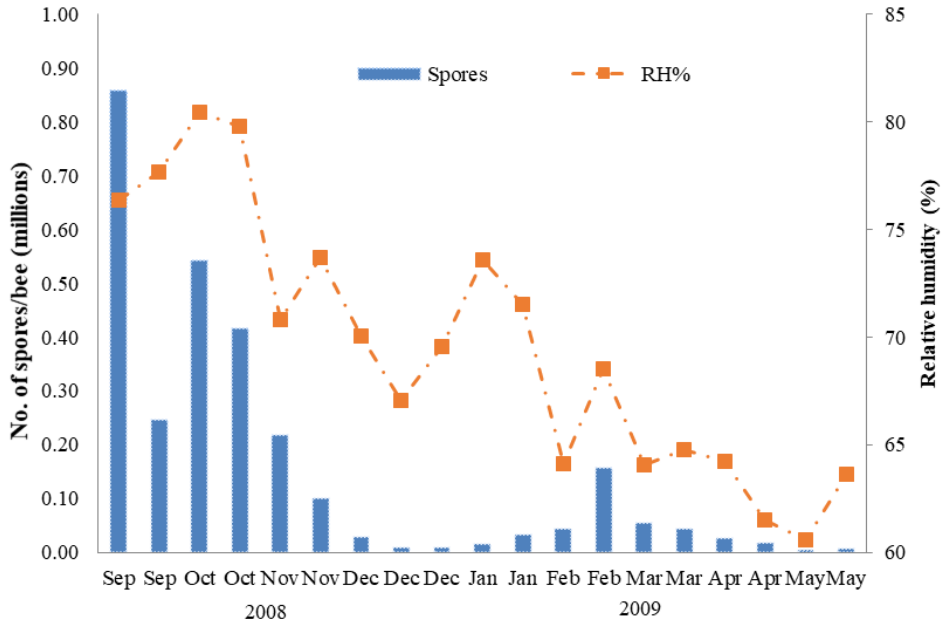
During October, there was an average of $542,188 \pm 1.03$ spores per bee, followed by November with an average of $217,813 \pm 0.32$ spores per bee, the month in which the level of infection began to decrease (Table 1).

Table 1: Number of *Nosema* spp. spores per bee, environmental temperature, relative humidity, and rainfall during the study period

	Spores/bee (millions) $\bar{X} \pm \bar{S}.D.$	Spores/bee Min - Max	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
September	0.86±2.23	50,000–9'147,500	28.9	76.4	3.2
September	0.25±0.20	19,000–722,500	28.3	77.6	11.3
October	0.54±1.03	120,000–4'355,000	26.7	80.4	3.9
October	0.42±0.75	57,500–3'190,000	26.7	79.8	1.2
November	0.22±0.32	3,750–1'332,500	24.0	70.8	0.0
November	0.10±0.06	47,500–272,500	23.5	73.7	0.3
December	0.03±0.05	5,000–192,500	22.3	70.1	0.0
December	0.01±0.00	5,000–17,500	24.1	67.1	0.4
December	0.01±0.01	2,500–47,500	25.3	69.6	0.0
January	0.02±0.03	0–127,500	25.0	73.6	0.4
January	0.03±0.09	0–395,000	21.5	71.5	0.8
February	0.04±0.07	0–192,500	23.0	64.1	0.0
February	0.16±0.58	0–2,550,000	25.1	68.5	0.0
March	0.06±0.10	0–335,000	24.1	64.1	0.1
March	0.04±0.06	0–252,500	26.3	64.8	0.3
April	0.03±0.05	0–200,000	27.8	64.2	0.0
April	0.02±0.03	0–110,000	28.5	61.5	0.0
May	0.00±0.01	0–25,000	29.8	60.6	0.0
May	0.01±0.01	0–22,500	29.6	63.6	0.0

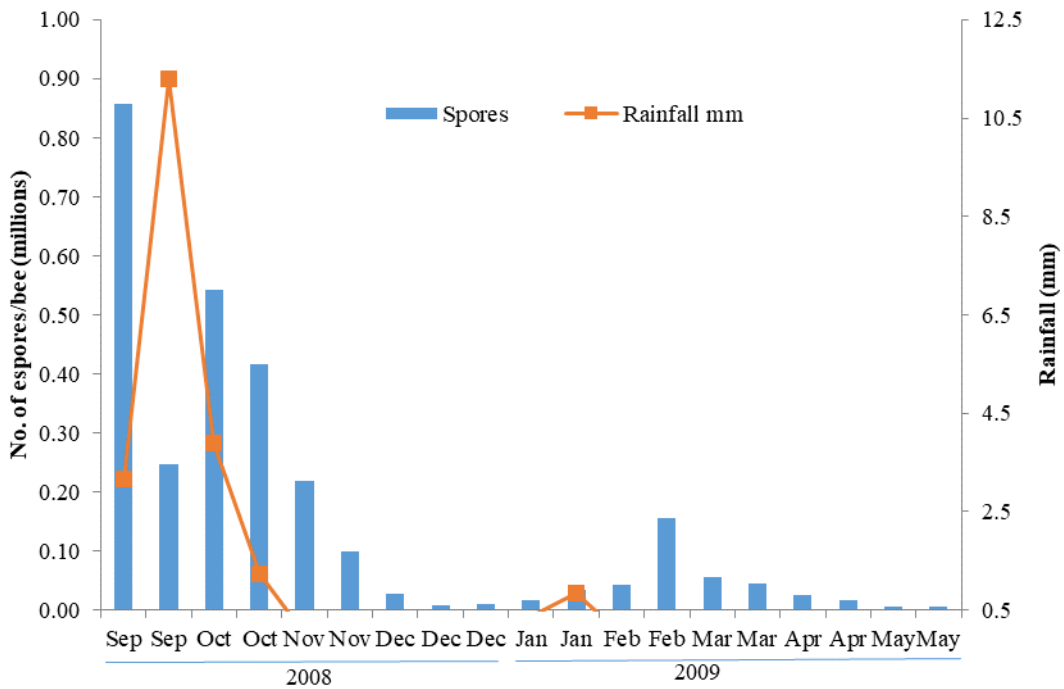
The fluctuation of *Nosema* spp. infection levels in Africanized bee colonies showed a positive correlation with relative humidity. There was an increase in the average number of spores per bee as relative humidity increased (Figure 1).

Figure 1: Number of *Nosema* spp. spores per bee in Africanized bee colonies and the percentage of relative humidity



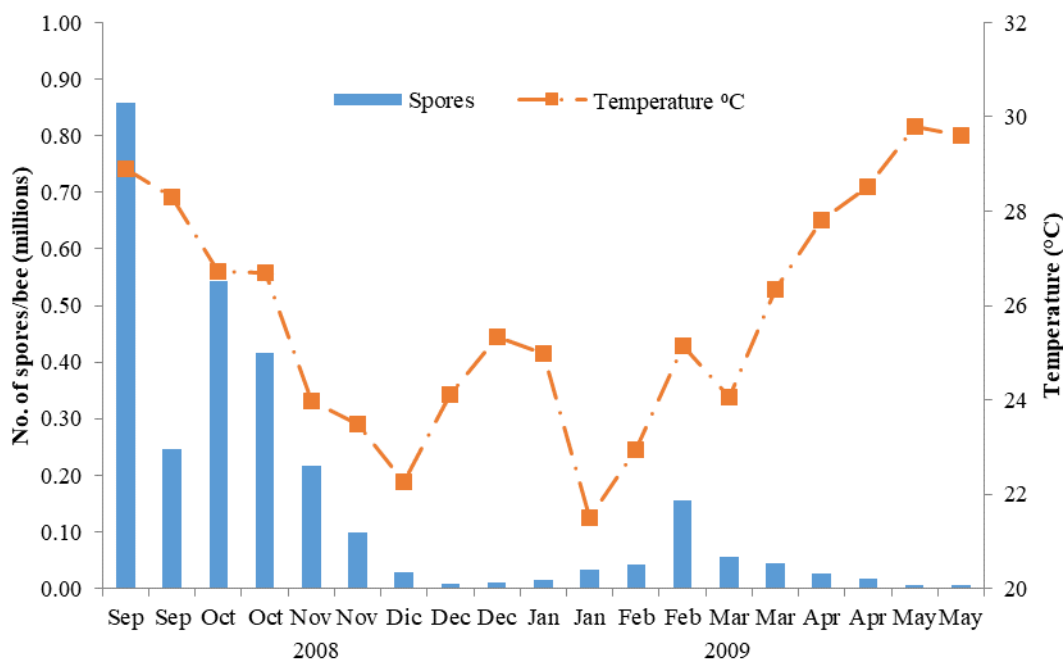
Similarly, it is possible to observe an influence of rainfall on the increase in the average number of spores per bee, since the number of spores increased as rainfall increased, contributing to their spread and multiplication (Figure 2).

Figure 2: Number of *Nosema* spp. spores per bee and rainfall (mm)



Regarding the environmental temperature recorded during the study period, it showed no correlation with the levels of *Nosema* spp. infestation in Africanized bee colonies under the environmental conditions of the site where the work was conducted (Figure 3).

Figure 3: Number of *Nosema* spp. spores per bee and environmental temperature (°C)



Pearson’s correlation analysis, used to determine the relationship between the number of *Nosema* spp. spores per bee and the variables of relative humidity, rainfall, and environmental temperature, revealed a positive correlation for relative humidity and rainfall (Table 2).

Table 2: Correlation of the number of *Nosema* spp. spores with relative humidity, rainfall, and environmental temperature

	Relative humidity	Precipitation	Temperature
Number of spores	0.698	0.450	0.295
Level of significance	0.0009	0.05	0.22
r ² (%)	48.6	20.2	8.67
n	19	19	19

r²= spore variability; n= number of samples.

Regarding the regression analysis, the correlation coefficient of the number of spores and relative humidity presented a moderately strong relationship, with a statistically significant

relationship ($P>0.0009$); in the case of precipitation, it indicated a relatively weak relationship, with a statistically significant relationship ($P>0.05$); and for temperature, it revealed a relatively weak relationship, without a statistically significant relationship ($P>0.22$).

The result obtained in the present study showed that the level of *Nosema* spp. infection is highly variable during most of the year; these results coincide with several studies^(11,20,21) where the fluctuation of nosemosis varies throughout the year, which reflects a variation throughout the months, even with different climatic conditions and bee subspecies.

Under the conditions of the region, specifically the area and months where the study was conducted, the highest infection levels occurred in the period of greater rainfall and high relative humidity, regardless of the amount of precipitation recorded in the evaluated months and historical records. This indicates that, during the rainy periods that corresponded to September and October, the spread and multiplication of spores among the bees of the colonies could be facilitated, in addition to the fact that outbreaks of the disease in the colonies may increase due to the scarcity of the flow of nectar and pollen during these periods; therefore, it is necessary to continue monitoring throughout the year to observe its behavior in the months not studied and to cover a greater number of samples to rule out differences in infection levels due to errors in the diagnostic technique, sampling, and biotic and abiotic factors.

Similarly, McAfeeet *et al*⁽²²⁾ found high levels of infection in the months of greatest rainfall, attributing the increase to limited foraging by bees, so that the disease can remain dormant throughout the year and manifest itself in sudden outbreaks and rapid contagion among bees, after prolonged periods of overcrowding as a result of cycles of persistent rainfall, cold, and intense winds⁽²³⁾.

Another study conducted in Costa Rica⁽¹⁵⁾ with Africanized bees found that the highest number of spores per bee occurred in July, October and November, spores were present in smaller quantities; however, this study only covered five months (July-November). This is contrary to the present research, which was conducted over nine months, finding a greater presence of spores between September and October, and from November, they tend to decrease. This pattern of spore decline is likely due to tropical climate conditions, as winter in the region does not reach very low temperatures. Therefore, there are no favorable conditions for the dissemination and multiplication of spores because the foraging and activities of bees are not limited. Bees can fly to evacuate without major problems; likewise, the risk conditions for contagion and dispersion of spores among the colony's individuals do not exist. Another factor to take into consideration is the origin of Africanized bees, which have various mechanisms of resistance to certain diseases⁽²⁴⁾; therefore, infection levels can be kept under control, compared to what happens with bees of European origin in countries

with extremely cold climates, reporting a disease more frequently and high prevalences of *N. apis*⁽¹⁴⁾, which is because cleaning flights are unpredictable and short, as well as due to the stress caused by low temperatures during the winter⁽¹⁵⁾.

Additionally, it must be considered that, when high prevalence and high levels of nosemosis infection are found, it may be due to the presence of the *N. ceranae* spore, which is a highly pathogenic disease that has parasitized honey bees, affecting Africanized bees; nevertheless, even with high levels of infection, bee colonies may or may not present clinical signs of the disease⁽²⁴⁾. For the present study, the maximum detected range of spores per bee was up to nine million spores per bee, so these levels are possibly due to the presence of *N. ceranae* in the region⁽¹⁹⁾; however, due to the type of bees (Africanized), the colonies may be affected, but also present greater resistance to diseases⁽²³⁾.

Under tropical climate conditions, it is crucial to know the times when the highest levels of infection occur in order to take preventive and control measures against this disease, providing some treatment when infection levels exceed one million spores per bee⁽⁵⁾; therefore, it is essential to initiate training programs for beekeepers.

In conclusion, nosemosis prevails during the study period, corresponding to September to May, and there is variability in the average number of spores per bee, which can be influenced by climatic conditions, as high levels of infection occurred during periods of greater rainfall. Nonetheless, high levels of infection could cause severe damage to the colony's population. Therefore, it is suggested to carry out more studies over several years, encompassing a larger number of samples and areas, to rule out differences in infection levels resulting from various biotic and abiotic factors.

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