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Nosemosis in Africanized Honey Bee Colonies (*Apis mellifera*) in the Tropical Conditions of Costa Rica: *Nosema apis* or *Nosema ceranae*

Nosemosis en colmenas de abejas africanizadas (*Apis mellifera*) en las condiciones tropicales de Costa Rica: *Nosema apis* o *Nosema ceranae*

Nosemose em colméias de abelhas africanizadas (*Apis mellifera*) nas condições tropicais da Costa Rica: *Nosema apis* ou *Nosema ceranae*

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Abstract

The presence of nosemosis in Africanized honey bees in Costa Rica was studied**.** A total of 75 samples of adult bees from different country regions were selected for molecular diagnosis of nosemosis. Prior to PCR tests, *Nosema* spp. spores were morphologically identified in most of the bee samples using a light microscopy at 40x magnification. According to molecular analyses, most of the bee samples were found to be infected with *Nosema ceranae*. However, colonies showed no clinical signs of infection at any time during the sampling period, none of them being infected with *Nosema apis*. Surprisingly, 29.3% of the bee samples tested PCR negative to nosemosis. The origin of the bee samples collected from apiaries located in four of the seven provinces of Costa Rica showed the microsporidium is widely spread throughout the main beekeeping areas of the country. The pathological consequences of *N. ceranae* in Africanized honey bee colonies have not been well determined. Because of reports of honey bee colony losses in Europe related to microsporidian infections, the virulence of *N. ceranae* in Africanized honey bees needs to be studied.

Keywords: *Nosema apis, Nosema ceranae*, nosemosis, Africanized honey bees, Costa Rica

Resumen

Se estudió la presencia del microsporidio *Nosema* spp. en colmenas de abejas africanizadas en Costa Rica. Se seleccionaron un total de 75 muestras de abejas adultas de diferentes zonas apícolas del país, para el diagnóstico molecular de nosemosis. Previamente a la prueba de PCR, las esporas de *Nosema* spp. se identificaron morfológicamente con el microscopio de luz a un aumento de 40x. Con base en el análisis molecular, se determinó que la mayoría de abejas estaban infectadas con *Nosema ceranae*, aun cuando las colmenas no mostraban signos clínicos de la infección durante el periodo de muestreo. Por otra parte, ninguna de las muestras estaba infectada con

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Nosema apis. Un hallazgo para resaltar es que un 29.3% de las muestras de abejas resultaron negativas a nosemosis mediante el examen de PCR. El origen de las abejas, las cuales se colectaron de apiarios ubicados en cuatro de las siete provincias de Costa Rica, indica que el microsporidio *N. ceranae* está ampliamente distribuido en las principales zonas apícolas del país. Aún no se conoce con exactitud las consecuencias patológicas de la presencia de *N. ceranae* en colmenas de abejas africanizadas. Sin embargo, debido a la pérdida de abejas melíferas reportada en Europa, relacionada a infecciones de microsporidios, la virulencia de *N. ceranae* en abejas africanizadas debe ser estudiada.

Palabras claves: *Nosema apis, Nosema ceranae*, nosemosis, abejas africanizadas, Costa Rica

Resumo
Foi estudada a presença do microsporídio *Nosema spp.* em abelhas africanizadas na Costa Rica. Um total de 75 amostras de abelhas adultas de diferentes regiões do país foram selecionadas para o diagnóstico molecular de nosemose. Antes dos testes de PCR, os esporos de *Nosema spp.* foram identificados morfológicamente na maioria das amostras de abelhas usando microscopia de luz com aumento de 40x. De acordo com análises moleculares, a maioria das amostras de abelhas estavam infectadas com *Nosema ceranae*. No entanto, as colônias não apresentaram sinais clínicos de infecção em nenhum momento durante o período da amostragem. Por outro lado, nenhuma das amostras estavam infectadas com *Nosema apis*. É válido ressaltar que 29,3% das amostras de abelhas testaram negativo no exame de PCR para nosemose. A origem das amostras das abelhas coletadas em apiários localizados em quatro das sete províncias da Costa Rica mostrou que o microsporídio está amplamente distribuído nas principais áreas apícolas do país. As consequências patológicas de *N. ceranae* em colônias de abelhas africanizadas ainda não é bem conhecida. Entretanto, devido a relatos de perdas de colônias de abelhas na Europa relacionadas a infecções por microsporídios, a virulência de *N. ceranae* em abelhas africanizadas precisa ser estudada.

Palavras-chave: *Nosema apis*, *Nosema ceranae*, nosemose, abelhas africanizadas, Costa Rica

Introduction

Nosemosis is the most widespread of adult bee diseases causing significant economic losses to beekeepers (Giersch et al., 2009). Honey bees, *Apis mellifera* (Hymenoptera: Apidae), are parasitized by two microsporidians, *Nosema apis* (Zander) (nosemosis type A) and *Nosema ceranae* (Fries) (nosemosis type C) (Fries et al., 1996). Both species of microsporidae infect the epithelial layer of the ventriculus and midgut of adult bees, causing digestive disorders and shortening the life span of bees, with a resulting decrease in bee population (Higes et al., 2006; Huang et al., 2007). In the case of *N. ceranae,* some studies suggest this microsporidium occurs in other tissues as well (Chen & Huang, 2010).

N. apis was the first described microsporidium in honey bees (Ritter, 2001). It is known to have infected honey bees worldwide (Matheson, 1993). *N. ceranae* was first reported in colonies of the Asian hive bee, *A. cerana* (Fries et al., 1996), but it was also found in *A. mellifera* colonies in both Taiwan and Europe (Higes et al., 2006). Recently, a new species, *Nosema neumanni* was found in Uganda (Chemurot et al., 2017).

Previously, nosemosis in Africanized honey bees (AHB) was attributed exclusively to *N. apis* (Matheson, 1993). However, it appears *N. ceranae* is an emerging pathogen that has increased its distribution in the past decade by jumping from Asian honey bees *A. ceranae* to *A. mellifera* worldwide (Chen et al., 2008; Higes et al., 2008; Klee et al., 2007). The presence of *N. ceranae* in Africanized bees in Costa Rica was confirmed in 2006 (Calderón et al., 2008). Nevertheless, little data are available about the spread of *N. ceranae* in the country (Calderón & Ramírez, 2013). Some studies have described *N. ceranae* to have a higher prevalence than *N. apis* in *A. mellifera*, although both are widely distributed (Paxton et al., 2007). However, studies of

Nosema spp. prevalence in Africanized honey bees in Costa Rica are scarce (Calderón & Ramírez, 2013). Furthermore, the pathological effects of *N. ceranae* in Africanized bees are not well understood, but due to reports of colony population depletion and high mortality in honey bee colonies connected to microsporidian infections (Higes et al., 2006), the virulence of *N. ceranae* in Africanized bees needs to be investigated.

The diagnosis of nosemosis has been made by detecting spores of *Nosema* spp. through microscopic analyses (Shimanuki & Knox, 2000). Nevertheless, spores produced by the two *Nosema* species are quite similar and very difficult to distinguish using light microscopy analysis. With the finding both *N. ceranae* and *N. apis* affect western honey bees (*A. mellifera*), proper molecular techniques are required to differentiate between these different species of microsporidia, because the spores of the two *Nosema* species cannot be really differentiate by their morphology (Fries et al., 1996). In addition, microscopic analyses are not as sensitive to detecting low levels of *Nosema* infection as molecular methods, such as PCR can be. Molecular techniques have been developed to improve accurate diagnoses of *Nosema* disease in the laboratory (Cueto et al., 2020; Higes et al., 2006; Klee et al., 2007; Martin-Hernández et al., 2007; Paxton et al., 2007). Here, we used PCR to characterize infections or co-infections by these two *Nosema* species in Africanized honey bee colonies. Prior to PCR analysis, spores were visually detected in most of the adult bee samples using light microscopy.

Materials and Methods

In this study, 532 adult bee samples of Africanized honey bee colonies (Spivak, 1991) from different areas of Costa Rica were randomly collected for *Nosema* spp. diagnosis. Most bee samples were collected from colonies with no clinical signs of the disease. Interestingly, reduced honey production had been reported by beekeepers in some of the sampled areas over the previous years.

Foragers and adult bees from the brood nest were collected for *Nosema* spp. analysis. Gathering the foragers implied closing the hive entrance for 30 minutes and collecting them as they arrived. Furthermore, a comb from the brood nest was used to collect adult bees inside the colony (considered to be representative of the house bees). Samples of at least 100 bees were collected per colony. Preliminary analysis was made at the Bee Pathology Lab of the Tropical Beekeeping Research Center (CINAT), Heredia, Costa Rica, to select positive and suspected samples to *Nosema* spp. The abdomens of thirty adult bees from each colony were macerated in 30 ml of distilled water and microscopically examined for the presence of *Nosema* spp. spores. The spores were identified under the coverslip using a light microscopy at 40x (400x). All bee samples belonged to Africanized colonies naturally infected with *Nosema* ssp., showing diverse infection levels. The spore concentration was determined by counting with a haemocytometer (Neubauer chamber). Once the presence of microsporidian spores was confirmed (high infection levels) or suspected (spore concentrations were found at very low levels), 75 samples from apiaries located in four out the seven provinces of Costa Rica (Table 1) were selected for molecular diagnosis (from the 217 positive ones).