Ciência Rural, Santa Maria, v.52:9, e20210473, 2022
http://doi.org/10.1590/0103-8478cr20210473
ISSNe 1678-4596
MICROBIOLOGY

Prevalence of *Nosema ceranae* in apiculture regions of Bahia State, Brazil

Vivian Marina Gomes Barbosa Lage[1*] Camila Dias Santana[2] Epaminondas Patrocínio[3] Rejane Peixoto Noronha[4] Ricardo Lopes de Melo[5] Cristiane de Jesus Barbosa[3] Suzana Telles da Cunha Lima[1]

[1]Laboratório de Bioprospecção e Biotecnologia, Instituto de Biologia, Universidade Federal da Bahia (UFBA), 40170-110, Salvador, BA, Brasil. E-mail: vivianmarina@hotmail.com. *Corresponding author.
[2]Laboratório de Biologia Molecular, Centro Tecnológico Agropecuário do Estado da Bahia (CETAB), Salvador, BA, Brasil.
[3]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) – Mandioca e Fruticultura, Cruz das Almas, BA, Brasil.
[4]Departamento de Sanidade Apícola, Agência Estadual de Defesa Agropecuária da Bahia (ADAB), Salvador, BA, Brasil.
[5]Instituto Federal de Educação, Ciência e Tecnologia Baiano (IFBA), Valença, BA, Brasil.

ABSTRACT: *Nosemosis is an important bee disease that is caused by microsporidia fungi of the Nosema genus, whose main etiological agents are Nosema apis and N. ceranae, both of which are found worldwide. In Brazil, the disease has been reported in several states but little is known about its occurrence and distribution in Bahia. This study identified the occurrence and distribution of nosemosis and its agents, N. apis and N. ceranae, in Apis mellifera L. bees collected from apiaries in the state of Bahia, Brazil. A total of 154 bee samples were collected and analyzed from 20 apiaries in six regions of the state. The hives sampled were evaluated for signs of the disease from December 2015 to July 2018. Molecular diagnosis was made using polymerase chain reaction (PCR). No signs of nosemosis were observed in the sampled apiaries, but from 154 samples analyzed via PCR, 96 were infected with N. ceranae. This pathogen was reported in samples from all six regions evaluated, and its occurrence in important apiculture regions of Bahia State is discussed in this study.*
Key words: *bee, beekeeping, nosemosis, parasite.*

Prevalência de *Nosema ceranae* em regiões apícolas do Estado da Bahia, Brasil

RESUMO: *A nosemose é uma importante doença das abelhas, sendo ocasionada por fungos microsporídios do gênero Nosema. Os principais agentes etiológicos desta doença são Nosema apis e N. ceranae, ambos bem difundidos mundialmente. No Brasil, a doença possui relatos em diversos estados, entretanto, pouco se sabe sobre sua ocorrência e distribuição na Bahia. O objetivo deste estudo foi identificar a ocorrência e distribuição da nosemose e de seus agentes, N. apis e N. ceranae, em abelhas Apis mellifera L. coletadas em apiários do estado da Bahia, Brasil. Foram analisadas 154 amostras de abelhas coletadas em 20 apiários de seis regiões apícolas do Estado. As colmeias amostradas foram avaliadas quanto aos sinais da doença no período de dezembro de 2015 a julho de 2018. O diagnóstico molecular foi realizado via reação em cadeia da polimerase (PCR). Não foram observados sinais da nosemose nos apiários amostrados e, das 154 amostras analisadas via PCR, 96 estavam infectadas por N. ceranae e N. apis não foi detectada. O patógeno N. ceranae foi encontrado em amostras das seis regiões avaliadas. A distribuição de N. ceranae em importantes regiões apícolas do estado da Bahia é discutida neste artigo.*
Palavras-chave: *abelhas, apicultura, nosemose, parasita.*

INTRODUCTION

Nosemosis is an important bee disease that is caused by parasites of the genus *Nosema*, currently classified as microsporidia fungi (ADL et al., 2005), which are intracellular parasites that develop in mucosal cells of the host intestine and are transmitted horizontally via the fecal-oral route (HUANG & SOLTER, 2013). Certain signs are associated with the disease, both in individual bees or within the colony, such as digestive and nutritional disorders, changes in eating behavior (from foraging to the amount of food ingested), hormonal and physiological changes, increased energy demand, changes in flight pattern and colony defense behavior, and premature death (GOBLIRSCH, 2018; LOURENÇO et al., 2021).

The disease has only two species involved in its etiopathogenesis, *Nosema apis*, which was initially identified in *Apis mellifera* (ZANDER, 1909), and *Nosema ceranae*, from the Asian bee *Apis*

Received 06.17.21 Approved 09.28.21 Returned by the author 11.29.21
CR-2021-0473.R2
Editor: Rudi Weiblen

cerana (FRIES et al., 1996). Both species parasitize *A. mellifera,* either individually or as co-infectors (MILBRATH et al., 2015).

The agents of nosemosis are distributed globally, but their occurrence and distribution in Brazil are still poorly understood (PIRES et al., 2016). Although, the first record of the pathogen in the country was from 2007 (KLEE et al., 2007), molecular analyses of bee samples collected in 1979 were recently performed that indicated the presence of microsporidia for at least the past three decades (TEIXEIRA et al., 2013). Articles on nosemosis occurrence in Brazil suggested that the disease is well disseminated in this country (SANTOS et al., 2014; LIMA et al., 2015; GUIMARÃES-CESTARO et al., 2016; CHAGAS et al., 2020).

A study by TEIXEIRA et al. (2013) investigated the occurrence and distribution of *N. apis* and *N. ceranae* in samples from 10 states of Brazil, including Bahia, where *N. ceranae* was detected. However, the number of samples identified in this state was minimal, with only one case report, and no information on the distribution of microsporidia in the main apiculture regions was evaluated.

The state of Bahia produces a significant amount of honey, with 3,942 tons in 2019 (IBGE, 2020). Considering the economic and social importance of this product, it is necessary to obtain information on the health of local bees. This study identified the occurrence and distribution of nosemosis and its agents, *N. apis* and *N. ceranae*, in *Apis mellifera* L. bees collected in apiaries from the state of Bahia, Brazil.

MATERIALS AND METHODS

Sampling

The sample group consisted of *A. mellifera* bees collected from 20 apiaries in six honey-producing municipalities of Bahia: Ribeira do Pombal, Brotas de Macaúbas, Inhambupe, Canavieiras, Ibotirama and Teixeira de Freitas. Each sample consisted of 30 adult bees collected at the entrance of the hives and deposited in containers containing 70% ethanol. A total of 154 samples were collected (Table 1). The harvest was conducted with the support of technicians from the State Agricultural Defense Agency of Bahia (ADAB) as part of the National Program of Apiculture Health (PNSAp).

Evaluation of hives

The 20 delimited apiaries were assessed over two and a half years (December 2015 to July 2018), with the help of the agricultural inspectors of ADAB. Hives were evaluated for signs of nosemosis that included the presence of feces deposited at the entrance of the colonies, changes in feeding behavior and the appearance of dead bees.

Molecular analysis

Molecular tests were carried out at the Molecular Biology Laboratory of EMBRAPA - Cassava & Fruit Advanced Field, located at the Agricultural Technology Center of Bahia State (CETAB) in Salvador, Bahia, Brazil.

DNA extraction

For bee total DNA extraction, the protocol described by DOYLE & DOYLE (1987) was used with modifications. Extraction was performed in duplicate from the abdomens of 15 adult bees. Samples were macerated in liquid nitrogen, and subjected to an extraction buffer (2% CTAB, 1.2M NaCl, 100 mM TrisHCl, 30 mM EDTA, 0,2% Mercaptoetanol, 0,3 mg/µl Proteinase K and ddH2O), followed by incubation in a water bath at 65 ºC for 1 h (vortexing every 15 min), homogenization with chloroform:isoamyl alcohol (24:1), centrifugation for 10 min at 10,000 rpm,

Table 1 - Municipalities evaluated in the state of Bahia, number of apiaries and samples collected.

Municipality	Number of apiaries	Number of samples
Brotas de Macaúbas	4	18
Canavieiras	3	18
Ibotirama	4	16
Inhambupe	2	33
Ribeira do Pombal	5	59
Teixeira de Freitas	2	10
Total	20	154

collection of the supernatant, final precipitation in isopropyl alcohol, centrifugation for 15 min at 14,000 rpm, and suspension in TE buffer (1M Tris-HCl and 0.5 M EDTA) with addition of RNAse (10mg/mL).

Changes from the original protocol were made for the maceration process (originally performed with plant tissue adapted here for animal tissue; and an increase in liquid nitrogen for maceration), the concentration of extractive solution reagents (1.4 mM NaCl ajusted to 1.2 mM, 20 mM EDTA changed to 30 mM, and the addition of proteinase Enzyme K), the incubation time (from 30 min and agitation every 10 min to 1 h with agitation every 15 min), the centrifugation step with chloroform:isoamyl alcohol (from 14,000 rpm for 5 min to 10,000 rpm for 10 min), and the DNA precipitation process (centrifugation from 7,500 rpm for 5 min to 14,000 rpm for 15 min).

DNA amplification

The detection of *N. apis* and *N. ceranae* was performed via PCR according to the adapted protocol of MARTÍN-HERNÁNDEZ et al. (2007). The 218MITOC-F and 218MITOC-R primers were used for *N. ceranae*, with positive fragments corresponding to 218bp, and 321APIS-F and 321APIS-R for *N. apis*, with positive fragments of 321bp (MARTÍN-HERNÁNDEZ et al., 2007). The concentration of reagents used for both protocols were modified (0.2 mM dNTPs, 1x Tris/KCl, 2.0 mM $MgCl_2$, 1U Taq, 0.5 mM *primers* and 5ng DNA). The reaction cycle was identical for both species, with changes to the denaturation and annealing temperatures (95 °C for 2 min x1; 95 °C for 30s, 55 °C for 30s and 72 °C for 60s x35; 72 °C for 5 min x1).

All analyses were performed in triplicates, including negative controls (uninfected samples) and positive controls (infected samples). PCR products were stained with ethidium bromide and subjected to electrophoresis in TBE Buffer with 2% agarose gel for 2 h at 110 volts. The analysis of bands patterns was obtained through readings under ultraviolet light, using a photo-documenter model LTB 20 x 20 HE of Loccus Biotechnology, with a molecular weight marker of 50 to 1,000 bp (Thermo Fisher Scientific). The positive control for *N. ceranae* came from the DNA bank of the Molecular Biology Laboratory of CETAB, and that for *N. apis* was provided by the Centro de Investigación Apícolay Agroambiental de Marchamalo, Spain.

RESULTS AND DISCUSSION

The hives evaluated presented no feces at the entrance of the colonies, changes in feeding behavior, or dead bees, which are characteristic of nosemosis (NAUG & GIBBS, 2009; GOBLIRSCH et al., 2013; PARIS et al., 2018). A total of 154 PCR samples were analyzed from the six delimited municipalities, 96 of which were positive for *N. ceranae* totaling 62.3%. *N. apis* was not detected in any of the samples.

The pathogen *N. ceranae* was reported in samples from all municipalities evaluated (Figure 1), with the highest and lowest occurrence, respectively, in the Inhambupe (100%) and Teixeira de Freitas (20%) municipalities.

The absence of indicators of nosemosis in the hives, considering the detection of *N. ceranae* via molecular diagnosis, has been reported in other studies, which suggested that this parasite may be present in bees for prolonged periods without producing signs in the individual or colony (PAXTON, 2010; FERNÁNDEZ et al., 2012). Microsporidia infections are considered common in bees; however, adverse conditions, such as coexistence with other pathogens (mites, bacteria and viruses), presence of pesticide residues, and management deficiencies (scarcity of nutritional resources and lack of shading in the colonies), are required to cause extreme bee death (CASTELLI et al., 2020; CHAGAS et al., 2020; FAITA et al., 2020).

An important factor that influences the lack of indicators in hives infected with nosemosis is the africanization of bees, since in Brazil the majority of bees originated from the crossing with European (*A. mellifera mellifera*) and African (*A. mellifera scutellata*) bees. Africanized bees appear to have greater resistance to pathogens than European bees (MAGGI et al., 2016; TIBATÁ et al., 2021). MENDOZA et al. (2013) compared African and European bee populations and reported that africanized bees had a lower rate of *N. ceranae* infection, as well as a higher abundancy and honey production rate.

The fact that *N. ceranae* was detected in samples from all regions evaluated (Figure 1) in this study reinforces the information reported in the literature regarding its wide distribution. The results of molecular analyses showed that *N. ceranae* was the only species of *Nosema* detected and that it is, copious in important beekeeping regions in the state of Bahia, which correlates with other reports worldwide.

The prevalence of *N. ceranae* infection in honey bees has been reported in different countries in Europe, Asia and America. In Italy, 38 apiaries were analyzed, with *N. ceranae* being detected in 28 of them (63.2%) and *N. apis* was absent (PAPINI et al.,

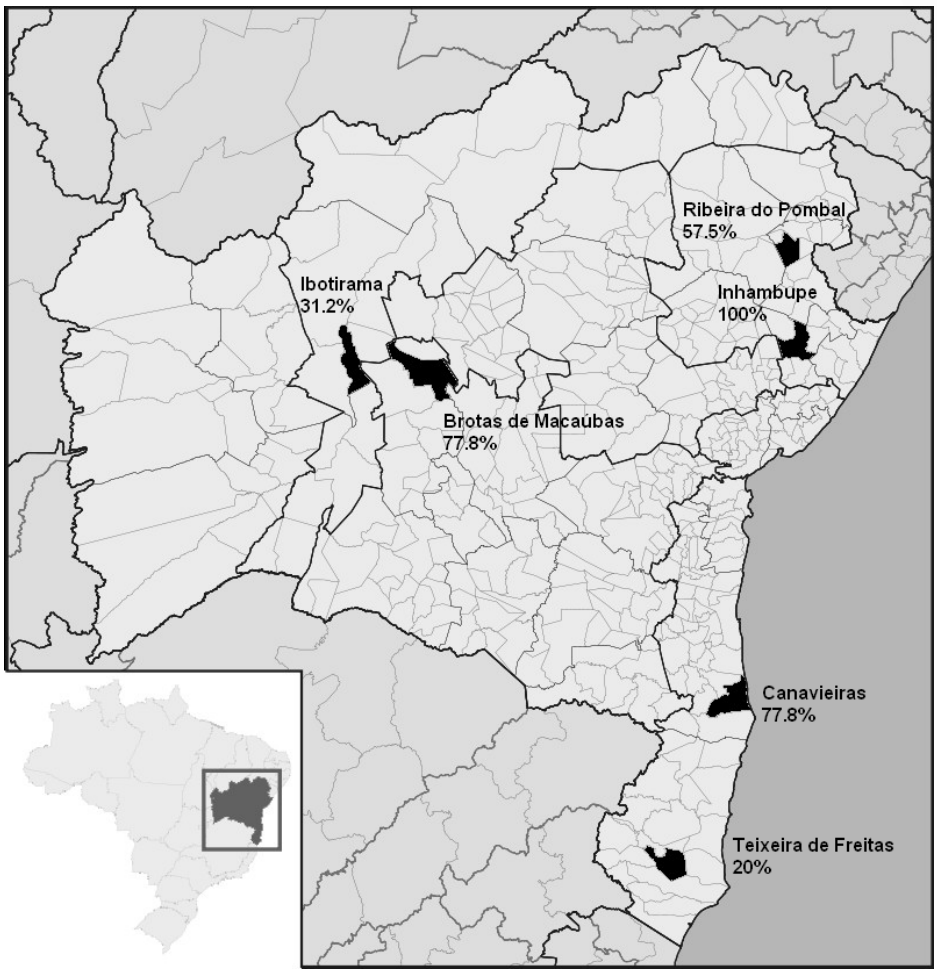

Figure 1 - Map of Bahia with the municipalities of the apiaries evaluated highlighted and the respective percentages of detection of *Nosema ceranae* via PCR found in this study.

2017). In France, 61 samples were evaluated, 59% positive for *N. ceranae,* 1.6% for N. *apis*, and 6.6% for co-infection cases (CHAUZAT et al., 2007). Iran had six positive samples analyzed via PCR (KHEZRI et al., 2018) and *N. ceranae* was detected in all of them. According to research in China (WANG et al., 2019), out of 69 positive samples evaluated (23.6%) 68 were *N. ceranae* and only one *N. apis*. In Canada, N. *ceranae* was reported in 41-91% of the samples evaluated and *N. apis* in 4-34% (EMSEN et al., 2016). Guerrero-Molina and collaborators (2016) also reported positive samples from 10 local colonies in Mexico, and only *N. ceranae* was detected.

Likewise, South America shows higher prevalence of *N. ceranae*. Out of 29 samples analyzed in Uruguay, 100% of infected bees were *N. ceranae* (INVERNIZZI et al., 2009). In Chile, samples from 12 Valparaiso apiaries were analyzed and 100% of them were infected with *N. ceranae* (BRAVO et al., 2014). Argentina had 38 municipalities evaluated and almost all samples analyzed were *N. ceranae*, but 6%, which were *N. apis* (MEDICI et al., 2012).

In Brazil, *N. ceranae* is consistently the species most associated with honeybee infection. TEIXEIRA et al. (2013) demonstrated that the pathogen was widely distributed in Brazil and was present in samples from 10 states, representing 98.82% of infected samples. Subsequently, these results were supported by other studies in which the pathogen was detected in the majority of the

samples evaluated, in different states, such as São Paulo with 85.2% (SANTOS et al., 2014) and 80% (GUIMARÃES-CESTARO et al., 2016), 96% in Mato Grosso (NASCIMENTO, 2016), 60% in Rio Grande do Norte (LIMA et al., 2015) and 57.6 % in Rio Grande do Sul and Santa Catarina (CHAGAS et al., 2020).

In this study, *N. ceranae* was also prevalent in Bahia state, being the sole species detected in more than 60% of the bees evaluated from important apicultural regions. Although, the pathogen occurrence in the state was previously reported by TEIXEIRA et al. (2013), no information was provided on their distribution among main bee-keeping counties, since the number of samples used in that study was small. The results presented here allow us to infer the distribution and potential importance of *N. ceranae* in apiculture in the state of Bahia.

Despite the absence of nosemosis signs in hives evaluated in this study, the presence of *N. ceranae* in all sampled regions indicates the risk that the disease poses for state beekeeping. Changes such as rising temperatures due to global warming, the unavailability of natural resources from prolonged droughts, the appearance of other pathogens, parasites, and exogenous predators, and the abusive use of pesticides, are considered determinants of the development of nosemosis indicators (PAXTON, 2010; PIRES et al., 2016) that can lead to a future epidemic of this disease. Understanding the distribution of the pathogen in the state allows for the development of management strategies by the ADAB program for bee health for preventive public policies, which include periodic monitoring of the disease.

CONCLUSION

Microsporidia *N. ceranae* was detected in 96 of the 154 samples evaluated (62.3%), while *N. apis* was not detected. *N. ceranae* was reported in samples from all six regions evaluated. The results obtained in this study indicate that the microsporidia *N. ceranae* is well disseminated in the apiaries of the state of Bahia, whereas *N. apis* is absent. These data corroborate the findings of other studies conducted in Brazil and worldwide. Although, no characteristic signs of the disease were recorded, attention is needed for possible future scenarios in which synergistic factors can contribute to the appearance of nosemosis indicators and subsequent damage to the local beekeeping industry.

ACKNOWLEDGMENTS

We thank the Programa de Pós-graduação em Biotecnologia (PPGBIOTEC) of UFBA, which supported this master's project, the Centro de Investigación Apícola y Agroambiental de Marchamalo, in Spain, for the positive controls of *N. apis*, Agência Estadual de Defesa Agropecuária da Bahia (ADAB), for providing the resources and project funding, Embrapa Mandioca e Fruticultura for technical support, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financing the master's scholarship.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of this manuscript. All authors critically revised and approved the final manuscript.

REFERENCES

ADL, S. M. et al. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. **Journal of Eukaryotic Microbiology**, v.52, p.399-451, 2005. Available from: <https://doi.org/10.1111/j.1550-7408.2005.00053.x>. Accessed: Jun. 08, 2021. doi: 10.1111/j.1550-7408.2005.00053.x.

BRAVO, J. et al. Identification of *Nosema ceranae* in the Valparaíso District, Chile. **Archivos de Medicina Veterinaria**, v.46, p.487-491, 2014. Available from: <http://dx.doi.org/10.4067/S0301-732X2014000300021>. Accessed: Jun. 08, 2021. doi: 10.4067/S0301-732X2014000300021.

CASTELLI, L. et al. Impact of Nutritional Stress on Honeybee Gut Microbiota, Immunity, and *Nosema ceranae* Infection. **Invertebrate Microbiology**, v.80, p.908-919, 2020. Available from: <https://doi.org/10.1007/s00248-020-01538-1>. Accessed: Jun. 08, 2021. doi: 10.1007/s00248-020-01538-1.

CHAGAS, D. B. et al. Black queen cell virus and *Nosema ceranae* coinfection in Africanized honey bees from southern Brazil. **Pesquisa Veterinária Brasileira**, v.40, n.11, 2020. Available from: <https://doi.org/10.1590/1678-5150-PVB-6678>. Accessed: Jun. 08, 2021. doi: 10.1590/1678-5150-PVB-6678.

CHAUZAT, M-P. et al. Presence of *Nosema ceranae* in French honey bee colonies. **Journal of Apicultural Research**, v.46, n.2, p.127-128, 2007. Available from: <https://doi.org/10.1080/00218839.2007.11101380>. Accessed: Sept. 21, 2021. doi: 10.1080/00218839.2007.11101380.

DOYLE, J. J.; DOYLE, J. L. A rapid DNA isolation procedure for small amounts offresh leaf tissue. **Phytochemical Bulletin**, v. 19, n.1, p. 11–15, 1987. Available from: <Doyle_plantDNAextractCTAB_1987.pdf (uncc.edu)>. Accessed: Jun. 08, 2021.

EMSEN, B. et al. Higher prevalence and levels of *Nosema ceranae* than *Nosema apis* infections in Canadian honey bee colonies. **Parasitology Research**, v.115, n.1, p.175-181, 2016. Available from: <https://doi.org/10.1007/s00436-015-4733-3>. Accessed: Jun. 08, 2021. doi: 10.1007/s00436-015-4733-3.

FAITA, M. R. et al. Glyphosate-based herbicides and *Nosema* sp. microsporidia reduce honey bee (*Apis mellifera* L.) survivability under laboratory conditions. **Journal of Apicultural Research**, v.59, n.4, p.332-342, 2020. Available from: <https://doi.org/10.1080/00218839.2020.1736782>. Accessed: Jun. 08, 2021. doi: 10.1080/00218839.2020.1736782.

FERNÁNDEZ, J. M. et al. Asymptomatic presence of *Nosema* sp. in Spanish commercial apiaries. **Journal of Invertebrate Pathology**, v.111, n.2, p.106-110, 2012. Available from: <https://doi.org/10.1016/j.jip.2012.06.008>. Accessed: Jun. 08, 2021. doi: 10.1016/j.jip.2012.06.008.

FRIES, I. et al. ***Nosema ceranae*** n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerane* (Hymenoptera, Apidae). **European Journal of Protistology**, v.32, n.3, p.356–365, 1996. Available from: <https://doi.org/10.1016/S0932-4739(96)80059-9>. Accessed: Jun. 08, 2021. doi: 10.1016/S0932-4739(96)80059-9.

GOBLIRSCH, M. *Nosema ceranae* disease of the honey bee (*Apis mellifera*). **Apidologie**, v.49, p.131-150, 2018. Available from: <https://doi.org/10.1007/s13592-017-0535-1>. Accessed: Jul. 29, 2021. doi: 10.1007/s13592-017-0535-1.

GOBLIRSCH, M. et al. Physiological and Behavioral Changes in Honey Bees (***Apis mellifera***) Induced by *Nosema ceranae* Infection. **Plos One**, v.8, n.3, 2013. Available from: <https://doi.org/10.1371/journal.pone.0058165>. Accessed: Jun. 08, 2021. doi: 10.1371/journal.pone.0058165.

GUERRERO-MOLINA, C. et al. *Nosema ceranae* is an old resident of honey bee (*Apis mellifera*) colonies in Mexico, causing infection levels of one million spores per bee or higher during summer and fall. **Journal of Invertebrate Pathology**, v.141, p.38-40, 2016. Available from: <https://doi.org/10.1016/j.jip.2016.11.001>. Accessed: Jul. 16, 2021. doi: 10.1016/j.jip.2016.11.001.

GUIMARÃES-CESTARO, L. et al. A scientific note on occurrence of pathogens in colonies of honey bee *Apis mellifera* in Vale do Ribeira, Brazil. **Apidology**, v.48, n.3, p.384-386, 2016. Available from: <https://doi.org/10.1007/s13592-016-0481-3>. Accessed: Jun. 08, 2021. doi: 10.1007/s13592-016-0481-3.

HUANG, W. F.; SOLTER, L. F. Comparative development and tissue tropism of *Nosema apis* and *Nosema ceranae.* **Journal of Invertebrate Pathology**, v.113, n.1, p.35-41, 2013. Available from: <https://doi.org/10.1016/j.jip.2013.01.001>. Accessed: Jun. 08, 2021. doi: 10.1016/j.jip.2013.01.001.

IBGE - Instituto Brasileiro de Geografia e Estatística. (2020) **Produção da pecuária municipal**. Available from: <https://cidades.ibge.gov.br/brasil/ba/pesquisa/18/0>. Accessed: Nov. 25, 2020.

INVERNIZZI, C. et al. Presence of *Nosema ceranae* in honeybees (*Apis mellifera*) in Uruguay. **Journal of Invertebrate Pathology**, v.101, n.2, p.150-153, 2009. Available from: <https://doi.org/10.1016/j.jip.2009.03.006>. Accessed: Jul. 16, 2021. doi: 10.1016/j.jip.2009.03.006.

KHEZRI, M. et al. Molecular detection of *Nosema ceranae* in the apiaries of Kurdistan province, Iran. **Veterinary Research Forum**, v.9, n.3, p.273-278, 2018. Available from: <https://doi.org/10.30466/vrf.2018.32086>. Accessed: Jun. 08, 2021. doi: 10.30466/vrf.2018.32086.

KLEE, J. et al. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis melifera.* **Journal of Invertebrate Pathology**, v.96, n.1, p.1-10, 2007. Available from: <https://doi.org/10.1016/j.jip.2007.02.014>. Accessed: Jun. 08, 2021. doi: 10.1016/j.jip.2007.02.014.

LIMA, T. S. et al. Meios de dispersão de *Nosema ceranae* em *Apis mellifera* Africanizada no município de Mossoró - RN. **Ciência veterinária nos trópicos**, v.18, n.2, p.193-196, 2015. Available from: <volume18_numero_2.pdf (rcvt.org.br)>. Accessed: Jun. 08, 2021.

LOURENÇO, A. P. et al. Immunity and physiological changes in adult honey bees (*Apis mellifera*) infected with *Nosema ceranae*: The natural colony environment. **Journal of Insect Physiology**, v.131, 2021. Available from: <https://doi.org/10.1016/j.jinsphys.2021.104237>. Accessed: Jun. 08, 2021. doi: 10.1016/j.jinsphys.2021.104237.

MAGGI, M. et al. Honeybee health in South America. **Apidologie**, v.47, p.835-854, 2016. Available from: <https://doi.org/10.1007/s13592-016-0445-7>. Accessed: Jun. 08, 2021. doi: 10.1007/s13592-016-0445-7.

MARTÍN-HERNÁNDEZ, R. et al. Outcome of colonization of *Apis mellifera* by *Nosema ceranae.* **Applied and Environmental Microbiology**, v.73, n.20, p.6331-6338, 2007. Available from: <https://doi.org/10.1128/AEM.00270-07>. Accessed: Jun. 08, 2021. doi: 10.1128/AEM.00270-07.

MEDICI, S. K. et al. Genetic variation and widespread dispersal of *Nosema ceranae* in *Apis mellifera* apiares from Argentina. **Parasitology research**, v.110, p.859-864, 2012. Available from: <https://doi.org/10.1007/s00436-011-2566-2>. Accessed: Sept. 21, 2021. doi: 10.1007/s00436-011-2566-2.

MENDOZA, Y. et al. *Nosema ceranae* and RNA viruses in European and Africanized honeybee colonies (*Apis mellifera*) in Uruguay. **Apidologie**, v.45, n.2, p.224-234, 2013. Available from: <https://doi.org/10.1007/s13592-013-0241-6>. Accessed: Jun. 08, 2021. doi: 10.1007/s13592-013-0241-6.

MILBRATH, M. O. et al. Comparative virulence and competition between *Nosema apis* and *Nosema ceranae* in honey bees (*Apis mellifera*). **Journal of Invertebrate Pathology**, v.125, p.9-15, 2015. Available from: <https://doi.org/10.1016/j.jip.2014.12.006>. Accessed: Jun. 08, 2021. doi: 10.1016/j.jip.2014.12.006.

NASCIMENTO, E. G. M. **Caracterização da apicultura e prevalência de *Nosema ceranae* na Mesorregião Norte Mato-Grossense**. 2016. Dissertação (Mestrado em Zootecnia). Universidade Federal de Mato Grosso, Brasil.

NAUG, D.; GIBBS, A. Behavioral changes mediated by hunger in honeybees infected with *Nosema ceranae.* **Apidologie**, v.40, p.595-599, 2009. Available from: <https://doi.org/10.1051/apido/2009039>. Accessed: Jun. 08, 2021. doi: 10.1051/apido/2009039.

PAPINI, R. et al. Prevalence of the microsporidian *Nosema ceranae* in honeybee (*Apis mellifera*) apiaries in Central Italy. **Saudi Journal of Biological Sciences**, v.24, n.5, p.979-982, 2017. Available from: <https://doi.org/10.1016/j.sjbs.2017.01.010>. Accessed: Jun. 08, 2021. doi: 10.1016/j.sjbs.2017.01.010.

PARIS, L. et al. Effects of the gut parasite *Nosema ceranae* on honey bee physiology and behavior. **Current Opinion in Insect Science**, v.26, p.149-154, 2018. Available from: <https://doi.org/10.1016/j.cois.2018.02.017>. Accessed: Jun. 08, 2021. doi: 10.1016/j.cois.2018.02.017.

PAXTON, R. J. Does infection by *Nosema ceranae* cause "Colony Collapse Disorder" in honey bees (*Apis mellifera*)? **Journal of Apicultural Research**, v.49, n.1, p.80-84, 2010. Available from: <https://doi.org/10.3896/IBRA.1.49.1.11>. Accessed: Jun. 08, 2021. doi: 10.3896/IBRA.1.49.1.11.

PIRES, C. S. S. et al. Enfraquecimento e perda de colônias de abelhas no Brasil: Há casos de CCD? **Pesquisa Agropecuária Brasileira**, v.51, n.5, p.422-442, 2016. Available from: <https://doi.org/10.1590/S0100-204X2016000500003>. Accessed: Jun. 08, 2021. doi: 10.1590/S0100-204X2016000500003.

SANTOS, L. G. et al. Honey Bee Health in Apiaries in the Vale do Paraíba, São Paulo State, Southeastern Brazil. **Sociobiology**, v.61, n.3, p.307-312, 2014. Available from: <http://dx.doi.org/10.13102/sociobiology.v61i3.307-312>. Accessed: Jun. 08, 2021. doi: 10.13102/sociobiology.v61i3.307-312.

TEIXEIRA, E. W. et al. *Nosema ceranae* has been present in Brazil for more than three decades infecting Africanized honey bees. **Journal of Invertebrate Pathology**, v.114, n.3, p.250–254, 2013. Available from: <https://doi.org/10.1016/j.jip.2013.09.002>. Accessed: Jun. 08, 2021. doi: 10.1016/j.jip.2013.09.002.

TIBATÁ, V. M. et al. Africanized honey bees in Colombia exhibit high prevalence but low level of infestation of *Varroa* mites and low prevalence of pathogenic viroses. **Plos One**, v.16, n.15, 2021. Available from: <https://doi.org/10.1371/journal.pone.0244906>. Accessed: Jun. 08, 2021. doi: 10.1371/journal.pone.0244906.

WANG, Q. et al. *Nosema ceranae*, the most common microsporidium infecting *Apis mellifera* in the main beekeeping regions of China since at least 2005. **Journal of Apicultural Research**, v.58, n.4, p.562-566, 2019. Available from: <https://doi.org/10.1080/00218839.2019.1632148>. Accessed: Jun. 08, 2021. doi: 10.1080/00218839.2019.1632148.