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Colonization patterns of *Nosema ceranae* in the Azores archipelago[#]

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Abstract:

Nosema ceranae and *Nosema apis* are pathogens of honey bees that cause nosemosis, a disease implicated in colony losses worldwide. They are obligate intracellular pathogens infecting the midgut epithelial cells of adult honey bees. Although *N. ceranae* was originally a pathogen specific of *Apis cerana*, currently, it is found in *Apis mellifera* throughout most of the world. Due to their confined environments, which limited pathogen transmission and dissemination, islands are unique places for epidemiological studies. There are only a few *Varroa destructor*-free and possibly even fewer *N. ceranae*-free honey bee sanctuaries in the world, with the Azores being one. Even though with the exception of Santa Maria and Flores, nosemosis has been present in the Azores since 2008, the causal species has yet to be identified. Hence, this study aimed to determine the prevalence and infection levels of *Nosema* spp. in the Azorean honey bees. In 2014/2015, 474 colonies were sampled on Faial, Flores, Pico, Graciosa, São Jorge, São Miguel, Santa Maria, and Terceira. Additionally, São Jorge, Santa Maria, Faial, and Terceira were re-sampled in 2020 with a total of 91 colonies. DNA was extracted, and the diagnosis and *Nosema* spp. loads were obtained by multiplex PCR and RT-qPCR. The findings indicate that *N. ceranae* appears to be the dominant species in the Azores. *N. apis* was only detected in 2014/2015 with a very low prevalence (5.1%). *N. ceranae* prevalence varied between 2.7%, on São Jorge, and 50.7%, on Pico. In 2020, *N. ceranae* positive colonies increased significantly on Terceira (57.1%) and São Jorge (50.0%). *N. ceranae* was not detected on Santa Maria in both periods. Average infection levels in positive *N. ceranae* colonies were usually medium to high ($>10^{-7}$ ng/ μ l), with São Jorge colonies displaying the greatest infection intensity ($>10^{-5}$ ng/ μ l). This study highlights the Azores archipelago as a unique place for beekeeping, with islands free of *N. ceranae* and *V. destructor*, which are two important stressors that afflict honey bees in the world.

Keywords: *Nosema apis*, *Nosema ceranae*, *Apis mellifera*, real-time qPCR, prevalence, infection levels

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