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Scientific note

A scientific note on occurrence of pathogens in colonies of honey bee *Apis mellifera* in Vale do Ribeira, Brazil

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In recent times, studies on honey bees have been intensified because of the decline in population of these insects, in various parts of the world, probably on account of the use of pesticides and/or by the action of pathogens (Doublet et al. 2015). On account of this, knowing the health profile of honeybee colonies with the help of preventive monitoring is important. This study evaluates the presence of *Nosema apis*, *Nosema ceranae*, *Paenibacillus larvae*, and *Varroa destructor* in colonies of the Vale do Ribeira region, State of São Paulo, Brazil.

The samples were collected in April 2013 (autumn season) from 15 apiaries in 10 municipalities of Vale do Ribeira region (Figure 1). All samples were properly packed in the field and transferred to the Honey Bee Health Laboratory of the Polo Regional Vale do Paraíba (LASA/APTA).

To determine the intensity of infection caused by the *Nosema* spp., the procedure described by Teixeira et al. (2013) was utilized, and this PCR conditions: denaturation at 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 50 s, and elongation at 72 °C for 5 min. For *P. larvae* analyses, samples of honey obtained from honeycombs were submitted to the official method of diagnosis (Brazil 2003) and the molecular method proposed by Guimarães-Cestaro et al. (2016). For analyses of *V. destructor*, the method proposed by De Jong et al. (1982) to analyze the infestation rate and Dietemann et al. (2013) to analyze brood combs was used. Adult mites were submitted for DNA extraction (Faza et al. 2013) and PCR reactions using primers suggested by Navajas et al. (2002), following these conditions: denaturation at 92 °C for 4 min, 35 cycles at 92 °C for 1 min, annealing at 52 °C for 90 s, and elongation at 72 °C for 90 s. Haplotypes J and K were identified according Anderson and Fuchs (1998).

Just the specie *N. ceranae* was detected and showed average of intensity of infection of 1,070,000 spores/bee (Table I, supplementary material). The occurrence of *N. ceranae* as a unique microscoporidium species in honey bee hives has also been reported in samples from other Brazilian regions (Santos et al. 2011, 2014), which reinforce the hypothesis that *N. ceranae* has replaced *N. apis* in various countries (Teixeira et al. 2013). In the samples analyzed here there is a variation in the number of *N. ceranae* spores, supporting the hypothesis that although *N. ceranae* is widely distributed in Brazil, it does not seem to have an infection pattern throughout the year (Teixeira et al. 2013). However, the high

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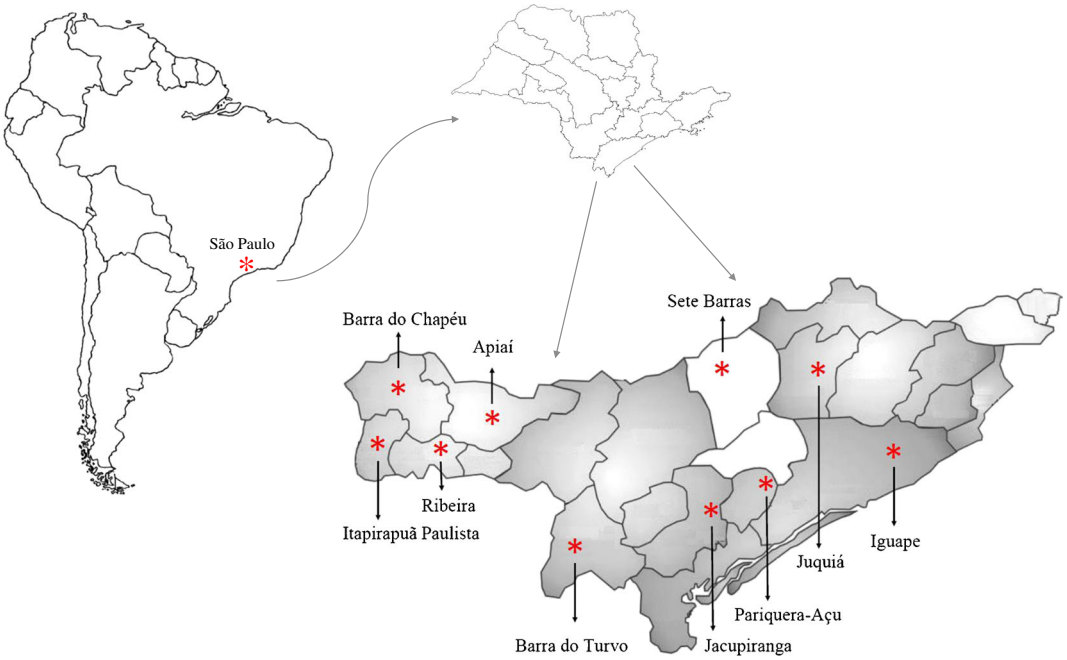

Figure 1. Municipalities in Vale do Ribeira region where the samples were collected: Apiaí (24° 30′ S 48 50′ O), Barra do Chapéu (24° 28′ S 49 01′ O), Barra do Turvo (24 45′ S 48 30′ O), Iguape (24° 42′ S 47 33′ O), Itapirapuã Paulista (20° 38′ S 47 13′ O), Jacupiranga (24° 41′ S −48° 00′ O), Juquiá (24° 19′ S 47 38′ O), Pariquera-Açu (24 42′ S 47 52′ O), Ribeira (24° 39′ S 49 00′ O), and Sete Barras (24° 23′ S 47 55′ O).

intensity of infection found in autumn may be explained by the cold temperature during this season, which results in the bees remaining in their hives, increasing the potentials of transmission and infection (Martín-Hernández et al. 2009).

All the 68 samples tested for the presence of *P. larvae* were negative, showing that, although the sampled municipalities were located about 200 km from the Municipality of Quatro Barras-PR, the more recent place with report of American Foulbrood in Brazil, this disease was controlled, as all contaminated hives in a 10-km radius around the outbreak were burned (MAPA 2006).

The mean rate of infestation by *V. destructor* was obtained in adult bees and cells of worker bees in brood comb (Table I, supplementary material). The fact that in autumn mites are found in the phoretic phase, feeding mainly on nurse bees may explain the higher infestation rates obtained in autumn compared to those in summer (Santos et al. 2011, 2014). Our findings about cells infested with offspring (from 0 to 5.12) support the hypothesis that females show reduced fertility in South America (Rosenkanz and Engels, 1994), where the authors found average number of 3.3–4.1. In addition, our data contradict the information given by Garrido et al. (2003) that the increased fertility in the *V. destructor* females has been attributed to haplotype K. In this study, all samples have haplotype K, without causing great damage to beekeepers (Moretto et al. 1991).

The microsporidian *N. ceranae* was present in 80% of samples, confirming the prevalence of this species in another region of the State of São Paulo, as well as the *V. destructor* mite, present in 98.8% of the samples of adult bees and 79% of the combs. Trade of bee products and their globalization issues can play an important role in the spread of pathogens and expansion assessments as presented in this study generate information on which grants to government guidelines, by through knowledge of the health of the national squad profile.

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Authors' contributions LGC: Carried out analysis of *Nosema* spp., *Varroa destructor* and *P. larvae* and contributed to write this article; JES: Carried out analysis of *Nosema* spp. and contributed to write this article; MLTMFA: Carried out the collections and analyzis of *Varroa destructor* and *P. larvae* and contributed to write this article; DM: Carried out analysis of *Nosema* spp. and contributed to write this article; EWT: Carried out the collections and contributed to write this article.

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