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# Honey Bee (*Apis mellifera*) Health in Stationary and Migratory Apiaries

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## Abstract

The practice of migratory beekeeping is based on moving honey bee (*Apis mellifera*) colonies between different locations to intensify agricultural production through improved pollination services. However, due to stress caused by exposure of bee hives to different environments, migratory beekeeping activities can lead colonies to greater susceptibility of these insects to pathogens and pests, thus leading to population decline and mortality. The aim of this study was to evaluate the health profile of apiaries that adopt two types of management (stationary and migratory), located in the central-eastern region of São Paulo state, Brazil, during two sampling periods, one in spring (October 2010), and one in autumn (May 2011). We collected 474 samples of honeycomb from the brood area, combs containing capped brood, adult bees that covered the brood area, and foraging bees, to evaluate the presence and prevalence of *Paenibacillus larvae*, *Varroa destructor, Nosema apis* and *N. ceranae*. Seasonality was identified as a determining factor in the health condition of Africanized *A. mellifera* colonies, causing a stronger effect on health than the type of management employed (stationary vs migratory beekeeping). The infection rates of *N. ceranae* were higher during the autumn in relation to the spring (387 ± 554 spores per bee in the spring and 1,167 ± 1,202 spores per bee in the autumn in stationary apiaries and 361 ± 687 spores per bee in the spring and 1,082 ± 1,277 spores per bee in the autumn in migratory apiaries). The same pattern was found for infestation rates of *V. destructor* (2.83 ± 1.97 in the spring and 9.48 ± 6.15 in the autumn in stationary apiaries and 3.25 ± 2.32 in the spring and 6.34 ± 6.58 in the autumn in migratory apiaries). These results demonstrate that the seasonality affects the health of *A. mellifera* colonies, but it does not depend on the type of management adopted (stationary or migratory).

## Introduction

In migratory beekeeping, honey bee (*Apis mellifera*) colonies are transported between locations so that they can pollinate crops, a practice that considerably increases agricultural production (Souza et al., 2007). However, the stress to honey bees due to the confinement they undergo for long periods and distances during migration, pathogens and pests, changing environments, intensive management,

poor nutrition, among other factors, have been linked to the weakening of the bees' immune system, making colonies more susceptible to disease and potential collapse (Naug et al., 2009; Bacandritsos et al., 2010). Honey bees can be affected by various pathogens and parasites including the the bacterium *Paenibacillus larvae*, microsporidian species in the genus *Nosema*, and the ectoparasitic mite *Varroa destructor* (Medina and Martin, 1999; Fries et al., 2010; Genersch et al., 2010).

The bacterium *P. larvae* is responsible for causing American foulbrood (AFB), considered the main cause of bee mortality in the world (Funfhaus et al., 2013). The contamination of bees with AFB occurs during feeding, when the larvae ingest food contaminated with bacterial spores (Garrido-Bailón et al., 2013). In Brazil, American foulbrood was first detected in 2001 in the municipality of Candelaria, Rio Grande do Sul, where samples of adult bees, honey, pollen and brood combs were collected and analyzed for AFB (Schuch et al., 2003), detecting the presence of bacterial spores in adult bees and honeycomb, as well as in imported honey and pollen samples. In 2006, clinical signs of the disease were reported in samples of the municipality of Quatro Barras, Paraná (MAPA, 2006).

Nosemosis, or disease caused by *Nosema* spp., can be caused by the microsporidia *N. apis* and *N. ceranae* after ingestion of spores from contaminated food or during the cleaning or foraging process (Higes et al., 2010; OIE, 2014). The infection affects intestinal epithelial cells (Fries et al., 2010) causing disorders in the digestive system, as well as decreased worker longevity, therefore reducing the population of bees in the colony (Eiri et al., 2015). Nosemosis can be found on five continents (Klee et al., 2007) and acts synergistically with pesticide contamination to lower bee health, a factor also considered responsible for the declining population of honey bees (Pettis et al., 2012). In Brazil, *N. ceranae* has been present since the 1970s (Teixeira et al., 2013), but the disease has not caused major problems in the country so far.

Just like nosemosis, infestations by the ectoparasitic mite *V. destructor* can also harm honey bee colonies, especially by transmitting different types of viruses (Le Conte et al., 2010). The mite feeds on honey bee hemolymph in its larval and adult stage, interfering with the flight performance and leading to a reduced lifespan (Rosenkranz et al., 2010), accounting for severe colony losses to beekeepers in many countries (Genersch et al., 2010; Martin et al., 2012).

Preventive monitoring to learn the health profile of honey bee colonies in Brazil is important, especially for commercial beekeeping operations .This study evaluated the presence and prevalence of *P. larvae*, *N. apis*, *N. ceranae* and *V. destructor*, in colonies from the east-central region of São Paulo state, Brazil, in two different seasons and under two different management systems (stationary vs. migratory).

**Material and Methods**

Sampling bees for analysis of the presence and prevalence of *P. larvae, N. apis, N. ceranae* and *V. destructor* in stationary and migratory honey bee colonies was based on Teixeira and Message (2010). We collected a total of 474 samples: 124 samples of forager bees collected at the entrance of the hive; 110 honeycomb samples from the brood area; 118 samples of comb containing capped brood; and 122 samples of adult bees present in the brood area.

Samples were collected during two different sampling periods, one in spring (Collection 1 - October 2010) and

one in autumn (Collection 2 - May 2011) in stationary and migratory apiaries in the east-central region of São Paulo state. Five municipalities were included in the study: Rio Claro (22º 24' S 47º 33' W), Ipeúna (22º 26' S 47º 43' W), Araras (22º 21' S 47º 23' W), Pirassununga (21º 59' S 47º 25' W) and Descalvado (21º 54' S 47º 37' W). Apiaries that were considered migratory referred to those that had just returned to the municipalities mentioned above from nearby municipalities (Corumbataí Analândia, Botucatu, São Simão, Tambaú, Luís Antônio, among others) after fulfilling pollination services in those locations.

Molecular analyses were performed to identify the bacterium *P. larvae* and microsporidia of the genus *Nosema*. For bacterial analysis, 20 ml of honey was diluted in 40 mL of distilled water and centrifuged at 2,518 $x$ g for 40 min. The supernatant was discarded and the pellet was resuspended in 1 ml of sterile distilled water, with subsequent homogenization and centrifugation at 10,000 × g for 20 min. The supernatant was again discarded and 600 µl of pellet was inoculated on agar PLA (Schuch et al., 2001). The remaining 400 µl of the honey solution (pellet) was used for DNA extraction, with the Qiagen DNeasy Plant Mini Kit® following the manufacturer's recommendations.

After extraction of genetic material, PCR was performed with a final volume of 20 µl using the following primer sequences (Piccini et al., 2002):
F 5´- CGA CGC GAC CTT GTG TTT CC– 3´
R 5´- TCA GTT ATA GGC CAG AAA GC – 3´

The program used for PCR consisted of an initial denaturation step at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 5 min (Puker, 2011).

To identify the species of microsporidia, thirty forager honey bees were pooled and macerated, using 1 ml of sterile distilled water per bee. The macerate was filtered and after constant agitation of the filtrate, a micropipette was used to remove a small aliquot of the suspension (10µl), which was deposited in a Neubauer chamber. The spore suspension was analyzed under light microscopy at 400x (Cantwell, 1970), to count the spores. The remainder of the spore suspension was centrifuged at 2,518g for 40 min at room temperature. The supernatant was discarded and the pellet was resuspended in 1 ml of sterile water. This suspension was centrifuged at 10,000g for 5 min, after which the supernatant was discarded and the pellet was submitted for DNA extraction employing a Qiagen DNeasy® Plant Mini Kit, according to the manufacturer's recommendations. After extraction of genetic material, the PCR reactions were performed with a final volume of 20 µl, using the primers presented by Martin-Hernandez et al. (2007):

*Nosema ceranae* (218 pb):
F 5´- CGG CGA CGA TGT GAT ATG AAA ATA TTA A –3´
R 5´- CCC GGT CAT TCT CAA ACA AAA AAC CG – 3´

*Nosema apis* (321 pb):
F 5´- GGG GGC ATG TCT TTG ACG TAC TAT GTA – 3´

R 5´- GGG GGG CGT TTA AAA TGT GAA ACA ACT ATG – 3´

The program used for PCR consisted initially of a denaturation step 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 extension at 72 °C for 5 min. After the PCR reaction, 5 µl was subjected to electrophoresis in 2% (w/v) agarose gel, stained with SYBR SAFE® in 1X TBE buffer (89mm Tris base, 89 mm boric acid and 2 mM EDTA). After electrophoresis, the gel was visualized with an E-Gel Imager (Life Technologies). The 100pb marker (Invitrogen) was used as a reference to determine the size of the fragments of interest.

The infestation rate of the mite *V. destructor* on adult honey bees was measured according to De Jong et al. (1982): approximately 200 adult honey bees were in plastic pots containing 70% ethanol, with enough volume to cover them. Subsequently, the containers were shaken in order to promote the detachment of possible parasites attached to the bees, which, when presented, are easily visible in the white plastic tray. Considering the number of mites as well as the number of bees per sample were obtained the infestation rates.

To know the infestation rate of the mite *V. destructor* on comb containing capped brood was utilized the method presented by Medina and Martin (1999) and Dietemann et al. (2013): 100 pupae with dark eyes were removed and evaluated for the presence of adults and / or offspring of mite per cell. The average number of adult female mites, infested cells, cells infested with offspring, viable offspring (females possibly become adults until the emergence of honey bee) and reproductive potential (viable offspring / number of adult females and males) were measured.

The data were submitted to analysis of variance according to the statistical model represented by $y_{ijk} = \mu + l_i + c_j + e_{ijk}$, where: $y_{ijk}$ = dependent variables; $\mu$ = general average; $l_i$ = effect of the ith site, $c_j$ = effect of j-th collection and $e_{ijk}$ = random error effect. The degrees of freedom relating to the sources of variation studied were decomposed into contrasts and evaluated by the F-test at 1% significance level. For comparing the infection rate by *Nosema* spp. and the infestation rate by *V. destructor* during the two collection periods, the analyses were performed using the GLM procedure of the SAS statistical package (1990). All analyses were carried out in the Honey Bee Health Laboratory (LASA) of the São Paulo State Agribusiness Technology Agency (APTA/SAA).

**Results**

Neither the bacterium *P. larvae* nor the microsporidian *Nosema apis* was detected by molecular analysis in any of the samples examined. Only *N. ceranae* was detected in the analyzed samples (Figure 1).
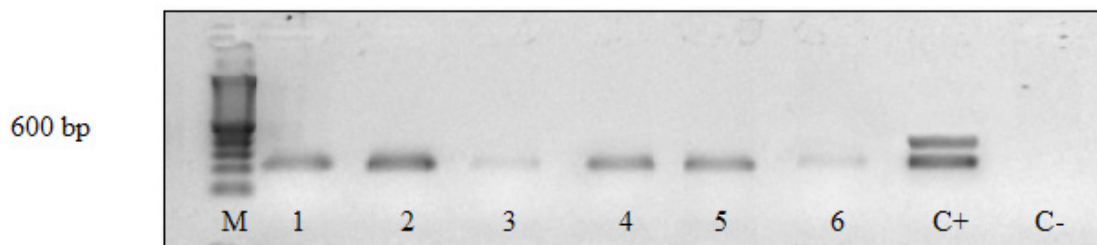


**Fig 1.** Agarose gel (2%) showing duplex *Nosema apis* and *N. ceranae* PCR products. M: 100 bp marker. Column 1- 6: samples showing *N. ceranae* amplification. Column C+: Positive control – *Nosema ceranae* (218 bp) and *Nosema apis* (321 bp). Column C-: Negative control.

The number of spores of *N. ceranae* per bee was significantly higher in the autumn compared to the spring. We did not find a statistical significance in the number of *N. ceranae* spores based on the type of management adopted (stationary vs migratory) (Table 1 and 3).

The levels of *V. destructor* infestation of adult bees covering the brood area, the average quantities of infested brood cells of workers, adult female mites per cell and cells containing descendants of the mite were significantly higher in spring of 2010 than in autumn of 2011 (Table 1, 2 and 3).

**Table 1.** Average number of spores of *Nosema ceranae* x 10³ per bee (or per milliliter) collected in entrance of the hives, and percentage of infestation of *Varroa destructor* mites in adult honeybees collected in brood area in two collection periods in stationary and migratory apiaries.

| | Collection 1 (October) | | Collection 2 (May) | |
|---|---|---|---|---|
| | Stationary Apiaries (n=33) | Migratory Apiaries (n=39) | Stationary Apiaries (n=29) | Migratory Apiaries (n=23) |
| Spores of *N. ceranae* (x 10³) | 387±554a | 361±687a | 1.167±1.202b | 1.082±1.277b |
| *V. destructor* (%) | 2.83±1.97a | 3.54±2.32a | 9.48±6.15b | 6.34±6.58b |

Means followed by different letters differ statistically (p< 0.01).

**Table 2.** Average number of adult females, descendants, infested brood cells (%), infested cells with descendants (%), total viable offspring and reproductive potential of the mite *Varroa destructor* in brood combs, in two collections, in stationary and migratory apiaries. Means followed by different letters differ statistically (p< 0.01).

| | Collection 1 (October) | | Collection 2 (May) | |
|---|---|---|---|---|
| | Stationary Apiaries (n=33) | Migratory Apiaries (n=37) | Stationary Apiaries (n=26) | Migratory Apiaries (n=22) |
| Adult Females (Total) | 1.68±2.28a | 1.31±1.97a | 3.58±3.40b | 2.87±4.03b |
| Descendants (Total) | .24±4.36c | 1.42±2.62d | 5.15±6.19c | 2.52±3.96d |
| Infested Cells (%) | 1.91±2.31e | 1.27±1.73e | 4.23±3.29f | 2.59±3.51f |
| Infested Cells with offspring (%) | 1.18±1.51g | 0.75±1.32g | 2.69±2.74h | 1.63±2.27h |
| Viable offspring (total) | 1.94±3.01i | 0.83±1.54j | 3.34±4.46bi | 1.00±1.57j |
| Reproductive Potential | 1.08±2.37k | 0.17±0.35l | 0.78±0.79k | 0.22±0.33l |

Means followed by different letters differ statistically (p< 0.01).

However, when comparing the number of descendants, number of viable offspring per cell, and the reproductive potential, no significant differences were observed between the collection periods (Table 2 and 3). Furthermore, the reproduction of the mites was significantly higher in stationary hives compared to migratory apiaries.

**Table 3.** Statistical results of infection of *N. ceranae* in adults honey bees and infestation of *V. destructor* in adult honey bees and brood cells.

| | Collection | | | Type of Management | | |
|---|---|---|---|---|---|---|
| | p value | F value | DF | p value | F value | DF |
| *Nosema ceranae* | 0.0002 | 15.21 | 1 | 0.9049 | 0.01 | 1 |
| *V. destructor* in adult honey bees | 0.0026 | 9.58 | 1 | 0.3772 | 0.79 | 1 |
| Adult female mites | 0.0016 | 10.50 | 1 | 0.0934 | 2.86 | 1 |
| Descendants | 0.0325 | 4.68 | 1 | 0.0042 | 8.54 | 1 |
| Infested cells | 0.0004 | 13.17 | 1 | 0.0144 | 6.17 | 1 |
| Infested cells with offspring | 0.0014 | 10.66 | 1 | 0.0346 | 4.57 | 1 |
| Viable offspring | 0.0904 | 2.92 | 1 | 0.0010 | 11.43 | 1 |
| Reproductive potential | 0.6467 | 0.21 | 1 | <.0001 | 16.99 | 1 |

## Discussion

Although the movement of hives between different locations is cited as one of the possible causes for the population decline of honey bee colonies, our results showed no statistically significant differences in infestation rates of pathogens analyzed in stationary and migratory apiaries. Our results are similar to those inferred by Stokstad (2007). Based on data from the United States, where this practice has been adopted for many years, the author infers that the movement of hives alone cannot explain the recent hive collapse problem (van Engelsdorp et al., 2013).

The difference (Table 1) observed in intensity of infection of *N. ceranae* between collections in October (spring) and May (autumn), both in stationary and migratory apiaries with a significant increase in the intensity of infection in the second collection, corroborates our earlier findings in the Vale do Paraíba region, where a higher intensity of infection was observed during the fall (Santos et al., 2014). Martin-Hernandez et al. (2009), studying bees in temperate regions, justified the higher intensities of infection found in the autumn based on the temperature drop, which leads to a prolonged period during which bees are confined in hives, increasing the risk of horizontal transmission of pathogens. Higes et al. (2008) also observed higher *N. ceranae* infection intensity in the cooler months and lower levels in early spring.

Analysis of European and Africanized bees has shown considerable prevalence of the species *N. ceranae* (Paxton et al., 2007; Teixeira et al., 2013), strengthening the hypothesis that *N. ceranae* overlap *N. apis* in infestation of honey bee colonies. The same was observed in this study, as in other recent studies (Santos et al., 2014; Guimarães-Cestaro et al., 2016).

Another analysis of the presence of honey bee pathogens in Turkey, Tozkar et al. (2015) showed also observed a higher prevalence of the species *N. ceranae* in relation to *N. apis*. In the same study, unlike the results obtained here, the authors noted higher levels of infection by *N. ceranae* in migratory colonies than stationary colonies.

According to the authors, it seems likely that the practice of migratory beekeeping allows the spread of disease, constituting a major threat to these insects.

Regarding the assessment of the infestation levels of the mite *V. destructor* on adult bees, there were considerably lower levels of *Varroa* in both sampling periods and types of management (p> 0.01), comparing with other studies conducted in similar climatic conditions (De Jong & Gonçalves, 1998; Lamb et al., 2014; Santos et al., 2014; Guimarães-Cestaro et al., 2016).

Several factors can explain the significant increase in *Varroa* infestation rates in autumn. Dooremalen et al. (2012), measured infestation rates in a temperate region and concluded that losses of colonies due to mites are higher during the colder months. Moretto et al. (1991) stated that the type of climate has a strong influence on the mite infestation rate, which is higher in colder regions. In that study, the authors found infestation rates of 3.5%, 5.11% and 11.37% in municipalities with respective average annual temperatures of 21 ºC (Ribeirão Preto, SP), 18 ºC (Rio do Sul, SC) and 13 ºC (São Joaquim, SC). Carneiro et al. (2014), in a yearlong evaluation of apiaries in Blumenau (SC), observed significant differences in mean values of mite infestation, with the highest rate occurring in winter. Moreover, the shortage of rain, decrease in the amount of pollen and consequent lack of brood in colonies should also be considered as causes of increased mite infestation in the autumn months (Moretto, 1997). Furthermore, defense mechanisms such as grooming behavior tend to increase as the brood number declines (Junkes et al., 2007).

Despite the differences observed in *Varroa* levels between the sampling periods (autumn and spring) and the management types (stationary and migratory), the rates of infestation by adult female mites can be considered low compared with the result of 19.8% obtained by Zhang et al. (2000) in New Zealand, or by Martin et al. (1994, 2001), ranging from 18 to 49% in Bulgaria, 15 to 40% England and 6 to 42% in the United Kingdom. According to van Engelsdorp et al. (2013), migratory colonies have higher risk of mortality and morbidity due to queen-related events (losses/replacement) and low brood quality.

The migratory hives from which we obtained samples had just returned from several locations where they had been intensively managed for honey production. As such, these hives had not been kept in conditions as satisfactory as the stationary apiaries with respect to the brood quantity and quality. This condition may have negatively influenced the reproductive capacity of the mite, explaining the smaller infestation rate in brood comb samples observed in migratory hives.

As argued by Correia-Marques et al. (2003), it is unfortunately virtually impossible to make an objective comparison with the various studies on reproduction of mites due to a lack of uniformity of the variables analyzed. According to Carneiro et al. (2014), despite the increase in reproductive capacity, infestation levels remain low, which

probably means that defense mechanisms, such as grooming and hygienic behavior, have allowed colonies of Africanized honey bees maintain low infestation rates in Brazil (De Jong, 1997; Carneiro et al., 2014.).

According to Van Engelsdorp et al. (2013), migratory colonies have higher risk of mortality and morbidity due to queen-related events (losses/replacement) and low brood quality. The migratory hives from which we obtained samples had just returned from several locations where they had been intensively managed for honey production. As such, these hives had not been kept in conditions as satisfactory as the stationary apiaries with respect to the brood quantity and quality. This condition may have negatively influenced the reproductive capacity of the mite, explaining the smaller infestation rate in brood comb samples observed in migratory hives.

In relation to infestation rates of mite *V. destructor*, the results obtained here corroborate with the inferences made by Correia-Marques et al. (2003). According the author, it is unfortunately virtually impossible to make an objective comparison with the various studies on reproduction of mites due to a lack of uniformity of the variables analyzed.

In Brazil, the infestation rates of mites are considered low, even though an increase in their reproductive capacity has been observed (Carneiro et al., 2014). Here we also observed low infection rates, as seen by Santos et al. (2014) and Guimarães-Cestaro et al. (2016) in the region of Vale do Paraíba and Vale do Ribeira, respectively and Carneiro et al. (2014) in Blumenau. These results may be related to defense mechanisms performed by Africanized Honey bees, such as hygienic behavior (De Jong et al., 1997; Carneiro et al., 2014).

The parameters evaluated in this study, in the region and periods studied, indicate that seasonality affects the health of colonies of Africanized *A. mellifera* but does not depend on the type of management adopted (stationary or migratory). The intensity of infection by *N. ceranae* and infestation by *V. destructor* were higher during the fall compared to the spring.

## Acknowledgments

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