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Honey Bee Health in Apiaries in the Vale do Paraíba, São Paulo State, Southeastern Brazil

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Abstract

Bee health is a growing global concern due to phenomena with as yet undefined causes, such as the sudden population decline of colonies that has been observed in apiaries in many countries, recently including Brazil. The main objective of this study was to assess the presence and/or prevalence of pathogens that afflict Africanized *Apis mellifera* bees in the Vale do Paraíba region of São Paulo state, Brazil. Three sampling periods were established: period 1 – August and September 2009 (winter/early spring); period 2 – December 2009 and January 2010 (summer); and period 3 – April and May 2010 (autumn). Samples were collected of honeycomb from the brood area, combs containing capped brood, adult bees that cover the brood and foraging bees, to evaluate the presence and prevalence of *Paenibacillus larvae*, *Varroa destructor* and *Nosema* sp. The results indicated that the intensity of infection by *Nosema ceranae* and infestation rates of *V. destructor* in the hives were low (mean $637x10^3$ spores of *Nosema ceranae*, 5.41% infestation of *Varroa* in adult bees and 4.17% infestation of *Varroa* in brood), with no detection of *P. larvae* spores in the samples. The prevalence of *N. ceranae* and *V. destructor* was high, at respective values of 85.2 and 95.7%. All told, 1,668 samples were collected from 438 hives, in 59 apiaries. These results demonstrate that although mites and microsporidia are widespread in the region's colonies, the Africanized bees are apparently tolerant to pathogens and parasites. However, the mechanisms related to defense against pathogens are not completely clear, and monitoring and prophylactic measures are essential to maintain the health of bee colonies.

Introduction

Although honey bees are the most frequently studied social insects due to their ecological and economic importance (Winston, 1987; Martin, 2001), a set of factors still not well understood has been affecting these arthropods in an increasing number of countries. Various organisms can parasitize honey bees in the immature or adult phase, possibly leading to collapse of the colony depending on the level of virulence (Bailey & Ball 1991; Ellis & Munn, 2005). There have been many reports of colony collapse in the Northern Hemisphere in recent years, prompting strong concern in the scientific community, particularly due to the importance of bees as pollinizers (Neumann & Carreck, 2010). This phenomenon, called Colony Collapse Disorder (CCD), was first identified in the United States in the winter of 2006, when 23% of apiaries were affected, with average colony losses of 45% (Van Engelsdorp et al., 2007). Besides the risks posed by CCD, another factor that must be considered is the potential problems caused by applying drugs to control diseases, because of the possibility of generating resistant populations and of contamination of bee products, with consequent risks to human health (Lodesani et al., 2008).

Although honey bees are afflicted by numerous parasites and pathogens, including viruses, bacteria, protozoa and mites (Bailey & Ball, 1991), in Brazil there are few

reports of diseases causing large-scale mortality. This pattern could be related to a series of biotic and abiotic factors typical of each region, among them the breed of bees used in local apiculture (Moretto, 1997). Africanized honey bees (AHB) have traits that favor resistance to parasites such as the mite *Varroa destructor*, among other pathogens (De Jong, 1996; Rosenkranz, 1999; Rosenkranz et al., 2010).

Nevertheless, in recent years honey production in some Brazilian regions has been declining, accompanied by observations of apparent weakening of colonies, with adult bees and brood in many cases presenting anomalous symptoms (Message et al., 2012). The explanation for this fact can be related both to increased virulence of parasites already present in the country and the introduction of new pathogenic agents, such as viruses (Teixeira et al., 2008).

Preventive monitoring of the levels of infestation and prevalence of harmful agents is important to maintain the health of apiculture in the country. In this study, we investigated the prevalence and incidence of *V. destructor, Nosema* sp. and *Paenibacillus larvae* in apiaries of southeastern Brazil. These agents have been indicated as responsible for causing large losses to apiculture worldwide.

Material and Methods

Samples were collected in 438 colonies of 59 apiaries, located in 13 municipalities in the region of Vale do Paraíba, São Paulo state. In this region, the climate is considered subtropical (Koeppen climate classification), with temperatures varying from 18ºC to 29ºC during the year.

Samples included pieces of honeycomb from the brood area, comb containing capped brood, adult bees covering the breeding area from brood comb, and forager bees collected at the entrance of the hive, to assess the presence and prevalence of *Paenibacillus larvae*, *Varroa destructor* and *Nosema* sp., respectively. We established three collection periods, to obtain samples from different moments of colony development as a function of the natural conditions and availability of food resources in the field (nectar and pollen flow): period 1 – August and September 2009 (winter/early spring); period 2 – December 2009 and January 2010 (summer); and period 3 – April and May 2010 (autumn). All the analyses were carried out in Pindamonhangaba, São Paulo, in the Honey Bee Health Laboratory of the São Paulo State Agribusiness Technology Agency (LASA/APTA). The collection of the samples for analysis of *Varroa destructor, Nosema* sp. and *Paenibacillus larvae* was based on Teixeira & Message (2010). Evaluations of *Varroa destructor* infestations were based on De Jong et al. (1982). The protocol for microbiological analyses developed by Schuch et al. (2001) and later considered as the Brazilian method (Brasil, 2003) was used to analyze samples for *P. larvae*, while the method of Cantwell (1970) was used to count the spores for *Nosema*. In order to identify the *Nosema* species, samples from all apiaries were submitted to duplex PCR assay (Martín-Hernández et al., 2007), using the primers 321APIS-FOR/REV, for identification of *N. apis* and 218MITOC-FOR/REV for *N. ceranae*. Positive and negative controls were used.

Analysis of variance was carried out considering a statistical model whose representation is given by: $y_{ijk} = \mu + l_i + c_j + e_{ijk}$, where: y_{ijk}= dependent variables; μ = overall mean; l_i = effect of the i^{th} site, c_j = effect of the j^{th} sample and e_{ijk} is the random effect of the error. The degrees of freedom for the sources of variation studied were decomposed into contrasts and evaluated through the F-test at a significance level of 1%. All analyses were performed using the GLM procedure of the SAS statistical package (2001).

Results

During the three sampling periods, 438 samples of foraging bees were collected at the hive entrances, and for other types of samples it was possible to collect 432 samples of adult bees present in the brood area, 368 comb pieces containing honey from the brood area and 430 samples of comb containing at least 100 older pupae. Table 1 shows the intensity of infection by the microsporidium *Nosema ceranae*, represented by the average number of spores per bee, as well as the average infestation rate (%) by the mite *V. destructor* in adult bees and in brood in apiaries located in 13 locations in the Vale do Paraíba: Cunha, Bananal, Lagoinha, Lorena, Monteiro Lobato, Natividade da Serra, Paraibuna, Pindamonhangaba, Redenção da Serra, São José dos Campos, São Luís do Paraitinga, Santo Antônio do Pinhal, Taubaté e Tremembé. Only the species *N. ceranae* was detected by molecular analysis (Fig. 1), and no *P. larvae* spores were detected in the honey samples.

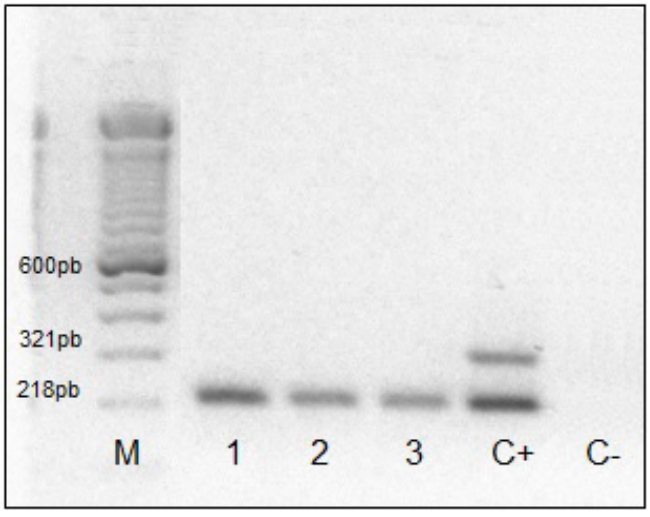

Fig. 1. 2% agarose gel showing duplex *Nosema apis* and *Nosema ceranae* PCR products. M: 100 bp marker. Column 1- 3: samples showing *N. ceranae* amplification. Column C+: Positive control – *Nosema ceranae* (218 bp) and *Nosema apis* (321 bp). Column C-: Negative control.

Table 1. Mean number of spores of *Nosema ceranae* per bee, mean infestation rate (%) by the mite *V. destructor* in brood worker bees and mean infestation rate (%) by *V. destructor* in brood in different municipalities*.

Municipality	No. of *Nosema* sp. spores (x10³)	*V. destructor* in adult bees (%)	*V. destructor* in brood (%)
Bananal	623 ± 188a	5.49 ± 0.97a	6.63 ± 1.34a
Cunha	485 ± 128a	3.53 ± 0.69a	2.93 ± 0.78a
Lagoinha	258 ±106a	5.87 ± 0.56a	5.65 ± 0.66a
Monteiro Lobato	623 ± 145a	5.92 ± 0.76a	5.70 ± 0.88a
Paraibuna	581 ± 99a	4.94 ± 0.51a	3.61 ± 0.61a
Pindamonhangaba (APTA)	1.655 ± 114b	4.74 ± 0.59a	3.81 ± 0.69a
Pindamonhangaba	424 ± 110a	6.75 ± 0.59a	3.30 ± 0.68a
Redenção da Serra	636 ± 153a	4.01 ± 0.79a	2.12 ±0.91a
São Jose dos Campos	459 ± 145a	4.26 ± 0.75a	2.52 ± 0.88a
Santo Antonio do Pinhal	607 ± 275a	4.17 ± 1.42a	1.14 ± 1.66a
São Luis do Paraitinga	369 ± 386a	5.66 ± 1.99a	2.90 ± 2.33a
Taubaté	1.055 ± 153b	4.90 ± 0.79a	3.55 ± 0.93a
Tremembé	658 ± 99a	5.82 ± 0.51a	4.65 ± 0.60a

* In collection period 2, it was not possible to obtain samples in five localities. †Means accompanied by different letters differ significantly (P<0.01).

Table 2. Mean number of spores of *Nosema ceranae* per bee, mean infestation rate (%) by the mite *V. destructor* in brood worker bees and mean infestation rate (%) by *V. destructor* in brood in different collection periods (1: winter 2009, 2: summer 2010, 3: autumn 2010).

Collection Periods	No. of *Nosema sp.* spores (x10³)	*V. destructor* in adult bees (%)	*V. destructor* in brood (%)
1	379 ± 62a	6.03 ± 0.32a	4.58 ± 0.37a
2	689 ± 87b	3.57 ± 0.45b	1.95 ± 0.53b
3	879 ± 74b	5.65 ± 0.39a	4.66 ± 0.46a
Mean	637 ± 36	5.41 ± 0.20	4.17 ± 0.23

† Means accompanied by different letters differ significantly (P<0.01).

The apiaries in Pindamonhangaba and Taubaté presented the highest infection intensities, differing significantly (P<0.001) from the other municipalities studied.

Table 2 reports the intensities of infection by the microsporidium in the three sampling periods. The first period (winter and early spring 2009) presented the least intense infection by *Nosema ceranae*, differing (P<0.001) from the intensities of periods 2 (summer 2010) and 3 (autumn 2010).

The results for the number of adult *V. destructor* mites as well as descendants in the 13 locations and three collection periods can be observed in Tables 1 and 2, respectively. The overall average infestation of *V. destructor* in adult bees was 5.41 ± 0.20 (Table 1). The infestation rates observed in period 2 were lower in relation to the other two periods (1 and 3), both in adult bees and brood. Therefore, there was a difference (P<0.01) of the infestation rates observed in these periods; but in all cases the infestation levels were low.

Discussion

This is the first comprehensive study (1,668 samples analyzed, collection periods during all seasons) conducted to identify the prevalence of three typical honey bee pathogens (*Nosema* sp., *Varroa destructor* and *Paenibacillus larvae*) in Brazil. Although *Nosema apis* was a highly problematic pathogen in the 1970s in the southern region of Brazil (Teixeira et al., 2013), it was not detected in the present study. In the first collection period (winter and early spring 2009), the number of spores detected per bee was very low, differing significantly (P<0.001) in relation to the other two periods (summer and autumn 2010).

Traver et al. (2011) also observed low levels of this pathogen in the winter, but in the autumn their results were opposite to ours, even though in the region of the United States studied by them the autumn temperatures are near those in the winter in São Paulo. In turn, Higes et al. (2008) observed higher intensities of infection by *N. ceranae* in the coldest months and lower intensities at the beginning of spring, while in Germany, Gisder et al. (2010) found that the intensity of infection by *Nosema* spp. was greater in spring than in autumn.

Although comparisons of infection intensity between different regions are highly contradictory, even considering data from temperate regions where the seasons are well defined, the climatic peculiarities of the regions under analysis must be considered, because they can affect the intensity of infection, even if only indirectly (Le Conte & Navajas, 2008). In Brazil, no pattern in the infection intensity of *Nosema*

ceranae has been observed during the year (Teixeira et al., 2013), considering the weather or region.

In the two places (Pindamonhangaba – SP and Taubaté – SP) where infection by *Nosema ceranae* was higher than at the other sites, the experimental apiaries are frequently managed for teaching/research purposes rather than production, so the frequent management practices (change of frames, common use of material between colonies, etc.) might have facilitated dispersion and/or made the bees more susceptible due to stress from frequent human intervention.

The low numbers of spores in our samples and the absence of collapse of any of the colony analyzed, in contrast to the observations of Higes et al. (2008), can possibly be credited to a higher tolerance of Africanized *A. mellifera* honey bees. In fact, many questions can be posed in relation to *Nosema ceranae*, mainly regarding the absence of clinical symptoms in infected colonies and the considerable variation of infection intensity within very short periods (D. Message, unpublished data).

In the state of São Paulo, considering the samples analyzed so far, the presence of the species *Nosema ceranae* has been confirmed in 100% of the cases where *Nosema* has been observed (Fig 1). According to Teixeira et al. (2013), even with the proof of the high prevalence of *N. ceranae* in the country's apiaries, the recommendation by researchers and technicians who work in the area of honey bee health is not to use chemical products, due to the inconsistency of the real effect of the presence of this pathogen in the colonies.

The first report of the species *Nosema ceranae* in Brazil was by Klee et al. (2007); these authors reported this species of microsporidium on four continents. According to various authors, *N. ceranae* jumped from *Apis cerana* to *Apis mellifera* probably in the 1990s, since then dispersing to most regions of the world (Fries et al., 1996; Higes et al., 2006; Klee et al., 2007; Paxton et al., 2007; Fries, 2010). Nevertheless, in Brazil, Teixeira et al. (2013) detected its presence in samples that had been collected in the southern state of Rio Grande do Sul more than three decades ago. In Asia, where it supposedly originated, recent articles have reported the presence of *N. ceranae* in Vietnam (Klee et al., 2007) and Iran (Nabian et al., 2011). In Europe, studies have confirmed its presence since 1998 (Fries et al., 2006; Paxton et al., 2007), in several countries: Spain (Fries et al., 2006; Higes et al., 2006; Klee et al, 2007; Martín- Hernandez et al., 2007), France (Chauzat et al., 2007), Germany (Klee et al., 2007), Sweden (Klee et al., 2007), Finland (Klee et al., 2007; Paxton et al., 2007), Denmark (Klee et al., 2007), Greece (Klee et al., 2007), Hungary (Tapaszti et al., 2009), Holland (Klee et al., 2007), United Kingdom (Klee et al., 2007), Italy (Klee et al., 2007), Serbia (Klee et al, 2007), Poland (Topolska &Kasprzak, 2007), Bosnia (Santrac et al., 2009) and Turkey (Whitaker et al., 2010). In Africa, Higes et al., (2009) reported *N. ceranae* in Algeria in 2008. In North America, this species has been present since 1995, affecting colonies mainly in the United States (Klee et al., 2007; Chen et al., 2008; Williams et al., 2008), as well as Canada (Williams et al., 2008) and Mexico (Guzmán-Novoa et al., 2011). In Central America, *N. ceranae* was found in Costa Rica by Calderón et al. (2010), and in South America it is present in Brazil and Argentina (Klee et al., 2007; Medici et al., 2012), Uruguay (Invernizzi et al, 2009) and Chile (Martínez et al., 2012). In Oceania, recent studies reported this species in New Zealand (Klee et al., 2007) and Australia (Giersch et al., 2009).

With respect to the mite *Varroa destructor*, the infestation rates were low, as also reported by Pinto et al. (2011) in Africanized bees in a region with similar environmental conditions to those in the present study, although with a smaller number of samples. On the other hand, in evaluating the infestation levels in the Forest Zone of the state of Minas Gerais, a region with a tropical climate with temperatures varying from 14 to 26ºC during the year (Koeppen climate classification), Bacha Junior et al. (2009) found an average value of 7.8% during the summer. Apparently, there is great variation in the infestation rates of this mite according to the region of the country and the respective climate (Moretto, 1997).

Showing the same tendency as the levels of infestation in adult bees, the rates of infestation by the mite in brood combs were low in comparison with those found in colonies without treatment in Bulgaria, England and the entire United Kingdom (18 to 49%, 15 to 40% and 6 to 42%, respectively) (Martin, 1994; 2001). Usually, when the temperature drops, especially during the autumn and the winter, the brood area shrinks, as a consequence of the reduced availability of food resources in the environment and the number of phoretic mites increases. This can explain the low number of the mites in the capped brood.

The high infertility rate of this mite on Africanized bees in Brazil (Message & Gonçalves, 1995; Rosenkranz, 1999; Calderón et al., 2010; Rosenkranz et al., 2010) could also explain the low infestation rate observed. However, Garrido et al. (2003), analyzing samples from different regions of Brazil, found predominance of the haplotype K, and also an increase in the mite's fertility rate.

The intensity levels of infection by *N.ceranae* and the rates of infestation by *V. destructor* observed in this study are low compared to other regions where chemical treatments are used for control in temperate zones, but the prevalence of these pathogens was high, considering that 85.2% of the hives were infected by the microsporidium and 95.7% presented infestation by mites.

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References

Bacha Júnior, G.L., Felipe-Silva, A.S., Pereira, P.L.L. (2009). Taxa de infestação por ácaro *Varroa destructor* em apiários sob georreferenciamento. Arquivos Brasileiros de Medicina Veterinária e Zootecnia, 61: 1471–1473.

Bailey, L.,Ball, B.V. (1991). Honey Bee Pathology. London: Academic Press, 208p.

Brasil (2003) Ministério da Agricultura e do Abastecimento. Instrução Normativa n° 62, de 26 de agosto de 2003. Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal. Anexo, Capítulo XIX Pesquisa de *Paenibacillus larvae* subsp. *larvae*. Diário Oficial da União, 18/09/2003.

Calderón, R.A., Van Veen J.W., Sommeijer, M.J., Sanchez, L.A. (2010). Reproductive biology of *Varroa destructor* in Africanised honey bees (*Apis mellifera*). Experimental and Applied Acarology, 50: 281–297.

Cantwell, G. R. (1970). Standard methods for counting nosema spores. American Bee Journal, 110: 222-223.

Chauzat, M.P., Higes, M., Martín-Hernandez, R., Meana, A., Cougoule, N., Faucon, J.P., (2007). Presence of Nosema ceranae in French honey bee colonies, Journal of Apicultural Research, 46: 127-128. DOI: 10.3896/IBRA.1.46.2.12.

Chen, Y., Evans, J.D., Smith, I.B., Pettis, J.S., (2008). *Nosema ceranae* is a long-present and wide-spread microsporidean infection of the European honey bee *(Apis mellifera)* in the United States. Journal of Invertebrate Pathology, 97: 186–188.

De Jong, D., Roma, D. A., Gonçalves, L.S. (1982). A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honey bees. Apidologie, 13: 297–306.

De Jong, D. (1996). Africanized honey bees in Brazil, forty years of adaptation and success. Bee World, 77: 67–70.

Ellis, J.D. & Munn, P. (2005). The worldwide health status of honey bees. Bee World, 86: 88-101.

Fries, I. (2010). *Nosema ceranae* in European honey bees (*Apis mellifera*). Journal of Invertebrate Pathology, 103: 573-579.

Fries, I., Feng, F., Silva, A., Slemenda, S.B., Pieniazek, N. J. (1996). *Nosema ceranae* sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). European Journal of Protistology, 32: 356-365.

Fries, I., Martín, R., Meana, A., García-Palencia, P., Higes, M. (2006) .Natural infections of *Nosema ceranae* in European honey bees. Journal of Apicultural Research, 45: 230-233.

Garrido C., Rosenkranz, P., Paxton, R.J., Gonçalves, L.S. (2003). Temporal changes in *Varroa destructor* fertility and haplotype in Brazil. Apidologie, 53: 535–541.

Giersch, T., Berg, T., Galea, F., Hornitzky, M. (2009). *Nosema ceranae* infects honey bees (*Apis mellifera*) and contaminates honey in Australia. Apidologie, 40: 117-123.

Gisder, S., Hedtke, K., Mockel, N., Frielitz, M., Linda, A., Genesrch, E. (2010). Five-Year Cohort Study of *Nosema* spp. in Germany: Does Climate Shape Virulence and Assertiveness of *Nosema ceranae*? Applied and Environmental Microbiology, 76:3032–3038. DOI: 10.1128/AEM.03097-09.

Guzmán-Novoa, E., Hamiduzzaman, M, M., Arechavaleta, M. E., Koleoglu, G. (2011). *Nosema ceranae* has parasited Africanized Honey bees in Mexico since at least 2004. Journal of Apicultural Research, 50: 167-169 DOI: 10.3896/IBRA.1.50.2.09.

Higes, M., Martín -Hernández R., Meana, A. (2006). *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. Journal of Invertebrate Pathology, 92: 93-95. DOI: 10.1016/j.jip.2006.02.005.

Higes, M., García-Palencia, P., Martín-Hernández, R., Botías, C., Garrido-Bailón, E., González-Porto, A.V., Barrios, L., Del Nozal, M.J., Berna, J. L., Jiménez, J. J., Palencia, P. G., Meana, A. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environmental Microbiology, 10: 2659-2669. DOI: 10.1111/j.1462-2920.2008.01687.x.

Higes, M., Matin-Hernandez, R., Garrido-Bailón E., Botías C., Meana A. (2009). First detection of *Nosema ceranae* (Microsporidia) in African Honey bees (*Apis melifera intermissa*). Journal of Apicultural Research, 48: 217–219. DOI: 10.3896/IBRA.1.48.3.12.

Invernizzi, C., Abud C., Tomasco, I.H., Harriet, J., Ramallo, G., Campa, J., Katz, H., Gardiol, G., Mendonça, Y.(2009). Presence of *Nosema ceranae* in honeybees (*Apis mellifera*) in Uruguay. Journal of Invertebrate Pathology, 101: 150-153

Klee J., Besana A.M., Genersch E., Gisder S., Nanetti, A., Tam D.Q., Chinh T.X., Puerta F., Ruz, J.M., Kryger P., Message D., Hatjina, F., Korpela, S., Fries, I., Paxton, R.J. (2007). Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of Invertebrate Pathology, 96: 1-10.

Le Conte, I.& Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. Revue Scientifique et Technique de L`Office Internationalndes Epizooies, 27 : 499-510.

Lodesani, M., Costa, C., Serra, G., Colombo, R., Sabatini, A. G. (2008). Acaricide residues in beeswax after conversion to organic beekeeping methods. Apidologie, 39: 324-333.

Martin, S.J. (1994) Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. Experimental and Applied Acarology, 18: 87–100.

Martin, S.J. (2001). *Varroa destructor* reproduction during the winter in *Apis mellifera* colonies in UK. Experimental and Applied Acarology, 25: 321–325.

Martín-Hernández, R., Meana, A., Prieto, L., Salvador, A.M., Garrido-Bailon, E., Higes, M. (2007). Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Applied Environmental Microbiology, 73: 6331-6338. DOI: 10.1128/AEM.00270-07.

Martínez, J., Leal, G., Conget, P. (2012). *Nosema ceranae* an emergent pathogen of *Apis mellifera* in Chile. Parasitological Research, 111: 601–607. DOI: 10.1007/s00436-012-2875-0.

Medici, S.K., Sarlo, E.G., Porrini, M.P., Braunstein, M., Eguaras, M.J.(2012). Genetic variation and widespread dispersal of *Nosema ceranae* in *Apis mellifera* apiaries from Argentina. Parasitol. Res., 110: 859-864. DOI: 10.1007/ s00436-011-2566-2.

Message, D., Gonçalves, L.S. (1995). Effect of size of worker brood cells of Africanized honey bees on infestation and reproduction of the ectoparasitic mite *Varroa jacobsoni* Oud. Apidologie, 26: 381- 386.

Message, D., Teixeira, E.W., De Jong, D. (2012). Situação da Sanidade das abelhas no Brasil. In: Imperatriz-Fonseca et al, Polinizadores no Brasil: Contribuição e perspectivas para a biodiversidade, uso sustentável, conservação e serviços ambientais. São Paulo, EDUSP.

Moretto, G. (1997). Defense of Africanized bee workers against the mite *Varroa jacobsoni* in Southern Brazil. American Bee Journal, 137: 746–747.

Nabian, S., Ahmadi, K., Nazem Shirazi, M.H., Gerami Sadeghin, A. (2011). First Detection of Nosema ceranae, a Microsporidian Protozoa of European Honeybees (*Apis mellifera*) In Iran. Iranian Journal of Parasitology, 6: 89-95.

Neumann, P. & Carreck, N.L. (2010). Honey bee colony losses. J Apic. Res., 49: 1-6. DOI 10.3896/IBRA.1.49.1.01

Paxton, R., Klee, J.S., Korpela, S., Fries, I. (2007). *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie, 38: 558–565. DOI: 10.1051/apido:2007037.

Pinto, F.A., Puker, A., Message, D., Barreto, L.M.R.C. (2011). *Varroa destructor* in Juquitiba, Vale do Ribeira, Southeastern Brazil: Sazonal Effects on the Infestation Rate of Ectoparasitic Mites in Honeybees. Sociobiology, 57: 511–518.

Rosenkranz, P. (1999). Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America. Apidologie, 30: 159–172.

Rosenkranz, P., Aumeier, P., Ziegelmann, B. (2010). Biology and control of *Varroa destructor.* Journal of Invertebrate Pathology, 103: 96-119. DOI: 10.1016/j.jip.2009.07.016.

Santrac, V., Granato, A., Mutinelli, F. (2009). First detection of *Nosema ceranae* in *Apis mellifera* from Bosnia and Herzegovina, Proc. Workshop "*Nosema* disease: lack of knowledge and work standardization" (COST Action FA0803) Guadalajara, http://www.coloss.org/news/nosema-workshop-proceedings-online (accessed on 01 Oct. 2013).

SAS - Statistical Analysis System (2001). SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC, USA.

Schuch, D.M.T., Madden, R.H.; Sattler, A., 2001. An improved method for the detection and presumptive identification *Paenibacillus larvae* spores in honey. Journal of Apicultural Research, 40: 59-64.

Tapaszti, Z., Forgách, P., Kövágó, C., Békési, L., Bakonyi, T., Rusvai, M. (2009). First detection and dominance of *Nosema ceranae* in Hungarian honeybee colonies. Acta Veterinaria Hungarica, 57: 383-388. DOI: 10.1556/AVet.57.2009.3.4.

Teixeira, E.W., Chen, Y., Message, D., Pettis, J., Evans, J.D. (2008). Virus infections in Brazilian honey bees. Journal of Invertebrate Pathology, 99: 117–119. DOI: 10.1016/j.jip.2008.03.014

Teixeira, E. W., Message, D. (2010). Manual Veterinário de Colheita e Envio de Amostras-Abelhas *Apis mellifera*. São Paulo: Editora Horizonte, OMS/OPAS/MAPA. p. 175-213, 2010.

Teixeira, E. W., Santos, L. G., Sattler, A., Message, D., Alves, M. L. T. M. F., Martins, M. F., Grassi-Sella, M. F., Francoy, T. M. (2013). *Nosema ceranae* has been present in Brazil for more than three decades infecting Africanized honey bees. Journal of Invertebrate Pathology, 114: 250-254. 2013. DOI: 10.1016/j.jip.2013.19.002.

Topolska, G. & Kasprzak, S. (2007). First cases of *Nosema ceranae* infection in bees in Poland. Medycyna Weterynaryjna Suppl., 63: 1504-1506.

Traver, B.E., Williams, M.R., Fell, R.D. (2011). Comparison of within hive sampling and seasonal activity of *Nosema ceranae* in honey bee colonies. Journal of Invertebrate Pathology, 109: 187-93.

VanEngelsdorp, D., Underwood, R. M., Caron, D., Hayes Jr. J. (2007). An estimate of managed colony losses in the winter of 2006-2007: a report commissioned by the Apiary Inspectors of America. American Bee Journal, 147: 599-603.

Whitaker, J., Szalanski, A. L., Kence, M. (2010). Molecular detection of *Nosema ceranae* and *Nosema apis* from Turkish honey bees. Apidologie, 41: 364-374. DOI: 10.1051/apido/2010045

Williams G.R., Shafer, A.B., Rogers, R.E., Shutler, D., Stewart, D.T. (2008). First detection of *Nosema ceranae*, a microsporidian parasite of European honey bees (*Apis mellifera*), in Canada and central USA. Journal of Invertebrate Pathology, 97: 189–192. DOI:10.1016/J.JIP.2008.04.005.

Winston, M. L. (1987). The Biology of the Honey Bee. Harvard University Press. Cambridge. London. UK.