

of the difficulty of collecting samples such as in our large country, but also because of the time and the cost of diagnosis techniques involved. The analyze of the presence of the spores of honeybee pathogens in honey has shown to be a good strategy for epidemiological studies and early detection before the expression of symptoms in the colony. Therefore it is important the standardization of techniques for rapid diagnosis to facilitate the safe performance of the epidemiological surveys, and controlling the spread of these microorganisms. Here, we it was standardized a multiplex PCR technique for simultaneous detection of four pathogens of *A. mellifera*: *A. apis*, *N. apis*, *N. ceranae* and *P. larvae* in honey. This technique was used in honey samples from some Brazilian states. Sterile honey samples (20 mL) were artificially contaminated with all selected pathogens. These positive samples were diluted in 30 mL sterile water followed by centrifugation. DNA from pellet was extracted using a commercial kit. A Multiplex PCR was standardized using specific primers and a common melting temperature. Recommendations of national legislation were used for preparation of honey solutions submitted to the developed technique. Also it were prepared samples of honey collected from brood area, extracted honey from supers by beekeepers and acquired from different commercial establishments used to validate the multiplex PCR, as well as to conduct a preliminary assessment of the distribution of pathogens (*A. apis*, *N. apis*, *N. ceranae* and *P. larvae*). The standard technique of this study was effective for diagnosing of the four pathogens in honey of *A. mellifera*: *A. apis*, *N. apis*, *N. ceranae* and *P. larvae*. Primers used in both PCR reactions (monospecific and multiplex) for DNA amplification of the target pathogens were precise and sensitive, resulting in products of expected sizes. The formation of nonspecific fragments and/or other artificial PCR products was not observed in multiplex PCR reactions. Thus, this method was suitable for simultaneous detection of the selected pathogens of *A. mellifera* extracted from honey, and probably can be used in other hive products with minor modifications. The selected pathogens were not found in the honey samples analyzed with this multiplex PCR standardized.

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Honey bee health and losses in Brazil

Sanidade apícola e perdas no Brasil. Sanidade apícola e perdas de colônias no Brasil

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In Brazil, though the Africanized honeybees are considered resistant and tolerant to most of bee pathogens and parasites, in recent years considerable losses have been reported in some localities, especially in the Southeast and Southern states. Since the phenomenon that is affecting honeybees around the world has been observed also in Brazil (with less intensity), we proposed a research project aiming to determine possible causes for such decline or losses, and in particular to test the likelihoods of specific predicted causative agent(s) for this condition. Alternative approaches, including genetic screening can be extremely useful and can accelerate important discoveries related to the current problem. Here it was present an overview about these activities and a summary (2009 to 2011) of the situation in the two regions. No symptoms of the American Foul Brood were detected and analysis of more than 600 honey samples presented negative results, using the Brazilian official method for microbiological detection of *Paenibacillus larvae* in honey (molecular techniques confirmed such absence). *Leptomonas apis*, and *Spiroplasma apis* were also detected in São Paulo State samples. One of the main sanitary problems of the Africanized bees (beekeeper loses, sometimes, all colonies during less than one-month period) is Brazilian Sacbrood-like disease, caused by the toxic effects of the *Stryphnodendron* spp pollen in the Atlantic Forest and Savannah regions (Sac Brood Virus has not been detected in these colonies and the symptoms are similar). Nowadays, the selection for resistance is under evaluation. Other brood mortality has been also observed during the last years showing anomalous symptoms. In adult bees different type of viruses such as ABPV, DWV, BQCV, IAPV have been detected, and previously CWV, FV and CBPV. Accidents with insecticides have been related. Collapse and mortality of adult bees have been detected in sugar cane areas where neonicotinoids normally are used, but in areas without sugar cane crop too. *Nosema ceranae* is present in many apiaries, showing high prevalence. The obtained results indicated that *N. ceranae* infection seems to suppress bees immune response of *A. mellifera* due the peptides transcription of the antimicrobes abaecina and himenoptaecina. *N. apis* was detected only in some localities of Santa Catarina and Paraná. *Varroa destructor* is present throughout apiaries, but causing no apparent direct economical damages. Replacement of the queens from beehives showing symptoms has been recommended, avoiding chemotherapeutic drugs usage. There is a critical need to increase the number of technicians and laboratories dedicated to bee pathology in several regions of the country in order to have a better control of the sanitary situation in the apiaries. Pathological, epidemiological, and widespread studies must be conducted as no single pathogen seems to be predominant in declining bee colonies.

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