Short Communication

Virus infections in Brazilian honey bees

Erica Weinstein Teixeira a,*, Yanping Chen b, Dejair Message c, Jeff Pettis b, Jay D. Evans b

a Agência Paulista de Tecnologia dos Agronegócios, SAA-SP, Av. Prof. Manoel Cesar Ribeiro, 1920. CP: 07, 12400-970 Pindamonhangaba, São Paulo, Brazil
b USDA-ARS Bee Research Laboratory, Beltsville, MD, USA

Abstract

This work describes the first molecular-genetic evidence for viruses in Brazilian honey bee samples. Three different bee viruses, Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), and Deformed wing virus (DWV) were identified during a screening of RNAs from 1920 individual adult bees collected in a region of southeastern Brazil that has recently shown unusual bee declines. ABPV was detected in 27.1% of colony samples, while BQCV and DWV were found in 37% and 20.3%, respectively. These levels are substantially lower than the frequencies found for these viruses in surveys from other parts of the world. We also developed and validated a multiplex RT-PCR assay for the simultaneous detection of ABPV, BQCV, and DWV in Brazil.

© 2008 Elsevier Inc. All rights reserved.
describe and validate a multiplex RT-PCR assay for the simultaneous detection of ABPV, BQCV, and DWV in honey bees from Brazil.

Samples were collected from 10 apiaries, located in the municipality Altinópolis, São Paulo State, Southeast Brazil. Samples were collected in April, 2007, coincident with population declines in that area. Adult bees that were covering the brood area were collected from 20 colonies in each apiary. Ten abdomens from each colony were combined in one centrifuge tube and ground in approximately 7 mL of RNA later® Tissue Collection (RNA stabilization solution, Ambion), prior to shipment to the United States. Samples spent approximately 3 days at room temperature and were otherwise maintained at −80 °C.

Total RNA isolation was performed using 25 μL of the supernatant from each collection above in the RNAqueuous®–96 kit (Ambion), following the manufacturer's instructions, performing a total of 192 compound samples, randomly sorted in two 96-well plates. Pure RNA was finally eluted in 50 μL of RNase-free water. The RNA concentrations and purity were evaluated by spectrophotometer (Pharmacia Biotech, GeneQuant).

First-strand cDNA synthesis was carried out using SuperScript™ II Reverse Transcriptase (Invitrogen), according to the manufacturer's instructions, using 8 μL of RNA extracted (corresponding to 32 ng RNA, approximately). Real-time RT-PCR assays were conducted as an initial diagnosis tool to assess both RNA quality (as described in Evans, 2006) and viral presence. PCR primers representing the six most common RNA honey bee viruses including ABPV, BQCV, CBPV, DWV, KBV, and SBV, were used in this screening, with PCR conditions as in Evans (2006). For amplification of BQCV, we used a previously published primer pair (Benjeddou et al., 2001) that is expected to generate a 700 bp product. For DWV, we designed a primer pair (DWV-F 5′-TGATGTCGCCCATA-3′; DWV-R 5′-TGAATTCAGTGTCGCCCATA-3′) to generate a 129 bp product, based on the full-genome sequence (GenBank Accession No. NC-004830). ABPV was diagnosed using primers developed by Benjeddou et al. (2001). After sequencing these products, new primers were designed matching the sequence from Brazilian honey bees each. A multiplex RT-PCR assay developed and standardized during a screening of RNAs from 192 compound samples of 10 colonies with at least two viruses: 9 (4.7%) with ABPV and DWV, 13 (6.8%) with BQCV and DWV, and 15 (7.8%) with ABPV and BQCV, and among them only 6 (3.1%) carried all three viruses.

This is the first published assay using molecular techniques to identify viruses in Brazilian honey bee samples and the first confirmation of DWV virus in Brazil. Our results indicate that viruses are considerably less prevalent in Brazilian bees compared to elsewhere. Antúnez et al. (2006) found viruses (CBPV, ABPV, BQCV, SBV, and DWV) to be frequent in apparently healthy colonies in Uruguayan honeybees (overall, 96% of samples contained at least one virus), with some association (e.g., ABPV) with Varroa destructor. In Brazil, although this mite has a large range, including 90% of São Paulo state (Gonçalves, 1984), Varroa levels are considered too low to require control procedures.

Mite levels differ across subspecies of Apis mellifera, and the fertility of female mites in AHB worker brood is considered low (Rosenkranz, 1999; Garrido et al., 2003) compared to other subspecies. Nevertheless, recent work suggests that the reproductive ability of Varroa destructor in Brazil is changing. Garrido et al. (2003) and Carneiro et al. (2007) observed that the fertility rates of female mites have increased in São Paulo and in Santa Catarina state (southeast and southern of Brazil, respectively). While Varroa mitos were not a specific focus of the current study; we did find mites in each of the analyzed colonies. An evaluation of colonies from this region in 2006 found infestation rates of 10.68 mites per 100 adult bees, on average, and that 9% of worker brood cells contained female mites (152/1700; unpublished data). According to Garrido et al. (2003), levels of Varroa mites in Brazil have increased since 1998 and are comparable to European levels. Along with host effects, this increased presence of mites is concordant with the dominance of what is believed to be a more virulent haplotype of Varroa destructor (Carneiro et al., 2007). Higher mite levels could certainly affect virus populations in Brazil, through the abilities of these mites to vector (Sumpter and Martin, 2004) these viruses. Indeed the recently described Varroa destructor-virus (VDV-1; Öngus et al., 2004), a close relative or variant of DWV (Berényi et al., 2007; Lanzl et al., 2006) appears to replicate while inside mite hosts, as it does in bees.
Our study is part of an Epidemiological Evaluation Program for bees in southeastern Brazil, where there has been an unusual decrease in adult bee population and significant honey bee mortality during the autumn. The purpose of that Program is to evaluate the prevalence of various parasites and pathogens of brood and adults bees, which could be involved directly or indirectly in such losses, and viruses, as a poorly understood but important factor in bee disease, are a central focus of concern.

Here, we described a nucleic acid detection method for viruses from Brazilian honey bees, and show that such tests can be carried out on colony-composite samples stored and shipped at room temperature. With RT-PCR such compound samples (10 bees in our case) are nevertheless able to identify viruses even at subclinical levels. The three viruses described here in Brazilian analyzed samples (ABPV, BQCV, and DWV) are three of 18 viruses isolated from honey bees (Bailey and Ball, 1991; Allen and Ball, 1996). ABPV is in the newly defined Dicistoviridae while BQCV and DWV remain as floating picorna-like viruses. Previously, Message et al. (1996) provided evidence for the presence of APV (=ABPV), BQCV (Black Queen Cell Virus), FV (Filamentous Virus), and CWV (Cloudy Wing Virus) in Brazil, using double-immune diffusion. Current techniques such as those described here can be used to determine both viral presence and correlation with disease.

Acknowledgments

We thank David De Jong for sample help and Dawn Lopez for laboratory assistance. This work was supported by National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology. Brazil (E.W.T.), and by USDA-NRI grant AG2004-36504-14277 (J.D.E.).

References


