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Scientific note

A scientific note on the first detection of black queen cell virus in honey bees (*Apis mellifera*) in Mexico

Ernesto Guzman-Novoa[1], Mollah Md. Hamiduzzaman[1], Adriana Correa-Benítez[2], Laura G. Espinosa-Montaño[2], José L. Uribe-Rubio[3]

[1]School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada
[2]Departamento de Medicina y Zootecnia en Abejas, FMVZ, UNAM, Cd. Universitaria, Mexico, DF 04510, Mexico
[3]Centro Nacional de Investigación en Fisiología Animal, INIFAP, Km 1 Carr. Colón, Ajuchitlán, Qro 76280, Mexico

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Honey bees (*Apis mellifera* L.) are infected by a variety of viruses of worldwide distribution (Allen and Ball 1996; Ellis and Munn 2005). Some of them, including deformed wing virus (DWV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), Kashmir bee virus, and Israeli acute paralysis virus (IAPV) have been associated with cases of bee mortality (Berthoud et al. 2010; Genersch and Aubert 2010). Thus, an increasing number of studies are being conducted to find out whether viruses are responsible for the massive loss of colonies recently experienced in many countries.

Mexico is a major honey producer, but despite its beekeeping importance, not much information exists for this country about the presence of viral diseases in honey bees. Of the more than 20 viruses known to infect the honey bee, the only viruses so far reported from bees collected in Mexico are CBPV (Bailey 1967), and more recently sacbrood virus, ABPV, DWV, and IAPV (Guzman-Novoa et al. 2012). Here, we report for the first time, the presence in Mexico of the black queen cell virus (BQCV).

Samples of worker pupae were collected in RNA Later (Invitrogen Canada, Burlington, Ontario, Canada) from five colonies kept by the Ministry of the Environment in Tlalpan, Distrito Federal, Mexico for different studies. It was noticed that some of the samples had a darker color than the typical white pearl color of healthy brood. Thus, they were tested for the presence of BQCV using reverse transcription polymerase chain reaction (RT-PCR) at the University of Guelph, in Guelph, Ontario, Canada.

Total RNA was extracted from 12 samples collected from five colonies (at least two samples per colony) by homogenizing five pupae per sample as per Chen et al. (2000); all pupae showed a dark color. All items that were used for macerating bees or mites, or extracting RNA, were thoroughly washed and then autoclaved prior to these procedures. The homogenates were extracted twice with chloroform and the RNA was precipitated using LiCl as described by Sambrook et al. (1989). The amount of total RNA extracted was determined with a spectrophotometer (Nanovue GE Healthcare;

Corresponding author: E. Guzman-Novoa, eguzman@uoguelph.ca
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Cambridge, UK). For cDNA synthesis, 2 μg of total RNA was reverse-transcribed using Oligo (dT) and M-MuLV RT with the RevertAid^{TM} H Minus First Strand cDNA Synthesis Kit (Fermentas Life Sciences; Burlington, Ontario, Canada), following the instructions of the manufacturer.

The primer sequences used to detect the BQCV were those reported by Benjeddou et al. (2001) with a slight modification to obtain a shorter PCR product and thus avoid hairpin loops. To modify the primers, the complete genome sequence of BQCV was obtained from the National Centre for Biotechnology Information. The sequence was aligned using CLUSTALX and the primers were redesigned by removing bases at the 5′ ends using Gene Runner (Version 3.05, Hastings Software Inc., New York, USA). The redesigned primers (F: GTCAGCTCCCACTACCTTAAAC and R: CAACAAGAAGAAACGTAAACCAC) were ordered from Laboratory Services of the University of Guelph.

The PCR reactions were done with a Mastercycler (Eppendorf, Mississauga, Ontario, Canada). Each 15 μL of reaction contained 1.5 μL of 10x PCR buffer (New England BioLabs; Pickering, Ontario, Canada), 0.5 μL 10 mM of dNTPs (Bio Basic Inc.; Markham, Ontario, Canada), 1 μL of 10 μM for forward and reverse primers, 0.2 μL 5 U/μL of *Taq* polymerase (New England BioLabs), 1 μL of the cDNA sample, and 7.8 μL of dd H_2O. The thermocycler was programmed to run at 94 °C for 3 min, followed by 35 cycles of 30 s at 94 °C, 60 s at 58 °C and 60 s at 72 °C, and a final extension step at 72 °C for 10 min. PCR products were separated by electrophoresis in 1.1 % agarose gels and stained with ethidium bromide. The amplified bands were captured in pictures using a digital camera with a Benchtop UV Transilluminator (BioDoc-It^{M} Imaging System; Upland, CA, USA). The 12 samples of the five colonies generated bands of 698 bp as expected for positive identification of BQCV (Fig. 1). To confirm identification, PCR products were purified using the EZ-10 Spin Column DNA Gel Extraction Kit (Bio Basic Canada Inc.; Markham, Ontario, Canada) and sequenced at the Laboratory Services of the University of Guelph. To verify specificity, the sequences were blasted with sequences of BQCV (GenBank accession no. AF183905) and all of them showed ≥97% identity.

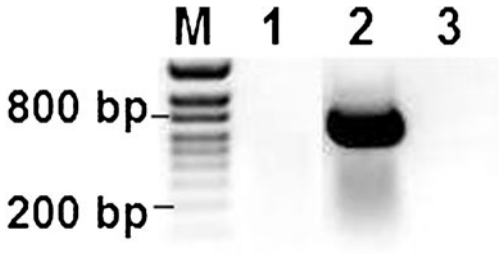

Figure 1. Detection of BQCV in Mexico: *1* control bees (negative to BQCV), *2* BQCV, *3* negative control (a reaction having no bee DNA with a pair of specific primers for BQCV). *M* (*far left*) is a 100 bp DNA ladder.

This is the first molecular report of BQCV infecting honey bees in Mexico. Further studies will be needed to determine the prevalence of honey BQCV in different regions of Mexico, as well as its impact on honey bee health.

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Note scientifique: découverte de la présence du virus de la cellule royale noire chez des abeilles (*Apis mellifera*) au Mexique

Eine wissenschaftliche Notiz zum ersten Nachweis des Black Queen Cell Virus bei Honigbienen (*Apis mellifera*) in Mexiko

REFERENCES

Allen, M., Ball, B.V. (1996) The incidence and world distribution of honey bee viruses. Bee World **77**, 141–162

Bailey, L. (1967) The incidence of virus diseases in the honey bee. Ann Appl Biol **60**, 43–48

Berthoud, H., Imdorf, A., Haueter, M., Radloff, S., Neumann, P. (2010) Virus infections and winter losses of honey bee colonies (*Apis mellifera*). J Apic Res **49**, 60–65

Benjeddou, M., Leat, N., Allsopp, M., Davison, S. (2001) Detection of acute bee paralysis virus and black queen cell virus from honeybees by reverse transcriptase PCR. Appl Environ Microbiol **67**, 2384–2387

Chen, C.Y.J., Jin, S., Goodwin, P.H. (2000) An improved method for the isolation of total RNA from *Malva pusilla* tissues infected with *Colletotrichum qloeosporioides*. J Phytopathol **148**, 57–60

Ellis, J.D., Munn, P.A. (2005) The worldwide health status of honey bees. Bee World **86**, 88–101

Genersch, E., Aubert, M. (2010) Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Vet Res. **41**:54, doi:10.1051/vetres/2010027

Guzman-Novoa, E., Hamiduzzaman, M.M., Espinosa-Montaño, L.G., Correa-Benítez, A., Anguiano-Baez, R., Ponce-Vázquez, R. (2012) First detection of four viruses in honey bee (*Apis mellifera*) workers with and without deformed wings and *Varroa destructor* in Mexico. J Apic Res **51**, 342–346

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY