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Short communication

DETECTION OF ISRAELI ACUTE PARALYSIS VIRUS (IAPV) AND *APIS MELLIFERA* FILAMENTOUS VIRUS (AMFV) IN HONEY BEES IN MEXICO

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Abstract

The recent alarming loss of honey bee colonies around the world is believed to be related to the presence of viruses. The aim of this study was to detect two major viral diseases, *Apis mellifera* Filamentous virus (AmFV) and Israeli Acute Paralysis Virus (IAPV) using Reverse Transcription - Polymerase Chain Reaction RT-PCR, in honey bees in Mexico. Adult and larvae honey bee samples were collected from asymptomatic colonies of six major beekeeping regions in the state of Chihuahua, Mexico. Both viruses were detected in both developmental stages of honey bees, IAPV at a higher prevalence (23.5%) as compared to AmFV, only in 0.9% of samples. However, this is the first report on AmFV infection in Mexican apiaries. Further studies are required to understand the AmFV and IAPV impact on colony loss in Mexico and to develop strategies for enhancing the control of viral diseases.

Keywords: *Apis mellifera* Filamentous Virus (AmFV), bee viruses, honey bee colony losses, Israeli Acute Paralysis Virus (IAPV), RT-PCR

INTRODUCTION

In recent years, significant losses of honey bee (*Apis mellifera* L.) colonies have been reported, and several studies have connected it to the presence of viruses (Cox-Foster et al., 2007; Carreck et al., 2010; Runckel et al., 2011; Cornman et al., 2012; Granberg et al., 2013). Israeli Acute Paralysis Virus (IAPV) has been strongly correlated with a new syndrome of honey bee losses observed in the United States, known as the Colony Collapse Disorder (CCD) (Cox-Foster et al., 2007). *Apis mellifera* Filamentous virus (AmFV) also has been related to dead bees and crawling bees at the entrance of hives resulting in colony losses (Clark, 1978; Sitaropoulou et al., 1989; Hou et al., 2016). Furthermore, AmFV infects most readily honey bee adults when these are infected with *Nosema apis* spores (Bailey, Ball, & Perry, 1983).

Viral bee diseases are major points of consideration in the world economy and Mexico is not an exception (Ellis & Munn, 2005). The high incidence and prevalence of bee diseases are affecting apiculture with seven different viruses in Mexico (Bailey, 1967; Guzman-Novoa et al., 2012; Guzman-Novoa et al., 2013; García-Anaya et al., 2016). Sitaropoulou et al. (1989) and Cox-Foster et al. (2007) studied how AmFV and IAPV infected and weakened bee colonies, while Ellis & Munn (2005) and Cox-Foster et al. (2007) studied their close relation with the presence of their counterparts in other North American countries. This may lead to the idea of its probable presence in Northern Mexico, hence the aim of this work was to evaluate for the presence of AmFV and IAPV through molecular techniques in northern Mexico.

MATERIAL AND METHODS

Samples were collected from 312 honey bee (*Apis mellifera*) colonies from six major beekeeping regions of the state of Chihuahua, Mexico during October 2013 and from April to June 2014. Five to eight apiaries for each region were analysed, and eight hives from each apiary were randomly sampled for a pool of ten

individual adult bees and ten larvae separately. Each sample was homogenized with a lysis buffer (0.8 M Guanidine thiocyanate, 0.4 M Ammonium thiocyanate, 0.1 M Sodium acetate, and 5% glycerol (v/v), 2% (v/v) Triton-X 100). The Genomic DNA Kit (Sigma–Aldrich, St. Louis, EU) and TRI Reagent (Sigma–Aldrich, St. Louis, EU) was used for DNA and RNA extraction, respectively, according to the manufacturer´s instructions. The cDNA synthesis was performed with the use of a RevertAid H Minus Reverse Transcriptase kit (Thermo Scientific, Lithuania, USA). The polymerase chain reaction (PCR) was performed using primers pairs for IAPV, AmFV and β-actin control, described by Yañez et al. (2015), Gauthier et al. (2015) and Chen et al. (2006), respectively.

The final volume was 25 µl including 2.5 µl 10× buffer, 1 µl of 0.4 mM dNTP mix, 1 µl of a stock 0.4 µM of each primer, 2 µl of the enzyme *Taq* polymerase solution and 2 µl of cDNA or DNA, according to the virus analyzed. PCR was carried out for IAPV and AmFV using a program of initial denaturing for 2 min at 95°C and 32 cycles of 95°C for 30 s, 52°C for 30 s and 72°C for 25 s, with a final extension step for 2 min at 72°C. For β-actin, PCR was performed using a program of initial denaturing for 2 min at 95°C and 35 cycles of 95°C for 30 s, 55°C for 20 s and 72°C for 40 s, with a final extension step for 2 min at 72°C. The quality of all samples was analysed by RT-PCR of β-actin internal control. Amplification products were observed in agarose gel (2.0%) stained with ethidium bromide. A 5000 bp ladder (Thermo Scientific, Lithuania, USA.) was included in each gel. PCR products were purified using the ZymoClean™ Gel DNA Recovery Kit (ZymoResearch, Orange, CA) according to the manufacturer´s protocols, and were sequenced at the CINVESTAV-Irapuato genomic services with an ABI 3730 XL DNA sequencing analyser (Applied Biosystems) and processed using the BigDye Terminator v3.1 Cycle sequencing kit (Thermo Fisher Scientific).

RESULTS AND DISCUSSION

The results of molecular diagnosis are shown in Tab. 1. The viruses IAPV and AmFV were detected in 26.9% of all adult bee and larvae colonies sampled from asymptomatic colonies. IAPV was detected at a higher prevalence with an occurrence of 25.9%. Guzman-Novoa et al. (2012) reported IAPV in a small sample of asymptomatic colonies. This finding is relevant due to its relation to colony losses (Cox-Foster et al., 2007). In recent years, colony losses have been reported in Mexico (Ministry of Agriculture and Livestock, 2010), but we are unaware of any studies showing a relationship between this virus and the colony losses. The exact nature of these losses is difficult to surmise, so more

Table 1.

Detection of AmFV and IAPV in honey bees from Northern Mexico

Region sampled[1] (Apiaries, Hives)	Date	Viruses[2]			
		AmFV		IAPV	
		Adult	Larvae	Adult	Larvae
DE (6, 48)	October -2013	0	0	0	0
CG (7, 56)	October -2013	1	0	46	6
RO (8, 64)	June-2014	0	0	0	0
CH (7, 56)	April-2014	0	0	0	0
PA (6, 48)	June-2014	0	0	18	11
JI (5, 40)	May-2014	1	1	0	0
No. (%) of positive hives		2 (0.6%)	1 (0.3%)	64 (20.5%)	17 (5.4%)

[1] DE, Delicias; CG, Casas Grandes; RO, Rosales; CH, Chihuahua; PA, Parral; JI, Jímenez.

[2] Numbers represent positive hive samples by region.

studies are needed to know its influence.

Our results constitute the first evidence for the presence of AmFV in *A. mellifera* in Mexico. Although its prevalence was low (0.9%), this finding is relevant due to its relationship with the microsporidian *N. apis* which has already been reported in winter in Northern Mexico (Casavantes, 2011). Furthermore, this relation can weaken the colony and cause bee mortality (Bailey et al., 1983; Sitaropoulou et al., 1989). The low prevalence could be because infected bees have a reduced life span (5 d post-infection) and are usually found at the entrance of the hive in crawling and dead bees (Clark, 1978; Sitaropoulou et al., 1989; Ball & Bailey, 1997). Moreover, its natural incidence is more prevalent during early spring (Hartmann et al., 2015) declining in summer and autumn with an incidence below the 5% (Clark, 1978), while peaks in winter with some mortality cases (Clark, 1978; Sitaropoulou et al., 1989; Hartmann et al., 2015). In this study, the honey bee samples were collected inside the hive in summer and autumn, which could have limited the detection of AmFV. The coinfection of these two viruses was not evident in all of the colonies sampled.

The presence of these viruses in North American countries e.g. the USA (Ellis & Munn, 2005; Cox-Foster et al., 2007) and now, in northern Mexico indicates that these viruses are potentially spreading worldwide. More investigations are needed to determine the impact of current and potential new viral diseases to provide a vital management for honey bee colonies to avoid dissemination of these viruses.

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