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RESEARCH ARTICLE

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Detection of deformed wing virus of honeybees in some apiaries in Syria

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ABSTRACT

Deformed wing virus (DWV) is one of the most important and distributable viruses that can infect honeybees. The presence of DWV has been reported in many countries. In this study, the presence of DWV was detected for the first time in apiaries from four different provinces in Syria by using RT-PCR and multiplex RT-PCR. We noted the high prevalence of DWV in apiaries which maybe had a main role in decline bee colonies. This is the first report of the presence of a honey bee virus in Syria. This is the first report about distribution of DWV in some apiaries in Syria.

Key words: DWV, honeybee viruses, RT-PCR, *Varroa destructor*, Syria.

INTRODUCTION

During the past few years, Syria had suffered a sharp decline in the number of bee colonies, where the number of beehives were reduced from 631,526 to 585,000 hives during 2011 to 2013 (http://faostat.fao.org). This reduction in the number of beehives occurred for many reasons; the most important is the unsecure situation in Syria over the past three years, which prevented many beekeepers to take care of their hives. In addition, there is a large-scale colony loss due to a bee disease syndrome called colony collapse disorder (CCD)[1, 2]

Honeybees are exposed to many pests and pathogens. Viruses were first identified as a new class of pathogens infecting honeybees at the beginning of the 20th century, which cause economic losses for beekeepers. At least 18 viruses have been reported to infect honeybees worldwide. Many viruses that infect and multiply in bees with high virus titers do not develop symptoms. This type of infection is called covert infection and is fairly typical of insect viruses[3, 4, 5, 6].

This study was initiated in 2010, which aimed to detect honeybee viruses in Syria, through samples collected from different beehives in several provinces. Among the most common symptoms observed were deformation and atrophy of the wings, and sometimes bulging abdomen, similar to those caused by deformed wing virus (DWV), which is one of the most important viruses that can infect honeybees [7-9]. Typical disease symptoms of DWV infection include shrunken and crumpled wings, decreased body size, and discoloration of adult bees. DWV was transmitted by *Varroa destructor* parasite during pupal stage, and it was detected in all other phases of bee life cycle, but without obvious symptoms [7-15].

The virus is currently distributed worldwide, but it was first isolated from a sample of symptomatic honeybees from Japan in the early 1980s and the viral genome was published in 2006 [8].

DWV has icosahedral particles 30 nm in diameter, and include a single positive-stranded RNA genome and three major structural proteins[16], originally classified as an insect picorna-like virus[17, 18], genus Ifla virus, with a typical picornavirus genome organization, consisting of a single open reading frame (ORF) in which the structural proteins are N-terminal to the non-structural proteins. DWV has many strains, the Italian strain is the most

important, with a genome size of 10,000 nt. [8]. DWV is closely related to Kakugovirus and Varroadestructor virus 1, which together form the deformed wing virus complex [3, 8].

MATERIALS AND METHODS

This study aimed to determine the prevalence of deformed wing Virus in Syria. Itcovered eight apiaries from Damascus, Homs, Lattakia and Tartus, which are the most important provinces for beekeepers in Syria (Fig. 1).

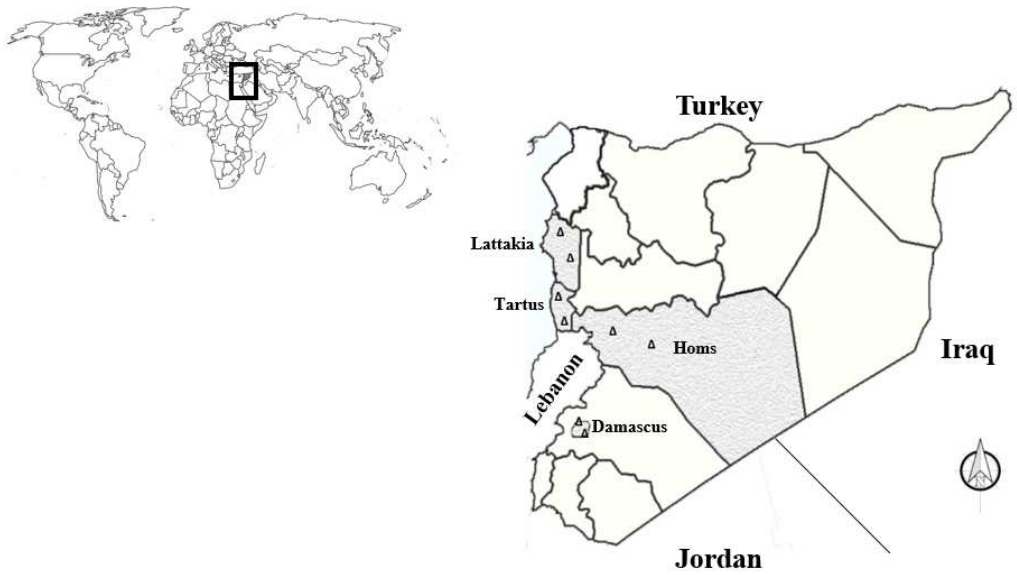

Fig 1.Maps of Syria and location of apiaries.

Two samples (30 worker bees) were collected from each apiary containing symptomatic and asymptomatic bees and stored at -20 °C until they were tested. Ten bees from each sample at random were homogenized in sterile ceramic mortars with sterile sand; thereafter, 1 ml cold phosphate-buffer-saline (PBS) was added. The homogenates were centrifuged at 2000 g for 15 min, and 140 µl of the supernatant was used for RNA extraction by employing the QIAamp® Viral RNA Mini Kit (Cat. no. 52904) according to the instructions of the manufacturer (QIAGEN GmbH, Germany).

DWV RNA was reverse transcribed, and two selected regions of the genome were amplified by two pairs of specific primers, F1:5'-TTTGCAAGATGCTGTATGTGG-3' and R1:5'-GTCGTGCAGCTCGATAGGAT-3' that amplify a 395 bp fragment[19], and F2: 5'-CTTACTCTGCCGTCGCCCA-3' and R2: 5'-CCGTTAGGAACT CATTATCGCG-3' that amplify a 194 bp fragment[10], by using a continuous RT-PCR and multiplex RT-PCR (mRT-PCR) method sand by employing a One Step RT-PCR kit according to the manufacturer recommendations (QIAGEN GmbH, Cat. no. 210210). The amplifications were performed in Cleaver CSL Gradient thermo cycler. The temperature profile for RT-PCR and for mRT-PCR were as follows: 30 min at 50 °C (reverse transcription) and 15 min at 95 °C (denaturation and HotStarTaq activation), followed by 40 cycles of amplification with 30 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C. Reactions were completed with a final elongation step for 7 min at 72 °C. RNA extracts from healthy bees without DWV infection were used as negative controls. The PCR products were electrophoresed on a 1.2% Tris-acetate-EDTAagarose gel and then stained with ethidium bromide. Bands were photographed by a MicroDOC System with UV Transilluminator (Cleaver Scientific Ltd). Fragment sizes were determined with reference to a 100-bp ladder (Promega).

RESULTS AND DISCUSSION

Figure 2 shows that all of symptomatic bee samples were infected with DWV, whereas asymptomatic bee samples collected from Damascus were infected with DWV, whereas the rest of asymptomatic bee samples from other locations were not infected (data not shown).

These results indicate that DWV is widespread among bee colonies in Syria and such results are similar to others conducted else where. In Jordan, infection rate with DWV was 92 % in Ajlon province [20]. Various studies confirmed that DWV is potentially responsible for bee colony collapse [21], but other studies considered that this virus is a poor pathogen[7, 22].

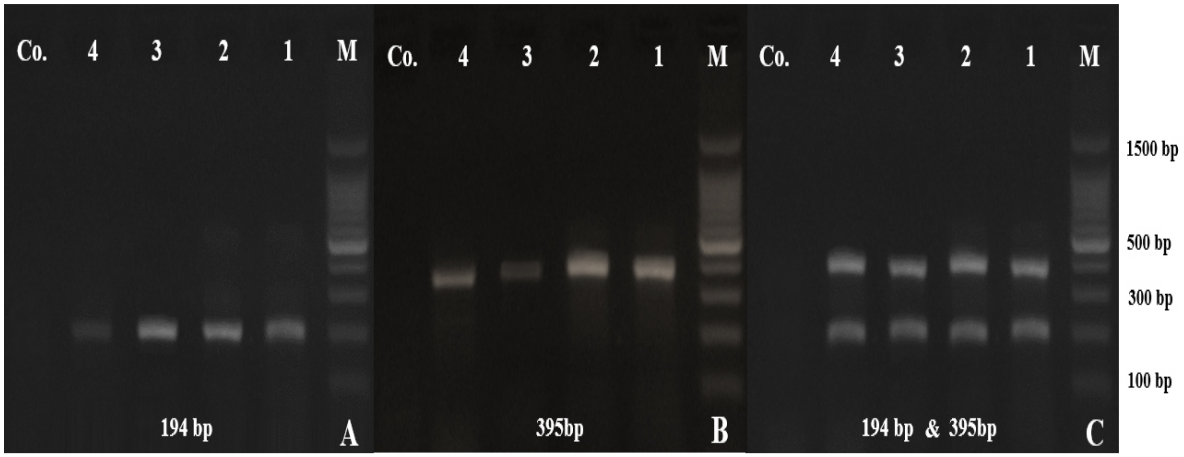

Figure 2: RT-PCR (A and B) and mRT-PCR (C)for symptomatic bees shows the amplification of a 395 bp and 194 bpfragments due to infection with deformed wing virus.M= Molecular weight ladder, Lane 1= Damascus,Lane 2=Homs,Lane 3=Tartus, Lane 4=Lattakia, and Co.= negative control sample.

CONCLUSION

This study suggest that the high prevalence of DWV among apiaries in Syria might have an impact on bee colonies, but more research is needed to confirm this. In addition, further research is needed to determine the complete genome of DWV from different apiaries in order to define the genetic relationships between colonies in Syria and other globally registered colonies.

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