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*Full Length Research Paper*

# First molecular detection and characterization of deformed wing virus (DWV) in honeybees (*Apis mellifera* L.) and mite (*Varroa destructor)* in Turkey

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**Deformed wing virus (DWV), a member of the genus iflavirus of the insect picorna-like viruses, is one of the most common honey bee viruses transmitted by the parasitic mite *Varroa destructor* during pupal stages, and is associated with wing deformities in adult honeybees. Although no data are available from Turkey up till now, DWV have been reported in many countries from all continents. In the present study, we tested the presence of DWV in *Apis mellifera L* and *V. destructor* from Ordu province of Turkey using one step reverse transcription-PCR (RT-PCR). We were able to demonstrate that all of the worker bees with deformed wings and the varroa mites but not healthy bees were infected with DWV. BLAST analysis of the sequences of PCR products showed 96-98% similarity to polyprotein gene of known DWV virus isolates.**

**Key words:** *Apis mellifera,* deformed wing virus, honeybee viruses, *Varroa destructor,* RT-PCR, Ordu, Turkey.

## INTRODUCTION

Honeybee, *Apis mellifera* L., is one of the most important economical insects, both by its various products and by contribution to the pollination of agricultural plants. However, many pathogens including honeybee viruses threaten honeybee hives and cause severe losses to apiculture. To date, approximately 18 honeybee viruses have been identified, which are distributed world-wide (Ball and Bailey and Ball 1991; Chen et al., 2004; Fievet et al., 2006; Chen and Reinhold, 2007). They usually cause unapparent infections and may not be perceived by beekeepers for many years.

Although honeybee viruses are reported from many countries (Allen and Ball, 1996; Chen et al., 2005; Lanzi et al., 2006; Berenyi et al., 2006; Berenyi et al., 2007; Forgách et al., 2008), there are very limited number of reports about viral honeybee diseases and their causative agents in Turkey. However, in many apiaries, body deformities such as severe wing deformities and bee deaths especially in *Varroa destructor* infested hives have been found in Turkey (Tutkun and Boşgelmez, 2003; Aydın et al., 2007). In addition, honeybee colony losses were reached to 35% in some provinces between autumn 2006 and spring 2007 especially in the southern and Northeastern part of Turkey and they suggested that these colony losses were related to irregular season and unknown factors (Giray et al., 2007).

Deformed Wing Virus (DWV), a positive stranded RNA virus, is very common virus infecting honeybee larvae and adults (Bailey and Ball 1991; Bowen-Walker et al., 1999). This virus is reported from almost all *V. destructor* infested hives and presence of wingless adult bees in a colony is accepted as an indicator of this virus (Lanzi et al., 2006; Nordström, 2003). Infected mites play signifycant role in transmission of the virus among honeybee hives (Ball and Allen, 1988; Bowen-Walker et al., 1999; Nordström, 2003; Baker and Schroeder, 2008). In addition to mite mediated transmission, vertical transmission of the virus from queens and drones to drone and worker offspring through unfertilized and fertilized eggs, respecttively, was demonstrated (Yue et al., 2007; de Miranda and Fries, 2008). In the presence of Varroa, DWV causes distinctive symptoms, such as wing deformities, inflated abdomens, reduction in emergence size, paralysis, and

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eventually deaths of honeybees (Bailey and Ball 1991; Bowen-Walker et al., 1999; Fievet et al., 2006; Lanzi et al., 2006; Berenyi et al., 2007).

Since wing deformities and DWV infection are associated with *Varroa* infestation (Ball and Allen, 1988; Bowen-Walker et al., 1999; Martin 2001, Yue et al., 2007) and symptoms become apparent by the presence of the *Varroa* mite, we performed a small size molecular survey to investigate presence of DWV infection on honeybees and *Varroa* samples collected from healthy and *V. destructor* infested hives from Ordu province where the beekeeping is the most developed.

## MATERIALS AND METHODS

### Sample collection

Honeybee and *V. destructor* samples were collected from 5 apiaries from Ordu province in spring 2007. Thirty honeybees (20 workers and 10 drones) were randomly sampled from 15 of the 220 apparently healthy hives. Fifty honeybees samples (30 workers and 20 drones) with wing and body deformities were collected from 25 of the 180 heavily *V. destructor* infested hives. Fifty *Varroa* samples were randomly sampled from 10 of 180 *V. destructor* infested hives. All samples were stored at -86°C immediately until RNA extraction.

### Total RNA extraction

Eighty frozen honeybees were crushed individually in 2 ml sterile eppendorf tubes containing 0.5 ml lysis buffer using sterile disposable plastic pestles. The homogenate was centrifuged at 5000 g for 5 min and the aqueous part of the bee homogenate was used for RNA extraction. Ten pools of five *Varroa* mites each were processed for RNA extraction as described as above. Total RNA from both honeybee and mite homogenates were extracted by using RNA easy Mini Viral RNA isolation kit (Qiagen, USA) according to the manufacturer's protocol. In all cases, elution volume of total RNA was 50 µl. All RNA samples (90) were quantified and stored at -86°C.

### Reverse transcriptase PCR

Presence of DWV was tested by one step RT-PCR using Ctherm One Step RT-PCR kit (Roche, USA) according to manufacturer's protocol using 0.5 µg total RNA. In all RT-PCR reactions the following cycling conditions used, 30 min at 60°C, 10 min 95°C followed by 35 cycle with 30 s at 94°C, 1 min at 54°C, 30 s at 72°C, and a final elongation step 10 min at 72°C. In all test, reaction volume was 25 µl. A PCR product of 395 bp was amplified using 0.2 µM DWV spe-cific forward (5'-TTTGCAAGATGCTGTATGTGG-3', position: 8561-8581; AY292384) and 0.2 µM reverse (5'-GTCGTGCAGCTCGA-TAGGAT-3', position: 8936-8955; AY292384) primers. In all tests $ddH_2O$ used as negative control. PCR products of 3-5 µl were ana-lyzed on a 1% agarose gel containing ethidium bromide, visualized on a UV transilluminator (Syngene, UK) and photographed using a digital camera (Sony, Japan).

### Gel purification, sequencing and genetic analysis of RT-PCR products

Several PCR products were selected randomly for sequencing to confirm their identity. The PCR products were gel purified using Qiagen DNA Gel Isolation Kit according to protocol recommended by manufacturer with minor modifications. The sequencing of purified RT-PCR products was performed by using DYEnamic ET Terminator Cycle Sequencing Kit (Amersham) using same primer pair used in RT-PCR on an ABI PRISM 310 Genetic Analyzer.

The DNA sequence similarity of DWV determined in this study was analyzed using Basic local alignment search tool (BLAST) of NCBI (Altschul et al., 1990). The translation of DWV sequence was performed using ExPASy translate tool (Gasteiger et al., 2003) and partial DWV polyprotein sequence observed was compared using protein BLAST of NCBI.

## RESULTS

### *Varroa* infestation and observation of DWV phenotype in apiaries

*V. destructor* infestation of honeybees is common in many provinces of Turkey (Aydın et al., 2007); however; there is no report for the presence of the DWV infection reported to date. In this study, 5 apiaries with total of 400 hives selected from Ordu province according to their *Varroa* infestation status in hives. Three of the five apiaries contained *Varroa* infested colonies whereas in the other two apiaries no *Varroa* infestation was observed. Therefore these were stated as healthy. DWV-like symptoms in some of the worker bees and drones were common in hives from *Varroa* infested apiaries but were not found in hives from healthy apiaries.

### Detection of DWV in honeybees and Varroa mites

All worker bees and drones from randomly selected hives infested with *V. destructor* mite were tested by RT-PCR randomly and they were found to be infected by DWV whereas; no DWV detected in any worker bees and drones tested from healthy hives (Figure 1A and B).

Ten *Varroa* mite pools collected from hives were found to be infected by DWV by the detection of 395 bp DNA band on agarose gels (Figure 1C) and presence of DWV was verified further by sequencing of PCR products.

### Genetic analysis of DWV sequence

The DWV infection in honey bees and *Varroa* mite samples determined by RT-PCR were further confirmed by sequencing of PCR products. The sequencing of randomly selected RT-PCR products from two worker bee and drones and two pools of varroa mites gave rise to an approximately 395 bp of sequence (not shown). Sequence of DWV from a worker bee (GenBank accession number: FJ011106) showed 98% sequence similarity to poylprotein gene of previously found DWV and Kakugo virus isolates according to BLAST analysis (Figure 2).

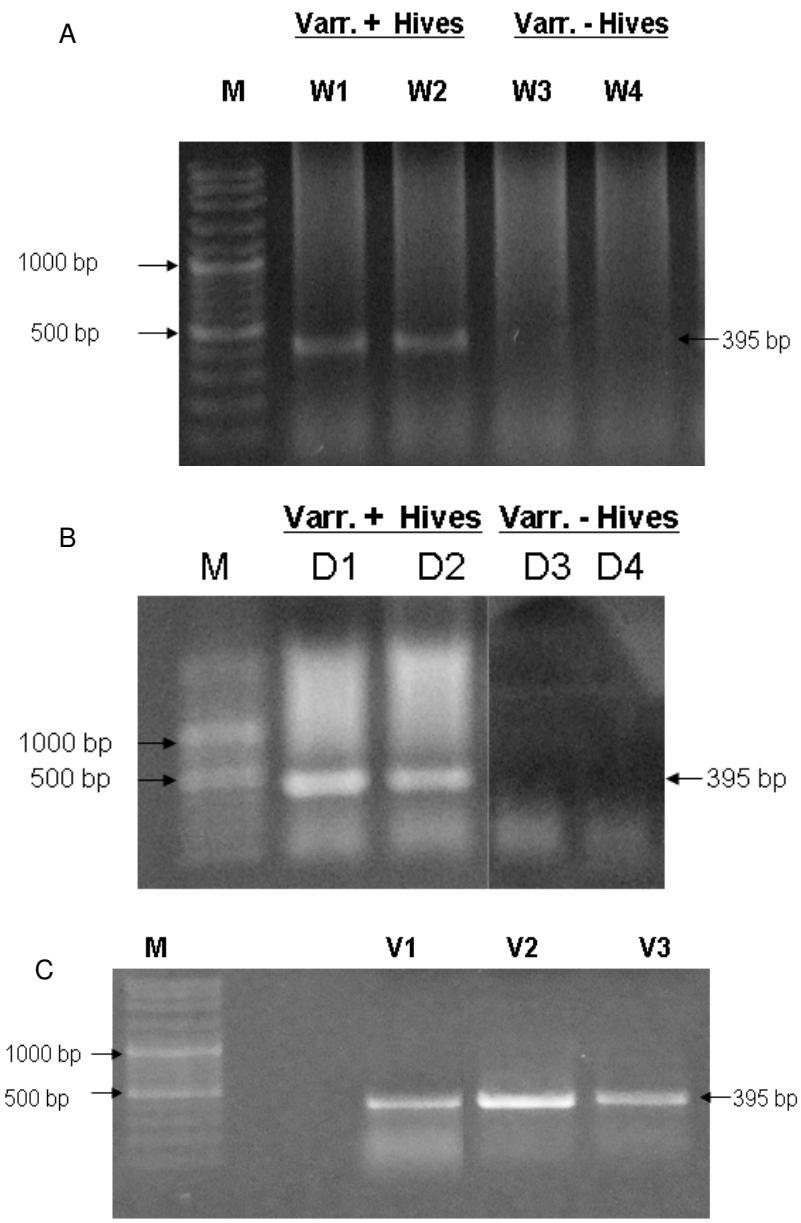


**Figure 1.** Detection of DWV in worker bees (A), drones (B) and varroa mites (C) by RT-PCR. Total RNA extracted from whole individual worker bees, drones and *Varroa* mites and analysed by one step RT-PCR. M; molecular weight standard, Varr.+; *Varroa* infested, Varr.-; Healthy Hives, W1-4; worker honeybees, D1-4; drones, V1-3; *Varroa* mite pools.

## DISCUSSION

Deformed Wing Virus (DWV) is a very common virus infecting honeybee larvae and adults and become one of the most common honeybee viruses in the world. DWV was reported from almost all *Varroa* infested hives from many countries (Chen et al., 2005; Lanzi, 2006; Berenyi et al., 2007) and is closely associated with *Varroa* infestation.

To date there is no detailed information about viral honeybee diseases in Turkey. However, symptoms like body such as wing deformities, shortened abdomens and discoloration which are common in DWV infected honeybees were reported in many apiaries in Turkey (Aydın et al., 2007). Therefore determination of association of honeybee diseases with DWV and other honeybee viruses would provide important and valuable information to explain some unexplained problems observed in

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FJ011106  1     TTTGCAAGATGCTGTATGTGGTGTGCCTGGTTTAGATGGGTTTGATTCGATATCTTGGAA  60
AY224602  3479  ............................................................  3538
AJ489744  8566  ............R...............................................  8625
AY292384  8561  ............................A...............................  8620

FJ011106  61    TACTAGTGCTGGTTTTCCTTTGTCTTCATTAAAGCCACCTGGAACATCAGGTAAGCGATG  120
AY224602  3539  ...............................................G............  3598
AJ489744  8626  ...............................................Y........Y....  8685
AY292384  8621  ...............................................G............  8680

FJ011106  121   GTTGTTTGACATTGAGCTACAAGACTCGGGATGTTATCTTTTGCGTGGAATGCGTCCCGA  180
AY224602  3599  ..........................................CC................  3658
AJ489744  8686  .........Y..............Y..............CY................  8745
AY292384  8681  ..........................................CC................  8740

FJ011106  181   ACTTGAGATTCAATTATCAACGACACAGTTAATGAGGAAAAAGGGAATAAAACCTCACAC  240
AY224602  3659  ............................................................  3718
AJ489744  8746  ............................................................  8805
AY292384  8741  ............................................................  8800

FJ011106  241   TATATTCACGGATTGTTTGAAAGATACTTGTTTGCCTGTTGAAAAATGTAGAATACCTGG  300
AY224602  3719  ............................................................  3778
AJ489744  8806  ............................................................  8865
AY292384  8801  ....................G.......................................  8860

FJ011106  301   TAAGACTAGAATATTTAGTATAAGTCCGGTACAGTTTACCATACCGTTTCGACAGTATTA  360
AY224602  3779  ............................................................  3838
AJ489744  8866  ............................................................  8925
AY292384  8861  ............................................................  8920

FJ011106  361   TTTAGACTTTATGGCGTCCTATCGAGCTGCACGAC  395
AY224602  3839  C.............A..................  3873
AJ489744  8926  C.............A..................  8960
AY292384  8921  C.............A..................  8955
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**Figure 2.** Sequence similarity of partial coding sequence of DWV polyprotein (GenBank accession number: FJ011106) detected in the present study with polyprotein sequences of previously detected DWV and Koguko viruses.

honeybees.

Here we demonstrated that DWV presents in adult honeybees (worker bees and drones) from *Varroa* mite infested hives, but not the ones from healthy hives. Therefore, it is proposed that symptoms like wing and body deformities seen in these hives in the area were actually originated from copresence of DWV and Varroa. Results shoved that *Varroa* mites collected from same colonies were also infected with DWV indicating that DWV may be transmitted by *Varroa* mites. Determination of DWV in only hives with *Varroa* infestation and in *Varroa* mites from same hives may support the common hypothesis that DWV is mostly spread by *Varroa* mites.

BLAST analyses of PCR products (Figure 2) found in the present work showed that the origin of Turkish DWV isolate is highly similar to DWV strains detected in the Europe. Since Tutkun and Boşgelmez (2003), reported that Varroa entered to Turkey from Balkan countries through transfer of *Varroa* infested hives in 1976, DWV may be spread to Turkey from Europe. Spread of the virus through the country is mostly due to mobile beekeeping practice which is very common in Ordu province.

In conclusion, presence of DWV in honeybees and *Varroa* mite is not restricted to countries where DWV is detected to date. This is the first study reporting the presence of DWV in honey bees and *V. destructor* mite in Turkey. Since *Varroa* mites also transmit other honeybee viruses (Chen et al., 2006), it is necessary to determine whether DWV and other viruses present and cause infection in other *Varroa* infested hives in Turkey. Therefore, an epidemiological survey on DWV and other honeybee viruses covering most part of the country needs to be done to show spatial or temporal dynamics in virus distribution.

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