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Prevalence of three honey bee viruses in Turkey

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ABSTRACT

In this study, 10 worker bees from each of 28 different apiaries were collected (a total of 280 honey bees) from 6 provinces of the Black Sea region in Turkey. These samples were tested by Reverse-Transcriptase PCR (RT-PCR), for three honey bee viruses: acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV) and black queen cell virus (BQCV). In addition, the samples were microscopically examined for *Varroa destructor*. In conclusion, CBPV, BQCV and *Varroa destructor* were identified in 25%, 21.42% and 53.57% of the bees respectively, but ABPV could not be detected in apiaries. This is the first report of CBPV, BQCV in Turkey.

Key words: honey bee viruses, chronic bee paralysis virus, black queen cell virus, acute bee paralysis virus, *Varroa destructor*, reverse-transcriptase PCR

Introduction

Turkey has great beekeeping potential with over 4.3 million beehives, the majority of which is located in the northern and western areas of the country (SIRALI, 2002).

One of the major causes of honeybee deaths are viral diseases. Chronic bee paralysis virus (CBPV) causes an infectious disease characterized by trembling, sightlessness and sometimes black individual scrawling at the hive entrance. Acute bee paralysis virus (ABPV) is a common infective agent of bees, frequently detected in apparently healthy colonies (ALLEN and BALL, 1996). Black queen cell virus (BQCV) can affect the brood of the honey bee (*Apis mellifera*) and typical sypmtoms are endangered dark coloured cell walls in queen cells. Virus can infect worker and dronebrood honey bees and clinical symptoms are rarely observed (SIEDE and BUCHLER).

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V. destructor could be detected in different stages of the honey bee, including larva, stern and adult stages (MORSE and FLOTTUM, 1997; SHIMANUKI and DAVID, 2000). Also, many studies have indicated that *Varroa destructor* was found to be associated with viral diseases and colony mortality (BALL and ALLEN, 1988; BAKONYI et al., 2006; MEDINA and MEJINA, 1999; SHIMANUKI and DAVID, 2000; TENTCHEVA et al., 2004**)**.

One of the most accurate methods for the diagnosis of honey bee RNA viruses is the reverse transcriptase-polymerase chain reaction (RT-PCR). This technique provides a quick, specific and sensitive diagnosis of viruses in samples. RT-PCR has been previously used for the detection of several honeybee viruses (BENJEDDOU et al., 2001; RIBIERE et al., 2002).

The aim of this study was the investigation of ABPV, CBPV, BQCV and *Varroa* parasites in apiaries with sudden honey bee deaths in the Black Sea region in Turkey.

Materials and methods

Samples. Ten worker bees (*Apis mellifera*) were randomly collected from each of 28 hives (total of 280) in which sudden honey bee deaths had drawn attention. These hives were located in 6 provinces in the Black Sea region of Turkey (Fig. 1). Honey bees were collected from June to August 2009 (the trimester in which sudden bee deaths had been seen). Samples were stored at -20 ºC until analyzed.

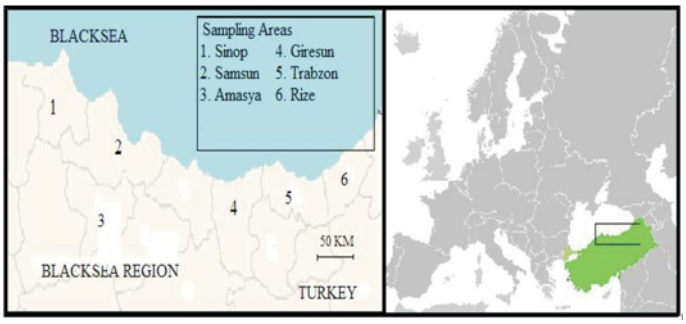

Fig. 1. Sampling area of honey bees in the Black Sea Region

RNA extraction. 10 worker bees that were collected from the same hive were accepted as a unique sample and the samples were placed in sterile plastic bags using sterile forceps. Afterwards, the wings and heads of the honey bees were ripped off and the abdomens and rib cages were homogenized (Heidolph Silent Crusher M/Germany) at 4 °C in 10 mL of phosphate-buffered saline (PBS). The mixture was centrifuged for 15 min at 4500 rpm and the supernatant was used for viral RNA extraction.

Viral RNA was extracted from the homogenized bee tissues using an acid guanidium-phenol-chloroform-isoamyl alcohol mixture as described by Chomcyznski and Sacch, 1987. Briefly, 400 µL supernatant from the bee homogenizat supernatants obtained was mixed in a denaturing solution of 4 M guanidinium isothiocyanate, 0.5% laurosylsarcosine (Sarcosyl), and 0.1 M β-mercaptoethanol in 25 mM sodium citrate (pH 7.0). After phenol/chloroform extraction, RNA was precipitated twice with isopropanol and washed with 70% ethanol. The RNA pellet was air-dried, resuspended in 20 µL of DEPC-treated water, and then stored in a deep freeze at -80 °C (CHOMCYNSZKI and SACCHI, 1987).

cDNA synthesis. First, the content of mixture I (containing 3.0 µL sterile distilled water, 0.5 µL random hexamer primer (0.2 µg/µL) and 3 µL RNA) was created. Following this, the tubes were placed into a thermal cycler and incubated for 5 minutes at 70 °C. Then, all the tubes were inserted into an ice box for cooling. The second step of the assay was as follows; following formation of mixture II (containing 2.0 µL 5x reaction buffer, 1.0 µL 10 mM dNTP mix and 0.5 µL (200 u/µL) M-MuLV reverse transcryptase), 3.5 µL mixture II was transferred to mixture I tubes and this was incubated at 48 °C for 45 minutes.

The selected primer sequences and expected size of the RT-PCR products are presented in Table 1 (BENJEDDOU et al., 2001; RIBIERE et al., 2002).

Reverse-Transcriptase PCR (RT-PCR) assays. Amplification was performed in a 0.25 µL (5 u/µL) Taq DNA polymerase, 3 µL $(NH_4)_2SO_4$, 10xTaq buffer, 2.4 µL 25 mM $MgCl_2$, 0.5 µL10 mM dNTP mix, 0.5 µL (10 pmol) sense primer, 0.5 µL (10 pmol) reverse primer and 19.85 µL distiled and 3 µL of cDNA (Fermentas).

Table 1. The primers used for ABPV, CBPV and BQCV detection.

Primer	Sequence (5'-3')	Length (bp)	Reference
ABPV1	TTATGTGTCCAGAGACTGTATCCA	900	Benjeddou et al. (2001)
ABPV2	GCTCCTATTGCTCGGTTTTTCGGT		
CBPV1	GTTGTCATGGTTAACAGGATACGAG	455	Ribiere et al. (2002)
CBPV2	TCTAATCTTAGCACGAAAGCCGAG		
BQCV1	TGGTCAGCTCCCACTACCTTAAAC	700	Benjeddou et al. (2001)
BQCV2	GCAACAAGAAGAAACGTAAACCAC		

PCR amplification was performed for one cycle at 95 °C for 2 minutes, 40 cycles at 95 °C for 30 seconds, at 55 °C for 1 minute and at 68 °C for 2 minutes; and a final extension with one cycle at 68 °C for 7 minutes. Positive and negative controls were used in each RT-PCR experiment. An aliquot of 10 µL of the reaction product was electrophoresed in a 1% agarose gel containing 0.5 g/mL ethidium bromid and visualized

by UV transillumination. A 100 bp marker (Fermentas) was used as the size standard for each gel lane (BEKESI et al., 1999).

Preparation of samples for V. destructor determination. Many methods have been developed for the diagnosis of Varroa. One of the methods is based on shaking the adult bee in 70% ethyl alcohol. In this study, 70% ethyl alcohol of added to 100 honey bees placed in a jar to kill the bees. The cover was tightly closed, and shaking for 10 minutes caused *V. destructor* mites to leave the bees. Separation of bees from the mites was achieved by a sieve with a mesh size of approximately 2-3 mm. Afterwards, using white paper, the number of *V. destructor* left on the filter was recorded. Under some circumstances, the *Varroa* mite may be confused with bee lice, so for confirmation *V. destructor* mites were identified by detecting the abdominal segment, the bottom of the wings and feather with a stereo microscope (KAR et al., 2006).

Results

28 hives were examined for Varroa, and 15 of 28 (53.57%) bee samples were determined positive for *V. destructor.* Alongside BQCV, CBPV positivity was also recorded for the 6 of 28 (21.42%) and 7 of 28 (25%) samples, respectively, but no ABPV positivity was detected in the sampled bees (Table 2).

The CBPV primers amplified a fragment of the predicted molecular weight (455 bp) from the CBPV genome and BQCV PCR amplicon was 700 bp, as predicted (Fig. 2).

The highest positivity rates determined were 66.66% for CBPV in region 1.50% for BQCV in region 6 and 100% for *V. destructor* in regions 3 and 5, in the examined area by RT-PCR assay (Table 2).

The obtained data revealed that *V. destructor,* CBPV and BQCV positivity is often described together.

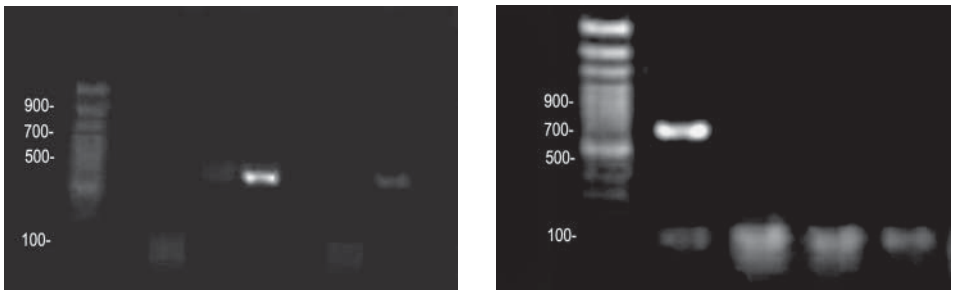

Fig. 2. RT-PCR results of samples for CBPV (455bp) and BQCV (700bp)

Table 2. ABPV, CBPV, BQCV and *Varroa* positivity, according to the sampling areas

Code of sampling region	No. of samples examined	RT-PCR (%)			*Varroa destructor*
		CBPV	BQCV	ABPV	
1	3	2 (66.66%)	0 (0%)	0 (0%)	0 (0%)
2	13	2 (15.38%)	4 (30.76%)	0 (0%)	6 (46.15%)
3	2	0 (0%)	1 (50%)	0 (0%)	2 (100%)
4	5	1 (20%)	0 (0%)	0 (0%)	3 (60%)
5	3	1 (33.33%)	0 (0%)	0 (0%)	3 (100%)
6	2	1 (50%)	1 (50%)	0 (0%)	1(50%)
Total	28	7 (25%)	6 (21.42%)	-	15 (53.57%)

Discussion

The critical component of honey bee disease for surveillance and control programs is the rapid and definite diagnosis of virus infections (CHEN et al., 2006). In this study, the presence of *V. destructor* and three honey bee viruses (BQCV, CBPV, ABPV) was related to the sudden deaths of the honey bees in the investigated area in Turkey.

The honey bee samples were found positive for BQCV (21.42%), CBPV (25%) and *V. destructor* (53.57%), but ABPV positivity was not determined. These data are the first demonstration of honey bee viruses in the Black Sea region of Turkey.

CHEN et al. (2006) detected BQCV and CBPV in queens and their offspring; including eggs, larvae and adult workers, but ABPV was not detected in queens and their offspring in the USA. Besides, MUZ and MUZ (2008) detected Deformed Wing Virus (DWV), ABPV and *V. destructor*-1 *virus* (VDV-1) in the East Mediterranean Region of Turkey. According to their data, all the colonies were positive for DWV and VDV-1, and 69% of colonies were positive for ABPV (MUZ and MUZ, 2008). ABPV (68%), BQCV (30%), and CBPV (10%) positivity was determined in Austrian honeybee colonies (BAKONYI et al., 2006). ANTUNEZ et al. (2005) found a high infection rate with CBPV and ABPV in Uruguay. TENTCHEVA et al. (2004) suggested that CBPV was found in 28% of the apiaries, ABPV was found in 58% of the apiaries and BQCV was found in 86% of the apiaries. The CBPV positivity mentioned above is concordant with the related data of our study, but ABPV was reported to be widespread in previous studies all over the world, while our data do not confirm this.

Previous studies have revealed that 89% of colonies in the Black Sea region, 6.2% of colonies in the Edirne area, 64.2% of colonies in the Thrace Region, 35% of colonies in the South Marmara Region, 32% of colonies in the Hatay area and all the colonies in the Toros mountain villages are infected with *V. destructor* in Turkey (ÇAKMAK et al., 2003; SAHINLER and GUL, 2005; OZKOK, 1995; YASAR et al., 2002; YILMAZ, 1999). In this study, *V. destructor* positivity was found (53.57%). These results were lower than in a

previous study that examined the same region. Otherwise, if the results are generalized according to Turkey, *V. destructor* positivity is similar to other investigated regions, apart from Edirne.

In conclusion, these results are the first notification of BQCV and CBPV in Turkey. We found that sudden honey bee deaths are probably related to BQCV, CBPV and *V. destructor,* and these infection factors are widespread in the areas investigated.

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SAŽETAK

Prikupljeno je po 10 radilica s 28 različitih pčelinjaka u šest provincija na području oko Crnoga mora u Turskoj. Uzorci su bili pretraženi lančanom reakcijom polimerazom uz prethodnu reverznu transkripciju (RT-PCR) na tri virusa medonosne pčele: virus akutne pčelinje paralize, virus kronične pčelinje paralize i virus crnih matičnjaka. Uzorci su povrh toga bili pretraženi na prisutnost parazita *Varroa destructor*. Virus kronične paralize bio je ustanovljen u 25%, virus crnih matičnjaka u 21,4% i *Varroa destructor* u 53,57% pčela dok virus akutne pčelinje paralize nije bio dokazan. Ovo je prvi dokaz prisutnosti virusa kronične pčelinje paralize i virusa crnih matičnjaka u Turskoj.

Ključne riječi: medonosna pčela, virus kronične pčelinje paralize, virus crnih matičnjaka, virus akutne pčelinje paralize, *Varroa destructor*, lančana reakcija polimerazom