Original Article

PREVALENCE AND MOLECULAR CHARACTERIZATION OF DEFORMED WING, ACUTE BEE PARALYSIS AND BLACK QUEEN CELL VIRUSES INFECTING HONEY BEES AND *VARROA* MITES

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Received: 24 February 2023; accepted: 18 May 2023

Abstract

Surveys were conducted in Malatya and Elazığ provinces of Eastern Türkiye between 2018-2019 to detect the presence and distribution of *Deformed wing virus* (DWV), *Acute bee paralysis virus* (ABPV) and *Black queen cell virus* (BQCV) infecting honey bees and *Varroa* mites. Thirty *Varroa* mites feeding on honey bees and 147 honey bees were collected from twenty-five apiaries of Malatya and fifteen apiaries of Elazığ. The collected samples were subjected to reverse transcriptase-polymerase chain reaction. All tested viruses were found to be present in the samples singly or as mixed infections. The prevalence of the viruses were 23.81%, 12.93%, and 10.20% for DWV, ABPV and BQCV, respectively. Five out of thirty *Varroa* mites tested for the viruses were found to be infected only with DWV. Randomly chosen sequences of each detected virus's partial polyprotein gene region were registered in GenBank under the accession numbers OP805878, OP805879, OP805880, OP805887, OP805888, OP805889, OP805890, OP805891 (DWV), OP805881, OP805882, OP805883, OP805884 (ABPV) and OP805885, OP805886 (BQCV). The phylogenetic tree of the viral isolates were compared with the world isolates. DWV isolates were found to be closely related to the UK, Lebanon, Türkiye, France, Germany and Israel isolates, whereas ABPV isolates were related to Türkiye, South Africa, Slovenia, Serbia, France, Hungary, Syria and USA isolates and BQCV isolates were related to China, South Korea, Czech Republic, France, Hungary, Poland, South Africa, Australia and Sweden isolates. The presence of DWV, ABPV and BQCV infections in Malatya and Elazığ provinces of Türkiye is revealed for the first time in this study.

Keywords: ABPV, BQCV, DWV, honey bee, Türkiye, *Varroa*

INTRODUCTION

Honey bee diseases and pests are major limitations in beekeeping worldwide with the rapid increase in bee deaths being a significant concern (Antúnez et al., 2006). Among honey bee pathogens, viruses pose the greatest threat to honey bee health. At least twenty-four viruses belonging to the *Dicistroviridae* and *Iflaviridae* families have been reported since their discovery as a new class of diseases in the early 20th century (de Miranda et al., 2013; Remnant et al., 2017; McMenamin et al., 2018).

Viruses can be transmitted at all phases of the honey bee life cycle: egg, larva, pupa, and adult. Bee viruses significantly distress honey bee health and shorten their lifetime under certain conditions. Viruses can seriously afflict bees, resulting in their death or causing the entire colony to collapse (Berenyi et al., 2006). Honey bee pathogens can cause disease simultaneously with environmental factors such as stress. Seven of these viruses, which cause severe illnesses and financial losses, pose a significant danger to beekeeping worldwide. These viruses are *Acute bee paralysis virus* (ABPV), *Deformed wing virus*

(DWV), *Black queen cell virus* (BQCV), *Israeli acute bee paralysis virus* (IAPV), *Sacbrood virus* (SBV), *Kashmir bee virus* (KBV) and *Chronic bee paralysis virus* (CBPV). While the most common honey bee viruses belong to the order Picornavirales, CBPV is an exception as it is not classified (Baker & Schroeder, 2008).

Deformed wing virus (DWV) was detected in the Ordu province and in queen bees in the Hatay province, where mass bee mortality occurred (Gülmez et al., 2009; Muz & Muz, 2009). Gümüşova et al. (2010) reported CBPV and BQCV for the first time in Black Sea Region of Türkiye. Özkırım & Schiesser (2013) collected seventy-one samples from Türkiye and reported *Israeli acute paralysis virus* (IAPV) in fifteen of the samples. Rüstemoğlu & Sipahioğlu (2019) investigated the presence of BQCV, DWV, IAPV, SBV, ABPV, KBV and CBPV in honey bees in the Hakkari province of Türkiye. KBV and IAPV were not detected in any of tested bee samples. The infection rates of BQCV, DWV, SBV, CBPV were 32%, 23%, 12% and 9%, respectively. Güller et al. (2021) detected DWV for the first time in honey bees from Türkiye's Erzincan province. Usta & Yıldırım (2022) determined the presence of DWV, BQCV and ABPV in honey bees in the Burdur province and determined the infection rates as 74.19% (23/31), 25.81% (8/31), 74.19% (23/31), respectively. Güller & Kurt (2022) reported the infection rates of SBV and BQCV as 7.03% and 11.70%, respectively in the Bingöl province of Türkiye, but ABPV infection was not detected in any of the surveyed apiaries.

The aim of this study is to ascertain the prevalence of DWV, ABPV, and BQCV in honey bees and *Varroa* mites in the Malatya and Elazığ provinces of Türkiye. It is also intended to carry out molecular characterisation of these economically significant viruses.

MATERIAL AND METHODS

Collecting honey bee and *Varroa* mite samples

Surveys were conducted during 2018 and 2019 to forty different apiaries in the Malatya (25 apiaries) and Elazığ (15 apiaries) provinces and honey bees and parasite *Varroa* mite feeding on the honey bees were collected. In total, 147 honey bee and thirty *Varroa* samples were obtained from Malatya (Battalgazi, Doğanşehir and Yeşilyurt districts) and Elazığ (Arıcak, Kovancılar, Sivrice and Palu districts). Eighty-four honey bee and fifteen *Varroa* mite samples were obtained from twenty-five different apiaries in Malatya province, while sixty-three honey bee and fifteen *Varroa* mite samples were taken from fifteen different apiaries in the Elazığ province. There were at least ten hives in each surveyed apiary. Honey bee and parasite *Varroa* mite samples were collected by sampling a representative number of bees from several hives in each apiary. In apiaries with ten to forty at least two samples were collected for every ten hives depending on size.

Honey bee samples showing paralysis, sudden death, change in colour (becoming dark brown to black), trembling, enlarged abdomens with wings dislocating, becoming hairless, dark to shiny black in colour were collected as well as the symptomless samples. Collected live honey bee and *Varroa* mite samples were taken into sterile tubes covered with cheesecloth in cold chain and were taken to the laboratory. They were numbered, photographed and classified according to the symptom type. Vector *Varroa* mites were collected via pliers under the microscope and taken into sterile micro tubes for RNA extraction.

Total RNA extraction and RT-PCR tests

Total RNA was extracted from live honey bee and *Varroa* mite samples using a commercial genomic RNA purification kit (GeneJET Plant RNA Purification Kit). Samples were prepared and extracted individually. Virus-specific primers were used to perform reverse transcription. DWV-specific primers used in the study (F-5'-TTG GTA TGC TCC GTT GAC TG-3', R-5'-ATT CCT CAG AAG TTG GTT TCG-3') were encoding an RdRP gene region with an expected band size of 488. BQCV-specific primers (F-5'-GAC AGC GTG CCA AAG AGA G-3', R-5'-GCG AAC CCG TCC AAT ACT TA-3') and ABPV-specific primers (F-5-GTA TGG AAG TGG GCT GAG GA-3', R-5-CGC GGT ACT AAA AAG CTA CGA-3') were encoding a part of the coat protein gene with an expected band size of 567 bp and 476 bp, respectively (Rüstemoğlu

& Sipahioğlu, 2019). To run RT-PCR, a total of 25 µl of reverse transcription mixture was used, which included 5 µl of 5x RT buffer, 1 µl of MMLV reverse transcriptase enzyme, 1 µl 100 nmol of each primer, 5 µl of dNTP (each 2.5 mM), 0.5 µl of RNase inhibitor, 7 µl RNase free water, and 2 µl total RNA.

PCR tests were performed according to Rüstemoğlu & Sipahioğlu (2019) with slight modifications. A 25 µL of PCR reaction volume containing 14.5 µl RNase free water, 2.5 µl 10x Go*Taq* Green Buffer (Promega, Madison, WI, USA), 0.5 µl Taq DNA Polymerase, 1 µl dNTP (20 mM each), 1.5 µl 25 mM MgCl, 1 µl of each forward and reverse primer (100 mM each), and 3 µl cDNA for DWV, BQCV and ABPV were prepared. Thermo Scientific Arktik Thermal Cycler (Waltham, MA, USA) was used to perform PCR with the following cycling parameters: initial denaturation at 94°C for 2 min followed by thirty-five cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 seconds for DWV and BQCV and 57°C for 30 seconds for ABPV, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The electrophoresis of fifteen µl of each amplicon was performed on 2% agarose gel in 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and stained with Pronasafe nucleic acid staining solution (CondaLab). As a marker, a DNA ladder was employed (100bp DNA ladder, Thermo Scientific).

Sequencing and phylogenetic analysis

Amplified PCR products were sequenced bidirectionally by a commercial firm (BM Labosis, Ankara/Türkiye) using the dideoxy chain termination reaction. The nucleotide sequences were compared to other isolates from the NCBI database online, revealing their phylogenetic relationships with viruses from throughout the world.

Randomly chosen viral isolates that were positive in RT-PCR tests (eight DWV, four ABPV and two BQCV isolates) were sequenced and compared to other viral isolates in the NCBI GeneBank, with differences and similarities investigated. Sequences of DWV, BQCV and ABPV generated in this study, as well as sequences of closely related species downloaded after a BLAST search from the GenBank database (http://www.ncbi.nlm.nih.gov/), were included in separate datasets in the Geneious Prime (GP) software (Version 2023.0.1). On the Geneious alignment, each dataset was individually aligned, and discrepancies were manually rectified. For phylogenetic inference, Maximum Likelihood analysis (ML) was used with 1000 bootstrap repetitions in the same software for each dataset. Phylogenetic analyses were done with Geneious tree builder.

RESULTS

Symptoms observed on the infected honey bees were deformation in the wings and black colour in the abdomen (Fig. 1 A-F) paralysis and sudden death. Alive *Varroa* mites feeding on honey bees were also observed (Fig. 1 G).

Molecular Studies

According to the RT-PCR tests performed with specific primers, thirty-five honey bee samples from the survey region were found to be infected with DWV, nineteen honey bee samples were found to be infected with ABPV, and fifteen honey bee samples were found to be infected with BQCV. Viruses were found in almost all of the symptomatic samples as well as the symptomless ones. Two of the thirty-five DWV positive samples, four of the nineteen ABPV positive samples, and three of the fifteen BQCV positive samples were asymptomatic. Almost all of the samples with enlarged abdomens with wings dislocating, trembling and paralysis were found to be infected with DWV. Honey bees found to be infected with BQCV showed change in colour (becoming dark brown to black) and ABPV positive honey bees had hairless abdomens and showing trembling, paralysis and death symptoms.

The infection rates were found to be 23.81%, 12.93% and 10.20% for DWV, ABPV and BQCV, respectively (Tab. 1). Based on the results of the viral-specific PCRs, out of 147 samples, four (2.72%) were found to be coinfected with DWV and BQCV, whereas one (0.68%) was found to be coinfected with DWV+BQCV+ABPV in Malatya province. No mixed infections were detected in Elazığ province. Totally 30 *Varroa* mite samples

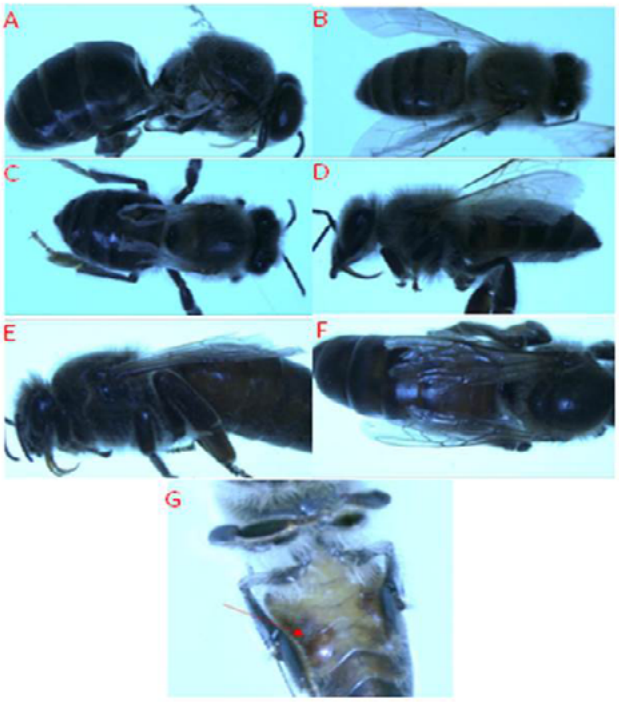


Fig. 1. A - Deformation of the wing on male honey bee. B - Abdomen blackening on female honey bee. C and D - Deformation of the wing and abdomen blackening on female honey bee. E and F - Abdomen blackening and wing deformation on queen bee. G - Live *Varroa* mite feeding on honey bee.

were also collected and tested against the mentioned viruses in both Malatya and Elazığ provinces in this study. Five out of thirty *Varroa* samples (two collected from Malatya and three collected from the Elazığ province) were found to be infected with DWV. The total infection rate of *Varroa* mites was found to be 16.66% for DWV. None of the samples tested were found to be positive for ABPV and BQCV (Tab. 1).

Randomly selected DWV positive isolates were sequenced and compared with other viral isolates in the NCBI GeneBank and differences and similarities with them were investigated. The resulting DWV sequences (OP805878, OP805879, OP805880, OP805887, OP805888, OP805889, OP805890, OP805891) were named as Malatya 1, Malatya 2, Malatya 3, Elazig 1, Elazig 2 (mite), Elazig 3, Elazig 4 and Elazig 5. Based on nucleic acid sequence similarity, the phylogenetic tree was divided into five major clusters. The isolates in this study were in the first group, together with DWV isolates from *A. mellifera* honey bees from the United Kingdom, Lebanon, Türkiye (Hakkari), France, Germany, and Saudi Arabia (Fig. 2).

Multiple alignments showed that DWV isolates revealed from this study has 96.34-98.06% nucleotide identity with the other global isolates retrieved from GenBank (Tab. 2). It was interesting that Malatya and Elazığ isolates were found to be similar with a Japan isolate accession no. B070959, which is a *Kakugo virus* (KV) (*Iflavirus)* with the similarity rate of 96.61% (Tab. 2).

When DWV isolates revealed in this study were compared with ten different isolates with the world and one isolate from Türkiye, it was observed that Elazığ 1 isolate had an insertion mutation in 107th nucleotide, Malatya 1 and Malatya 2 isolates had five deletions in 62nd-63rd nucleotides and six frameshift mutations, 32 deletions and 29 frameshift mutations in Malatya 3, Elazığ 2, Elazığ 3 and Elazığ 4 isolates in 128th and 129th nucleotides. There was no mutation in Elazığ 5 isolate. There was one insert mutation in Elazığ 1 isolate. In total thirty-seven deletions in Malatya 1 (two mutations), Malatya 2 (three mutations), Malatya 3 (eight

Table 1.

DWV, ABPV and BQCV infections in honey bee and *Varroa* mite samples in the Malatya and Elazığ provinces

Honey bee samples								
Province			**DWV**		**ABPV**		**BQCV**	
		TS	IS	IR (%)	IS	IR (%)	IS	IR (%)
Malatya	Battalgazi	35	10	28.57	10	28.57	9	25.71
	Yeşilyurt	15	11	73.33	1	6.67	3	20.00
	Doğanşehir	34	6	17.65	4	11.76	2	5.88
Total		84	27	32.14	15	17.86	14	16.67
Elazığ	Arıcak	8	-	-	-	-	1	12.50
	Kovancılar	4	-	-	1	25.00	-	-
	Palu	29	8	27.59	3	10.34	-	-
	Sivrice	14	-	-	-	-	-	-
	Keban	8	-	-	-	-	-	-
Total		63	8	12.70	4	6.35	1	1.59
General Total		147	35	23.81	19	12.93	15	10.20
Varroa mite samples								
Province			**DWV**		**ABPV**		**BQCV**	
		TS	IS	IR(%)	IS	IR	IS	IR
Malatya		15	2	13.33	-	-	-	-
Elazığ		15	3	20.00	-	-	-	-
Total		30	5	16.66	-	-	-	-

TS stands for tested sample, IS stands for infected sample, and IR is for infection rate.

mutations), Elazığ 2 (eight mutations), Elazığ 3 (eight mutations) and Elazığ 4 (eight mutations) mutations were observed. There was also thity-five frameshift mutations in Malatya 1, (four mutations), Malatya 2 (two mutations), Malatya 3 (seven mutations), Elazığ 2 (seven mutations), Elazığ 3 (eight mutations) and Elazığ 4 (seven mutations) isolates (data not shown).

ABPV sequences (OP805881, OP805882, OP805883 and OP805884) were named as Malatya 1, Malatya 2, Malatya 3 and Malatya 4 and their multiple alignments showed that these isolates revealed 96.76% and 96.70% nucleotide identity with Ankara and Tekirdağ (Türkiye) isolates and 89.62-96.46% with the other world isolates retrieved from GenBank (Tab. 3). Based on nucleic acid sequence similarity, the phylogenetic tree was divided into two major clusters. Malatya 1 and Malatya 2 isolates were in the first group forming another branch different than the other isolates, whereas Malatya 3 and Malatya 4 were in the second group distinct from all the other isolates (Fig. 3). The similarities and differences were determined by comparing the base sequence of coat protein genes with other ABPV isolates deposited in GeneBank and a shift mutation in the 96th nucleotide of ABPV Malatya 1 isolate, and an insertion mutation and a shift mutation in 98th nucleotide of ABPV Malatya 2 isolate were observed, whereas forty-four deletion mutations and twenty shift mutations were observed in the 164th and 167th nucleotides of ABPV Malatya 3 and ABPV Malatya 4 isolates (data not shown).

BQCV sequences (OP805885 and OP805886) were named as Malatya 1 and Malatya 2 in the GenBank/EMBL database and their multiple alignments showed that BQCV isolates revealed

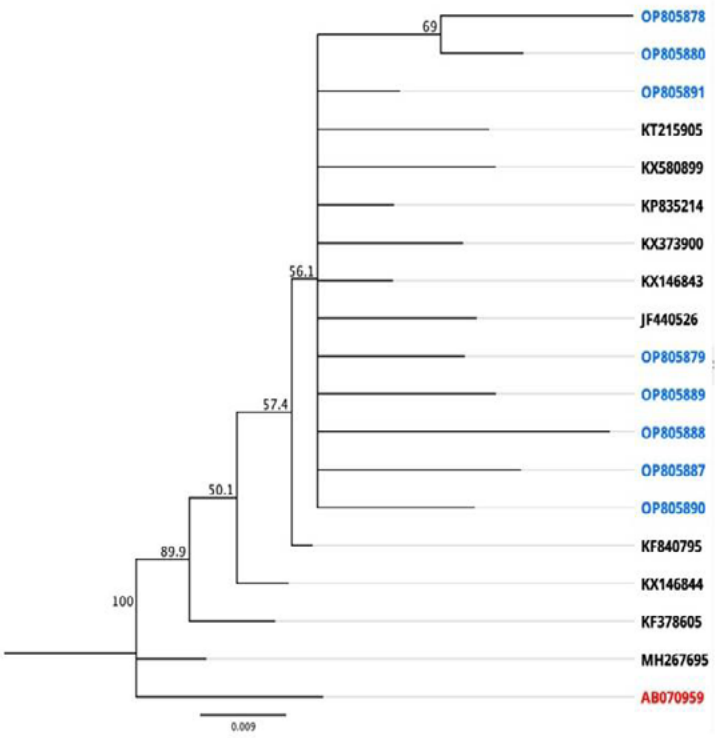


Fig. 2. Phylogenetic tree of DWV (Malatya 1, Malatya 2, Malatya 3, Elazığ1, Elazığ 2, Elazığ 3, Elazığ 4 and Elazığ 5) isolates.
A *Kakugo virus* (in with red color) was included as an outgroup from Japan. The sequences from this study are blue in color.

96.78% nucleotide identity with another Turkish isolate from Hakkari province and 87.40-90.21% with the other world isolates retrieved from GenBank (Tab. 4). Based on nucleic acid sequence similarity, the phylogenetic tree was divided into six major clusters. The isolates revealed in this study were in the second group together with the Türkiye (Hakkari) isolate of BQCV from *A. mellifera* honey bee (Fig. 4).

Also, the BQCV isolates obtained in this study were compared via CP gene sequence with the other isolates deposited in the GeneBank and the similarities and differentiations were determined. According to the results, seven shift mutations were observed in the 525th nucleotide of Malatya 1 isolate, and fifteen shift mutations and one deletion mutation were observed in the 527th nucleotide in BQCV Malatya 2 and BQCV Elazığ 1 isolates (data not shown).

DISCUSSION

When honey bee diseases are not detected, their control can be difficult, especially when it comes to viruses. The RT-PCR technique is a specific, confidential and sensitive method used to detect RNA viruses such as picorna-like insect viruses. A reverse transcriptase PCR procedure to detect *Acute bee paralysis virus* (ABPV) and *Black queen cell virus* (BQCV) was provided by Benjeddou et al. (2001). Hereby we also used the RT-PCT method for the detection of DWV, ABPV and BQCV. *Deformed wing virus* (DWV) is the most common and well-studied insect disease in the world (Chen et al., 2005; Yue & Genersh, 2005; Tentcheva et al., 2006; Teixeira et al., 2008; and Zvokelj et al., 2020). DWV, ABPV and BQCV viruses were found in Thailand, Uruguay, Austria and France as a result of investigating decreases in bee colonies and losses in honey production (Tentcheva et al., 2006; Bereyni et al., 2006; Chantawannakul et al., 2006).

Table 2.

Similarity rates of DWV isolates with other isolates from the world

Country	Organism	Host	Accession number	Similarity rate (%)	Reference
Israel	DWV	*Apis mellifera*	JF440526	98.06	Zioni et al., 2011
Germany	DWV	*Varroa destructor*	KX146843	97.88	Grau et al., 2017
France	DWV	*Apis mellifera*	KX373900	97.51	Dalmon et al. ,2017
United Kingdom	DWV	synthetic construct	KT215905	97.06	Ryabov, 2015 (unpublished)
Japan	*Kakugo virus*	*Apis mellifera*	AB070959	96.61	Fujiyuki et al., 2004
Germany	DWV	*Varroa destructor*	KX146844	96.34	Grau et al., 2017
Turkey	DWV	*Apis mellifera*	KP835214	97.09	Rüstemoğlu & Sipahioğlu, 2019
Africa	DWV	*Apis mellifera adansonii*	KF378605	96.19	Cornelissen et al., 2014 (unpublished)
Lithuania	DWV	*Apis mellifera*	KF840795	97.09	Skrodenyte-Arbaciauskiene et al., 2014 (unpublished)
Sweden	DWV	*Apis mellifera*	MH267695	97.31	Thaduri et al., 2018
Türkiye	DWV	*Apis mellifera*	OP805878		
Türkiye	DWV	*Apis mellifera*	OP805880		
Türkiye	DWV	*Apis mellifera*	OP805888		
Türkiye	DWV	*Apis mellifera*	OP805887		
Türkiye	DWV	*Apis mellifera*	OP805890		
Türkiye	DWV	*Apis mellifera*	OP805879		
Türkiye	DWV	*Apis mellifera*	OP805889		
Türkiye	DWV	*Varroa* sp.	OP805891		

Martin & Bretell (2019) correlated the global spread of DWV to its ectoparasitic mite *Varroa destructor*. They reported that DWV is now the most widespread virus infecting honey bees in thirty-two different countries. Also, DWV has been detected in three orders of Arachnida and sixty-five arthropod species belonging to eight insect orders. *Varroa* mites are the ectoparasit vector transmitting it globally. The interaction among the host (honey bee), the vector acar (*Varroa* mites) and the pathogen has a great importance in DWV epidemiology, so these acars were tested in most of the studies (Ryabov et al., 2014; Wilfert et al., 2016; Manley et al., 2019; Posada-Florez et al., 2019). Simeunović et al. (2014) monitored the presence of *Acute bee paralysis virus* (ABPV) and *Deformed wing virus* (DWV) in fifty-five honey bee colonies from different regions of Serbia. It was reported that inefficient *Varroa* control may be the reason for high frequency infection of ABPV and mite transmission the reason for DWV. We found out that DWV was the most prevalent virus as with the infection rate of 23.81% in the survey area. We also tested the *Varroa* mites for the presence of the three viruses, but only DWV was found in the tested samples.

ABPV is a common virus infecting bees and occurs worldwide except South America (Allen & Ball, 1996). de Miranda et al. (2010) reported that *Israeli acute paralysis virus* (IAPV), *Kashmir bee virus* (KBV) and *Acute bee paralysis virus* (ABPV) are widely spread in honey bee colonies. Antunez et al. (2005) determined CBPV and ABPV by RT-PCR for the first time and reported that these viruses cause widespread infection

Table 3.

Similarity rates of ABPV isolates with other isolates from the world

Country	Organism	Host	Accession number	Similarity rate (%)	Reference
Türkiye (Ankara)	ABPV	*Apis mellifera*	EU779947	96.76	Muz & Muz, 2009
South Africa	ABPV	*Apis mellifera*	AF150629	93.56	Govan et al., 2000
Türkiye (Tekirdağ)	ABPV	*Apis mellifera*	KY465554	96.72	Muz & Muz 2009
Slovenia	ABPV	*Apis mellifera*	HQ877397	96.46	Jamnikar Ciglenecki & Toplak, 2013
Serbia	ABPV	*Apis mellifera*	KM001899	95.56	Cirkovic, 2018
France	ABPV	*Vespa velutina nigrithorax*	MN565031	94.75	Dalmon et al., 2019
Hungary	ABPV	*Apis mellifera*	AF486072	93.72	Bakonyi et al., 2002
Syria	ABPV	*Apis mellifera*	MH378788	93.30	Barhoum & Mouhanna, 2018 (unpublished)
Slovenia	ABPV	*Apis mellifera*	HQ877382	96.46	Jamnikar Ciglenecki & Toplak, 2013
USA	ABPV	*Apis mellifera*	AF264688	89.62	Evans et al., 2001
Hungary	ABPV	*Apis mellifera*	AY053385	94.30	Bakonyi et al., 2002
Türkiye	ABPV	*Apis mellifera*	OP805881		
Türkiye	ABPV	*Apis mellifera*	OP805882		
Türkiye	ABPV	*Apis mellifera*	OP805883		
Türkiye	ABPV	*Apis mellifera*	OP805884		

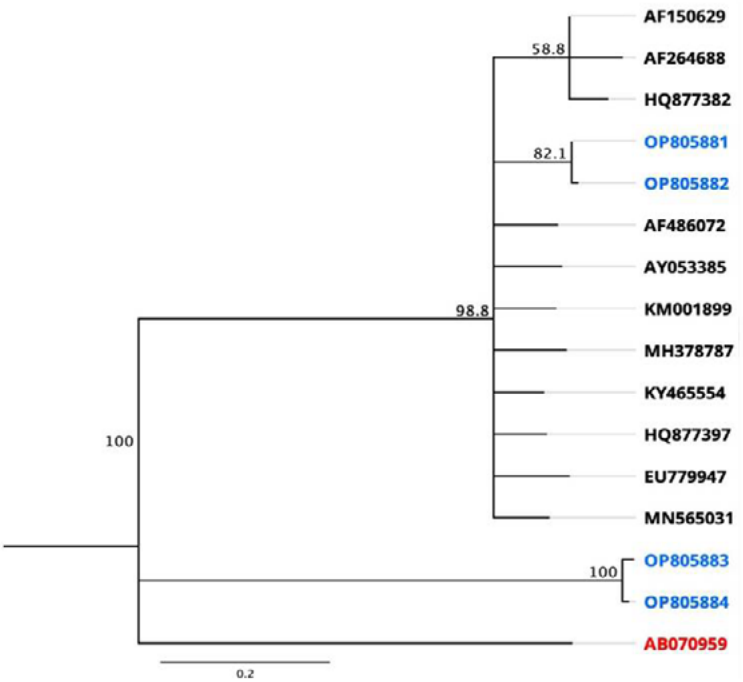


Fig. 3. Phylogenetic tree of ABPV isolates (Malatya 1, Malatya 2, Malatya 3 and Malatya 4).
A *Kakugo virus* (in with red color) was included as an outgroup from Japan. The sequences from this study are blue in color.

Table 4.

Similarity rates of BQCV isolates with other isolates from the world

Country	Organism	Host	Accession number	Similarity rate (%)	Reference
China	BQCV	*Apis mellifera*	MG799370	87.86	Sun et al., 2013 (unpublished)
South Korea	BQCV	*Apis mellifera*	JX149531	87.40	Reddy et al., 2013
Czech Republic	BQCV	*Apis mellifera*	KY243932	87.91	Spurny et al., 2017
France	BQCV	*Vespa velutina nigrithorax*	MN565034	87.98	Dalmon et al., 2019
Hungary	BQCV	*Apis mellifera*	EF517515	89.02	Tapaszti et al., 2009
Turkey (Hakkari)	BQCV	*Apis mellifera*	KP835213	96.78	Rüstemoğlu & Sipahioğlu, 2019
Poland	BQCV	*Apis mellifera*	EF517520	90.02	Tapaszti et al., 2009
South Africa	BQCV	*Apis mellifera*	AF183905	89.34	Leat et al., 2000
Australia	BQCV	*Apis mellifera*	KY465685	90.21	Roberts et al., 2017
Sweden	BQCV	*Apis mellifera*	MH267694	89.81	Thaduri et al., 2018
Türkiye	BQCV	*Apis mellifera*	OP805881		
Türkiye	BQCV	*Apis mellifera*	OP805882		
Türkiye	BQCV	*Apis mellifera*	OP805883		
Türkiye	BQCV	*Apis mellifera*	OP805884		

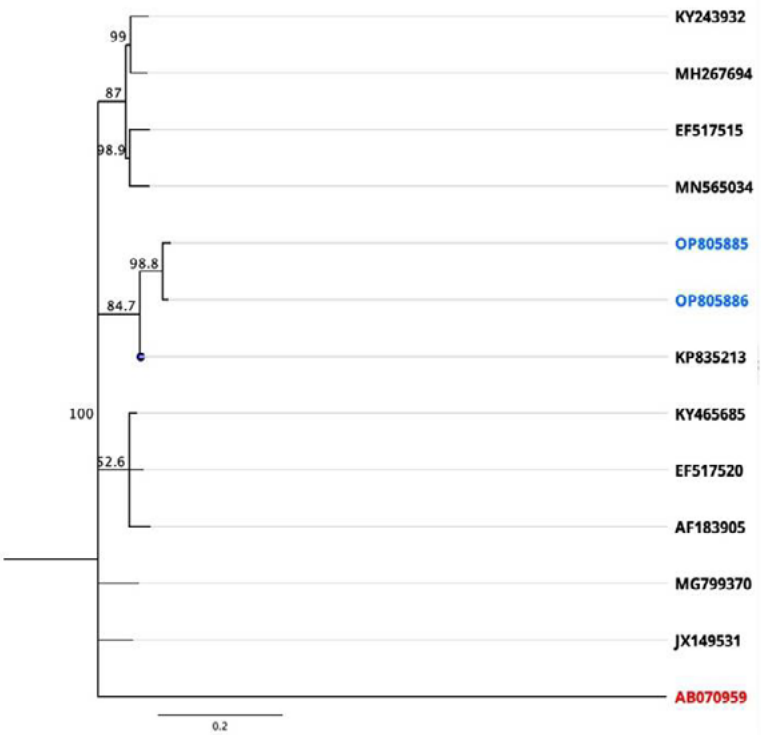


Fig. 4. Phylogenetic tree of BQCV (Malatya 1 and Malatya 2) isolates.
A *Kakugo virus* (red in color) was included as an outgroup from Japan. The sequences from this study are blue in color.

in Uruguayan honey bees. ABPV was discovered to be the second most common honey bee virus in this study with a 12.93% infection rate.

Berenyi et al. (2006) collected honey bees which showed sudden colony collapse, decrease in population, paralysis or dark colouring. They detected *Chronic bee paralysis virus* (CBPV), *Black queen cell virus* (BQCV), *Acute bee paralysis virus* (ABPV), *Sacbrood virus* (SBV), *Deformed wing virus* (DWV) and *Kashmir bee virus* (KBV) in Austria by RT-PCR. They found DWV as the most prevalent virus with an infection rate of 91%, followed by ABPV, SBV, and BQCV (68%, 49%, and 30%, respectively). KBV was not found in any of the samples and CBPV was present with a quite low rate. Mixed infections were detected in most of the samples. We also detected mixed infections in honey bee samples tested.

Leat et al. (2000) found *Black queen cell virus* (BQCV) in South African honey bees. Although BQCV has the lowest infection rate (10.20%) in our survey area, it is a prevalent virus in Türkiye (Gumusova et al., 2010; Karapınar et al., 2018; Kalaycı et al., 2020; Muz & Muz, 2018; Güller, 2021).

The DWV sequences revealed in this study were identical with one another. They showed 96.34-98.06% nucleotide identity with UK, Lebanon, Türkiye (Hakkari), France, Germany and Israel isolates. Although BQCV isolates revealed in this study were not in the same cluster with the other world isolates in the phylogenetic tree, they showed 87.40-90.21% nucleotide identity with China, South Korea, Czech Republic, France, Hungary, Poland, South Africa, Australia and Sweden isolates. ABPV sequences in this study showed 96.76% and 96.70% nucleotide identity with Ankara and Tekirdağ isolates from Türkiye and 89.62-96.46% nucleotide identity with South Africa, Slovenia, Serbia, France, Hungary, Syria and USA isolates from the world.

Beekeeping activities are increasing day by day in Türkiye and in the world. Many factors are affecting beekeeping activities and they are interconnected. Bacterial, fungal, parasitic and viral factors increase their sensitivity when environmental and nutritional conditions are unfavourable. Chemical use also has a detrimental effect on bee health. The sterilization of the hives and the materials used in the hives with insufficient maintenance causes the formation of viral diseases and causes losses. These viruses are transmitted horizontally and vertically by physical and biological vectors as well as by feeding, contact and fecal contamination. Moreover, because mostly migratory beekeeping is carried out, transmission occurs from infected colonies to healthy colonies easily. Honey-bee viruses mostly cause more damage in colonies infected with *Varroa* spp. It is recommended to take cultural precautions in beekeeping activities and to find solutions to virus-based losses in the colony. In addition to breeding healthy bees and colonies, *Varroa* mites, insect and ant control plays an important role inr preventing viral infections. There are many control methods used against *Varroa* mites, which survives in all life stages of honey bees. In recent studies, essential oils and oxalic and lactic acids are used extensively. Chemical control has an impact on both bee and human health due to high pesticide residues in honey and wax production. All of these measures and suggestions will result in better disease and pest control, as well as longer-lasting generations.

ACKNOWLEDGEMENT

This research is a part of M. Sc. study of Songül Aydın and supported by a grant from the Research Fund of Malatya Turgut Özal University (Project no: 191403).

Conflict of interest

The authors declare no conflict of interest.

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