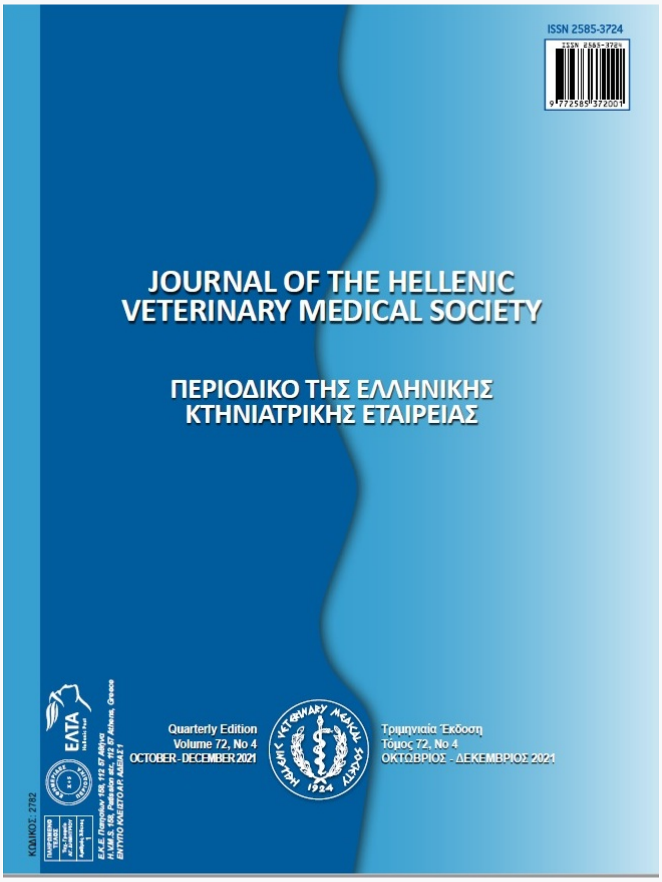
Journal of the Hellenic Veterinary Medical Society

Vol 72, No 4 (2021)



The phylogenetic analysis of RNA-dependent RNA polymerase gene of chronic bee paralysis virus in Turkey

AA ÇAGIRGAN, Y YILDIRIM, A USTA

doi: 10.12681/jhvms.29385

To cite this article:

ÇAGIRGAN, A., YILDIRIM, Y., & USTA, A. (2022). The phylogenetic analysis of RNA-dependent RNA polymerase gene of chronic bee paralysis virus in Turkey. *Journal of the Hellenic Veterinary Medical Society*, *72*(4), 3415–3422. https://doi.org/10.12681/jhvms.29385

J HELLENIC VET MED SOC 2021, 72(4): 3415-3422
ΠΕΚΕ 2021, 72(4): 3415-3422

Research article
Ερευνητικό άρθρο

The phylogenetic analysis of RNA-dependent RNA polymerase gene of chronic bee paralysis virus in Turkey

A.A. Çagirgan[1*], Y. Yildirim[2], A. Usta[2]

[1]Izmir/Bornova Veterinary Control Institute, Department of Virology, Bornova, Izmir, Turkey

[2]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Virology, Burdur, Turkey

ABSTRACT: Chronic bee paralysis virus (CBPV) is a major contributor to global bee colony losses. However, among the RNA viruses infecting honeybees, CBPV is still not classified. In this study, samples of asymptomatic adult honey bees were randomly collected from 45 apiaries in three provinces (Burdur, Antalya, and Isparta) in Turkey. Five of the 45 samples were determined to be positive by reverse transcription polymerase chain reaction (RT-PCR). In addition, three positive samples from Burdur, Isparta, and Antalya were confirmed by sequencing. We constructed a phylogenetic tree that was divided into five main groups based on the RNA-dependent RNA polymerase gene region. The Turkish strains TUR/BUR/CBPV1, TUR/ISPT/CBPV2, and TUR/ANT/CBPV3 and the Turkish strains from obtained previous studies formed a different cluster. The sequence homology results of a phylogenetic analysis showed that the Turkish strains shared 97-98.9% of their nucleotide identity and had 96.9-99% similarity rates with each other. The strains obtained in this study and the Turkish strains detected in previous studies were also 81-84.6% similar to European, Chinese, and Uruguay strains. This research underlines the presence of CBPV in apparently healthy Turkish bee colonies and the remarkable differences in Turkish CBPV strains. Further investigation is needed to identify the molecular characterization, complete genome sequence, and pathogenesis of Turkish CBPV strains.

Keywords: honey bee, chronic bee paralysis virus, RT-PCR, phylogenic analysis, Turkey

Corresponding Author:
Abdurrahman Anil Çagirgan, Ankara street No:172/155 post code 35010 Bornova-Izmir, Turkey
E-mail address: a.anilcagirgan@gmail.com

Date of initial submission: 06-10-2020
Date of acceptance: 24-03-2021

INTRODUCTION

Honey bees, which contribute greatly to agriculture and economics, are one of the most important pollinators in the world (Morse and Colderone, 2000; Chauzat et al., 2003; Celli and Maccagni, 2003). However, the number of honey bee colonies has decreased significantly in recent years because of a large number of biotic and abiotic stressors (vanEngelsdorp et al., 2011; Cornman et al., 2012). Bacteria, parasites, and viruses are some of the main threats to honey bee health, among which viruses have caused significant colony losses (Brutscher et al., 2016; Gisder et al., 2016; Tehel et al., 2016).

CBPV is one of the most common viruses affecting honey bee colonies. First detected by Bailey et al. (1963), CBPV is an infectious and contagious disease that causes a high mortality ratein adult honey bees. CBPV is transmitted by two main routes: through physical contact between adult healthy bees and infected individuals (Bailey et al., 1983) and oral contact between healthy bees and infectious particles in the feces of paralyzed bees (Ribiere et al., 2007). In particular, this virus causes individual black, hairless bees to stand at the hive entrance and leads to clusters of trembling, flightless, crawling bees (Chen and Siede, 2007; Dittes et al., 2020). Bees with symptoms from CBPV die within 6 days, and inapparent virus infections can persist in colonies (Youssef et al., 2015).

The symmetry, size, and genomic structure of CBPV are completely different from other bee viruses. For example, CBPV has anisometric particles that measure 20 nm in width and 30-60 nm in length (Bailey et al., 1968). In addition, it is an unclassified polymorphic, segmented, non-enveloped, positive single-stranded RNA virus, with a viral particle composed of two major RNAs (RNA1 [3674 nt] and RNA2 [2305 nt]) and three minor RNAs (RNA 3a, 3b, and 3c). It also has seven overlapping open reading frames (ORFs), with three on RNA1 and four on RNA2. The RNA-dependent RNA polymerase is encoded by RNA1-ORF3 (Olivier et al., 2008). The structural proteins hSP and pSP are thought to be encoded by RNA2-ORF2 and RNA2-ORF3, respectively (Chevin et al., 2015). To date, CBPV has not been classified by the International Committee on Taxonomy of Viruses (ICTV) (http://www.ictvonline.org). It was stated by Oliver et al (2008) that only ORF3 on RNA1 showed similarities with the Nodaviridae and Tombusviridae families. However, Lake Sinai Virus, which is a novel honey bee virus, and CBPV are thought to belong to a new viral family (Schuster et al., 2014).

CBPV is commonly detected in bee colonies worldwide, such as France (Tentcheva et al., 2004), Uruguay (Antunez et al., 2005), Australia (Berenyi et al., 2006), Hungary (Forgach et al., 2008), Denmark (Nielsen et al., 2008), China (Wu et al., 2015; Li et al., 2017), Iran (Ghorani et al., 2017) and Turkey (Cagirgan et al., 2020; Kalayci et al., 2020). In addition to agarose gel immune-diffusion assay (AGID) (Ball, 1999), RT-PCR (Ribiere et al., 2002, Blanchard et al., 2007a), multiplex RT-PCR (Sguazza et al., 2013; Cagirgan and Yazici, 2020), and real-time RT-PCR (Blachard et al., 2007b) are used for the diagnosis of CBPV. However, it is possible to detect CBPV by RT-PCR test, even in the case of inapparent infections (Tentcheva et al., 2004; Blanchard et al., 2007a; Cagirgan et al., 2018).

This study aims to investigate the presence of CBPV in asymptomatic adult honey bees in the southern cities of Burdur, Isparta, and Antalya in Turkey and construct its phylogenetic tree based on the RNA-dependent RNA polymerase gene region.

MATERIAL AND METHODS

Ethics Statement

No ethics committee approval document was required for this study. The study involved the *Apis mellifera* which is neither a protected nor endangered species.

Samples and Preparation

Samples of alive asymptomatic adult bees were randomly collected from 45 apiaries in three provinces (Antalya [n=17], Isparta [n=8], and Burdur [n=20]) of Turkey (Figure 1). The samples were collected between March and November 2019. The samples were taken from three hives from each apiary. A pool consisting of 30 adult bees was created for each apiary. These pools were homogenized with 9 ml of Eagle's Minimum Essential Medium (Sigma Aldrich, UK). Then, the homogenates were centrifuged at 5000 rpm at 4ºC for 30 minutes.

Following this step, 200 µl of the supernatant were taken for RNA extraction after centrifuging the homogenates. This step was carried out using the High Pure Viral RNA Kit (Roche, Germany) according to the manufacturer's instructions. The extracted RNA was stored at -20°C until testing.

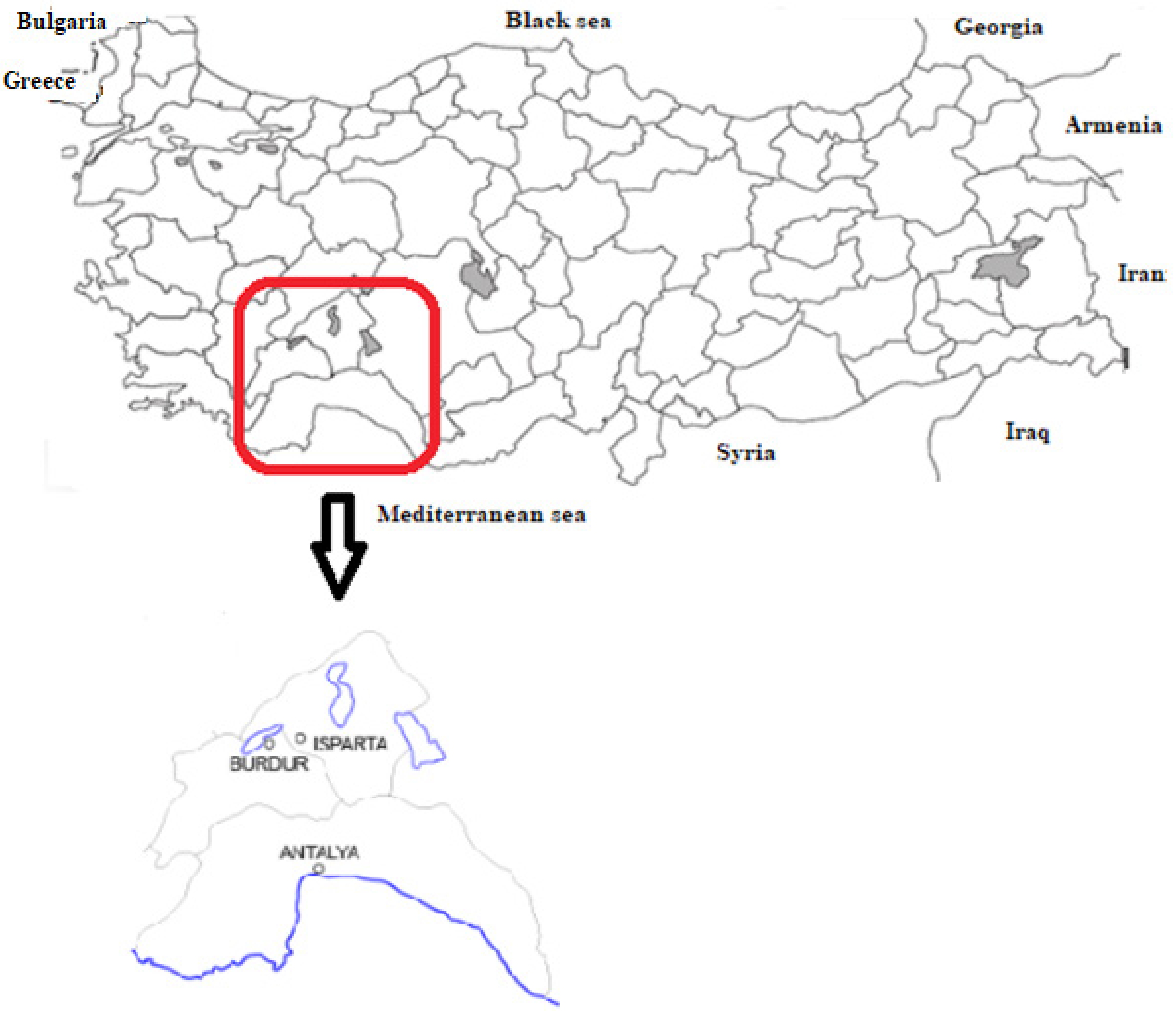


Figure 1. The geographical location of the three proviences of Turkey where honeybee samples were collected.

RT-PCR

An RT-PCR for the partial genes was conducted using the oligonucleotide sense and antisense primers (5'-TCAGACACCGAATCTGATTATTG-3' 1921-1933 and 5'-CCGGAGACAAAGGTCATCAT-3' 3445-3426) targeting a 1525-bp fragment of the RNA-dependent RNA polymerase gene of the CBPV, as described by Blanchard et al. (2009). For the amplification, an Xpert One-Step RT-PCR Kit (Grisp Research Solutions, Porto, Portugal) was used. The total reaction volume was 25 µl, and the final concentration of the primer was 0.4 mM.

The thermocycler was programmed with the following steps and cycling times. First, one cycle at 45ºC for 15 minutes was performed for the reverse transcription, followed by the initial denaturation at 95ºC for 3 minutes. This step was followed by another denaturation at 95ºC for 10 seconds, annealing at 55ºC for 10 seconds. Finally, an extension at 72ºC for 15 seconds was sustained for 35 cycles, with a final extension at 72ºC for 1 minute. The final PCR products were analyzed in a 1.5% agarose gel electrophoresis (containing ethidium bromide) and visualized under a UV light source.

The PCR products were sequenced in the Forward and Reverse directions by a Microsynth AG (Balgach, Switzerland). A phylogenetic analysis of the partial RNA-dependent RNA polymerase gene region targeting a 1290-bp region was performed using 32 CBPV sequences from Turkey and other geographical regions of the world. These additional sequences were taken from National Center for Biotechnology Information (NCBI). The nucleotide sequence results were analyzed and assembled, and the obtained sequences were aligned using DNADynamo DNA Sequence Analysis Software. To interpret the results of the phylogenetic analysis, an SDT a stand-alone program was used (Muhire et al., 2014). A graphical port demonstrated pairwise identity scores using a color-coded pairwise-identity matrix and enabled the visualization of the proximity among sequences in a dataset. Phylogenetic analyses were constructed the MEGA X (Kumar et al., 2018) with Maximum likelihood trees based on the Tamura-Nei parameter (TN93+G) model with 1,000 bootstrap replicates for the determination of genetic distances between nucleotide sequences. GenBank accession numbers were taken for three different strains obtained in this study.

RESULTS AND DISCUSSION

The results revealed that 5 of the 45 samples were CBPV positive. The basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI) was used for searching the obtained nucleotide sequences. The TUR/BUR/CBPV1, TUR/ISPT/CBPV2, and TUR/ANT/CBPV3 strains detected in this study and the Turkish strains detected in previous studies formed a separate branch in phylogenetic tree (Figure 2). Based on the non-structural RNA-dependent RNA polymerase gene region, the tree was divided into five main groups. According to phylogenetic analysis, the sequence homology results showed that the Turkish strains shared 97-98.9% of the nucleotide identity and 96.9-99% similarity rates with each other (Figure 3). All of the strains obtained in this study and the Turkish strains detected in previous studies showed 81-84.6% similarities with European, Chinese, and Uruguayan strains.

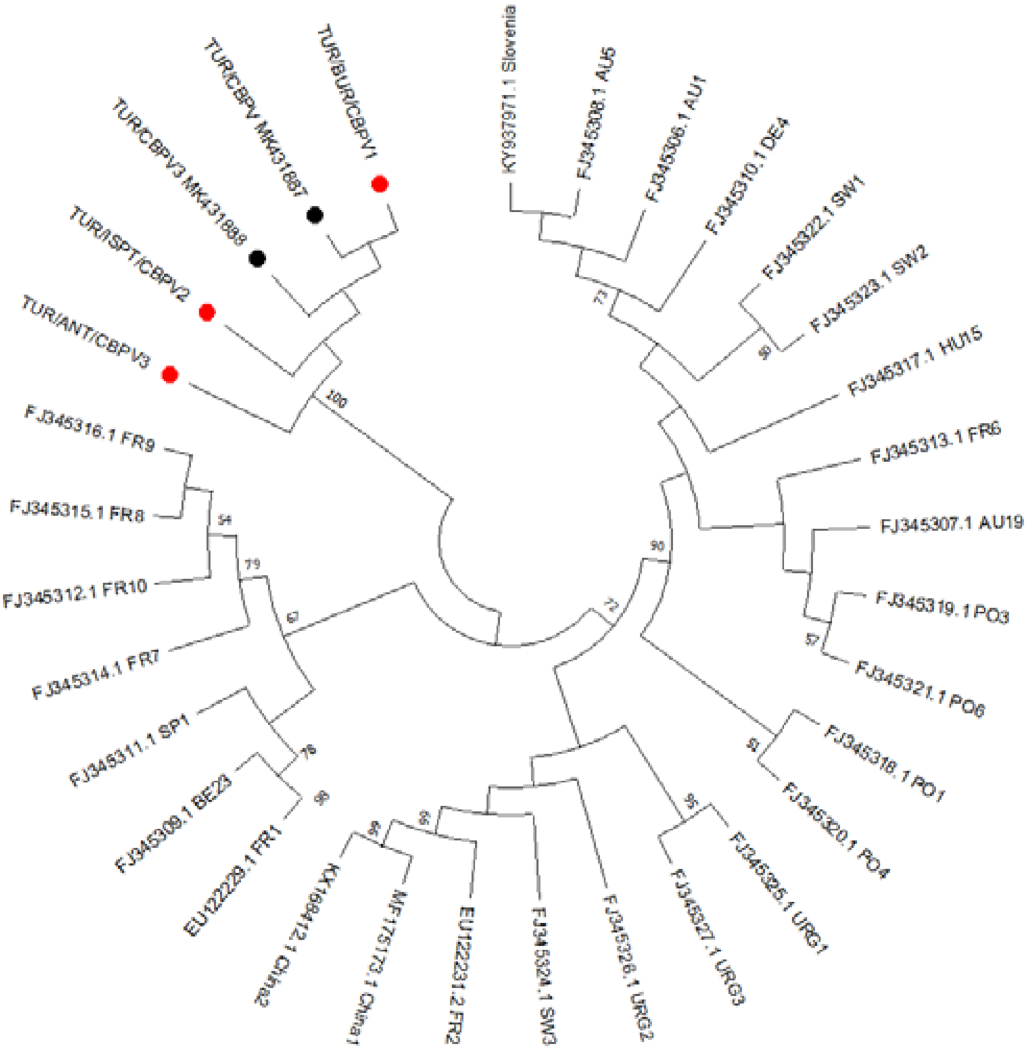
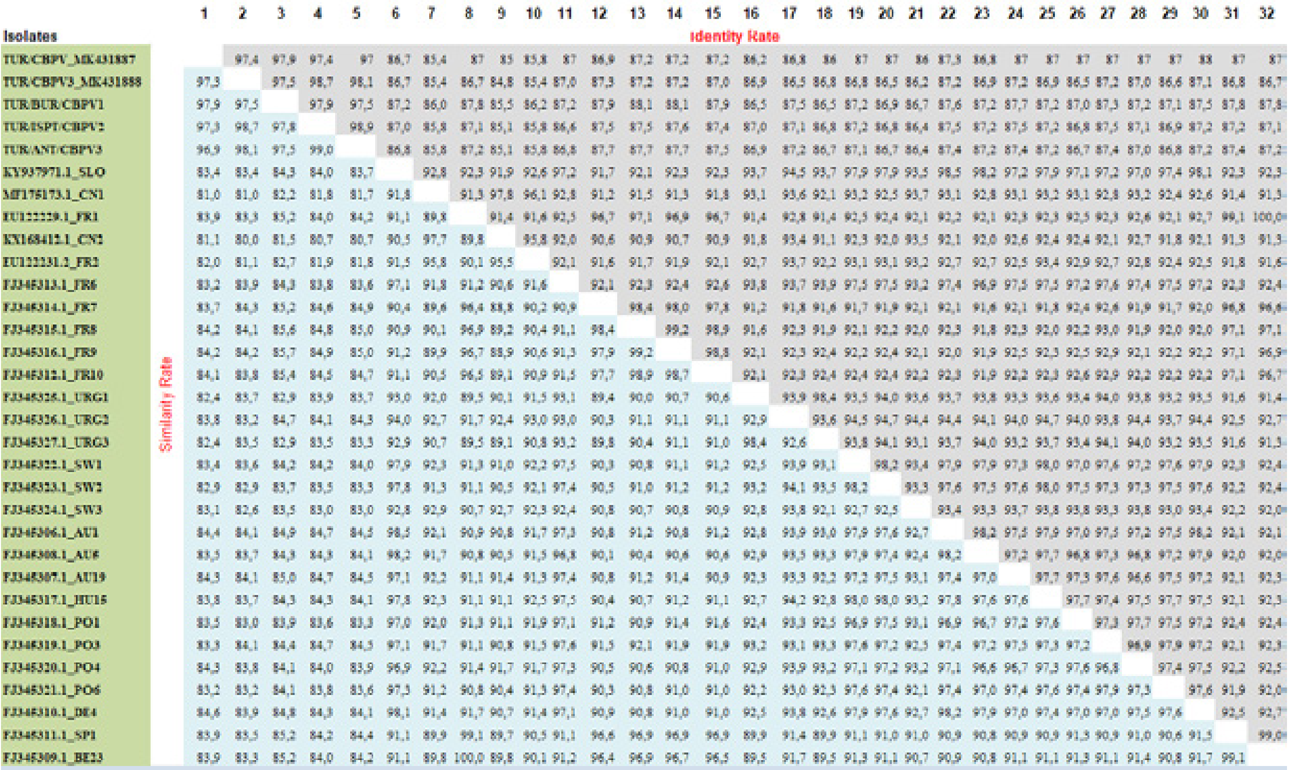


Figure 2. The phylogenetic tree constructedusing Maximum Likelihood (ML) method with Tamura-Nei parameters, included in the MEGA-X software, on the aligment of the 1290 bp of RNA-dependent RNA polymerase sequences of 32 CBPV strains.



Identity Rate

Similarity Rate

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
TUR/CBPV_MK431887		97,4	97,9	97,4	97	86,7	85,4	87	85	85,8	87	86,9	87,2	87,2	87,2	86,2	86,8	86	87	87	86	87,3	86,8	87	87	87	87	87	87	88	87	87
TUR/CBPV3_MK431888	97,3		97,5	98,7	98,1	86,7	85,4	86,7	84,8	85,4	87,0	87,3	87,2	87,2	87,0	86,9	86,5	86,8	86,8	86,5	86,2	87,2	86,9	87,2	86,9	86,5	87,2	87,0	86,6	87,1	86,8	86,7
TUR/BUR/CBPV1	97,9	97,5		97,9	97,5	87,2	86,0	87,8	85,5	86,2	87,2	87,9	88,1	88,1	87,9	86,5	87,5	86,5	87,2	86,9	86,7	87,6	87,2	87,7	87,2	87,0	87,3	87,2	87,1	87,5	87,8	87,8
TUR/ISPT/CBPV2	97,3	98,7	97,8		98,9	87,0	85,8	87,1	85,1	85,8	86,6	87,5	87,5	87,6	87,4	87,0	87,1	86,8	87,2	86,8	86,4	87,5	87,2	87,5	87,2	86,8	87,5	87,1	86,9	87,2	87,2	87,1
TUR/ANT/CBPV3	96,9	98,1	97,5	99,0		86,8	85,8	87,2	85,1	85,8	86,8	87,7	87,7	87,7	87,5	86,9	87,2	86,7	87,1	86,7	86,4	87,4	87,2	87,4	87,2	86,7	87,4	87,0	86,8	87,2	87,4	87,2
KY937971.1_SLO	83,4	83,4	84,3	84,0	83,7		92,8	92,3	91,9	92,6	97,2	91,7	92,1	92,3	92,3	93,7	94,5	93,7	97,9	97,9	93,5	98,5	98,2	97,2	97,9	97,1	97,2	97,0	97,4	98,1	92,3	92,3
MF175173.1_CN1	81,0	81,0	82,2	81,8	81,7	91,8		91,3	97,8	96,1	92,8	91,2	91,5	91,3	91,8	93,1	93,6	92,1	93,2	92,5	93,7	93,1	92,8	93,1	93,2	93,1	92,8	93,2	92,4	92,6	91,4	91,3
EU122229.1_FR1	83,9	83,3	85,2	84,0	84,2	91,1	89,8		91,4	91,6	92,3	96,7	97,1	96,9	96,7	91,4	92,8	91,4	92,3	92,4	92,1	92,2	92,1	92,3	92,3	92,5	92,3	92,6	92,1	92,7	99,1	100,0
KX168412.1_CN2	81,1	80,0	81,5	80,7	80,7	90,5	97,7	89,8		95,8	92,0	90,6	90,9	90,7	90,9	91,8	93,4	91,1	92,3	92,0	93,5	92,1	92,0	92,6	92,4	92,4	92,1	92,7	91,8	92,1	91,3	91,3
EU122231.2_FR2	82,0	81,1	82,7	81,9	81,8	91,5	95,8	90,1	95,5		92,1	91,6	91,7	91,9	92,1	92,7	93,7	92,2	93,1	93,1	93,2	92,7	92,7	92,5	93,4	92,9	92,7	92,8	92,4	92,5	91,8	91,6
FJ345313.1_FR6	83,2	83,9	84,3	83,8	83,6	97,1	91,8	91,2	90,6	91,6		92,1	92,3	92,4	92,6	93,8	93,7	93,9	97,5	97,5	93,2	97,4	96,9	97,5	97,5	97,2	97,6	97,4	97,5	97,2	92,3	92,4
FJ345314.1_FR7	83,7	84,3	83,2	84,6	84,9	90,4	89,6	96,4	88,8	90,2	90,9		98,4	98,0	97,8	91,2	91,8	91,6	91,7	91,9	92,1	92,1	91,6	92,1	91,8	92,4	92,6	91,9	91,7	92,0	96,8	96,6
FJ345315.1_FR8	84,2	84,1	85,6	84,8	85,0	90,9	90,1	96,9	89,2	90,4	91,1	98,4		99,2	98,9	91,6	92,3	91,9	92,1	92,2	92,0	92,3	91,8	92,3	92,0	92,2	93,0	91,9	92,0	92,0	97,1	97,1
FJ345316.1_FR9	84,2	84,2	85,7	84,9	85,0	91,2	89,9	96,7	88,9	90,6	91,3	97,9	99,2		98,8	92,1	92,3	92,4	92,2	92,4	92,1	92,0	91,9	92,5	92,3	92,5	92,9	92,1	92,2	92,2	97,1	96,9
FJ345312.1_FR10	84,1	83,8	85,4	84,5	84,7	91,1	90,5	96,5	89,1	90,9	91,5	97,7	98,9	98,7		92,1	92,3	92,4	92,4	92,4	92,2	92,3	91,9	92,2	92,3	92,6	92,9	92,2	92,2	92,2	97,1	96,7
FJ345325.1_URG1	82,4	83,7	82,9	83,9	83,7	93,0	92,0	89,5	90,1	91,5	93,1	89,4	90,0	90,7	90,6		93,9	98,4	93,5	94,0	93,6	93,7	93,8	93,3	93,6	93,4	94,0	93,8	93,2	93,5	91,6	91,4
FJ345326.1_URG2	83,8	83,2	84,7	84,1	84,3	94,0	92,7	91,7	92,4	93,0	93,0	90,3	91,1	91,1	91,1	92,9		93,6	94,5	94,7	94,4	94,4	94,1	94,0	94,7	94,0	93,8	94,4	93,7	94,4	92,5	92,7
FJ345327.1_URG3	82,4	83,5	82,9	83,5	83,3	92,9	90,7	89,5	89,1	90,8	93,2	89,8	90,4	91,1	91,0	98,4	92,6		93,8	94,1	93,1	93,7	94,0	93,2	93,7	93,4	94,1	94,0	93,2	93,5	91,6	91,3
FJ345322.1_SW1	83,4	83,6	84,2	84,2	84,0	97,9	92,3	91,3	91,0	92,2	97,5	90,3	90,8	91,1	91,2	92,5	93,9	93,1		98,2	93,4	97,9	97,9	97,3	98,0	97,0	97,6	97,2	97,6	97,9	92,3	92,4
FJ345323.1_SW2	82,9	82,9	83,7	83,5	83,3	97,8	91,3	91,1	90,5	92,1	97,4	90,5	91,0	91,2	91,2	93,2	94,1	93,5	98,2		93,3	97,6	97,5	97,6	98,0	97,5	97,3	97,3	97,5	97,6	92,2	92,4
FJ345324.1_SW3	83,1	82,6	83,5	83,0	83,0	92,8	92,9	90,7	92,7	92,3	92,4	90,8	90,7	90,8	90,9	92,8	93,8	92,1	92,7	92,5		93,4	93,3	93,7	93,8	93,8	93,3	93,8	93,0	93,4	92,2	92,0
FJ345306.1_AU1	84,4	84,1	84,9	84,7	84,5	98,5	92,1	90,9	90,8	91,7	97,3	90,8	91,2	90,8	91,2	92,8	93,9	93,0	97,9	97,6	92,7		98,2	97,5	97,9	97,0	97,5	97,2	97,5	98,2	92,1	92,1
FJ345308.1_AU5	83,5	83,7	84,3	84,3	84,1	98,2	91,7	90,8	90,5	91,5	96,8	90,1	90,4	90,6	90,6	92,9	93,5	93,3	97,9	97,4	92,4	98,2		97,2	97,7	96,8	97,3	96,8	97,2	97,9	92,0	92,0
FJ345307.1_AU19	84,3	84,1	85,0	84,7	84,5	97,1	92,2	91,1	91,4	91,3	97,4	90,8	91,2	91,4	90,9	92,3	93,3	92,2	97,2	97,5	93,1	97,4	97,0		97,7	97,3	97,6	96,6	97,5	97,2	92,1	92,3
FJ345317.1_HU15	83,8	83,7	84,3	84,3	84,1	97,8	92,3	91,1	91,1	92,5	97,5	90,4	90,7	91,2	91,1	92,7	94,2	92,8	98,0	98,0	93,2	97,8	97,6	97,6		97,7	97,4	97,5	97,7	97,5	92,1	92,3
FJ345318.1_PO1	83,5	83,0	83,9	83,6	83,3	97,0	92,0	91,3	91,1	91,9	97,1	91,2	90,9	91,4	91,6	92,4	93,3	92,5	96,9	97,5	93,1	96,9	96,7	97,2	97,6		97,3	97,7	97,5	97,2	92,4	92,4
FJ345319.1_PO3	83,3	84,1	84,4	84,7	84,5	97,1	91,7	91,1	90,8	91,5	97,6	91,5	92,1	91,9	91,9	93,2	93,1	93,3	97,6	97,2	92,5	97,4	97,2	97,5	97,3	97,2		96,9	97,9	97,2	92,1	92,3
FJ345320.1_PO4	84,3	83,8	84,1	84,0	83,9	96,9	92,2	91,4	91,7	91,7	97,3	90,5	90,6	90,8	91,0	92,9	93,9	93,2	97,1	97,2	93,2	97,1	96,6	96,7	97,3	97,6	96,8		97,4	97,5	92,2	92,5
FJ345321.1_PO6	83,2	83,2	84,1	83,8	83,6	97,3	91,2	90,8	90,4	91,3	97,4	90,3	90,8	91,0	91,0	92,2	93,0	92,3	97,6	97,4	92,1	97,4	97,0	97,4	97,6	97,4	97,9	97,3		97,6	91,9	92,0
FJ345310.1_DE4	84,6	83,9	84,8	84,3	84,1	98,1	91,4	91,7	90,7	91,4	97,1	90,9	90,8	91,0	91,0	92,5	93,8	92,6	97,9	97,6	92,7	98,2	97,9	97,0	97,4	97,0	97,0	97,5	97,6		92,5	92,7
FJ345311.1_SP1	83,9	83,5	85,2	84,2	84,4	91,1	89,9	99,1	89,7	90,5	91,1	96,6	96,9	96,9	96,9	89,9	91,4	89,9	91,1	91,0	91,0	90,9	90,8	90,9	90,9	91,3	90,9	91,0	90,6	91,5		99,0
FJ345309.1_BE23	83,9	83,3	85,2	84,0	84,2	91,1	89,8	100,0	89,8	90,1	91,2	96,4	96,9	96,7	96,5	89,5	91,7	89,5	91,3	91,1	90,7	90,9	90,8	91,1	91,1	91,3	91,1	91,4	90,8	91,7	99,1	

Figure 3. Detailed nucleic acid identity and similarity rates for 32 CBPV strains

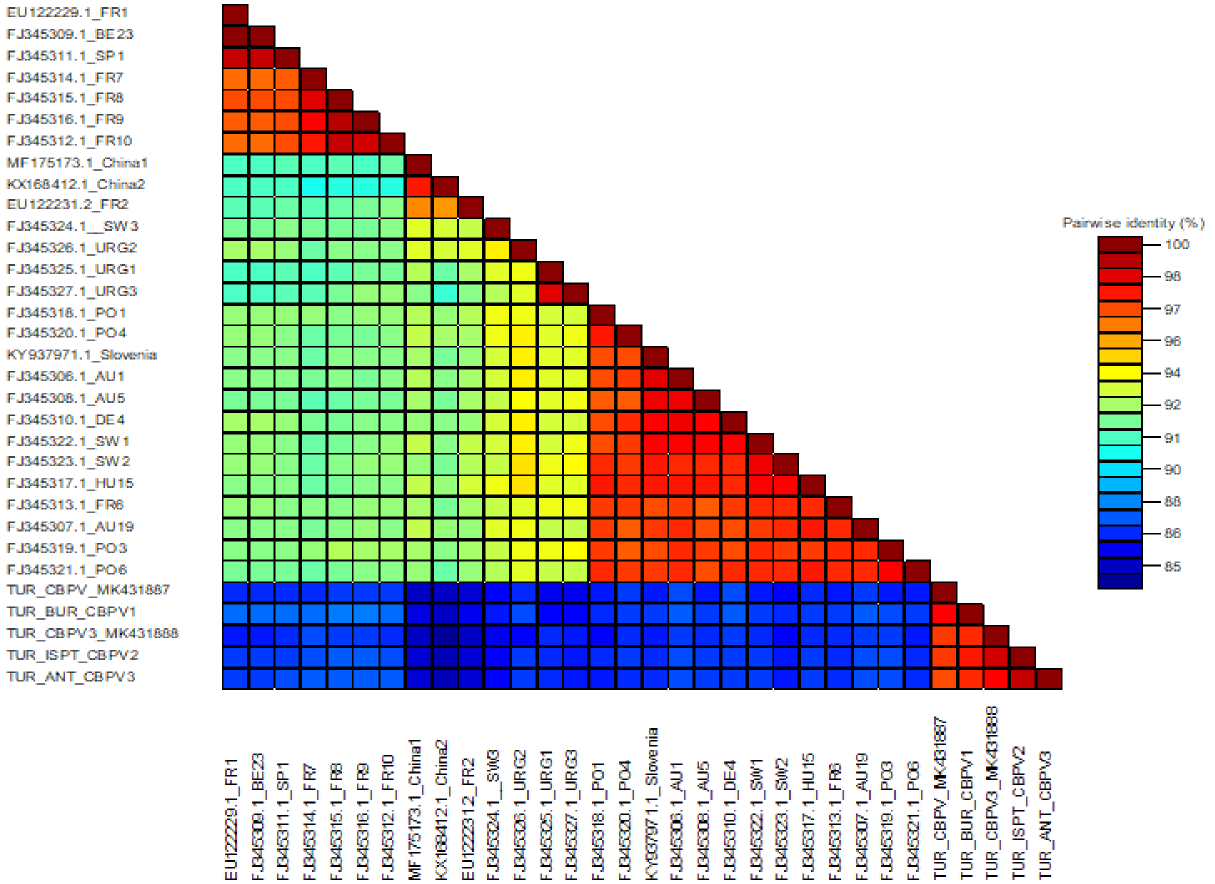


Figure 4. SDT color-coded matrix of nucleotide pairwise identity scores performed by alignment of a 1290 bp fragment RNA-dependent RNA polymerase gene for 32CBPV strains. Each colored square symbolizes a percentage identity score between two sequences

A total of 32 CBPV strains were analyzed using the Sequence Demarcation Tool (SDT) program. The nucleotide pairwise identity changed between the interval of 100% to 85%. The identity showed in different colors: from maroon to dark red, to red, orange-red, orange, yellow, yellow-green, aquamarine, turquoise, and blue to navy blue. The CBPV strains reported in this study largely showed as blue (~86-88%) and dark blue (~85%) (Figure 4). According to the SDT graphic, the Turkish CBPV strains exhibited high differences compared to European, Uruguayan, and Chinese strains.

Honeybees play an important role in pollination and, as such, offer both ecological and economic contributions. Turkey, with its 7.9 million honeybee colonies (FAOSTAT, 2018), is one of the most important honey producers in the world. One of the major challenges that Turkish beekeepers face is viruses. CBPV is common in Turkish bee colonies and the cause of many colony losses (Kalayci et al., 2020).

In France, CBPV was sporadically detected in adult bees sampled from 360 healthy colonies using the RT-PCR diagnosis method. CBPV was found in 28% of 36 apiaries (Tentcheva et al. 2004). Previous studies in Europe show that the prevalence of this virus was low in Austria, Denmark, and Croatia, respectively (Berenyi ve ark., 2006; Nielsen ve ark., 2008; Gajger ve ark., 2014). Ghorani et al. (2015) reported a finding of CBPV in 3 out of 89 (3.3%) apiaries in Iran.

The first molecular detection of CBPV in Turkey was reported by Gumusove et al. (2010), who detected the virus in 7 of 28 (25%) samples from the Black Sea region in Turkey. CBPV was previously reported in 1.8% of 111 apparently healthy colonies in seven provinces of the Aegean Region (Cagirgan, 2018). In addition, CBPV was identified in 14 of 76 (18.4%) apiaries complaining of colony losses in Turkey (Kalayci et al., 2020). In this study, CBPV was detected in 5 of 45 (11.1%) apiaries.

The nucleotide similarity rates of the three CBPV strains obtained from the Burdur, Isparta, and Antalya provinces were 96.9-99%. Their similarities with the strains obtained from different countries are shown in Figure 3, which details that the strains obtained from different countries and the Turkish strains ranged in similarity from 81-84.6%. This range can be explained by the fact that CBPV is a segmented and variable virus. In addition, the genomic variability of honeybee viruses, such as the Sacbrood virus in Turkey has been previously described (Kalayci et al., 2019; Yildirim et al., 2020). The genetic variability of CBPV was also reported in a previous study by Cagirgan (2018).

The phylogenetic tree based on the RNA-dependent RNA polymerase gene region was first constructed by Blanchard et al. (2009). In the phylogenetic evaluation, CBPV strains formed four different groups: A, B, C, and D. Strains obtained from the same countries took place in different branches on the phylogenetic tree. Strains of the same country were included in different groups. Turkish strains formed the E group in the study conducted by Cagirgan (2018). In that study, the Turkish strains were separated into a different branch. This difference suggests that Turkish CBPV strains generate a new group in addition to the groups formed by Blachard et al. (2009). In a study based on the RNA-dependent RNA polymerase gene region, the strains from Iran formed Group 1 and Group 2 (Ghorani et al., 2015). While CBPV reveals genetic differences even in the same countries, the Turkish strains were not separated into different groups.

In conclusion, this study underlines the presence of CBPV in apparently healthy colonies in Turkey and remarkable differences between Turkish CBPV strains, which divide into different branches of the phylogenetic tree, from the CBPV strains obtained in other countries. Further investigation of CBPV strains is necessary to reveal their geographical distribution and genetic variability, to evaluate the global distribution of this virus, and to investigate the role of CBPV in disease outbreaks in Turkey.

REFERENCES

Antunez K, Alessandro BD, Corbella E, Zunino, P (2005) Detection of chronic bee paralysis virus and acute bee paralysis virus in Uruguayan honeybees. J Invertebr Pathol. 90: 69-72.

Bailey L, Gibbs AJ, Woods RD (1968) The purification and properties of chronic bee-paralysis virus. J Gen Virol 2: 251-260.

Bailey L, Ball BV, Perry JN (1983) Honeybee paralysis: its natural spread and its diminished incidence in England and Wales. J Apic Res 22: 191-195.

Bailey L, Gibbs AJ, Woods RD (1963) Two viruses from adult honey bees (Apis mellifera Linnaeus). Virology 21: 390-395.

Ball BV (1999) Paralysis In: Bee disease diagnosis. Options Mediteranneennes: pp 81-89.

Berenyi O, Bakonyi T, Derakhshifar I, Koeglberger H, Nowotny N (2006) Occurrence of six honeybee viruses in diseased austrian apiaries. Appl Environ Microbiol. 72: 2414-2420.

Blanchard P, Olivier V, Iscache AL, Celle O, Schurr F, Lallemand P, Ribière M (2007a) Improvement of RT-PCR detection of chronic bee paralysis virus (CBPV) required by the description of genomic variability in French CBPV isolates. J Invertebr Pathol 97: 182-185.

Blanchard P, Ribière M, Celle O, Lallemand P, Schurr F, Olivier V, Iscache AL, Faucon JP (2007b) Evaluation of a real-time two-step RT-PCR assay for quantitation of Chronic bee paralysis virus (CBPV) genome in experimentally-infected bee tissues and in life stages of a symptomatic colony. J Virol Methods 141(1): 7-13. doi: 10.1016/j.jviromet.2006.11.021.

Blanchard P, Schurr F, Olivier V, Celle O, Antùnez K, Bakonyi T, Berthoud H, Haubruge E, Higes M, Kasprzak S, Koeglberger H, Kryger P, Thiéry R, Ribière M (2009) Phylogenetic analysis of the RNA-dependent RNA polymerase (RdRp) and a predicted structural protein (pSP) of the Chronic bee paralysis virus (CBPV) isolated from various geographic regions. Virus Res 144(1-2): 334-8. doi: 10.1016/j.virusres.2009.04.025.

Brutscher LM, McMenamin AJ, Flenniken ML (2016) The buzz about honey bee viruses. PLoS Pathog 12: e1005757 4.

Cagirgan AA, Yazici Z (2020) Development of a multiplex RT-PCR assay for the routine detection of sevenRNA viruses in *Apis mellifera*. J Virol Methods 281: 113858. doi:10.1016/j.jviromet.2020.113858

Cagirgan AA, Yildirim Y, Usta A (2020) The investigation of Israil acute bee paralysis virus, sacbrood virus, kashmir bee virus and chronic bee paralysis virus in honeybees (*Apis mellifera*). Eurasian J Vet Sci 36: 2, 96-10. doi:10.15312/EurasianJVetSci.2020.265

Cagirgan, AA (2018) Ege bölgesinde virus nedenli arı hastalıklarının multipleks polimeraz zincir reaksiyonu ile araştırılması (Investigation of virus-induced bee diseases in Aegean Region with multiplex polymerase chain reaction). https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp (accessed 9 September 2020).

Gumusova Okur S, Albayrak H, Kurt M, Yazici Z (2010) Prevalence of three honey bee viruses in Turkey. Vet Arhiv 80 (6): 779-785.

Celli G, Maccagnani B (2003) Honey bees as bioindicators of environmental pollution. Bull Insectology 56:137-139.

Chauzat MP, Cauquil L, Roy L, Franco S, Hendrikx P, Ribière-Chabert M (2013) Demographics of the European apicultural industry. PLoS One 13: 8(11): e79018. doi: 10.1371/journal.pone.0079018.

Chen YP, Siede R (2007) Honey Bee Viruses. Adv Virus Res 70: 33-80.

Chevin A, Coutard B, Blanchard P, Dabert-Gay AS, Ribière-Chabert M, Thiéry R (2015) Characterisation of structural proteins from chronic bee paralysis virus (CBPV) using mass spectrometry. Viruses 23: 7(6): 3329-44. doi: 10.3390/v7062774.

Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS, vanEngelsdorp D, Evans JD (2012) Pathogen webs in collapsing honey bee colonies. PLoS One 2012: 7(8): e43562. doi: 10.1371/journal.pone.0043562.

Dittes J, Schäfer MO, Aupperle-Lellbach H, Mülling CKW, Emmerich IU (2020) Overt Infection with Chronic Bee Paralysis Virus (CBPV) in Two Honey Bee Colonies. Vet Sci22: 7(3):142.

Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), 2018. Data of Turkey beehives in 2018. http://www.fao.org/faostat/en/#data/QA (accessed 03 August 2020).

Forgach P, Bakonyi T, Tapaszti Z, Nowotny N, Rusvai M (2008) Prevalence of pathogenic bee viruses in Hungarian apiaries: situation before joining the European Union. J Invertebr Pathol. 98: 235-238.

Gajger IT, Kolodziejek J, Bakonyi T, Nowotny N (2014) Prevalence and distribution patterns of seven different honeybee viruses in diseased colonies: a case study from Croatia. Apidologie 45: 701-706.

Ghorani M, Ghalyanchi Langeroudi A, Madadgar O, Rezapanah M, Nabian S, Khaltabadi Farahani R, Maghsoudloo H, Forsi M, Abdollahi H, Akbarein H (2017) Molecular identification and phylogenetic analysis of chronic bee paralysis virus in Iran. Vet Res Forum 8(4): 287-292.

Gisder S, Genersch E (2016) Viruses of commercialized insect pollinators. J Invertebr Pathol doi:10.1016/j.jip.2016.07.010 5.

Kalayci G, Cagirgan AA, Kaplan M, Pekmez K, Beyazit A, Ozkan B, Yesiloz B, Arslan F (2020) The role of viral and parasitic pathogens affected by colony losses in Turkish apiaries. Kafkas Univ Vet Fak Derg 26(5): 671-677. doi: 10.9775/kvfd.2020.24154

Kalayci G, Cagirgan AA, Pekmez K, Ozkan B, Kaplan M (2019) Molecular detection and phylogenetic analysis of the honeybee (Apis mellifera) sacbroodvirus in Turkey. Turk J Vet Anim Sci 43: 551-554.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35: 1547-1549.

Li BB, Deng S, Yang DH, Hou CS, Diao QY (2017) Complete sequences of the RNA 1 and RNA 2 segments of chronic bee paralysis virus strain CBPV-BJ detected in China. Arch Virol 8: 2451-2456.

Morse RA, Calderone NW (2000). The value of honey bees as pollinator of US crops in 2000. Bee Cult 128: 2-15.

Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PloS one 9: e108277. doi:10.1371/journal.pone.0108277

Nielsen SL, Nicolaisen M, Kryger P (2008). Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (*Apis mellifera*) in Denmark. Apidologie 39: 310-314.

Olivier V, Blanchard P, Chaouch S, Lallemand P, Schurr F, Celle O, Dubois E, Tordo N, Thiéry R, Houlgatte R, Ribière M (2008) Molecular characterisation and phylogenetic analysis of Chronic bee paralysis virus, a honey bee virus. Virus Res 132(1-2): 59-68. doi: 10.1016/j.virusres.2007.10.014.

Ribière M, Lallemand P, Iscache AL, Schurr F, Celle O, Blanchard P, Olivier V, Faucon JP (2007) Spread of infectious chronic bee paralysis virus by honeybee (*Apis mellifera* L.) feces. Appl Environ Microbiol 73(23): 7711-6. doi: 10.1128/AEM.01053-07.

Ribiere M, Triboulot C, Mathieu L, Aurieres C, Faucon JP, Pepin M (2002) Molecular diagnosis of chronic bee paralysis virus infection. Apidologie 33: 339-351.

Schuster S, Zirkel F, Kurth A, van Cleef KW, Drosten C, van Rij RP, Junglen SA (2014) Unique nodavirus with novel features: mosinovirus expresses two subgenomic RNAs, a capsid gene of unknown origin, and a suppressor of the antiviral RNA interference pathway. J Virol 88(22): 13447-59. doi: 10.1128/JVI.02144-14.

Sguazza GH, Reynaldi FJ, Galosi CM, Pecoraro MR (2013) Simultaneous detection of bee viruses by multiplex PCR. J Virol Methods 194(1-2): 102-6. doi: 10.1016/j.jviromet.2013.08.003.

Tehel A, Brown MJF, Paxton RJ (2016) Impact of managed honey bee viruses on wild bees. Curr Opin Virol 19: 16-22 6.

Tentcheva D, Gauthier L, Zappulla N, Dainat B, Cousserans F, Colin ME, Bergoin M (2004) Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. Appl Environ Microbiol 70: 7185-7191.

vanEngelsdorp D, Hayes JJ, Underwood RM, Caron RM, Pettis J (2011) A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. J Apic Res 50: 1-10.

Wu YY, Jia RH, Wang Q, Dai LP, Diao YQ, Xu FS, Wang X, Zhou T (2015). Multiple virus infections and the characteristics of chronic bee paralysis virus in diseased honey bees (*Apis mellifera* L.) in China. J Apic Sci 59: 95-106. doi:10.1515/JAS-2015-0026

Yildirim Y, Cagirgan AA, Usta A (2020) Phylogenetic analysis of sacbrood virus structural polyprotein and non-structural RNA dependent RNA polymerase gene: differences in Turkish strains. J Invertebr Pathol 176: 107459.

Youssef I, Schurr F, Goulet A, Cougoule N, Ribiere-Chabert M, Darbon H, Thiery R, Dubois E (2015) RNA 1 and RNA 2 genomic segments of chronic bee paralysis virus are infectious and induce chronic bee paralysis disease. J immunol Res. 423493. doi:10.1155/2015/423493