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Türkiye'nin Bingöl ili Bal Arı Kolonilerinde Deforme kanat virüsü ve Kronik arı felci virüsü'nün Prevalansı

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Öne Çıkanlar:
- Bal arısı virüsleri
- Moleküler karakterizasyon

Anahtar Kelimeler:
- DWV
- CBPV
- Yayılım
- Türkiye
- Apis mellifera

ÖZET:

Bal arılarını infekteleyen virüsler dünyanın her yerinde bal arılarının her gelişim evresinde infeksiyona neden olan ve ekonomik zarara yol açan hastalıklardır. Bu araştırmada dünya çapında yaygın olan iki bal arısı virüsünün (deforme kanat virüsü [DWV] ve kronik arı paraliz virüsü [CBPV]) varlığı ve yaygınlığını belirlemek için bir survey çalışması gerçekleştirilmiştir. Viral RNA'yı belirlemek amacıyla Bingöl ili sınırlarındaki 9 farklı lokaliteden toplam 128 arılık ziyaret edilmiştir. Toplanan 384 bal arısı örneği her virüse özgü genom spesifik primerler kullanılarak moleküler olarak (revers-transkriptaz polimeraz zincir reaksiyonu, RT-PCR) testlenmiştir. 128 arılığın 28'i (%21.87) DWV için pozitif reaksiyon vermiştir fakat hiçbir arılıkta CBPV patojeni tespit edilmemiştir. Pozitif örneklerden rastgele seçilen birinin klonlanarak ortaya çıkarılan 702 bazlık nükleotit dizisi gen bankasına MZ357973 ulaşım numarasıyla kaydedilmiştir. NCBI veri tabanındaki BLASTn analizine göre, Bingöl ilinde belirlenen DWV etmeninin nükleotit dizisi aynı virüsün diğer izolatlarıyla kıyaslandığında %77.82-98.45 arasında değişen nükleotit benzerliği göstermiştir. Ayrıca, farklı orijinlerden izolatların kullanıldığı filogenetik ağaca dayanarak bizim izolatımız Türkiye'den Erzincan-DWV izolatıyla yakın filogenetik ilişki içerisinde olduğu tespit edilmiştir. Literatür taramalarına göre, bu çalışma Bingöl ilinde DWV ve CBPV yaygınlığını ve varlığını DNA temelli yöntemlerle ortaya koyan ilk kayıt niteliğindedir

Prevalence of Deformed wing virus and Chronic bee paralysis virus in Honey Bee Colonies of Bingöl province, Turkey

Highlights:
- Honeybee viruses
- Molecular characterization

Keywords:
- DWV
- CBPV
- Prevalence
- Turkey
- Apis mellifera

ABSTRACT:

Viruses infecting honey bees are diseases that cause infection and economic damage in every developmental stage of honey bees all over the world. In this study, a survey was conducted to determine the presence and prevalence of two worldwide common honeybee viruses (deformed wing virus [DWV] and chronic bee paralysis virus [CBPV]). 128 apiaries from 9 different localities in the province of Bingöl were visited to determine viral RNA. Collected 384 honey bee samples were tested molecularly (reverse-transcriptase polymerase chain reaction, RT-PCR) using genome-specific primers specific to each virus. In molecular tests, 28 of 128 apiaries (21.87%) gave a positive reaction for DWV, but no CBPV pathogen was detected in any apiary. The 711 bp nucleotide sequence revealed by cloning one of the randomly selected positive samples was registered in the gene bank with the accession number MZ357973. According to the BLASTn analysis in the NCBI database, the nucleotide sequence of the DWV agent determined in Bingöl Province showed nucleotide similarity between 77.82-98.45% when compared to other isolates of the same virus. In addition, our isolate was found to be in a close phylogenetic relationship with the Erzincan-DWV isolate from Turkey, based on the phylogenetic tree using isolates from different origins. According to our literature screening, this study is the first record that reveals the prevalence and the presence of DWV and CBPV in Bingöl Province with DNA-based approaches.

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INTRODUCTION

Honey bee (Apis mellifera L.) colonies are invaded by pathogens and parasites such as bacteria (Melissococcus plutonius, Paenibacillus larvae), fungi (Aspergillus spp., Ascosphaera apis), protozoa (Nosema spp.), and parasitic insects (Acarapis woodi, Varroa destructor, Tropilaelaps spp.). Besides these, honeybee viruses also create stress for the colonies by affecting their morphology, physiology, and behavior, and causing colony weakening and collapse (Genersch and Aubert, 2010).

Most known viruses can be found in single or multiple forms, leading to latent and persistent infections in honey bees at the colony or individual level (Bailey et al., 1981; Gauthier et al., 2007). The DWV, responsible for most colony losses, is a 30 nm icosahedral particle comprising a single, positive-stranded RNA genome typical for iflaviruses, a genus of the Iflaviridae family in the order Picornavirales (Adams et al., 2014). The virus, which is widespread worldwide, is well-known among all honeybee viruses (Ai et al., 2012). Today, DWV is encountered in adults, pupae, and larvae of A. mellifera and A. cerena, as well as in Varroa mites (Posada-Florez et al., 2019), Tropilaelaps mercedesae (Forsgren et al., 2009), Bombus terrestris (Reynaldi et al., 2013). DWV can be transmitted by queens and drones vertically (de Miranda and Fries, 2008) or horizontally from larval food (Möckel et al., 2011). Although the virus causes benign and asymptomatic infections, typical symptoms such as wing anomalies, short and swollen abdomen, and color changes occur if the virus is transmitted to the pupae via varroa. High mite infestations and viral load shorten the lifespan of honey bees, eventually resulting in death (Bailey and Ball, 1991; Yang and Cox-Foster, 2007; de Miranda and Genersch, 2010).

Chronic bee paralysis is a viral disease caused by Chronic bee paralysis virus that causes severe symptomatology, including loss of colonies. The virus, which has a global distribution, causes large worker bee losses, mostly in strong colonies. The disease is characterized by two different types: The disease is characterized by two different types: paralysis, which includes tremor, ataxia, and flightlessness, and hairlessness syndrome, which includes black hairless individuals with a short abdomen. Both syndromes can affect a colony simultaneously (Budge et al., 2020). Infected symptomatic honey bees clump in front of the hive, eventually dying in about a week. This leads to colony collapse or a decrease in honey production because of a weakened colony. The agent has a worldwide distribution, including in Asia, Europe, and North America, with an increasing incidence of CBPV (Traynor et al., 2016; Porrini et al., 2016; Li et al., 2017).

To date, different studies on honeybee viruses have been carried out in different regions of Turkey and five different viruses with RNA genomes (DWV, SBV, BQCV, ABPV, CBPV) have been reported (Rüstemoğlu and Sipahioğlu, 2016; Karapınar et al., 2018; Rüstemoğlu and Sipahioğlu, 2019; Rüstemoğlu, 2020). This study intends to figure the presence and prevalence of two viral agents important for beekeeping in worker honey bee samples collected from apiaries of Bingöl Province (Turkey) in 2020, using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method.

MATERIALS AND METHODS

Sampling

From April to September in 2020, 128 apiaries were visited from 8 regions within the borders of Bingöl Province (Table 1). According to the randomized plots trial design, symptomatic and asymptomatic live worker honey bees were sampled. Three samples taken from apiaries were placed in plastic bottles and brought to the laboratory in a cold chain and stored at -80°C until total RNA extraction.

Table 1. Chart showing the number of apiaries and samples collected in the surveyed regions

District	No. of inspected apiaries	No. of collected Samples
Centre	44	132
Adaklı	12	36
Kiğı	11	33
Karlıova	10	30
Solhan	19	57
Yedisu	11	33
Genç	17	51
Yayladere	4	12
Total	**128**	**384**

Presence of Viral Infections

Total RNA Extraction (TRE), complementary DNA (cDNA) synthesis, and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) methods were employed to verify DWV and CBPV. TRE was done according to the silica-based method reported by Foissac et al., (2001). For cDNA synthesis, the commercially available kit was handled according to the manufacturer's instructions. In PCR tests, primer sets specific to the coat protein gene (CP) were used, as reported by Chen et al., (2004) for DWV and Ribiere et al., (2002) for CBPV (Table 2).

Each 25 µl PCR mix had 2 µl of cDNA, 17.3 µl of RNAase free water, 1.5 µl of MgCl2 (25 mM), 0.5 µl of dNTPs (10 mM), 2.5 µl of 10× PCR buffer (Promega, Carlsbad, USA), 0.2 µl of Taq DNA Polymerase (10U/µl) (Thermo, USA), and 0.5 µl of forward and reverse primer (100µM each). The specific primers practiced in PCR tests and thermocycling parameters are shown in Table 2.

Table 2. Chart showing expected amplicon, temperature cycles, and specific primers used in PCR assays

Virus	Primer sets	Product	Cycling	
DWV	F-5'-ATCAGCGCTTAGTGGAGGAA-3'	711 bp	94°C 2 min	
	R-5'-TCGACAATTTTCGGACATAC-3'		**94°C 30s**	
CBPV	F- 5'-AGTTGTCATGGTTAACAGGATACGAG-3'		**55°C 60s**	**40 cycling**
	R- 5'-TCTAATCTTAGCACGAAAGCCGAG-3'	455 bp	**68°C 2 min**	
			72°C 5 min	

For the reliability of PCR, DWV isolate previously got from honeybee (Apis mellifera L.) by Güller et al. (2021), was used as a positive control. Asymptomatic honeybee was served as the negative control. All PCR tests were completed on Eppendorf Mastercycler (Hamburg, Germany). Fifteen µl of PCR product and 1500 bp DNA marker were separated on a 1% agarose gel and viewed in a UV transilluminator (Syngene™ UV Transilluminator 2020LM).

Molecular Cloning, Sequencing, and Phylogeny

The amplified viral genome was purified using a GeneJET Gel Extraction Kit (Thermo, USA) and immediately cloned into the prokaryotic plasmid vector (pGEM-T Easy, Madison, USA) using DNA ligase enzyme (Promega, USA), followed by transformed into competent cell *E. coli* (Promega, USA). Potential recombinant plasmids (50 µl) purified from recombinant bacteria were sequenced by New Generation Sequencing (NGS) (Sentebiolab, Turkey), and CP-DNA sequence of the DWV-Bingöl isolate was submitted to GenBank (National Center for Biotechnology Information).

Twenty-one isolates from various countries and hosts were used to determine the phylogenetic relationships of the 711 bp sequence detected in Bingöl Province. The molecular phylogeny was revealed using the Neighbor-joining algorithm by 1000 re-sampling analysis. The black queen cell virus (BQCV) (AF183905.1) was chosen as an outsource for better branching.

RESULTS AND DISCUSSION

Viral Infection Incidence of Surveyed Regions

Viruses affecting honeybees (*A. mellifera* and *A. cerena*) are dangerous infectious diseases that cause an economic loss for the beekeeping industry worldwide. PCR is a fast and dependable technique for analyzing honey bee viruses (Haddad et al., 2017). In the present study, the PCR assays were performed and DWV and CBPV were investigated from the collected samples. DWV, a common virus today, has infected at least 55% of colonies/apiaries on average in 32 countries (Martin and Brettell, 2019). In many surveys, different virus incidence of both viruses was reported over the world, such as Austria (Berenyi et al., 2006), Brazil (Weinstein-Teixeira et al., 2008), Denmark (Nielsen et al., 2008), France (Tentcheva et al., 2004), Spain (Antunez et al., 2012), Thailand (Sanpa and Chantawannakul, 2009), Jordan (Haddad et al., 2008), and Greece (Bacandritsos et al., 2010). In the present research, 384 worker honey bee samples were gathered from 128 apiaries from the province of Bingöl. Virus-specific tests showed that the overall infection rate for DWV was 21.87%, in 28 of 128 apiaries (Table 3). Compared to studies conducted worldwide, the DWV outputs of this study (21.87%) were lower than in most countries, including Serbia (76.4%) (Simeunović et al., 2014), China (approx. 40-95%) (Chen et al., 2021), Chile (42%) (Rodríguez et al., 2014), Austria (91%) (Berenyi et al., 2006), Denmark (55%) (Nielsen et al., 2008), and France (97%) (Tentcheva et al., 2004), but higher than Brazil (20.3%) (Weinstein-Teixeira et al., 2008) and Spain (18.6%) (Antunez et al., 2012). No CBPV infections were found in our tests. This is because the viral infection causes honey bee deaths outside the hive in a short time, but the surveys in the current study were sampling from alive individuals of the colony. In addition, viral agents are rarer or have a less frequency than other common viral pathogens in studies conducted in Turkey and around the world (Allen et al., 1996; Berènyi et al., 2006; Toplak et al., 2013).

Table 3. Localities of DWV-infected samples detected in Bingöl Province of Turkey

Districts	No. of surveyed apiaries	No. of DWV-positive samples	Incidence (%)
Centre	44	5	11.30
Karlıova	19	2	10.50
Solhan	17	5	29.40
Genç	12	3	25
Yedisu	10	4	40
Adaklı	11	5	45.40
Yayladere	4	2	50
Kiğı	11	2	18.18
Total	**128**	**28**	**21.87**

In Turkey, viruses infecting honey bees have been well-studied and detailed using different primer sets from various districts. In most studies, DWV is the predominant virus, consistent with our study, and the infection frequency is relatively low compared to other studies. Likewise, CBPV was undetected or had a low prevalence. In the Black Sea region (Sinop, Samsun, Amasya, Giresun, Trabzon, Rize), Gumusova et al. (2010) reported the rate of CBPV and BQCV infection as 25% and 21%, and no ABPV infection was found in the surveyed areas. Samples of 76 apiaries with colony loss complaints from Bursa, Kütahya, Manisa, İzmir, Aydın, Muğla, and Adana were tested against different honeybee viruses. DWV virus was reported as the most common virus, with a rate of 44.7%. Other viruses were detected in ABPV 35.5%, BQCV 28.9%, SBV 22.3%, CBPV 18.4% and IAPV 6.5% (Kalaycı et al., 2020). In 260 adult honey bees from 26 beehives in Van province, Karapınar et al., (2018) detected DWV and BQCV with prevalence rates of 69.23% and 88.46%, but ABPV and CBPV could not be found in any apiaries. Rüstemoğlu (2019), Cagirgan et al., (2020), and Güller et

al., (2021) reported that the frequency of DWV infection is 38% for Şanlıurfa, Diyarbakır, Mardin, Siirt and Şırnak provinces, 88% for Burdur Province, and 50% for Erzincan province. Of adult bees from 111 apiaries sampled from Aegean of Turkey (İzmir, Aydın, Muğla, Manisa, Kütahya, Uşak, Denizli), 22 (19.8%) and 2 (1.8%) were positive for DWV and CBPV (Cagirgan and Yazici, 2021).

Primer sets amplifying different DNA fragments are widely used for the correct identification of honeybee viruses. DNA fragments of 488 bp, 269 bp, 395 bp, and 618 bp were got using different primers designed for various gene regions by Gülmez et al. (2009), Rüstemoğlu and Sipahioğlu (2019), Usta and Yıldırım (2021), and Cagirgan and Yazici (2021), respectively. In our study, the primer sets specific to the CP gene amplified 711 bp DNA fragments for DWV (Fig. 1), which agrees with other researchers using the same primer sets (Chen et al., 2004; Desai et al., 2012).

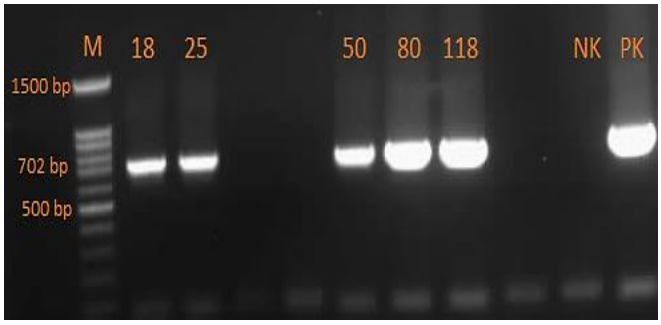

Figure 1. Agarose gel image exhibiting DWV-positivity determined in RT-PCR tests applied to honey bee samples from Bingöl province; M: 100-1500 bp marker; 18, 25, 50, 80, 118; Positive samples; NK: negative control; PK: positive control

Molecular Phylogeny of Bingöl-DWV Isolate

A casually selected one of the DWV-positive isolates was successfully cloned and the CP gene partial nucleotide sequence was revealed. The sequence (711 bp) identified was denominated as Bingol 13, registered in the GenBank database under the acc. No. MZ357973. To establish phylogenetic relationships and nucleotide similarity, 21 different DWV isolates from distinct regions of the world were selected from the NCBI database (Table 4).

Table 4. Chart showing accession number, origin, host, gene, nucleotide similarity of different isolates of DWV in the NCBI database

No	Acces. no.	Country	Host	Genome	Nucleotide identity
1	MW962982	Turkey	*A. melifera*	Polyprotein	% 98.45
2	AY224602	France	*A. melifera*	Polyprotein	% 97.75
3	KU847397	Australia	European honeybee	Structural protein	% 97.33
4	AY292384	USA	*A. melifera*	Polyprotein	% 97.33
5	MW962981	Turkey	*A. melifera*	Polyprotein	% 97.33
6	HM067438	England	*A. melifera*	Polyprotein	% 97.33
7	MN746311	Sweden	*A. melifera*	Polyprotein	% 97.05
8	LN851545	Syria	*A. melifera*	Polyprotein	% 97.05
9	KJ437447	England	*A. melifera*	Polyprotein	% 96.91
10	MT096518	Spain	Insect metagenome	Polyprotein	% 96.91
11	JQ413340	Chile	Honey bee	Polyprotein	% 96.77
12	MK262743	Spain	*A. melifera*	Complete genom	% 96.62
13	MG831200	USA	Honey bee	Polyprotein	% 96.20
14	KY909333	Italy	*Vespa crabro*	Polyprotein	% 95.92
15	MH165180	China	*A. melifera*	Polyprotein	% 95.78
16	JX878305	South Korea	*A. melifera*	Polyprotein	% 95.64
17	MF036686	Chile	*A. melifera*	Polyprotein	% 95.36
18	MF770715	China	*A. melifera*	Polyprotein	% 95.36
19	MN538209	Holland	*A. melifera*	Polyprotein	% 77.50
20	KX783225	Belgium	*A. melifera*	Polyprotein	% 77.25
21	MT747987	Italy	*A. melifera*	Polyprotein	% 77.22

BLAST analyzes showed that the nucleotide identity of the CP gene sequence of DWV-Bingöl isolate varied from 98.45-77.22% with other isolates identified in other locations around the world. Our DWV isolate indicated nucleotide similarity with the lowest (77.22%) Italian isolate (MT747987) and the highest (98.45%) Turkish isolate (MW962982) (Table 4). Computer-based nucleotide sequence consensus revealed that 9 substitutions were detected in Bingol 13 isolate, throughout the CP gene, corresponding to nearly 1.2% (Table 5).

Table 5. Chart showing the nucleotide substitutions of Bingol 13 DWV isolate (MZ357973) based on the consensus in the CP gene region

Order	Consensus	Substitution	Order	Consensus	Substitution
287	T	C	**502**	T	C
288	C	T	**512**	T	A
305	T	C	**605**	A	G
311	A	G	**647**	T	C
458	T	C			

The created phylogenetic tree confirmed the results of BLAST analysis that DWV-Bingöl isolate is in the same cluster and genetically similar to the other Turkey isolate (Erzincan isolate, MW962982) (Fig. 2). The phylogenetic dendrogram was divided into three main clades. Isolates from Holland, Belgium, and Italy from the European continent were in group 2, and isolates from China (Asia) and Chile (South America) from different origins were in group 3. DWV isolates from different countries from the Asian, American and European continents (group I) showed closer phylogenetic interrelationship, which resulted in same clusters, because of high nucleotide similarity (95.64% - 98.45%). All these results show the groups formed in the phylogenetic tree are not dependent on the geographical origin. The reason for this is most likely because of the spread of honeybee pathogens via the international trade in queen bees, beekeeping tools and equipment, and honey bee products (pollen, honey, etc.) (Mutinelli, 2011; Di Pasquale et al., 2013).

Using contaminated tools and equipment and the infected queen bee handles colony collapse and the spread of honeybee viruses amongst colonies and apiaries. Some specific insect vectors, especially Varroa destructor, also play a crucial role in the transmission of honeybee viral agents and decreased honeybee health (Chen, 2011; Di Pasquale et al., 2013). Two ant species (*Camponotus vagus* and *Formica rufa*) were described as critic reservoir hosts for CBPV in France by Celle et al., (2008).

It has been recorded that DWV is infectious in 65 species of arthropods, including eight orders of insects and three orders of Arachnida, except bees. It triggers infection in wild bee species (*Colletes inaequalis* and *Megachile rotundata*) (Dolezal et al., 2016) and ant (Messor concolor) (Rüstemoğlu, 2020). In the investigation conducted in Argentina, it was reported that the transmission of ABPV was caused by a parasitic infestation of *Braula schmitzi* for the first time (Avalos et al., 2019).

Toplak et al., (2013) reported that Nosema ceranae, a protozoan pathogen of honeybees, and CBPV have a synergistic effect on the death of honeybees in vitro.Israel acute paralysis virus (IAPV) and Black queen cell virus positivity were determined in large wax moth (Galleria mellonella) larvae employing the RT-PCR method carried out in Apis cerana japonica colonies of Japan in 2012 (Traiyasut et al., 2016). BQCV and IAPV were examined in darkling beetles (Alphitobius diaperinus) (Li et al., 2016), Moku virus in wasps (Vespa spp) (Highfield et al., 2020), and DWV in small hive beetle (Aethina tumida) (Eyer et al., 2009). The presence of Nosema and varroa in Bingöl Province was announced by Kutlu and Gül, (2020) and Kutlu and Ekmen, (2003). During the surveys of the current study, foreign visitors such as ants, Vespa, and small hive beetles were encountered around and inside the hive. In the light of current literature reviews, combating potential vector insect species is of

critical importance in terms of control and management of honeybee viruses and sustainable beekeeping activities.

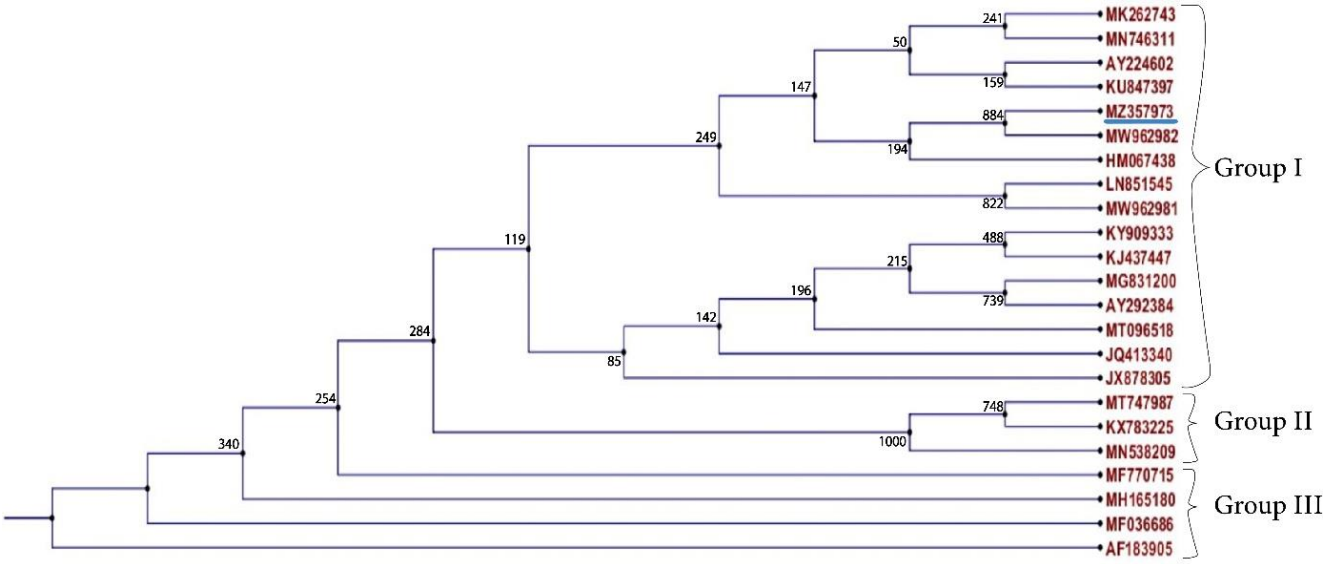

Figure 2. Radial phylogenetic tree of DWV-Bingöl isolate (MZ357973) (shown underlined) with other DWV isolates based on the nucleic acid sequence of the CP gene using the neighbor-joining method with 1000 bootstrap analysis

CONCLUSION

In this study, honey bee samples were analyzed using the PCR technique. Examined colonies of Bingöl Province (Turkey) were positive for DWV (21.87%) with varying incidences and negative for CBPV (0%). The absence of CBPV is an advantage for this region, but it is important to pay attention to routine hygiene and basic care practices to prevent the spread of honeybee diseases and later economic losses.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author’s Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Adams, M. J., Lefkowitz, E. J., King, A. M. Q., & Carstens, E. B. (2014). Recently agreed changes to the statutes of the international committee on taxonomy of viruses. *Archives of Virology*, 159, 175-180.

Ai, H., Yan, X., & Han, R. (2012). Occurrence and prevalence of seven bee viruses in Apis mellifera and Apis cerana apiaries in China. *Journal of invertebrate pathology*, 109(1), 160-164.

Allen MF, Ball BV, (1996). The incidence and world distribution of honey bee viruses. *Bee World*, 77: 141–162.

Antúnez, K., Anido, M., Garrido-Bailon, E., Botias, C., Zunino, P., Martínez-Salvador, A., ... & Higes, M. (2012). Low prevalence of honeybee viruses in Spain during 2006 and 2007. Research in *Veterinary Science*, 93(3), 1441-1445.

Avalos, J., Rosero, H., Maldonado, G., & Reynaldi, F. J. (2019). Honey bee louse (Braula schmitzi) as a honey bee virus vector?. *Journal of Apicultural Research*, 58(3), 427-429.

Ayşegül, U.S.T.A., & Yıldırım, Y. (2022). Investigation of deformed wing virus, black queen cell virus and acute bee paralysis virus infections by using reverse transcriptase-polymerase chain reaction (RT-PCR) technique in honey bees. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*. https://doi.org/10.33988/auvfd.824882.

Bacandritsos, N., Granato, A., Budge, G., Papanastasiou, I., Roinioti, E., Caldon, M., ... & Mutinelli, F. (2010). Sudden deaths and colony population decline in Greek honey bee colonies. *Journal of invertebrate pathology*, 105(3), 335-340.

Bailey, L., Ball, B. V., & Perry, J. N. (1981). The prevalence of viruses of honey bees in Britain. *Annals of Applied Biology*, 97(1), 109-118.

Bailey L, Ball BV, 1991. Honeybee pathology. Academic Press, London, United Kingdom.

Berényi, O., Bakonyi, T., Derakhshifar, I., Köglberger, H., & Nowotny, N. (2006). Occurrence of six honeybee viruses in diseased Austrian apiaries. *Applied and environmental microbiology*, 72(4), 2414-2420.

Budge, G. E., Simcock, N. K., Holder, P. J., Shirley, M. D., Brown, M. A., Van Weymers, P. S., ... & Rushton, S. P. (2020). Chronic bee paralysis as a serious emerging threat to honey bees. *Nature Communications*, 11(1), 2164.

ÇAĞIRGAN, A. A., & Yazici, Z. (2021). The prevalence of seven crucial honeybee viruses using multiplex RT-PCR and theirphylogenetic analysis. *Turkish Journal of Veterinary & Animal Sciences*, 45(1), 44-55..

Cagirgan, A. A., Yildirim, Y., & Usta, A. (2020). Phylogenetic analysis of deformed wing virus, black queen cell virus and acute bee paralysis viruses in Turkish honeybee colonies. *Medycyna Weterynaryjna*, 76(08)..

Celle, O., Blanchard, P., Olivier, V., Schurr, F., Cougoule, N., Faucon, J. P., & Ribière, M. (2008). Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. *Virus research*, 133(2), 280-284..

Chen, G., Wu, Y., Deng, J., Wen, Z., Wang, S., Chen, Y., ... & Zheng, H. (2021). Seasonal variation of viral infections between the eastern honey bee (Apis cerana) and the western honey bee (Apis mellifera). *Microbiologyopen*, 10(1), e1162.

Chen, Y. (2011). Viruses and viral diseases of the honey bee, Apis mellifera. Recent Advances in Entomological Research: From Molecular Biology to Pest Management, 105-120.

Chen, Y., Smith, I. B., Collins, A. M., Pettis, J. S., & Feldlaufer, M. F. (2004). Detection of deformed wing virus infection in honey bees, Apis mellifera L., in the United States. *American Bee Journal*, 144(7), 557-559..

De Miranda, J. R., & Genersch, E. (2010). Deformed wing virus. *Journal of invertebrate pathology*, 103, S48-S61..

De Miranda, J. R., & Fries, I. J. J. O. I. P. (2008). Venereal and vertical transmission of deformed wing virus in honeybees (Apis mellifera L.). *Journal of invertebrate pathology*, 98(2), 184-189.

Desai, S. D., Eu, Y. J., Whyard, S., & Currie, R. W. (2012). Reduction in deformed wing virus infection in larval and adult honey bees (Apis mellifera L.) by double-stranded RNA ingestion. *Insect molecular biology*, 21(4), 446-455.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., ... & Alaux, C. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter?. *PloS one*, 8(8), e72016.

Dolezal, A. G., Hendrix, S. D., Scavo, N. A., Carrillo-Tripp, J., Harris, M. A., Wheelock, M. J., ... & Toth, A. L. (2016). Honey bee viruses in wild bees: viral prevalence, loads, and experimental inoculation. *PloS one*, 11(11), e0166190.

Eyer, M., Chen, Y. P., Schäfer, M. O., Pettis, J., & Neumann, P. (2009). Small hive beetle, Aethina tumida, as a potential biological vector of honeybee viruses. *Apidologie*, 40(4), 419-428.

Svanella-Dumas, L., Dulucq, M. J., Candresse, T., Gentit, P., & Foissac, X. (2000, July). Polyvalent detection of fruit tree tricho, capillo and foveaviruses by nested RT-PCR using degenerated and inosine containing primers (PDO RT-PCR). In XVIII International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops-Top Fruit Diseases 550 (pp. 37-44).

Forsgren, E., De Miranda, J. R., Isaksson, M., Wei, S., & Fries, I. (2009). Deformed wing virus associated with Tropilaelaps mercedesae infesting European honey bees (Apis mellifera). *Experimental and Applied Acarology*, 47, 87-97.

Gauthier, L., Tentcheva, D., Tournaire, M., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2007). Viral load estimation in asymptomatic honey bee colonies using the quantitative RT-PCR technique. *Apidologie*, 38(5), 426-435.

Genersch, E., & Aubert, M. (2010). Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). *Veterinary research*, 41(6).

Güller, A., Mustafa, U. S. T. A., Çakar, G., & Zeynelabidin, K. U. R. T. (2021). Molecular characterization of Deformed wing viruses identified in honeybee (Apis mellifera L.) colonies in Erzincan province of Turkey. *Avrupa Bilim ve Teknoloji Dergisi*, (27), 186-192.

Gülmez, Y., Bursalı, A., & Tekin, S. (2009). First molecular detection and characterization of deformed wing virus (DWV) in honeybees (Apis mellifera L.) and mite (Varroa destructor) in Turkey. *African Journal of Biotechnology*, 8(16).

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

Haddad, N., Al-Gharaibeh, M., Nasher, A., Anaswah, E., Alammari, Y., & Horth, L. (2018). Scientific note: molecular detection of pathogens in unhealthy colonies of Apis mellifera jemenitica. *Apidologie*, 49(1), 84-88.

Haddad, N., Brake, M., Migdadi, H., & de Miranda, J. R. (2008). First detection of honey bee viruses in Jordan by RT-PCR. *Jordan Journal of Agricultural Sciences*, 4(3), 242-247.

Highfield, A., Kevill, J., Mordecai, G., Hunt, J., Henderson, S., Sauvard, D., ... & Schroeder, D. C. (2020). Detection and replication of Moku virus in honey bees and social wasps. *Viruses*, 12(6), 607.

Kalayci, G., Cagirgan, A. A., Kaplan, M., Pekmez, K., Beyazit, A., Ozkan, B., ... & Arslan, F. (2020). The role of viral and parasitic pathogens affected by colony losses in Turkish apiaries. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 26(5).

Karapınar, Z., Oğuz, B., Dinçer, E., & Öztürk, C. (2018). Phylogenetic analysis of black queen cell virus and deformed wing virus in honeybee colonies infected by mites in Van, Eastern Turkey. *Medycyna Weterynaryjna*, 74 (7), 460-465.

Kutlu, M.A. & Ekmen, F. (2003). Presence of Nosema disease and infection rate in honey bees (Apis mellifera L.) in Bingöl region. *Technical Beekeeping*, 79: 24-26.

Kutlu, M. A. & Gül, A. (2020). Bingöl ilinde yaşanan koloni kayıpları (arı ölümleri), nedenleri ve öneriler. *Türk Tarım ve Doğa Bilimleri Dergisi*, 7(4), 867-873.

Li, Z., Huang, S., Huang, W. F., Geng, H., Zhao, Y., Li, M., ... & Su, S. (2016). A scientific note on detection of honeybee viruses in the darkling beetle (Alphitobius diaperinus, Coleoptera: Tenebrionidae), a new pest in Apis cerana cerana colonies. *Apidologie*, 47, 759-761.

Li, B., Deng, S., Yang, D., Hou, C., & Diao, Q. (2017). Complete sequences of the RNA 1 and RNA 2 segments of chronic bee paralysis virus strain CBPV-BJ detected in China. *Archives of virology*, 162, 2451-2456.

Martin, S. J., & Brettell, L. E. (2019). Deformed wing virus in honeybees and other insects. *Annual review of virology*, 6, 49-69.

Mockel, N., Gisder, S., & Genersch, E. (2011). Horizontal transmission of deformed wing virus: pathological consequences in adult bees (Apis mellifera) depend on the transmission route. *Journal of General Virology*, 92(2), 370-377.

Mutinelli, F. (2011). The spread of pathogens through trade in honey bees and their products (including queen bees and semen): overview and recent developments. *Revue Scientifique et Technique-OIE*, 30(1), 257.

Nielsen, S. L., 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(3), 310-314.

Porrini, C., Mutinelli, F., Bortolotti, L., Granato, A., Laurenson, L., Roberts, K., ... & Lodesani, M. (2016). The status of honey bee health in Italy: Results from the nationwide bee monitoring network. *PLoS One*, 11(5), e0155411.

Posada-Florez, F., Childers, A. K., Heerman, M. C., Egekwu, N. I., Cook, S. C., Chen, Y., ... & Ryabov, E. V. (2019). Deformed wing virus type A, a major honey bee pathogen, is vectored by the mite Varroa destructor in a non-propagative manner. *Scientific reports*, 9(1), 12445.

Reynaldi, F. J., Sguazza, G. H., Albicoro, F. J., Pecoraro, M. R., & Galosi, C. M. (2013). First molecular detection of co-infection of honey bee viruses in asymptomatic Bombus atratus in South America. *Brazilian journal of biology*, 73, 797-800.

Ribière, M., Triboulot, C., Mathieu, L., Aurières, C., Faucon, J. P., & Pépin, M. (2002). Molecular diagnosis of chronic bee paralysis virus infection. *Apidologie*, 33(3), 339-351.

Rodríguez, M., Vargas, M., Antúnez, K., Gerding, M., Ovídio Castro, F., & Zapata, N. (2014). Prevalence and phylogenetic analysis of honey bee viruses in the Biobío Region of Chile and their association with other honey bee pathogens. *Chilean journal of agricultural research*, 74(2), 170-177.

Rüstemoğlu, M., & Sipahioğlu, H. M. (2016). Occurrence and molecular characterization of acute bee paralysis virus (ABPV) in honeybee (Apis mellifera) colonies in Hakkari province. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*. 26 (2), 174 – 182.

Rüstemoğlu, M. (2019). Uluslararası Arıcılık Araştırmaları ve Sürdürülebilir Kırsal Kalkınma Stratejileri Kongresi. 11-13 Ekim 2019. Bingöl, Türkiye.

Rüstemoğlu, M., & Sipahioğlu, H. M. (2019). Occurrence and prevalence of six honey bee viruses in Hakkari (Turkey) and their genomic divergence. *Munis Entomology & Zoology*, 14(2), 574-583.

Rüstemoğlu, M. (2020). Isolation And Characterization Of Two Important Honeybee-Killing Virus Species, Deformed Wing Virus (Dwv) And Black Queen Cell Virus (Bqcv) From *Messor concolor* Ants (Hymenoptera: Formicidae). *Applied Ecology and Environmental Research*. 18(5):7407-7415.

Sanpa, S., & Chantawannakul, P. (2009). Survey of six bee viruses using RT-PCR in Northern Thailand. *Journal of Invertebrate Pathology*, 100(2), 116-119.

Simeunović, P., Stevanović, J., Vidanović, D., Nišavić, J., Radović, D., Stanišić, L., & Stanimirović, Z. (2014). A survey of deformed wing virus and acute bee paralysis virus in honey bee colonies from Serbia using real-time RT-PCR. *Acta Veterinaria*, 64(1), 81-92.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & Bergoin, M. (2004). Prevalence and seasonal variations of six bee viruses in Apis mellifera L. and Varroa destructor mite populations in France. *Applied and environmental microbiology*, 70(12), 7185-7191.

Toplak, I., Jamnikar Ciglenečki, U., Aronstein, K., & Gregorc, A. (2013). Chronic bee paralysis virus and Nosema ceranae experimental co-infection of winter honey bee workers (*Apis mellifera* L.). *Viruses*, 5(9), 2282-2297.

Traiyasut, P., Mookhploy, W., Kimura, K., Yoshiyama, M., Khongphinitbunjong, K., & Chantawannakul, P. (2016). First detection of honey bee viruses in wax moth. *Chiang Mai Journal of Science*, 43(4).

Traynor, K. S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., ... & Vanengelsdorp, D. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie*, 47, 325-347.

Teixeira, E. W., Chen, Y., Message, D., Pettis, J., & Evans, J. D. (2008). Virus infections in Brazilian honey bees. *Journal of Invertebrate Pathology*, 99(1), 117-119.

Yang X , Cox-Foster D, (2007). Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of Apis mellifera in correlation with viral incidence and microbial challenge. *Parasitology*, 134:405.