Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org

RESEARCH ARTICLE

Impact of deformed wing virus master variants (DWV-A, DWV-B, and DWV-C) in managed honey bee colonies of Türkiye

Mustafa Emin Oz*[1], Oguzhan Avci [2], Muge Dogan [1]

[1]Konya Veterinary Control Institute, Molecular Microbiology Laboratory, Konya, Türkiye
[2]Selçuk University, Veterinary Faculty, Department of Virology, Konya, Türkiye

Received: 25. 05.2023, Accepted: 15.08.2023

*oz.mustafaemin@gmail.com

Türkiye'de yer alan bal arısı kolonilerinde deforme kanat virüsü ana varyantlarının (DWV-A, DWV-B ve DWV-C) etkisi

Eurasian J Vet Sci, 2023, 39, 3, 124-131
DOI: 10.15312/EurasianJVetSci.2023.407

Öz

Amaç: Bu çalışmada, Türkiye'nin İç Anadolu ve Akdeniz Bölgelerinde yer alan bal arısı kovanlarında deforme kanat virüsü (DWV) ana varyantlarının belirlenmesi amaçlandı. Ayrıca arı kovanlarında sirküle olan DWV genotiplerinin bal arısı kovanlarında gözlenen klinik belirtilerle ilişkisi araştırıldı.

Gereç ve Yöntem: Bu çalışma için Aksaray, Isparta, Karaman, Konya ve Niğde illerinden 2019 yılı ilkbahar-yaz ve sonbahar sezonlarında aynı 25 kovandan yetişkin bal arıları toplandı. DWV'ye özgü nükleik asit ve DWV genotipleri, sırasıyla DWV gerçek zamanlı RT-PCR tahlili ve ABC tahlili ile tespit edildi.

Bulgular: DWV enfeksiyonu örnekleme yapılan her mevsimde tespit edildi. Örneklenen bir çok kolonide klinik bulgu görülmezken, bazı arılıklarda kanatlarda şekil bozukluğu, titreme, felç, karında şişlik, verim kaybı ve ölü arılar gözlemlendi. Erişkin bal arılarında DWV-A, DWV-B ve DWV-C yaygınlıkları sırasıyla %62, %82 ve %24 idi. Arı kovanlarında tespit edilen baskın genotip, DWV-B ana varyantıydı (%98). Ayrıca DWV-A ana varyantının virüs yükü, kışlama kayıpları görülen bal arısı kovanlarının tamamında yüksekti.

Öneri: Bu çalışmada, Türkiye'de sirküle olan DWV ana varyantlarının mevcut durumu ve bal arısı kolonileri üzerindeki etkileri ile ilgili veriler ilk kez rapor edildi. Böylece Türkiye arı kovanlarında yılın her mevsiminde değişen oranlarda verim kayıplarına neden olan DWV'nin dikkatle izlenmesi gerektiği önerilmektedir.

Anahtar kelimeler: Bal arısı, DWV ana varyantları, gerçek zamanlı RT-PCR, klinik belirtiler

Abstract

Aim: This study aimed to determine the deformed wing virus (DWV) master variants in managed honey bee hives in Central Anatolia and the Mediterranean Regions of Türkiye. Also, the relationship of DWV genotypes circulating in the apiaries with clinical signs observed in honey bee hives was investigated.

Materials and Methods: For this study, adult honey bees were collected from the same 25 hives in the spring-summer and autumn seasons of 2019 from the provinces of Aksaray, Isparta, Karaman, Konya and Nigde. DWV-specific nucleic acid and DWV genotypes were detected by DWV real-time RT-PCR assay and ABC assay, respectively.

Results: Deformed wing virus infection was detected in each sampling season. While many colonies were without any clinical signs, in some of the apiaries where samples were collected, wing deformity, trembling, paralysis, swelling in the abdomen, loss of productivity, and dead bees were observed. The prevalences of DWV-A, DWV-B, and DWV-C in adult honey bees were 62%, 82%, and 24%, respectively. The dominant genotype detected in bee hives was the DWV-B master variant (98%). Also, the virus load of the DWV-A master variant was high in all of the honey bee hives with wintering losses.

Conclusion: In this present study, data on the current status of DWV master variants circulating in Turkey and their impacts on honey bee colonies are reported for the first time. Thus, it is thought that DWV, which causes yield losses at varying rates in every season of the year in Turkish bee hives, should be carefully monitored.

Keywords: Clinical signs, DWV master variants, honey bee, real-time RT-PCR

CITE THIS ARTICLE: *Oz, Avci and Dogan 2023. Impact of deformed wing virus master variants (DWV-A, DWV-B, and DWV-C) in managed honey bee colonies of Turkey Eurasian J Vet Sci, 39, 3, 124-131*

Introduction

The activities of managed honey bee (*Apis mellifera*) colonies are significant economically and ecologically (Venturini et al 2017, Lester 2021). Thanks to its rich floral biodiversity and more than 8 million honey bee hives, Turkey is among the most important countries for honey beekeeping (Doğanay 2017, TÜİK 2022). The critical decline of the honey bee population in recent years is worrying (Kevill et al 2019). Especially RNA viruses are among the causes of distress observed in honey bee hives (Kevill et al 2019, Brasesco et al 2021).

The deformed wing virus (DWV) is a member of the genus Iflavirus of the family Iflaviridae, belonging to the order Picornavirales. The single-stranded positive-sense RNA genome has a single open reading frame (ORF) (Valles et al 2017). Furthermore, DWV has three genotypes (Type A-B-C) that differ in their pathogenic effects and genetic diversity (Brettell et al 2017). DWV was previously reported as one of the severe honey bee viruses for hive health (ANSES 2011). Although subclinical DWV infections are common in hives (Mouret et al 2013), typical symptoms of disease caused by DWV are wing deformity, paralysis, abdominal swelling, discolouration, decreased lifespan, and death (Chen and Siede 2007, Ryabov et al 2016).

Having more than one strain or genotype of virus species is one of their well-defined characteristics (Piot and Smagghe 2022). DWV is one such virus that represents three genotypes or master variants (Martin et al 2012, Mordecai et al 2016, Martin and Brettell 2019). The DWV type-A master variant includes the original DWV genotype (Lanzi et al 2006) and the Kakugo virus (Fujiyuki et al 2004). Initially reported as Varroa destructor virus-1 (VDV-1) (Ongus et al 2004) in the taxonomy, the virus was assigned as the DWV type-B master variant (Mordecai et al 2016). Distinguished from the other two master variants, DWV type-C is the last master variant of the DWV species complex (Mordecai et al 2016). Infections of the DWV-A and DWV-B variants have been associated with overwintering colony death. Moreover, co-infection with DWV-B and Varroa destructor can be critical for a honey bee colony (Genersch et al 2010, Dainat et al 2012). It has also been reported that DWV-B may be more virulent than DWV-A (McMahon et al 2016, McMenamin and Flenniken 2018).

DWV is common in managed honey bee colonies globally (Cirkovic et al 2018, Hassanyar et al 2019, Avci et al 2022). Varroa mite is an effective vector for the transmission of DWV and mediates the worldwide spread of this virus (Berenyi et al 2006, Wilfert et al 2016). At the end of the summer, the prevalence of DWV increased with the mite infestation in the hives (De Miranda et al 2013), and it was reported that the adult honey bees with normal appearance were short-lived (Martin 2001). Thus, DWV infections can cause wintering losses or colony extinction in the spring (Ball 2001, Dainat et al 2012, Nazzi et al 2012).

Explaining the evolution of DWV and so the effect of its genotypic diversity on the honey bee is a biological process that may take many years (Martin and Brettell 2019). Some studies focused on exploring the selection or effects of DWV master variants to elucidate this dynamic process (Ryabov et al 2017, Brasesco et al 2021, De Souza et al 2021). Also, studies are concentrating on DWV prevalence and phylogenetic analysis in Turkey, and the prevalence rate of DWV has been reported to be between 1.8% and 84% (Kalayci et al 2020, Çağırgan and Yazici 2021, Avci et al 2022, Usta and Yıldırım 2022). However, there are no reports on the genotypic diversity of DWV yet. This research aimed to investigate and genotype the impacts of DWV on managed honey bee health to explain the influence of DWV variants at the colony level (representing apiaries) in the Central Anatolia and Mediterranean Regions of Turkey and to contribute to the dynamic nature of DWV variants.

Material and Methods

Material

Honey bee samples

The adult honey bees were collected from 25 hives at a 95% confidence interval and 5% expected prevalence, using the multistep sampling method in Aksaray, Isparta, Karaman, Konya, and Nigde provinces, where beekeeping is common. The samples of at least 30 adult honey bees were collected from the same colonies during the spring/summer and autumn months of 2019. Samples were kept on dry ice in sterile laboratory tubes during carry to the laboratory and stored at −80 °C until virus nucleic acid isolation. Clinical signs observed in the study colonies were recorded to understand the influence of DWV master variants at the colony level.

Method

Viral RNA isolation

Seven adult honey bees from each hive were placed in sterile centrifuge tubes and homogenized in 7 ml of PBS. These samples were centrifuged at 4000 rpm at 4°C for 10 min two times to obtain supernatants. Viral RNA was isolated from these supernatants according to the manufacturer's recommendations using an IndiSpin Pathogen Kit (Indical Bioscience, Germany).

DWV real-time RT-PCR assays

The DWV-specific RNA was specified with real-time RT-PCR using a QuantiNova Pathogen +IC Kit (Qiagen, Germany)

with a previously reported assay (Chantawannakul et al. 2006). The DWV real-time RT-PCR assay was carried out in a 20 µl reaction volume, containing 5 µL 4X Pathogen Master Mix, 5 µL template RNA,1 µL TaqMan probe (5 pmol/µL), 0.8 µL forward primer (10 pmol/µL), 0.8 µL reverse primer (10 pmol/µL), and 7.4 µL PCR-grade water. The assay conditions were as follows: reverse transcription at 50 °C for 10 min; reaction initial activation at 95 °C for 2 min followed by 40 cycles of denaturation at 95 °C for 5 sec, and primer annealing and extension at 60 °C for 30 sec.

Samples detected as DWV-positive were then analysed for DWV master variants with ABC assay (Kevill et al 2017), using a QuantiNova Pathogen +IC Kit. The reaction mixture and thermal cycling conditions for ABC real-time RT-PCR assay are shown in Table 1. Also, the primer sets used in the study are listed in Table 2.

Exogenous internal control assays

To consider the in-analysis reliability of the DWV real-time RT-PCR assay and ABC assay, the exogenous internal control assays were performed, in which both viral RNA isolation and RT-PCR efficiency were controlled. The exogenous internal control assay was based on visualising a commercially available synthetic RNA (200 bp) amplification from the QuantiNova Pathogen +IC Kit.

Results

Clinical signs

Deformed wing virus master variants infection was detected in each sampling season, and Varroa infestation was also observed in these hives. While many colonies were without any clinical signs, in some of the apiaries where samples were collected, wing deformity, trembling, paralysis, swelling in the abdomen, loss of productivity, and dead bees were observed. Additionally, no overt infections appeared in larvae and pupa samples, except for Varroa infestation, and the queen bees were also healthy in the colonies. The assays results of the study samples and the clinical signs observed in the apiaries are shown in Table 3.

Prevalence of DWV master variants

The result of DWV real-time RT-PCR and ABC assay revealed prevalence patterns and master variants of DWV in five provinces. The DWV real-time RT-PCR showed that the prevalence of DWV was 42/50 (84%) of the adult honey bees. Also, the prevalence rate of DWV-A, DWV-B, and DWV-C master variants was 62% (31/50), 82% (41/50) and 24% (12/50), respectively (Table 4).

Table 1. ABC real-time RT-PCR assay reaction mixture and thermal cycling conditions

Reagent	Volume (µl)	Thermal cycling conditions	Temperature (°C)	Time	Cycle
4X Pathogen Master Mix	5	Reverse transcription	50	10 min	1
Primer fwd (10 pmol/µl)	0.8	PCR initial activation	95	2 min	1
Primer rev (10 pmol/µl)	0.8	Denaturation	95	5 sec	
EvaGreen dye	1	Annealing/ Extension	58.5* and 61.5**	30 sec	40
RNA template	5				
PCR-grade water	7.4				
Total volume	20				

* DWV-A and DWV-B primers annealing
** DWV-C primer annealing

Table 2. Primers used in this study

Virus	Primer	Primer sequence (5'-3')	Reference
DWV	DWV958F	CCTGGACAAGGTCTCGGTAGAA	Chantawannakul et al 2006
	DWV9711R	ATTCAGGACCCCACCCAAAT	
	DWV9627T	FAM-CATGCTCGAGGATTGGGTCGTCGT-TAMRA	
	DWVnew-F1	TACTAGTGCTGGTTTTCCTTT	
DWV Type A	DWVA-R1	CTCATTAACTGTGTCGTTGAT	Kevill et al 2017
DWV Type B	DWVB-R1	CTCATTAACTGAGTTGTTGTC	
DWV Type C	DWVC-R1	ATAAGTTGCGTGGTTGAC	

Table 3. Real-time RT-PCR assays results of the study samples and the clinical signs

Sample	Season	Clinical signs				C_T value of DWV[a]	C_T value of DKV-A[b]	C_T value of DKV-B[b]	C_T value of DKV-C[b]
		Varroa	Wing deformity	Death	Other signs				
Isparta-Bee-İ1	Spring/summer	+	-	+	-	22.92	21.40	29.60	No C_T
Isparta-Bee-S1*	Autumn	+	+	+++	+	6.95	11.99	29.78	26.62
Isparta-Bee-İ2	Spring/summer	+	-	-	-	30.34	30.77	29.53	No C_T
Isparta-Bee-S2	Autumn	++	-	+	+	15.54	30.35	16.80	No C_T
Isparta-Bee-İ3	Spring/summer	+	-	-	-	21.82	30.96	22.48	No C_T
Isparta-Bee-S3	Autumn	-	-	-	-	33.12	No C_T	33.84	No C_T
Isparta-Bee-İ4	Spring/summer	+	-	-	-	27.81	28.08	29.47	No C_T
Isparta-Bee-S4	Autumn	+	-	-	-	27.40	29.98	29.70	No C_T
Isparta-Bee-İ5	Spring/summer	+	-	-	-	29.19	28.75	28.90	No C_T
Isparta-Bee-S5	Autumn	+	-	-	+	22.27	32.77	23.99	No C_T
Konya-Bee-İ1	Spring/summer	+	+	-	+	23.83	No C_T	22.14	25.21
Konya-Bee-S1	Autumn	+	-	-	-	32.68	No C_T	33.57	No C_T
Konya-Bee-İ2	Spring/summer	+	-	-	-	27.88	No C_T	28.44	No C_T
Konya-Bee-S2	Autumn	+	-	-	-	29.57	No C_T	27.12	No C_T
Konya-Bee-İ3	Spring/summer	+	-	-	-	22.86	33.16	22.12	No C_T
Konya-Bee-S3	Autumn	-	-	-	-	32.29	No C_T	29.90	No C_T
Konya-Bee-İ4	Spring/summer	+	-	-	-	29.57	32.40	27.52	24.56
Konya-Bee-S4	Autumn	+	+	-	+	20.68	29.10	21.46	28.04
Konya-Bee-İ5	Spring/summer	+	+	++	+	14.30	15.00	28.77	27.15
Konya-Bee-S5*	Autumn	++	+	+++	+	12.39	12.13	26.47	23.49
Aksaray-Bee-İ1	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-S1	Autumn	-	-	-	-	31.69	No C_T	32.00	No C_T
Aksaray-Bee-İ2	Spring/summer	-	-	-	-	33.73	No C_T	32.40	No C_T
Aksaray-Bee-S2	Autumn	-	-	-	-	33.42	No C_T	34.15	No C_T
Aksaray-Bee-İ3	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-S3	Autumn	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-İ4	Spring/summer	-	-	-	-	30.70	No C_T	29.78	No C_T
Aksaray-Bee-S4	Autumn	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Aksaray-Bee-İ5	Spring/summer	+	-	-	-	29.96	33.14	28.95	No C_T
Aksaray-Bee-S5	Autumn	+	-	-	-	19.52	30.80	20.16	No C_T
Karaman-Bee-İ1	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-S1	Autumn	++	+	-	-	24.94	29.45	24.70	No C_T
Karaman-Bee-İ2	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-S2	Autumn	+	-	-	-	24.63	28.08	24.56	No C_T
Karaman-Bee-İ3	Spring/summer	+	-	-	-	25.68	30.44	26.74	28.83
Karaman-Bee-S3*	Autumn	+++	+	+++	+	8.49	10.39	27.41	29.25
Karaman-Bee-İ4	Spring/summer	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-S4	Autumn	-	-	-	-	No C_T	No C_T	No C_T	No C_T
Karaman-Bee-İ5	Spring/summer	++	-	-	-	22.65	29.07	24.00	No C_T
Karaman-Bee-S5	Autumn	++	-	-	+	23.08	29.80	22.40	No C_T
Nigde-Bee-İ1	Spring/summer	+	-	-	-	28.40	30.16	31.34	No C_T
Nigde-Bee-S1	Autumn	+	+	+	+	14.77	16.90	28.99	No C_T
Nigde-Bee-İ2	Spring/summer	-	-	-	-	15.73	21.62	22.41	24.26
Nigde-Bee-S2	Autumn	++	-	-	+	26.70	29.06	25.26	No C_T
Nigde-Bee-İ3	Spring/summer	+	-	-	-	26.92	29.05	27.10	No C_T
Nigde-Bee-S3	Autumn	++	-	+	+	12.90	29.79	14.08	No C_T
Nigde-Bee-İ4	Spring/summer	+	-	-	-	27.60	25.16	No C_T	20.36
Nigde-Bee-S4*	Autumn	+	+	+++	+	10.53	12.58	31.34	22.60
Nigde-Bee-İ5	Spring/summer	++	+	+	+	11.88	32.17	12.47	No C_T
Nigde-Bee-S5	Autumn	++	+	-	+	12.70	No C_T	13.00	26.18

*: Hive with wintering losses, a: Chantawannakul et al (2006), b: Kevill et al (2017), (+): Low, (++): Moderate, (+++): High, (-): Not observed, CT: Threshold Cycle, Other signs: paralysis, swelling in the abdomen, loss of productivity, wintering losses

Monitoring of exogenous internal control

The exogenous internal control assay allowed monitoring of the efficiency of the RNA isolation, and the reaction inhibitors and reverse transcription steps were monitored. Thus, the assays revealed an absence of PCR inhibitors or no trouble with the pathogen nucleic acid isolation or assay reaction. The Ct values of the exogenous internal control assay were in a range of 27 ± 3.

Discussion

The combined effect of biological and environmental potential stress factors is critical in reducing the honey bee population (Annoscia et al 2018, Brodschneider et al 2018, Bartlett et al 2021). Especially honey bee viruses can be determinant of hive distress, yield reduction and wintering losses (Dainat et al 2012, Martin et al 2012). However, it is difficult to identify potential causes of colony losses during the winter when hive inspection is infrequent (Kevill et al 2019).

Table 4. Season-based percentage prevalence of DWV master variants

Provinces	Year	Season	Number of hive	Prevalence of DWV	DWV-A	DWV-B	DWV-C
Aksaray	2019	Spring/summer	5	60% (6/10)	20% (1/5)	60% (3/5)	0% (0/5)
		Autumn	5		20% (1/5)	60% (3/5)	0% (0/5)
Karaman		Spring/summer	5	60% (6/10)	40% (2/5)	40% (2/5)	20% (1/5)
		Autumn	5		80% (4/5)	80% (4/5)	20% (1/5)
Konya		Spring/summer	5	100% (10/10)	60% (3/5)	100% (5/5)	60% (3/5)
		Autumn	5		40% (2/5)	100% (5/5)	40% (2/5)
Nigde		Spring/summer	5	100% (10/10)	100% (5/5)	100% (5/5)	40% (2/5)
		Autumn	5		80% (4/5)	80% (4/5)	40% (2/5)
Isparta		Spring/summer	5	100% (10/10)	100% (5/5)	100% (5/5)	20% (1/5)
		Autumn	5		80% (4/5)	100% (5/5)	0% (0/5)
Prevalence of spring/summer			25		64% (16/25)	80% (20/25)	28% (7/25)
Prevalence of autumn			25		60% (15/25)	84% (21/25)	20% (5/25)
Prevalence of DWV master variants			50		62% (31/50)	82% (41/50)	24% (12/50)

Turkey has a rich biodiversity and suitable climatic conditions for honey beekeeping (Muz and Muz 2018, Avci et al 2022). In recent years, managed honey bee viruses, a potential threat in apiaries of Turkey, have been investigated (Kalayci et al 2020, Çağırgan and Yazici 2021). However, data on the biology and epidemiology of honey bee viruses are limited. Beekeeping activities in the Mediterranean and Central Anatolia Regions, which this study focuses on, contribute to Turkey's ecology and economic dynamics.

DWV is a common honey bee virus in Europe and Turkey (Martin and Brettell 2019, Avci et al 2022). Generally, it has a low virulence compared to the infection pattern it creates in hives (Loope et al 2019). However, DWV can be devastating to bee hives after being transmitted between honey bees for several generations via Varroa infestation (Martin and Brettell 2019) and is one of the causes of overwintering loss in bee hives (Kevill et al 2019). Virus and mite infections observed together in honey bees can create a devastating and complex disease profile and cause new genotypes to circulate for a virus (Yang and Cox-Foster 2005, Kevill et al 2019). Varroa may also be the determinant in the selection of DWV genotypes (Martin et al 2012), and the dynamics of bee biology may play a role in the evolution of DWV master variants (Martin and Brettell 2019). Moreover, investigating the possible interaction of DWV master variants with other honey bee viruses may be critical for the evolution of DWV genotypes and elucidating their effects on bee health.

In the last decade, although studies have been reported on the epidemiology and infections caused by DWV master variants (McMahon et al 2016, Mordecai et al 2016, Tehel et al 2019), the effect of the genotypes of this virus on virulence or bee biology has not yet been comprehensively explained. However, it has been reported that the DWV-A genotype may be a more virulent variant compared to DWV-B. Moreover, the prevalence and viral load of the DWV-A variant were found to be higher than the other master variants in hives with overwintering losses (Kevill et al 2019). The DWV-B and DWV-C genotypes were generally detected at low viral load (Kevill et al 2019), and their effect was not devastating in managed honey bee hives (Mordecai et al 2016).

While the circulating DWV-C variant is infrequent among the honey bee (Mondet et al 2020), DWV-A and DWV-B are common master variants in managed hives (De Souza et al 2021). Initially, DWV-A was the major genotype (Ryabov ve ark 2017). However, in recent years it has been reported that the DWV-B variant is predominant and may be effective in the DWV genotypes selection (Kevill et al 2019).

DWV is one of the most common honey bee viruses detected in Turkey (Kalayci et al 2020). However, DWV genotypes have not yet been identified in managed honey bee hives. The DWV prevalence in the Central Anatolia and Mediterranean Regions was 84% (42/50) in adult honey bees. Hence, the prevalence of DWV determined in this study was consistent with the results of studies previously reported in Turkey (Avci et al 2022, Usta and Yıldırım 2022). Moreover, the prevalence of DWV in Aksaray, Konya, Karaman, Isparta, and Nigde was %60 (6/10), %100 (10/10), %60 (6/10), %100 (10/10), and %100 (10/10), respectively (Table 4). DWV infections usually peak in the autumn (Dainat et al 2012). However, the seasonal prevalence rates of DWV infections in Spring/Summer and Autumn were similar, with 80% (20/25) and 88% (22/25), respectively.

The prevalences of DWV-A, DWV-B, and DWV-C in hives were 62%, 82%, and 24%, respectively (Table 4). Also, the DWV master variants prevalences in the sampled provinces and seasons are shown in Table 4. In this study, DWV-B appeared to be the dominant genotype. The DWV-B genotype dominance (98%) detected in managed honey bee hives was consistent with the overall pattern reported by Kevill et al (2019) in bee hives in England and Wales.

Generally, the viral load of DWV is high in adult honey bees with deformed wing syndrome (Martin and Brettell 2019). However, the effect of DWV master variants on this syndrome is unclear (Brettell et al 2017, Tehel et al 2019).

Moreover, this syndrome can be an essential indicator of wintering losses in hives (Dainat and Neumann 2013). In this study, deformed wing syndrome was observed in colonies with wintering losses and some other honey bee hives.

Typically, the viral load of DWV is low in asymptomatic honey bees without Varroa infestation (Martin and Brettell 2019). Also, high Ct values were detected in DWV (ABC) real-time RT-PCR assays, indicating a low viral load, except in hives with overt infection in this study.

For this study, adult honey bees sampled had high Ct values (low viral load) in DWV real-time RT-PCR. Thus, the DWV load can be considered low for almost a year. However, the Ct value of DWV-A was notably lower (high viral load) in colonies with wintering loss (16%). Moreover, it was determined that the DWV-B genotype was the dominant variant (98%) at low viral load throughout the year in the surviving hives (84%) after the overwintering period. Previously, it has been reported that the incidence of wintering losses is low in honey bee hives with high DWV-B prevalence and dominance (McMahon et al 2016, Mordecai et al 2016, Natsopoulou et al 2017, Gisder et al 2009).

For a honey bee colony to be exposed to the devastating effects of DWV, the viral load of DWV-B must exceed the host tolerance threshold (Kevill et al 2019). Also, multiple infections of honey bees with DWV master variants can make the devastating effect on the hives obvious (Brasesco et al 2021). In this study, the more virulent DWV-A genotype was detected together with the DWV-B genotype in the bee hives with colony loss. Moreover, the fact that wintering losses were observed in colonies with high loads of DWV-A once again revealed the devastating effect of this variant on honey bees.

Conclusion

In conclusion, a report on the current status of DWV master variants circulating in Turkey and their impact on honey bee colonies is presented for the first time. In this study, it has been speculated that DWV, which causes varying levels of yield losses in every season of the year for the apiaries of Türkiye, should be carefully monitored. In particular, further studies in which whole genome sequences of DWV main variants and recombinations are determined to determine the role of the circulating DWV complex in colony losses will contribute to the elucidation of the ongoing evolutionary dynamism of DWV.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

Funding

This study was supported by the Scientific Research Projects Coordination Unit, Selcuk University (Project Number: 21401044).

References

Annoscia D, Brown S, Di Prisco G, De Paoli E, et al., 2018. Haemolymph removal by the parasite Varroa destructor can trigger the proliferation of the Deformed Wing Virus in mite infested bees (Apis mellifera), contributing to enhanced pathogen virulence. Bio Rxiv, 257667.

ANSES: European Union Reference Laboratory for honeybee health, 2011. Guidelines for a pilot surveillance project on honey bee colony losses. https://ec.europa.eu/food/sites/food/files/animals/docs/la_bees_health_annex_i_pilot_project_en.pdf/ Date of access: 20.09.2022

Avci O, Oz ME, Dogan M, 2022. Silent threat in honey bee colonies: infection dynamics and molecular epidemiological assessment of black queen cell virus in Turkey. Arch Virol, 167(7), 1499-1508.

Ball BV, 2001. Viruses and varroa. Bee Craft, 83(11), 20-22.

Bartlett LJ, Boots M, Brosi BJ, de Roode JC, et al., 2021. Persistent effects of management history on honeybee colony virus abundances. J Invertebr Pathol, 179, 107520.

Berenyi O, Bakonyi T, Derakhshifar I, Köglberger H, et al., 2006. Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl Environ Microbiol, 72(4), 2414-2420.

Brasesco C, Quintana S, Di Gerónimo V, García MG, et al., 2021. Deformed wing virus type a and b in managed honeybee colonies of Argentina. Bull Entomol Res, 111(1), 100-110.

Brettell LE, Mordecai GJ, Schroeder DC, Jones IM, et al., 2017. A comparison of deformed wing virus in deformed and asymptomatic honey bees. Insects, 8(1), 28.

Brodschneider R, Gray A, Adjlane N, Ballis A, et al., 2018. Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. J Apic Res, 57(3), 452-457.

Chantawannakul P, Ward L, Boonham N, Brown MA, 2006. Scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in varroa mites collected from a Thai honeybee (Apis mellifera) apiary. J Invertebr Pathol, 91(1), 69-73.

Chen YP, Siede R, 2007. Honey bee viruses. Adv Virus Res, 70, 33-80.

Cirkovic D, Stevanovic J, Glavinic U, Aleksic N, et al., 2018. Honey bee viruses in Serbian colonies of different strength. Peer J, 6, e5887.

Çağırgan AA, Yazici Z, 2021. The prevalence of seven crucial honeybee viruses using multiplex RT-PCR and their phylogenetic analysis. Turk J Vet Anim Sci, 45(1), 44-55.

Dainat B, Evans JD, Chen YP, Gauthier L, et al., 2012. Predictive markers of honey bee colony collapse. Plos one,

7(2), e32151.

Dainat B, Neumann P, 2013. Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. J Invertebr Pathol, 112(3), 278-280.

De Miranda JR, Bailey L, Ball BV, Blanchard P, et al., 2013. Standard methods for virus research in Apis mellifera. In: The Coloss Beebook Standard methods for Apis mellifera pest and pathogen research. Eds: Dietemann V, Ellis JD, Neumann P, Volume II: J Apicult Res, 52(4), 5-43.

De Souza FS, Allsopp MH, Martin SJ, 2021. Deformed wing virus prevalence and load in honeybees in South Africa. Arch Virol, 166(1), 237-241.

Doğanay A, 2017. Genel arıcılık, In: Bal arısı yetiştiriciliği ürünleri hastalıkları. Ed; Doğanay A, Aydın L, Birinci Baskı, Dora Basın Yayın Dağıtım, Bursa, pp; 21-55, 121-141.

Fujiyuki T, Takeuchi H, Ono M, Ohka S, et al., 2004. RNA-like virus identified in the brains of aggressive worker honeybees. J Virol, 78(3), 1093-1100.

Genersch E, Ohe von der W, Kaatz H, Schroeder A, et al., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie, 41(3), 332-352.

Gisder S, Aumeier P, Genersch E, 2009. Deformed wing virus: replication and viral load in mites (Varroa destructor). J Gen Virol, 90(2), 463-467.

Hassanyar AK, Huang S, Li Z, 2019. Prevalence of bee viruses in Apis cerana cerana populations from different locations in the Fujian Province of China. Microbiol Open, 8(9), e00830.

Kalayci G, Cagirgan AA, Kaplan M, Pekmez K, et al., 2020. The role of viral and parasitic pathogens affected by colony losses in Turkish apiaries. Kafkas Univ Vet Fak Derg, 26(5), 671-677.

Kevill JL, Highfield A, Mordecai GJ, Martin SJ, et al., 2017. ABC assay: method development and application to quantify the role of three DWV master variants in overwinter colony losses of European honey bees. Viruses, 9(11), 314.

Kevill JL, de Souza FS, Sharples C, Oliver R, et al., 2019. DWV-A lethal to honey bees (Apis mellifera): A colony level survey of DWV variants (A, B, and C) in England, Wales, and 32 states across the US. Viruses, 11(5), 426.

Lanzi G, de Miranda JR, Boniotti MB, Cameron CE, et al., 2006. Molecular and biological characterization of deformed wing virus of honeybees (Apis mellifera L.). J Virol, 80(10), 4998-5009.

Lester P, 2021. Healthy Bee Sick Bee, In: The influence of parasites, pathogens, predators and pesticides on honey bees, First Edition, Victoria University Press, Melbourne, pp; 8-9.

Loope KJ, Baty JW, Lester PJ, Wilson Rankin EE, 2019. Pathogen shifts in a honeybee predator following the arrival of the Varroa mite. Proc R Soc B, 286(1894), 20182499.

Martin SJ, 2001. The role of Varroa and viral pathogens in the collapse of honey bee colonies: a modelling approach. J Appl Ecol, 38(5), 1082-1093.

Martin SJ, Highfield AC, Brettell L, Villalobos EM, et al., 2012. Global honey bee viral landscape altered by a parasitic mite. Science, 336(6086), 1304–1306.

Martin SJ, Brettell LE, 2019. Deformed wing virus in honey bees and other insects. Annu Rev Virol, 6, 49-69.

McMahon DP, Natsopoulou ME, Doublet V, Fürst M, et al., 2016. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. Proc R Soc Lond B, 283(1833), 20160811.

McMenamin AJ, Flenniken ML, 2018. Recently identified bee viruses and their impact on bee pollinators. Curr Opin Insect Sci, 26, 120-129.

Mondet F, Beaurepaire A, McAfee A, Locke B, et al., 2020. Honey bee survival mechanisms against the parasite Varroa destructor: a systematic review of phenotypic and genomic research efforts. Inter J Parasitol, 50(6-7), 433–447.

Mordecai GJ, Wilfert L, Martin SJ, Jones IM, et al., 2016. Diversity in a honey bee pathogen: first report of a third master variant of the Deformed Wing Virus quasispecies. ISME J, 10(5), 1264–1273.

Mouret C, Lambert O, Piroux M, Beaudeau F, et al., 2013. Prevalence of 12 infectious agents in field colonies of 18 apiaries in Western France. Revue de Med Vet, 164(12), 577-582.

Muz D, Muz MN, 2018. A molecular epidemiological study of black queen cell virus in honeybees (Apis mellifera) of Turkey: the first genetic characterization and phylogenetic analysis of field viruses. Apidologie, 49(1), 89-100.

Natsopoulou ME, McMahon DP, Doublet V, Frey E, et al., 2017. The virulent, emerging genotype B of Deformed wing virus is closely linked to overwinter honeybee worker loss. Sci Rep, 7(1), 1-9.

Nazzi F, Brown SP, Annoscia D, Del Piccolo F, et al., 2012. Synergistic parasite pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. Plos Path, 8(6), e1002735.

Ongus JR, Peters D, Bonmatin JM, Bengsch E, et al., 2004. Complete sequence of a picorna-like virus of the genus iflavirus replicating in the mite Varroa destructor. J Gen Virol, 85(12), 3747-3755.

Piot N, Smagghe G, 2022. Critical view on the ımportance of host defense strategies on virus distribution of bee viruses: What can we learn from SARS-CoV-2 variants? Viruses, 14(3), 503.

Ryabov EV, Fannon JM, Moore JD, Wood GR, et al., 2016. The Iflaviruses Sacbrood virus and Deformed wing virus evoke different transcriptional responses in the honeybee which may facilitate their horizontal or vertical transmission. Peer J, 4, e1591.

Ryabov EV, Childers AK, Chen Y, Madella S, et al., 2017. Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. Sci Rep, 7(1), 1-10.

Tehel A, Vu Q, Bigot D, Gogol-Döring A, Koch P, et al., 2019. The two prevalent genotypes of an emerging infectious disease, deformed wing virus, cause equally low pupal mortality and equally high wing deformities in host honey

bees. Viruses, 11(2), 114.

Usta A, Yıldırım Y, 2022. Investigation of deformed wing virus, black queen cell virus, and acute bee paralysis virus infections in honey bees using reverse transcriptase-polymerase chain reaction (RT-PCR) method. Ankara Univ Vet Fak Derg, 697(1), 303-311.

TÜİK: Türkiye İstatistik Kurumu, 2022. https://www.tuik.gov.tr/ Date of access: 26.4.2023

Valles SM, Chen YP, Firth AE, Guerin DMA, et al., 2017. ICTV Report Consortium, ICTV virus taxonomy profile: Iflaviridae. J Gen Virol, 98(4), 355-356, 527-528.

Wilfert L, Long G, Leggett HC, Schmid-Hempel P, et al., 2016. Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. Science, 351(6273), 594-597.

Venturini E, Drummond FA, Hoshide AK, Dibble AC, et al., 2017. Pollination reservoirs in lowbush blueberry (Ericales: Ericaceae). J Econ Entomol, 110(2), 333-346.

Yang B, Peng G, Li T, Kadowaki T, 2013. Molecular and phlylogenetic characterization of honey bee viruses, Nosema microsporidia, protozoan parazites and parasitic mites in China. Ecol Evol, 3(2), 298-311.

Author Contributions

Motivation/Concept: MEO, OA, MD; Design: MEO, OA; Control/Supervision: OA; Data Collection and/or Processing: MEO, MD; Analysis and/or Interpretation: MEO, OA, MD; Literature Review: MEO, OA; Writing the Article: MEO, OA, MD; Critical Review: OA.

Ethical Approval

Selcuk University Experimental Research and Application Center, Animal Experiments Ethics Committee dated 28.12.2020 meeting numbered 2020/12 and 2020/118 Number Ethics Committee Decision.