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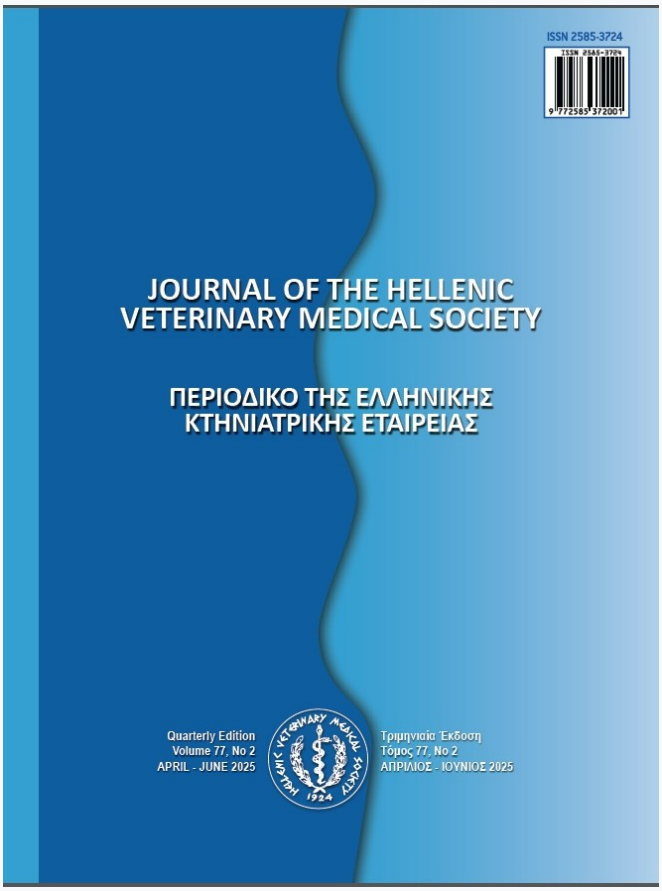

The first case of Co-infection of Sacbrood virus and Varroa destructor virus-1 in honey bees (Apis mellifera L., 1758)

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Case report
Κλινικό περιστατικό

The first case of Co-infection of Sacbrood virus and Varroa destructor virus-1 in honey bees (*Apis mellifera* L., 1758)

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ABSTRACT: Viral infections can occur at all developmental stages of honey bees and are of great concern for the beekeeping industry as they cause significant economic losses in beehives. The aim of this study is to investigate sudden bee mortalities and colony losses in an apiary. In 2022, high levels of bee mortality were observed in an apiary in Kırıkhan district of Hatay province. In order to determine the cause of bee mortality, adult worker bee samples (n = 50) were collected from hives within the apiary. Collected bee samples were processed for RNA extraction. One step real time RT-PCR methods were applied for the detection of Sacbrood virus (SBV) and Varroa destructor virus-1 (VDV-1). The one step real time RT-PCR analyses revealed the presence of mixed infection with SBV and VDV-1 viruses in the apiary. As far as we know, this study is the first report of a mixed infection of SBV and VDV-1 in honey bees. Additional comprehensive studies are needed to better understand the epidemiology of mixed infections caused by viruses in honey bees.

Keyword: Honey bee; SBV; VDV-1; mixed infection; Türkiye.

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INTRODUCTION

Honey bees are arguably the most significant living species on Earth in terms of maintaining ecological balance and agricultural food production due to their ability to pollinate flowering plants, ensuring biodiversity (Martin, 2001; Genersch, 2010; Parveen et al., 2022). However, the sudden decrease in the population of these highly vital species, along with the recent loss of colonies, are cause for concern (Aizen and Harder, 2009; Potts et al., 2010). Increasing numbers of global honey bee mortalities and colony losses have been reported in various parts of the world, particularly in the United States and Europe (Wyszkowska et al., 2019; Lu et al., 2020). Habitat loss, pesticides, bee pathogens and numerous stress factors are considered to be contributing factors to the decline in honey bee populations (Potts et al., 2010). Infections caused by bee pathogens, especially viruses, can be observed in all developmental stages of honey bees leading to significant losses in beehives and are of great concern for the beekeeping sector (Morawetz et al., 2019; Kalayci et al., 2020; Ullah et al., 2021).

The majority of bee viruses are known to be RNA viruses and are classified in the *Iflaviridae and Dicistroviridae* families (de Miranda, 2008). At least twenty-four different viruses have been reported to date in honey bees (de Miranda et al., 2013; Remnant et al., 2017; McMenamin and Flenniken, 2018), several of these viruses have been reported to pose a substantial threat to beekeeping worldwide (Tantillo et al., 2015; McMenamin and Flenniken, 2018). Prominent viruses that cause severe diseases and economic losses in honey bee colonies; Deformed wing virus (DWV), Varroa destructor virus-1 (VDV-1), Sacbrood virus (SBV), Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), Black queen cell virus (BQCV), Slow bee paralysis virus (SBPV), Kashmir bee virus (KBV), Lake Sinai virus (LSV) and Chronic bee paralysis virus (CBPV) (Tantillo et al., 2015; McMenamin and Flenniken, 2018; Ullah et al., 2021). The present study aims to report on the findings from an apiary with high rates of bee mortality.

CASE REPORT

In March 2022, an apiary conducting a fixed beekeeping practice in Hatay province was visited due to complaints of sudden bee mortalities and colony loss. The apiary was equipped with 50 hives.

Clinical symptoms, including flightless, crawling, paralysis, and wing abnormalities, were observed among the adult bees infected by VDV-1 (Figure 1). Samples of adult worker bees (n = 50) showing

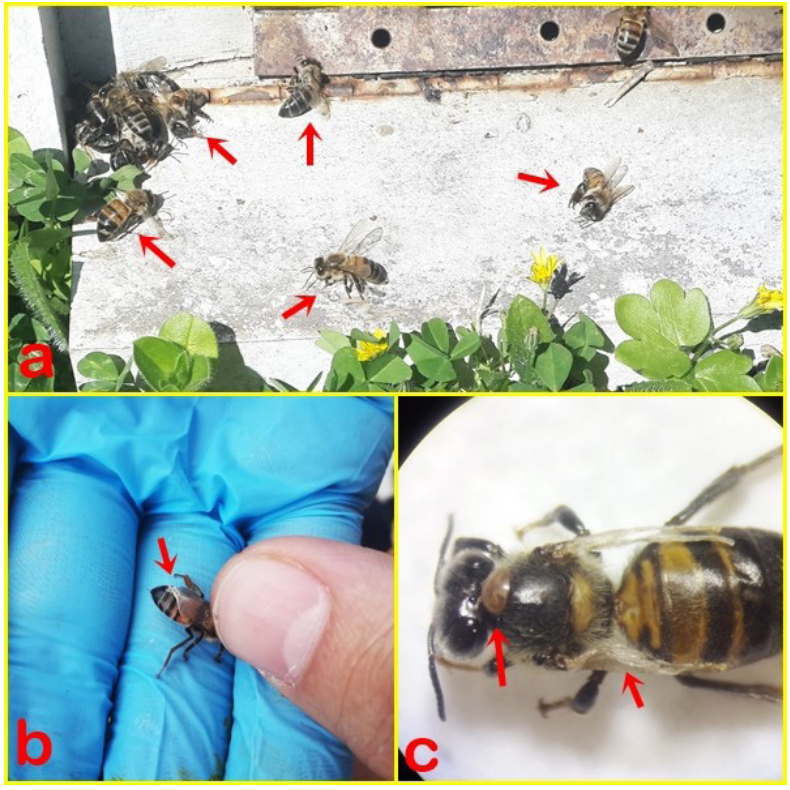

Figure 1. Clinical signs detected in sampled bees; a) Paralysis b) Deformed wing c) Bee infected with Varroa mite and showing signs of deformed wing (marked with red arrows)

clinical symptoms were collected from 10 hives in the facility selected by random sampling method, with 5 samples from each hive. It has been reported that this sample size is sufficient for the molecular detection of infectious agents affecting 45% or more of the individuals within a colony (Daughenbaugh et al., 2015; Cavigli et al., 2016). Samples collected in sterile falcon tubes were immediately transferred to the laboratory under cold chain and stored at -80 °C until further analysis.

Bee samples were pooled in sterile falcon tubes containing phosphate-buffered saline (PBS) before being crushed utilizing a TissueRuptor device (Qiagen, Hilden, Germany). The homogenates were then centrifuged at 5000 rpm for 30 minutes at 4 °C and 50 µl of supernatant was taken for RNA extraction. RNA extraction was conducted with the use of a commercial kit (High Pure Viral Nucleic Acid Kit, Roche, Germany) in accordance with the manufacturer's instructions. Obtained extracts were stored at -80°C until further analysis. The RNA extracts were analysed for the presence of SBV and VDV-1. These viruses were selected for analysis, based on the clinical symptoms of the afflicted bees. Probe and primer pairs as defined by Schurr et al. (2019) were employed for VDV-1 virus-specific RNA detection, and probe and primer pairs as defined by Blanchard et al. (2014) were employed for SBV detection (Table 1). One step real time RT-PCR master mix was prepared using a commercial kit (AgPath-ID One-Step RT-PCR, ThermoFisher Scientific, MA, USA). Amplification conditions: reverse transcription at 45°C for 10 minutes, initial PCR activation at 95°C for 10 minutes and 40 cycles at 95°C for 15 seconds and 60°C for 1 minute (binding/extension). Real time RT-PCR cycle threshold value (Ct) equal or lower than 35 was considered as positive (de Miranda et al., 2013). Throughout the analysis, sterile nuclease-free water was used as a negative control while positive RNA samples of SBV and VDV-1 isolates previously confirmed by sequence analysis (obtained from Department of Parasitology, Veterinary Faculty, Hatay Mustafa Kemal University, Antakya, Türkiye) were used as positive controls.

DISCUSSION

Viruses that affect honey bees threaten the beekeeping industry worldwide, causing significant economic losses (Kevill et al., 2017). SBV and DWV, the most widespread honey bee viruses globally, are the most prominent viruses associated with colony collapse disorder (Kevill et al., 2017; Li et al., 2019; Wei et al., 2022). According to reports, honey bees can become infected with multiple viruses, making it possible to identify mixed infections (Chen et al., 2006). In the present study, SBV and VDV-1 mixed infection was detected in honey bees. As far as we know, this study is the first report of a mixed infection of SBV and VDV-1 in honey bees.

SBV was the first virus identified in honey bees and remains one of the most prevalent honey bee viruses worldwide (Wei et al., 2022). SBV is a member of the *Iflavirus* genus of the *Iflaviridae* family and has positive-sense, single-stranded RNA genome (Valles et al., 2017). SBV infection can occur in both larval and adult stages (Ball and Bailey, 1997). Infected larvae are unable to pupate and take on a sac-like appearance. In adult bees, where there are no obvious clinical symptoms, it causes a latent/persistent infection with shortened lifespan (Berényi et al., 2006; de Miranda, 2008).

Studies carried out in honey bees throughout the world showed that the prevalence of SBV varies between 40.2% and 90.5% (Tentcheva et al., 2004; Choe et al., 2012; Tlak Gajger et al., 2014). In Türkiye, it has been reported that the prevalence of SBV in apiaries varies between 2.7% and 22.3% (Kalayci et al., 2020; Cagirgan and Yazici, 2021). The differ-

Table 1. Primers and probes used in one step real time RT-PCR analyses

Primer/ Probe	Sequence (5'-3')	Concentration	Reference
VDV-1-F	GGTCTGAAGCGAAAATAG	1200 nM	Schurr et al. (2019)
VDV-1-R	CTAGCATATCCATGATTATAAAC	1200 nM	
VDV-1-P	Fam-CCTTGTCCAGTAGATACAGCATCACA-Tamra	400 nM	
SBV-F	AACGTCCACTACACCGAAATGTC	320 nM	Blanchard et al. (2014)
SBV-R	ACACTGCGCGTCTAACATTCC	320 nM	
SBV-P	Fam-TGATGAGAGTGGACGAAGA-MGB	200 nM	

ence in SBV rates detected in honey bees in various countries and regions may be associated with the number of sampled bees, the method of analysis used and the awareness of beekeepers against diseases.

VDV-1 is classified as the DWV-B genotype of DWV and has positive-sense, single-stranded RNA genome within the genus *Iflavirus* of the family *Iflaviridae* (Ongus et al., 2004; Lanzi et al., 2006). VDV-1 causes wing deformities in developing honey bees and a shortened life span in infected adult bees (Benaets et al., 2017; Brettell et al., 2017).

VDV-1 was detected at very high levels of 66% in the United States (Ryabov et al., 2017), 47% in Argentina (Brasesco et al., 2021), in 82% and 98% of adult honey bees and hives in Türkiye, respectively (Oz et al., 2023). In addition, it was reported that the province of Hatay had a high rate of virus infection during the colony losses in 2010-2011 (Tozkar et al., 2015). The current research also detected VDV-1 in an apiary in Hatay province. This result shows that VDV-1 is still circulating in honey bees located in Hatay province.

Mixed infections in bees can lead to severe colony collapse disorders. Studies conducted throughout the globe have reported mixed infections including SBV and DWV-A viruses in honey bees in Argentina (Sguazza et al., 2013), Croatia (Tlak Gajger et al., 2014), Austria (Berényi et al., 2006), France (Tentcheva et al., 2004), Iran (Moharrami and Modirrousta, 2018), Denmark (Nielsen et al., 2008), USA (Chen et al., 2004), Jordan (Haddad et al., 2008). Although there are studies in Türkiye in which SBV and DWV-A viruses have been detected together (Rüstemoğlu and Sipahioğlu, 2019; Kalayci et al., 2020; Kızıltepe et al., 2023; Muz and Muz, 2023a; Muz and Muz, 2023b), the present study was the first to detect SBV and VDV-1 in honey bees.

In conclusion, the results of this study showed that SBV and VDV-1 mixed infection can cause high rates of bee mortality in honey bees. Recent studies have shown that virus interactions play a role in colony collapse disorder (Li et al., 2019; Wei et al., 2022). The way of transmission plays a crucial role in the dynamics of viral infections and in the evolutions of host-pathogen interactions. The spread of the infection can occur through the robbing and drifting of virus-infected colonies between hives. Further comprehensive research is necessary to fully understand the epidemiology of mixed viral infections in honey bees.

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