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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ISRAEL ACUTE BEE PARALYSIS VIRUS PREVALENCE IN APIARIES WITH COLONY LOSS IN TÜRKİYE

İsrail Akut Arı Felci Virüsü'nün Türkiye'de Koloni Kayıplı Arılıklardaki Yaygınlığı

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ABSTRACT

Honeybees are indispensable pollinator insects for vegetative pollination and biodiversity. Moreover, they serve medicinal importance with products such as honey, propolis, pollen, and royal jelly. Sudden bee deaths and colony collapse disorder (CCD) threaten the sustainability of colony health. Honeybee viruses, parasites, and pathogens trigger colony losses and CCD. This study investigated the presence and prevalence *of Israeli acute bee paralysis virus* (IAPV) in apiaries with sudden bee deaths, colony losses, and CCD-like problems in 16 provinces in different eco-geographic regions of Türkiye between 2011- 2021. Samples were tested for the coexistence of honeybee pathogens with IAPV. The sampled apiaries were evaluated for other bee pathogens such as *Acute bee paralysis virus, Black queen bee virus, Chronic bee paralysis virus, Deformed wing virus, Kashmir bee virus, Lake Sinai virus, Sacbrood virus,* Varroa mites, and *Nosema sp*. analyzed. Pathogen-specific RT-PCR assay was used for bee viruses. IAPV positivity was found to be 52.5% in apiaries. 97.5% of the sampled apiaries were positive for at least one pathogen. According to the results of this study, the presence of IAPV in apiaries suffering from colony loss and CCD-like problems was higher than in previous reports, and viruses of different species, *Nosema sp.,* and varroa infestation were found to be frequently encountered. The results suggest that the coexistence of IAPV and multiple pathogens may be effective in colony losses.

Keywords: IAPV, Honeybee Viruses, CCD, Colony Losses, Türkiye

ÖZ

Bal arıları bitkisel tozlaşma ve biyoçeşitliliğin devamında vazgeçilmez polinatör böceklerdir. Ayrıca bal, propolis, polen arısütü gibi ürünleriyle tıbbi öneme sahiptirler. Ani arı ölümleri ve koloni çöküş bozukluğu (CCD) koloni sağlığının sürdürülebilirliğini tehdit eder. Bal arısı virüsleri, parazit ve patojenleri koloni kayıplarını ve CCD-benzeri problemleri tetikler. Bu araştırmada 2011- 2021 yılları arasında Türkiye'de farklı eko-coğrafik bölgelerdeki 16 ilde ani arı ölümleri, koloni kayıpları ve CCD yaşanan arılıklarda *İsrail akut arı felci virüsünün* (IAPV) varlığı ve yaygınlığının tespiti amaçlanmıştır. Örnekler patojenlerinin IAPV ile bir arada bulunması açısından test edildi. Örneklenen arılıklar arı patojenlerinden *Akut arı felci virüsü, Siyah kraliçe arı virüsü, Kronik arı felci virüsü, Deforme kanat virüsü, Kaşmir arı virüsü, Lake Sinai virüsü, Sakbrood virüs*, Varroa akarları ve *Nosema sp.* yönünden analiz edildi. Arı virüsleri için patojen spesifik RT-PCR testleri kullanıldı. Örneklenen arılıkların %97,5'i en az bir patojen yönünden pozitif bulunurken, IAPV pozitifliği %52,5 olarak tespit edildi. Araştırma sonuçlarına göre, koloni kaybı ve CCD-benzeri problemler yaşanan arılıklarda IAPV'nin yaygınlığının önceki raporlara kıyasla daha yüksek olduğu belirlendi. IAPV pozitif arılıklarda farklı türlerdeki virüsler,

Nosema sp. **ve varroa enfestasyonuna rastlanıldığı tespit edildi. Sonuçlar IAPV ile birlikte çoklu patojen pozitifliğinin koloni kayıplarında etkili olabileceğini düşündürmektedir.**

Anahtar Kelimeler: IAPV, Bal Arısı Virüsleri, CCD, Koloni Kaybı, Türkiye

GENİŞLETİLMİŞ ÖZET

Amaç: Bu araştırma kapsamında Türkiye'de 2011-2021 yılları arasında koloni kaybı ve koloni çöküş bozukluğu şikayetleri olan arılıklarda İsrail akut arı felci virüsü (IAPV) varlığı ve yaygınlığını tespit etmek amaçlandı. Ayrıca örneklenen arılıklarda IAPV ile birlikte eş zamanlı bulunan bal arısı patojenleri araştırıldı.

Giriş: Bal arıları tarımsal biyoçeşitliliğinin ve üretimin devamlılığında kritik rolleri bulunan ekonomik yönden önemli polinatör böceklerdir. Çevresel faktörler, zirai ilaçlar, stres faktörleri, patojen ve parazitler arı sağlığı ve koloni sağlığının sürekliliğini tehdit ederler. 2006 yılından itibaren başta A.B.D. başta olmak üzere ülkemiz dahil dünya ülkelerinde görülen ani koloni kayıpları ve koloni çöküş bozukluğu (CCD) küresel bir sorun haline gelmiştir. "İyi arıcılık uygulamaları" kapsamında CCD ve kış kayıplarının nedenlerinin ve risk faktörlerinin araştırılması koloni sağlığının yönetilmesine katkı sağlayacaktır. Farklı viral enfeksiyonlar ile Varroa akarlarının bir arada bulunması CCD için yüksek risk olarak gösterilmektedir. *İsrail akut arı felci virüsü* (IAPV) ilk olarak 2004 yılında CCD'li arılıklarda izole edilmiş ve koloni kayıplarıyla ilişkilendirilmiştir. Yapılan çalışmalarda, IAPV tek başına CCD nedeni olarak gösterilmese de koloni kayıpları ve CCD vakalarında rol oynayabileceği ve diğer faktörlerin varlığında koloni sağlığını olumsuz etkileyeceği vurgulanmıştır.

Gereç ve Yöntem: Araştırmada 2011-2021 yılları arasında Türkiye'nin farklı eko-coğrafik bölgesinden 16 ilde koloni kayıpları ve CCD benzeri şikayetleri olan arılıklardan örneklemeler yapıldı. Bu amaçla toplam 120 arılıktan canlı arı örnekleri ve yavrulu petek örnekleri toplandı. Bu örnekler başta IAPV olmak üzere arı patojenlerinden *Akut arı felci virüsü* (ABPV), *Siyah kraliçe arı virüsü* (BQCV), *Kronik arı felci virüsü* (CBPV), *Deforme kanat virüsü* (DWV), *Kaşmir arı virüsü* (KBV), *Lake Sinai virüsü* (LSV), *Sakbrood virüs* (SBV), Varroa akarları ve *Nosema sp.* yönünden analiz edildi. Arı virüsleri patojen spesifik RT-PCR testleri kullanılarak araştırıldı. Arılıklardaki şikayetler kaydedildi.

Bulgular ve Sonuç: Yapılan RT-PCR sonuçlarına göre IAPV pozitifliği %52,5 (n=63) olarak tespit edildi. Test edilen arılıklar %97,5 oranında en az bir patojen yönünden pozitif bulundu. Örneklenen arılıklarda DWV ve BQCV sırasıyla %80 (n=96) ve %57,5 (n=69) oranlarıyla en yaygın arı virüsleri olarak belirlendi. Diğer arı virüslerinin pozitifliği LSV, CBPV, ABPV ve SBV, sırasıyla %30 (n=36), %15 (n=18), %10 (n=12) ve %7,5 (n=9) oranlarında olduğu tespit edildi. Örnekler KBV yönünden negatif bulundu. Arılıklarda *Nosema sp.* pozitifliği %32,5 (n=39) olarak belirlenirken Varroa enfestasyonu arılıkların %91,6'sında farklı seviyelerde kaydedildi. Koloni kayıplarının yaşandığı arılıkların patojen varlığı yönünden birden fazla virüs türü ile enfekte olduğu tespit edildi. IAPV varlığı diğer patojenlerle birlikte miks enfeksiyonlar içerisinde kaydedildi. Yapılan 10 yıllık araştırmada IAPV pozitifliği Türkiye'den bildirilen raporlara göre yüksek bulundu. Arılıklardaki şikayetlere göre ani gelişen koloni kaybı, yoğun kış kaybı yaşanan veya CCD şikayetleri bulunan arılıklardaki IAPV pozitifliği %44,4- 58,8 değişen oranlarda yüksek bulunurken, bu arılıklardaki pozitiflikler arasındaki farklılık istatistiki olarak anlamlı değildi. Sonuç olarak, kış kayıpları, koloni kayıpları yaşanan arılıklarda yüksek oranlarda IAPV varlığı tespit edildi. Arılıklarda koloni sağlığının sürekliliği sağlanması yönünde yapılacak iyi arıcılık uygulamaları kapsamında patojen varlığının araştırılması ve mücadelesi kapsamında stratejilerin geliştirilmesi önemli katkılar sunacaktır.

INTRODUCTION

Honeybees are among the most significant economically colonized pollinator insects, but various factors threaten the sustainability of honey bee colonies at ever-increasing levels (Mcmenamin and Genersch 2015). Colony Collapse Disorder (CCD) has caused serious significant global concerns since 2006 on colony health. CCD is a phenomenon that the disappearance of worker bees has characterized despite a queen, enough food, brood cells, and few nurse bees exist in a colony (VanEngelsdorp et al. 2007). Matching of variables like multiple pathogens, agricultural chemicals, and stress factors trigger colony losses and CCD.

Scientific results have reported that synergizing two or more factors may contribute to colony population declines (Cox-Foster et al. 2007). Within the scope of "good beekeeping practices," it has been accepted that investigating and revealing the causes of colony collapses and winter losses will contribute to easier management of colony health (Williams et al. 2010). Varroa mite is one of the majority factors causing the deterioration of bee health and they act as biological and mechanical vectors of many viruses. The coexistence of different viral infections and Varroa mites in colonies is considered a high risk for CCD (Muz 2008; De Miranda et al. 2011).

Honeybee viruses are known most common bee pathogens by their effects on colony health (Mcmenamin and Genersch 2015). They can show the covert infections specified with asymptomatic bees and overt infection with symptomatic bees in the colony (Hails et al. 2008). Some reports highlight the evidence that some bee viruses can trigger CCD (Cox-Foster et al.2007; Genersch et al. 2010; Corman et al. 2012). Many viruses have been reported from apiaries to date worldwide (Corman et al. 2012; Muz and Muz 2021). Israeli acute paralysis virus (IAPV) was first identified in CCD-affected colonies in 2004 (Maori et al. 2007). Since then, the association and interaction of IAPV with single or multiple variables remain an interesting research priority. Horizontal and vertical transmission routes were approved in IAPV transmission in honeybee colonies. IAPV can infect honeybees during separate biological stages, primarily in the digestive, nervous systems, and hypopharyngeal glands (De Miranda et al. 2010; Chen et al. 2014). Thus IAPV-infected colonies have asymptomatic or symptomatic bees suffering from trembling wings, paralysis, darkened body, and death (Maori et al. 2007). IAPV is an RNA virus classified in the Dicistroviridae subgroup in the Picornaviridae family.

Türkiye's suitable geographical location is so reasonable for beekeeping. Türkiye is remarkable in global ranking with its hive number and the produced honey amount. But colony losses seriously cause product losses to inconvenience beekeepers in most countries such as Türkiye (Çakmak 2012; 2016). Honeybee viruses have been reported in colony-lossed apiaries in Türkiye (Gülmez et al. 2009, Muz and Muz 2009; 2018, Kalaycı et al. 2020). Although the record of IAPV existence has been reported previously in Türkiye, its role and the inter-pathogen interrelationship on colony losses are not clear (Özkırım and Schiesser 2013; Rüstemoğlu and Sipahioğlu 2019; Çağırgan et al. 2020; Kalaycı et al. 2020; Çağırgan and Yazıcı 2021). In this study, the share of IAPV in pathogen distribution was investigated according to beekeeper complaints based on colony loss between 2011-2021, and some other pathogens were also tested.

MATERIAL AND METHODS

Sampling Area and Sample Collection:

The samples represent 16 provinces of different eco-geographic regions in Türkiye. The source points are Afyonkarahisar, Ağrı, Antalya, Balıkesir, Bursa, Düzce, Edirne, Erzurum, Giresun, Hatay, İstanbul, Mersin, Muğla, Sivas and Tekirdağ. The sampling period was between April and September of 2011-2021. In the study, the sample numbers were determined by considering the existence of colony loss and CCD-like complaints, the amount of hives in the sampled provinces. The sampling covered 120 apiaries with sudden bee deaths, brood deaths, unexpected high winter losses, severe colony losses, or CCD-like complaints. There were different symptoms in the sampled apiaries. The specimen from each colony per apiary included at least 20 alive nurser bees and brood comb (15 cm x 15 cm in size). Each apiary's worker bee, larva, and pupa samples were transferred to sterile tubes for molecular analysis. Bee samples for molecular tests were stored at -80 °C until analysis.

Homogenization, Nucleic Acid Extraction, and PCR Method

RNA was isolated from bee samples for use in molecular analyses. Briefly, sample pools with five bees were homogenized in 5 ml of PBS. Then, the obtained supernatant after centrifugation at 3000 rpm for 5 minutes was used in the extraction protocols. According to the kit's protocol, a commercial kit (GeneJET RNA Purification Kit, Thermo) was used for RNA extraction. The cDNA synthesis reaction was performed using the commercial kit (RevertAid First Strand cDNA Synthesis Kit) according to its protocol for cDNA synthesis. *Acute bee paralysis virus* (ABPV), *Black queen cell virus* (BQCV), *Chronic bee paralysis virus* (CBPV), *Deformed wing virus* (DWV), IAPV, *Kashmir bee virus* (KBV*), Lake Sinai virus* (LSV), *Sacbrood virus* (SBV) were analyzed in PCR tests. The PCR mixture was prepared as 5u Taq polymerase (Dream Taq polymerase, Thermo), 10x Taq buffer, 3mM MgCl2, 300 pmol dNTP mix, and

sterile water, in total 30 µl. ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV, SBV specific forward and reverse primers previously reported in the literature for each pathogen were added to the mixture (Stoltz et al., 1995; Chen et al., 2004; Berenyi et al., 2006; Palacious et al 2008) (Table 1). PCR reaction conditions included 5 min first denaturation at 95 °C, followed by 40 cycles of 30" denaturation at 95 °C, 30" annealing step between 48-60 ° C, and 30" elongation step at 72 ℃. The amplified DNA products were run on a 1% ethidium bromide gel using the agarose electrophoresis method. Positive samples were detected using a UV transmitter under UV light.

The nurse bees and sealed broods from each colony were used for varroa detection. The colonies infested more than seven mites were considered high, with 4-6 mites considered moderately, and those infested with 1-3 were considered mildly infested. To diagnose *Nosema sp* spores; the abdomen of ten nurse bees were crushed in a mortar with 10 ml of distilled water, and a drop of homogenate was examined under the light microscope between the lamellae.

To determine the colony losses, the answers given by the beekeepers were analyzed and the colonies were divided into three groups accordingly. It is the first group in which winter losses are at the lowest level, but symptomatic bees appear quickly at the beginning of spring. The symptoms of this group were recorded as trembling on the wings of the adult bees, deformity of the wings, paralysis, blackening, and brown/black spots in the larva and pupa samples. The second group had both high winter losses and severe colony losses. In the third group, there were only CCD-like complaints.

Table 1 The primer pairs used in RT-PCR protocols.

Virus	Amplified Region	Primer Sequence (5'-3')	Expected Length (bp)	Reference
ABPV	Capsid protein	F: GTGCTATCTTGGAATACTAC R: AAGGYTTAGGTTCTACTACT	618	Berenyi et al. 2006
BQCV	Nonstructural polyprotein	F: AGTAGTTGCGATGTACTTCC R: CTTAGTCTTACTCGCCACTT	472	Berenyi et al. 2006
CBPV	RdRp	F: TGTCGAACTGAGGATCTTAC R: GACCTGATTAACGACGTTAG	315	Berenyi et al. 2006
DWV	Helicase	F: ATCAGCGCTTAGTGGAGGAA R: TCGACAATTTTCGGACATCA	702	Chen et al. 2004
IAPV	IGR	F:GGTTGGCTGTGTGTCATCAT R:CGATGAACAACGGAAGGTTT	767	Palacious et al. 2008
KBV	Nonstructural polyprotein	F: GATGAACGTCGACCTATTGA R: TGTGGGTTGGCTATGAGTCA	415	Stoltz et al. 1995
SBV	Structural protein	F: ACCAACCGATTCCTCAGTAG R: CCTTGGAACTCTGCTGTGTA	487	Berenyi et al. 2006

Statistical Evaluation

The distribution of pathogen positivity in apiaries and the statistical evaluation of the relationship between this distribution were evaluated using the SPSS IBM (Version 25) program. For this purpose, independent Sample T-test, and correlation analysis were used. The apiaries were divided into three groups. Apiaries from which symptomatic bees were sampled constituted the first group. The second group sampled asymptomatic bees with colony losses and unexpectedly high winter losses. And apiaries with CCD-like complaints constituted the third group.

RESULTS

Sampling was performed in 16 provinces with a total of 120 apiaries in different years. Sampling was also repeated in additional times in provinces where colony loss complaints and beekeeping activities are intense (Figure 1). ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV, and Nosema sp. were analyzed in this research. Sampling was performed in 16 provinces of 120 apiaries over different years. Sampling was repeated in areas with intense colony loss complaints and beekeeping activities (Figure 1). The results showed 97.5 % positivity (n=117) of at least one pathogen in the apiaries. In terms of the bee virus's positivity, DWV was the prevalent pathogen with an 80% (n=96) rate, followed by BQCV and IAPV positivity with 57.5% (n=69) and 52.5% (n=63) rates, respectively (Figure 2). The LSV, CBPV, ABPV, and SBV positivity were detected at 30% (n=36), 15% (n=18), 10% (n=12), and 7.5% (n=9), respectively (Figure 2A). The samples were negative for KBV. Nosema sp. positivity was found to be 32.5% (n=39). Varroa infestation was determined at different levels at 91.6% of apiaries (n=110). IAPV-positive apiaries were all Varroa-positive. Varroa levels were at low, medium, and high levels determined in IAPV-positive apiaries (n=63) with 57.1 %, 28.6%, and 14.3 %, respectively. Compared to IAPV existence, the Varroa levels were statistically insignificant ($p<0.05$).

Only three apiaries were free of tested pathogens. The DWV has often been detected all years in the apiaries, although DWV occurrence is statistically ($p<0.05$) insignificant with the IAPV positivity over the years. Regarding the coexistence of the most prevalent pathogens in IAPV-positive apiaries were IAPV+DWV (n=51), IAPV+ BQCV (n=33) and IAPV+ Nosema sp. (n=24) combinations followed (Figure 2B). The coexistence of IAPV with SBV was not found. IAPV and other investigated pathogens were statistically insignificant ($p<0.001$, $p<0.05$) (Table 2). The existence of BQCV, LSV, CBPV, and *Nosema sp*. was found to be low-significant statistically in apiaries ($p<0.05$). Single pathogen positivity was in nine apiaries while multiple pathogens' existence was noted with dual (n=27, 23.1%), triple (n=45, 38.5%), and tetrad (n=36, 30.8%) in pathogen positive apiaries (Figure 2C). At least one pathogen positivity was also detected in all IAPV-positive apiaries. IAPV positivity was seen in 12 provinces and all sampled years (2011-2021).

Table 2. Correlations between tested variables

		Mn.	Sd.	1	2	3	4	5	6	7	8	9
1	IAPV	0,5250	0,50147	1								
2	DWV	0,8000	0,40168	0,150	1							
3	BQCV	0,5750	0,49642	-0,008	-0,177	1						
4	ABPV	0,1000	0,30126	0,150	-0,042	0,118	1					
5	SBV	0,0750	0,26450	-0,109	0,142	-0,139	-0,095	1				
6	LSV	0,3000	0,46018	-0,033	,191*	0,011	-,218*	-,186*	1			
7	CBPV	0,1500	0,35857	-0,021	-,315**	,220*	0,093	0,146	-0,122	1		
8	Nosema sp	0,3250	0,47034	0,126	-,320**	-0,051	-0,053	0,005	-,221*	-0,142	1	
9	Apiary status	1,8750	0,75105	-0,092	0,084	-0,008	0,056	-,206*	,255**	-0,023	-0,098	1

*: $p<0.05$, **: $p<0.01$

According to colony losses complaints, three groups was the first group consisted of 51 apiaries, the second group was 42 apiaries and the third included 27 apiaries. IAPV positivity was found as 58.8 % (n=30), 50 % (n=21), and 44.4 % (n=12) rates in the first, second, and third groups, respectively. Among groups, the positivity of IAPV was statistically insignificant ($p<0,05$). In the first group, darkened bees and brown/black spotted were noted in larvae and pupae brood samples in 27 (52.9%) apiaries. Fifteen (55.6%, 15/27) of these apiaries were positive for IAPV. IAPV tested positive in 36 (85.7%) of 42 apiaries in paralyzed bees were recorded.

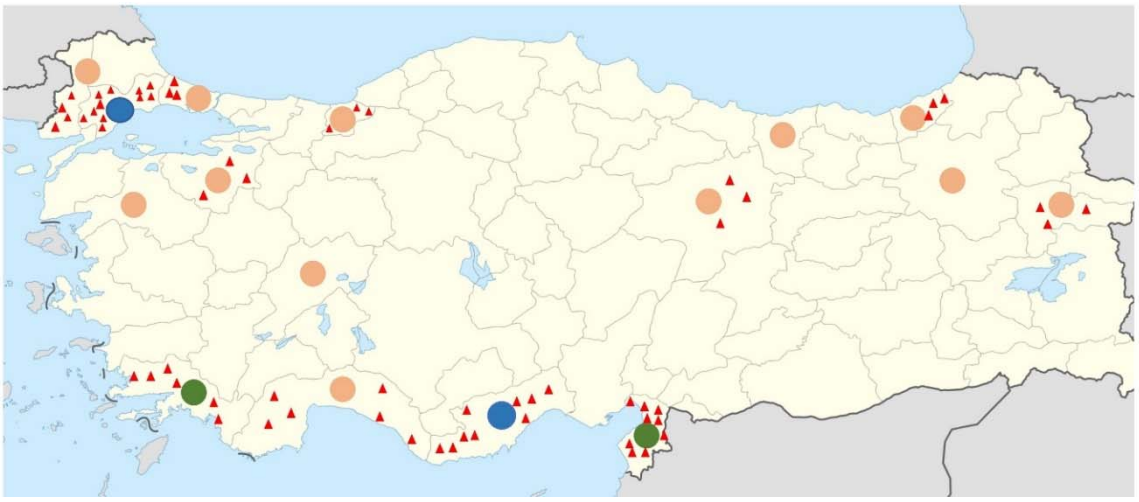

Figure 1: Sample area and sampling years are shown with different color circles. The orange circle: 1-3 years sampled, the green circle; 4-5 years sampled, blue circle; 6-8 years sampled. The IAPV-positive apiaries are marked with a red triangle in the sampled provinces.

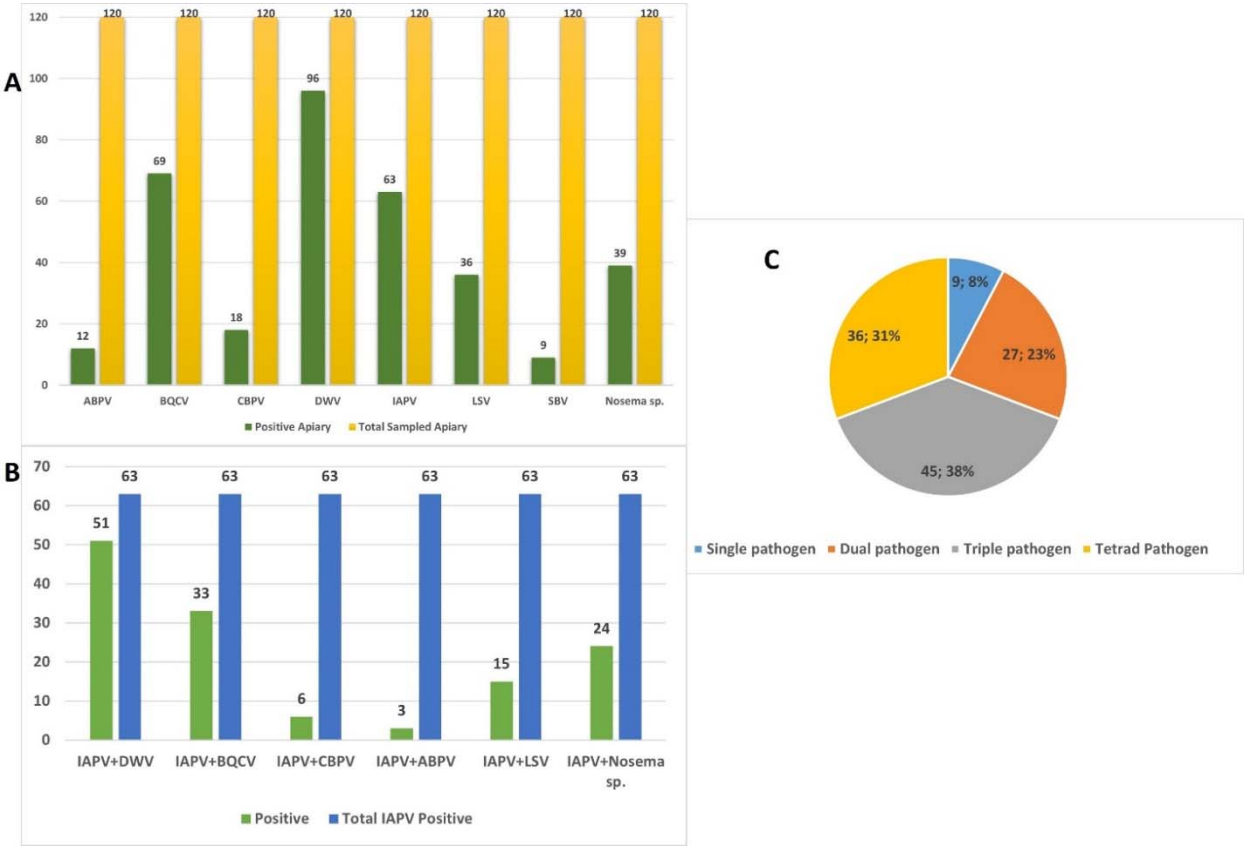

Figure 2: The results of pathogens' positivity of apiaries in this research. A. The distribution of pathogen positivity in sampled apiaries. B. The coexistence of pathogens and IAPV in positive apiaries. C.The distribution of single and multiple pathogens in positive apiaries.

DISCUSSION

Honeybee viruses may induce covert infections in worker and queen bees of healthy-looking colonies. As sick and clinically symptomatic bees may suffer from more severe problems depending on different factors. (Chen et al. 2005). Stress factors like increased mite loads and elongated overwintering periods trigger the viral multiply resulting in colony declines and collapse (Chen et al. 2014). Deformed wing virus (DWV) load increased significantly after Varroa infestation and caused colony collapses reported (Martin et al. 2012). IAPV, one of the Varroa-born viruses, can be transmitted through trophallaxis, fecal-oral route, robbing, and horizontal and vertical transmission (Amiri et al. 2019). All of these pose increased risks and severe threats to colony health. IAPV has been reported globally since it was first detected in symptomatic colonies with losses in Israel apiaries (Maori et al. 2007; Chen et al. 2014). Although initial results pointed to IAPV infection effects in CCD-like symptoms (Cox-Foster et al. 2007), the subsequent reports could not verify this relevance (Vanengelsdorp et al. 2009; McMenamin and Genersch 2015). Instead, IAPV infection in the colony is an effective factor in colony productivity and bee health (Hou et al. 2014). The IAPV, common in asymptomatic colonies, risks colony health and production. (De Miranda et al. 2010).

This study investigated some honeybee pathogens in 120 colonies in 16 provinces of five ecogeographic regions, where colony loss and CCD-like complaints were reported during 2011-2021. At least one pathogen was detected in 117 (97.5 %) of 120 problematic colonies with loss reported, while IAPV was detected in 63 (52.5%). Previously reported two IAPV records ranged from 6.5 to 21,12% in the Türkiye (Özkırım and Schiesser 2013; Kalaycı et al. 2020). The IAPV prevalence was higher than the results of previous reports spread over 10-year sampling. Results indicated that IAPV exists in different developmental stages of honeybees. IAPV was detected higher, especially in symptomatic adult bees with paralysis and brown/black darkened brood samples. The IAPV infection rate was also higher in larvae and pupae samples than in adult bees compared to previous reports (Maori et al. 2007; Chen et al. 2014). The rate of IAPV infection was reported to increase in weak colonies (Chen et al. 2014); IAPV does not restrict to cause only CCD but also an increase in bee deaths.

Experimental studies show that IAPV is also spread by close contact and virus particles are easily transmitted to bees topically (Amiri et al. 2019). It emphasized a positive association between IAPV infection, virus load, and mite infestation (Di Prisco et al. 2011). In this study, mite infestation was determined in all IAPV-positive apiaries. Although mite levels do not appear statistically significant for IAPV positivity, to possibly have negative effects on colony health. The mite-infested colonies under the effects of various stress factors, the virus multiplies rapidly, and the viral load increases, leading to possible bee death and colony collapse (Chen et al. 2004). High IAPV titer is highly lethal in worker bees and pupae, and may present with typical symptoms of trembling wings, progressive paralysis, and nerve dysfunction similar to experimental infections (Hou et al. 2014). In this study, the IAPV load was not analyzed but, high rates (85.7%) of IAPV positivity were noted in paralyzed bees sampled. It suggests that symptomatic overt viral infections would be an increasing risk for bee health and colony decline.

DWV has been reported to be linked to global honeybee colony losses. DWV and BQCV are two of the most prevalent viruses in apiaries in Türkiye and the world (Kalaycı et al 2020; Muz and Muz 2009; 2018; 2022). Although the prevalence of ABPV, CBPV, and SBV is mostly lower, it has also been reported from apiaries in different geographic regions in Türkiye (Gümüşova-Okursoy et al. 2010; Kalaycı et al. 2020; Çağırgan and Yazıcı 2021; Güller and Kurt 2022; Muz and Muz 2022). Current results revealed that multiple honeybee virus infections are common in colony losses and may play a critical role in colony health. Compatible with previous studies, DWV was determined the most prevalent virus followed by BQCV positivity in the second and IAPV in the third in sampled apiaries in this study. IAPV was described as the most common viral agent after DWV and BQCV in bee colonies worldwide. The status of IAPV infections is directly related to colony survival (Chen et al 2014). IAPV positivity in all three groups sampled (according to colony complaints) in the current study ranged from 44.4 to 58.8%. IAPV may be an influencing factor in intense winter losses, colony declines, and CCD cases.

Nosema ceranae prevalence was highly reported in local colony losses (Muz et al 2010; Ostroverkhova et al 2020). The threat posed by unexpected colony losses is compounded by the fact that bee colonies often suffer from several pathogens simultaneously

which negatively affect colony health (Martín-Hernández et al 2007; Berthoud et al 2010; Muz et al 2010; Botias et al 2013). Numerous studies have shown a correlation between *N.ceranae* infections and colony declines, but some do not confirm this relationship (William et al 2010; Ostroverkhova et al 2020). While the *Nosema sp* positivity is defined as 32.5% in our research results, it is similar to the previous epidemiological studies in Türkiye (Ivgın-Tunca et al .2016; Tosun and Yaman 2016; Ütük et al 2016; Muz and Muz 2022), while some local reports where beekeeping is intense (Muz et al 2010; Kartal et al. 2021) were reported at a lower rate. The results suggest that multiple pathogen positivity may be effective in colony losses. In conclusion, implementing the necessary control and treatment strategies to combat pathogens in colony losses is regularly essential. Complete and timely maintenance of the colonies in spring and winter can also contribute to the fight against pathogens.

Conclusion

In this study, IAPV investigated in apiaries with colony loss and CCD complaints in different eco-geographic regions in Türkiye by 10-year fieldwork. The IAPV positivity was determined at higher rates than other reports from Türkiye. The worker bee and brood samples were positive for IAPV, and more than one pathogen coexisted. Multiple viral infections, Varroa mite and *Nosema sp.* exist prevalent in apiaries. The results suggest that multiple pathogen positivity may be effective in colony losses. The presence of high varroa mites can trigger many viral infections to threaten colony health. The fight against pathogens must be carried out periodically to protect colony health.

Authors' contributions: Concept – DM planned the concept and designed the research. MNM worked on field study. DM and MNM worked on laboratory analysis, processing/interpretation of data, and writing the manuscript. All authors have read and approved the final manuscript.

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