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Honey Bee Pathogen Prevalence and Interactions in the Ağrı Province of Türkiye

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Abstract

This study examines the prevalence and interactions of bacterial, viral, and parasitic pathogens in honey bee (*Apis mellifera* L.) colonies in Ağrı Province, Türkiye. To iden-tify pathogens, samples collected from beekeeping enterprises across different regi-ons were analyzed using laboratory techniques, including bacterial culture isolation and one-step RT-PCR. No positivity was detected for *Paenibacillus larvae*, the causa-tive agent of American Foulbrood (AFB). Among viral pathogens, the Black Queen Cell Virus (BQCV) was found in 100% of the samples. In comparison, *Apis mellifera* Filamentous Virus (*Am*FV) and Sacbrood Virus (SBV) were detected in 90.9%, Defor-med Wing Virus (DWV) in 72.7%, *Varroa destructor* Virus-1 (VDV-1) in 18.2%, and Acute Bee Paralysis Virus (ABPV) and Israeli Acute Bee Paralysis Virus (IAPV) in 9.1% of the samples. Chronic Bee Paralysis Virus (CBPV) and Kashmir Bee Virus (KBV) were undetected. *Nosema apis* tested negative, whereas *Nosema ceranae* showed a 27.3% positivity rate, with 100% consistency between molecular and microscopic analyses. No *Varroa* mites were found in any of the colonies. These findings provide valuable insights into the prevalence of honey bee pathogens in Ağrı province, offering a fou-ndation for future research and disease management strategies.

Introduction

Honey bees (*Apis mellifera* L.) are among the most crucial pollinators, playing a vital role in maintaining ecosystem balance and supporting agricultural production. The health and size of honey bee colonies worldwide are critical for the efficiency of farming processes. Bees' contribution to pollination significantly impacts the yield of many fruits, vegetables, and other crops, making them indispensable to the farming economy (Klein et al., 2007). A global decline in honey bee colonies has emerged as a major concern in recent years. This decline is attributed to factors such as pesticide use, habitat loss, climate change, and, most notably, the emergence of bacterial, viral, and parasitic diseases (VanEngelsdorp & Meixner, 2010; Gürler et al., 2025).

One of the most significant bacterial infections affecting honey bees is American Foulbrood (AFB), caused by *Paenibacillus larvae*. This contagious disease results in substantial losses for the beekeeping industry worldwide (Rosario & Miller, 2024). Over 30 honey bee viruses have been identified globally (Remnant et al., 2017), with eight commonly found in honey bee colonies. These include the Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Kashmir Bee Virus (KBV), Israeli Acute Paralysis Virus (IAPV), Sacbrood Virus (SBV), Acute Bee Paralysis Virus (ABPV), and *Varroa destructo*r Virus-1 (VDV-1). In contrast to RNA viruses, research on DNA viruses in honey bees has been relatively limited. One such DNA virus is *Apis mellifera* Filamentous Virus (*Am*FV) (Wang et al., 2019). Among parasitic factors,

the *Varroa destructor* and *Nosema* species are the most significant parasites, posing serious threats to honey bee health. The impact of these pathogens and parasites on honey bee populations is closely linked to climatic conditions and regional environmental factors (Yang et al., 2013; Beaurepaire et al., 2020; Sabahi et al., 2020).

The province of Ağrı, located in Türkiye's Eastern Anatolia Region, shares geographical characteristics with many high-altitude areas where beekeeping is practiced. Ağrı's rugged terrain and topographical variations within a narrow area allow for a longer beekeeping season. In addition to altitude differences, the province boasts a rich flora, with plants flowering at various times of the year and an abundance of local flowers, making Ağrı a favorable location for beekeeping. As is well known, pesticide use reduces the nectar sources that honey bees rely on, leading to a decline in colony productivity and necessitating more intensive migratory beekeeping. However, in Ağrı, the relatively low level of agricultural pest control activities provides a significant advantage in preserving both honey quality and the bee population (Kaya, 2008; Yakan, 2021; Sümbül, 2024).

This study aims to reveal the distribution of common pathogen species, infection rates, and potential interactions between pathogens in honey bee colonies within the borders of Ağrı province in Türkiye's Eastern Anatolia Region. Supported by molecular diagnostic methods and field observations, this research aims to map the health risks to bees in the region, thereby contributing to local beekeeping practices and the global literature on pathogen ecology. The findings are expected to provide insights into understanding pathogen dynamics related to climatic stress factors and to aid in developing sustainable beekeeping strategies.

Materials and Methods

Study area and sample collection

In June 2024, samples were collected from beehives located in 11 different apiaries in Ağrı province. The locations of the sampling areas are shown in Fig 1. In each apiary, one colony was selected for sampling bees. Then, in each colony, 200 live adult worker bees were randomly collected from the beehives, placed in a Falcon tube, and transported to the laboratory under a cold chain. The samples were then stored at +4 °C until analysis.

During the fieldwork, it was ensured that each apiary was located at a minimum of 20 km from each other.

Diagnosis of American Foulbrood (AFB)

Cultures were inoculated onto media to isolate and identify *P. larvae* in diagnosing AFB. For inoculation, 15 adult

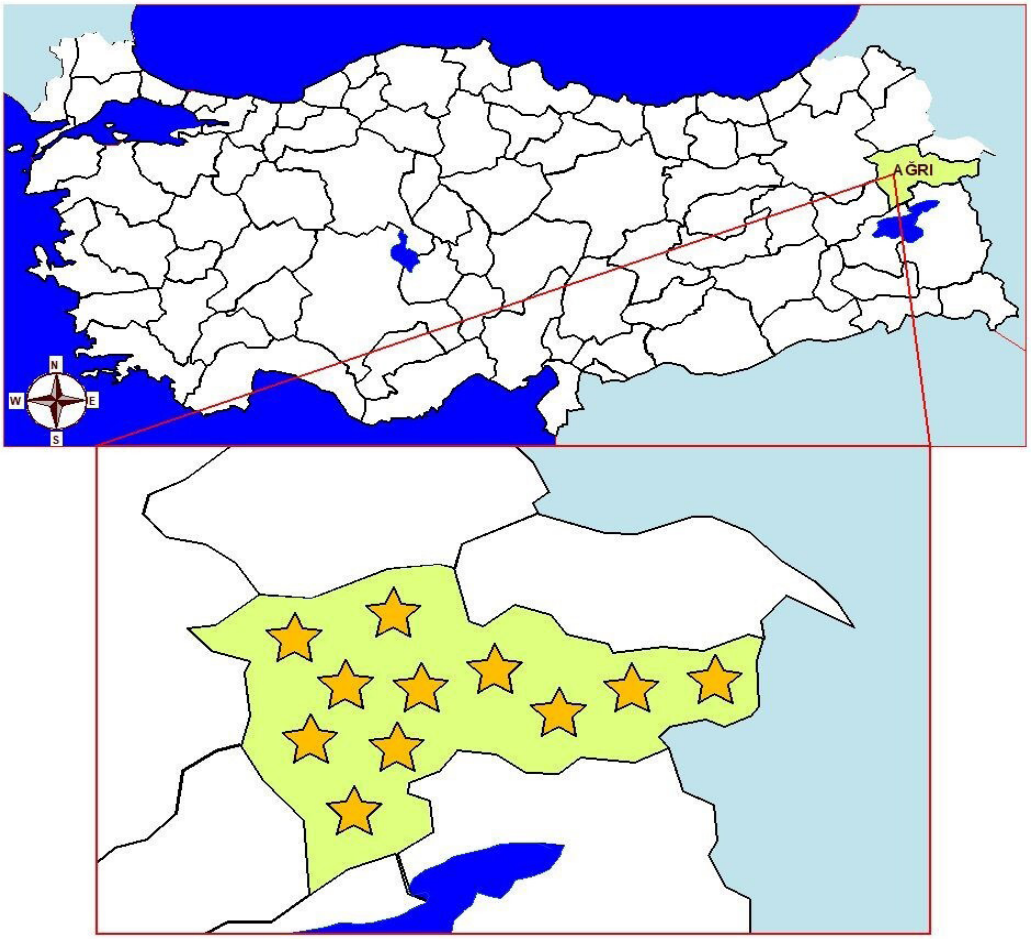

Fig 1. Distribution of beekeeping regions sampled in Ağrı province.

bee samples were selected from each hive, transferred into 3 ml of PBS, and homogenized in a Bead Ruptor Elite (Bead Mill Homogenizer, Sku 19-042E, Omni International, USA). The prepared samples were incubated in a water bath at 80 ºC for 10 minutes to kill non-spore-forming bacteria. For the isolation of *P. larvae*, Brain Heart Infusion agar (BHIA, Thiamine-supplemented), Columbia Sheep Blood Agar (CSA), and MYPGP agar (10 g Mueller-Hinton broth, 15 g yeast extract, 3 g K_2HPO_4, 1 g Na-pyruvate, 20 g Agar, and 20 ml 10% glucose) (Dingman & Stahly, 1983) were used. To inhibit bacteria from the genera *Bacillus* and *Brevibacillus*, nalidixic acid (Hornitzky & Clark, 1991) and pipemidic acid (Alippi, 1995) were added to the culture medium, as with other *Paenibacillus* species. All inoculated plates were incubated at 37 °C with 5-7% CO_2 for 2-5 days. The bacterial colonies observed on the Petri dishes were examined under a light microscope (Olympus, BX53, Japan) after Gram staining, carbol fuchsin, and nigrosin staining. Additionally, a catalase test was also performed as a biochemical test (Gochnauer, 1973; Dingman & Stahly, 1983; Hornitzky & Karlovskis, 1989; OIE, 2018; Karaoğlu et al., 2023).

Viral Nucleic Acid extraction from tissue

Fifteen adult bee samples collected from the same hive were placed into 7 mL cryogenic tubes, and 3 mL of phosphate-buffered saline (PBS) (SIGMA, 806544-500ML, USA) was added to each tube. The samples were homogenized using an automated homogenizer (Bead Ruptor Elite, Bead Mill Homogenizer, SKU 19-042E OMNI International, USA). Following homogenization, the samples were centrifuged at 4000 rpm for 15 minutes at +4 °C. Total viral nucleic acids were extracted from the resulting supernatants, while the remaining samples were stored at -20 °C for future use. Total viral nucleic acids were extracted using a commercial kit (High Pure Viral Nucleic Acid Kit, REF: 11858874001, Roche, Germany) by the manufacturer's protocol. The extracted total viral nucleic acid samples were preserved at -20 °C until further analysis.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) mixture

The one-step RT-PCR test's reaction mixtures and thermal conditions were applied similarly for nine viral diseases. Although *Am*FV is a DNA virus and the other viral agents (ABPV, BQCV, CBPV, DWV-A, DWV-B, IAPV, SBV, and KBV) are RNA viruses, one-step RT-PCR tests were performed under the same thermal conditions using the same mix components (excluding primers) in separate tubes. A commercial kit (Rotor-Gene Probe RT-PCR Kit, 204574, QIAGEN, Germany) was used for the one-step RT-PCR test. Although the kit was designed for RNA-based tests, it was considered that processing the samples obtained from the same hive in a single run would minimize potential nucleic acid losses caused by freeze-thaw-related thermal stress. This approach was possible because the DNA integrity remained intact during the reverse transcription (RT) step, and subsequent stages allowed amplification from either cDNA or DNA. The reaction mixture was prepared by combining 12.5 µL of 2X Rotor-Gene Probe RT-PCR Master Mix, 0.8 µL of each 10 pmol forward and reverse primers, 0.25 µL of Rotor-Gene RT Mix, and 5.65 µL of ultra-pure water. 5 µL of the suspected total nucleic acid sample was added, making the total reaction volume 25 µL (Stoltz, 1995; Benjeddou et al., 2001; Bakonyi et al., 2002; Chen et al., 2006; Gauthier et al., 2007; Palacios et al., 2008; Locke et al., 2012; Sguazza et al., 2013). The prepared reaction mixtures were transferred to the Thermal Cycler (T100 Thermal Cycler, 621BR38781, BIO-RAD, Singapore). The Thermal Cycler performed RT and PCR steps with the same thermal conditions. To convert RNAs to complementary DNAs and then inactivate the reverse transcriptase enzyme, the samples were incubated at 50 °C for 30 minutes, followed by 95 °C for 15 minutes. After this, for 40 cycles, the following thermal conditions were applied: denaturation at 95 °C for 30 seconds, annealing at 55 °C for 60 seconds, and extension at 72 °C for 60 seconds. Finally, a final extension was performed at 72 °C for 7 minutes to complete the reaction. The resulting PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized under UV light. Using positive and negative controls, the RNA bands for the following nine diseases were examined under the ultraviolet transilluminator: ABPV (398 bp), BQCV (700 bp), CBPV (774 bp), DWV (702 bp), KBV (415 bp), SBV (342 bp), IAPV (767 bp), *Am*FB (551 bp), and VDV-1 (413 bp). The results were considered positive if bands were present for these RNA markers. The negative control should show no bands. The results were evaluated according to the amplicon sizes provided in Table 1.

Microscopic examination and analysis of Nosema spp.

The abdomens of sixty adult bees were separated into two distinct groups: the first group, consisting of thirty worker bee abdomens, was allocated for microscopic examination, while the second group, comprising another thirty worker bee abdomens, was designated for molecular analysis. To measure the presence of *Nosema* spp. spores, the abdomens of 30 bees from each hive (using sterilized materials for each hive) were placed into petri dishes. Then, 15 mL of distilled water (0.5 mL per bee) was added. After thoroughly crushing the abdomens, 0.1 mL of the resulting mixture was placed between a microscope slide and a coverslip. The presence of *Nosema* spp. spores was examined under a light microscope (Olympus, BX53, Japan) at 40x magnification.

Molecular analysis

The bee abdomen samples from the second pool were placed into 7 mL cryogenic tubes, with 3 mL of phosphate-buffered saline (PBS) added. These tubes were then processed in an automated homogenizer (Bead Ruptor Elite, Bead Mill

Table 1. Primer sequences used in this study.

*Viruses	Primers	Amplicon size (bp)	References
ABPV	ABPV-F: 5'- CAT ATT GGC GAG CCA CTA TG-3' ABPV-R: 5'- CCA CTT CCA CAC AAC TAT CG -3'	398 bp	Bakonyi et al., 2002
BQCV	BQCV-F (5'-TGGTCAGCTCCCACTACCTTAAAC-3') BQCV-R (5'-GCAACAAGAAGAAACGTAAACCAC-3')	700 bp	Benjeddou et al., 2001
CBPV	CBPV-F: 5'- AACCTGCCTCAACACAGGCAAC -3' CBPV-R: 5'- ACATCTCTTCTTCGGTGTCAGCC -3'	774 bp	Sguazza et al., 2013
DWV	DWV-F: 5'- ATCAGCGCTTAGTGGAGGAA -3' DWV-R: 5'- TCGACAATTTTCGGACATCA -3'	702 bp	Chen et al., 2006
KBV	KBV-F: 5'-GATGAACGTCGACCTATTGA-3' KBV-R: 5 -TGTGGGTTGGCTATGAGTCA-3'	415 bp	Stoltz et al., 1995
SBV	SBV- F: 5'-CGTAATTGCGGAGTGGAAAAGATT-3' SBV- R: 5'-AGATTCCTTCGAGGGGTACCCTCATC-3'	342 bp	Sguazza et al., 2013
IAPV	IAPV-F: 5'-CGATGAACAACGGAAGGTTT-3' IAPV-R: 5'-ATCGGCTAAGGGGTTTGTTT-3'	767 bp	Palacios et al., 2008
*Am*FV	AmFV-F:5'-CAGAGAATTCGGTTTTTGTGAGTG -3' AmFV-R:5'-CATGGTGGCCAAGTCTTGCT-3'	551 bp	Gauthier et al., 2015
VDV-1	VDV-1 F:5'- GCCCTGTTCAAGAACATG-3' VDV-1 R:5'-CTTTTCTAATTCAACTTCACC-3'	413 bp	Locke et al., 2012

*ABPV (Acute Bee Paralysis Virus); BQCV (Black Queen Cell Virus); CBPV (Chronic Bee Paralysis Virus); DWV (Deformed Wing Virus); KBV (Kashmir Bee Virus); SBV (Sacbrood Virus); IAPV (Israeli Acute Paralysis Virus); *Am*FV (*Apis mellifera* Filamentous Virus); VDV-1 (*Varroa destructor* Virus-1).

Homogenizer, SKU 19-042E, Omni International, USA) and subsequently centrifuged at 4000 rpm for 10 minutes at 4 °C using a Nüve NF 200 centrifuge (Türkiye). DNA was extracted using a commercially available DNA extraction kit (PureLink Genomic DNA Mini Kit, Invitrogen), adhering to the manufacturer's protocol. The extracted DNA samples were preserved at -20°C until further analysis. Multiplex-PCR was conducted according to the OIE guidelines. The SSU rRNA gene region was amplified using specific primers: for *Nosema ceranae*, MITOC FOR 5'-CGGCGACGATGTGATATGAAAATATTAA-3' and MITOC REV 5'-CCCGGTCATTCTCAAACAAAAAACCG-3'; for *Nosema apis*, APIS FOR 5'-GGGGGCATGTCTTTGACG TACTATGTA-3' and APIS REV 5'-GGGGGGCGTTTAAAA TGTGAAACAACTATG-3' (OIE, 2018). The PCR mixture, totaling 50 µL, included: 23.5 µL of DNase- and RNase-free sterile distilled water, 5 µL of 10X Taq Buffer ($(NH_4)2SO_4$), 6 µL of $MgCl_2$ (25 mM), 2 µL of dNTPs (10 mM), 2 µL of each primer (*N. ceranae* and *N. apis* - 10 pmol), 0.5 µL of Taq DNA polymerase (1.25 IU) (Thermo Scientific), and 5 µL of DNA template. The PCR protocol consisted of an initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 50 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes. The resulting PCR products were analyzed via 1.5% agarose gel electrophoresis and visualized under UV light. Positive results were indicated by bands at 321 bp for *N. apis* and 218-219 bp for *N. ceranae*. Positive virus DNA samples and accession numbers used in this study, respectively: ABPV (OP504103), BQCV (OK345070), DWV (OP504101), *Am*FV (OP642035), CBPV (EU122229), KBV (OP504105), SBV (OP504108), IAPV (OP504110), and VDV-1 (OP504112). These positive virus DNAs were used as positive controls in PCR.

Varroa diagnosis

To investigate the presence of varroosis in the colonies, approximately 100 adult bee samples collected from each colony were examined according to the instructions described in the OIE Terrestrial Manual (OIE, 2021). The morphological identification of *V. destructor* was performed by detection using the alcohol washing method (Oliver, 2020). The abdomen segment gaps and the undersides of the wings of all the bees were specifically checked.

Results and Discussion

American Foulbrood is a highly contagious and fatal disease affecting honey bees (*Apis mellifera* L.), caused by the bacterium *P. larvae* (Solmaz et al., 2021). This disease poses a significant threat to the global beekeeping industry and has been the focus of numerous studies in Türkiye. In our study, no positive results for *P. larvae* were detected in the suspicious samples collected from all hives within the borders of Ağrı Province. The prevalence of AFB varies regionally worldwide, with different rates reported in various studies. For instance, the highest AFB positivity rates in the Northwest Pakistan region were 39% in Kohat, 37% in Bannu, and

34.5% in Karak (Anjum et al., 2015). In Türkiye, Şık et al. (2022) reported a 30.7% positivity rate for *P. larvae* in AFB-suspected samples analyzed at the Ankara Etlik Veterinary Control Center between 2015 and 2020. Another study in the Southern Marmara region reported an AFB prevalence rate of 31.3% (Mayack & Hakanoğlu, 2022). In the Black Sea Region, the AFB positivity rate was 4.83% (Akpınar et al., 2023). In our study, the absence of *P. larvae* in the suspicious samples from Ağrı Province, compared to the higher prevalence rates reported in other regions, suggests that local differences and various factors such as climate, biodiversity, soil and plant health, colony management practices, bee race, and genetic diversity-should be taken into consideration when evaluating disease prevalence.

The prevalence of ABPV has been investigated in various studies conducted in different regions. A study in the Veneto region of Italy reported that ABPV showed high prevalence, particularly during the spring season, alongside other pathogens. During this period, the weakening of the bees‘ immune systems was identified as a significant factor in the spread of ABPV (Bordin et al., 2022). Additionally, Nogueira et al. (2024) demonstrated that infection rates within the same apiary can vary significantly across different sampling dates within a short period of just two months. This finding suggests that the spread of infection may have a dynamic and time-dependent nature. The relationship between ABPV and *V. destructor* mites is also a crucial area of research. *Varroa* mites are considered the primary vectors for ABPV transmission. Numerous studies have demonstrated that this virus is prevalent in colonies infested with *Varroa* mites. Similarly, a study conducted in the United Kingdom (UK) emphasized that *Varroa* mites play a critical role in the spread of ABPV infections (Gregory et al., 2005). A study conducted in Pakistan found that 39% of the samples from the Kohat region were positive for ABPV, revealing that the virus exhibits a higher prevalence in certain regions (Anjum et al., 2015). The prevalence of ABPV infections in Türkiye has been reported at varying rates. While a study conducted on adult bees in beekeeping operations in the Aegean region did not detect the pathogen (Gümüşova et al., 2010), other studies reported a 1.27% prevalence (Beyazıt et al., 2012) and a 35.5% prevalence (Kalaycı et al., 2020). Additionally, ABPV infections were reported at rates of 2.2% in Hakkari (Rüstemoğlu & Sipahioğlu, 2016), 3.6% in the Aegean Region (Çağırgan, 2018), and 74.19% in the Burdur province (Usta & Yıldırım, 2020), while no presence of the infection was detected in the Van province (Karapınar et al., 2018). A literature review indicates that previously reported prevalence rates in Türkiye ranged from 1.27% to 74.19%, representing a broad spectrum. In a study conducted across Türkiye, the Acute Bee Paralysis Virus (ABPV) was reported to have a prevalence of 6.4% (Akpınar et al., 2024a). Another study by Bozdeveci et al. (2024) found that the prevalence of ABPV in the Sivas province was 7.5%. In this study, the prevalence of ABPV was found to be 9.1% (Fig 2A), which falls within the range of the rates reported in the literature. Although ABPV infection is generally reported at high prevalence rates in the literature, the 9.1% prevalence observed in this study indicates a lower frequency. This difference might be attributed to factors such as the study‘s geographical region, the health status of the bees, and the prevalence of *Varroa* mites. For instance, since the presence of *Varroa* mites plays a significant role in the spread of ABPV, a lower prevalence of *Varroa* mites could impact the prevalence of ABPV. The results of our study reveal that ABPV has a lower prevalence in honey bee colonies in the eastern province of Ağrı, Türkiye. Comparing these findings with the literature suggests that while ABPV generally exhibits high prevalence, regional differences and various environmental factors can significantly influence its spread.

Black Queen Cell Virus is a significant viral pathogen in honey bees that causes widespread damage to colonies globally. Studies conducted in Europe and Türkiye have revealed that this virus exhibits high prevalence in commercial and wild colonies. Research in various European countries has reported high prevalence rates of BQCV. In Switzerland, a study by Hartmann et al. (2015) found BQCV present at a rate of 64%. Similarly, in Austria, the prevalence of the virus was reported to reach as high as 70% (Roberts et al., 2013). Additionally, Rodriguez et al. (2012) reported an 82% positivity rate in Chile. In Türkiye, studies have reported varying prevalence rates for BQCV. Gümüşova et al. (2010) found a prevalence of 21.42%, Oğuz et al. (2017) reported 88.5%, Karapınar et al. (2018) found 88.46%, Aydın (2020) reported 16%, and Kalaycı et al. (2020) detected 28.9%. A study by Şık et al. (2022) conducted at the Ankara Etlik Veterinary Control Center between 2015 and 2020 found a BQCV prevalence of 80%. Similarly, a study by Mayack and Hakanoğlu (2022) reported a BQCV prevalence of approximately 60% in the Marmara region. In our study, however, the prevalence of BQCV was found to be 100% (Fig 2B). Considering that BQCV prevalence typically ranges between 50% and 80% in previous studies (Mayack & Hakanoğlu, 2022; Akpınar et al., 2024a), our findings indicate a very high infection rate in the region. This elevated prevalence suggests the potential influence of factors such as local beekeeping practices, environmental conditions, or the genetic characteristics of the bee populations. In conclusion, the prevalence of BQCV in Türkiye aligns with the high rates reported in similar European studies. However, the 100% prevalence observed in our study suggests that the impact of this virus in the region may be severe. Future studies should investigate the reasons behind this high prevalence rate and develop preventative measures for beekeeping management strategies.

Chronic Bee Paralysis Virus is a significant pathogen in honey bees, with varying prevalence rates reported globally. The prevalence of CBPV infections depends on numerous factors, including regional conditions, climate,

beekeeping practices, the genetic structure of bee populations, and other stress factors. Studies conducted in Türkiye and worldwide have demonstrated that CBPV occurs at different rates. Globally, the prevalence of CBPV shows considerable variation. A study conducted in Iran reported CBPV in 7.5% (12/160) of samples collected from 160 apiaries across 23 provinces (Moharrami & Modirrousta, 2015). In Denmark, CBPV was detected in only four of the examined apiaries (Nielsen et al., 2008). A study in Uruguay documented the first detection of CBPV in South America (Antnez et al., 2005). In Türkiye, a study conducted in three provinces (Burdur, Antalya, and Isparta) found CBPV in 11% (5/45) of the samples collected from 45 apiaries (Çağırgan et al., 2022). Additionally, Gümüşova et al. (2010) reported a prevalence rate of 25%, while Kalaycı et al. (2020) found CBPV in 18.4% of the samples. In this study, the prevalence of CBPV in Ağrı was 0% (Fig 2C). This outcome might be attributed to several factors. The cold climate and high altitude of Ağrı could act as limiting factors for the spread of CBPV. Furthermore, traditional beekeeping practices in the region and the limited introduction of external colonies or materials may have restricted the entry of the virus. Successful control of *Varroa destructor*, which plays a role in the transmission of CBPV, might also have prevented the spread of the virus in the area.

Deformed Wing Virus is a disease commonly found in bee colonies and can cause severe damage, especially to honey bees. This disease is caused by a viral infection from a pathogen known as DWV. DWV infection negatively affects colony health and productivity, leading to losses in the beekeeping industry. In the UK, it has been reported that DWV infection is high, with a large portion of colonies (50-80%) infected with DWV. This rate varies depending on the effectiveness of *Varroa* control (Kevill et al., 2021). Infection rates increase in areas where the spread of *Varroa* mites cannot be controlled (Parveen et al., 2022). Studies conducted in Türkiye have shown a prevalence of 74.19% in Burdur province (Usta & Yıldırım, 2022), 69.23% in Van province (Karapınar et al., 2018), 25.2% in the Aegean Region (Çağırgan, 2018), and 23.3% in Hakkari province (Rüstemoğlu & Sipahioğlu, 2016). DWV and the associated DWV are significant problems for bee colonies worldwide, and the prevalence generally varies depending on regional conditions, the genetic makeup of the bees, the spread of *Varroa* mites, and beekeeping management practices. The finding of a 72.7 % prevalence of DWV (Fig 2D) in our study in Ağrı is a notable result compared to other regional, national, and international studies. This rate suggests that the disease may have spread through the effects of environmental conditions.

Apis mellifera Filamentous Virus is a double-stranded DNA virus that infects bees. Studies conducted in China have shown that the prevalence of *Am*FV ranges from 10% to 80% (Hou et al., 2016), while in eight provinces of Argentina, it was determined to be 61% (19/31) (Quintana et al., 2020).

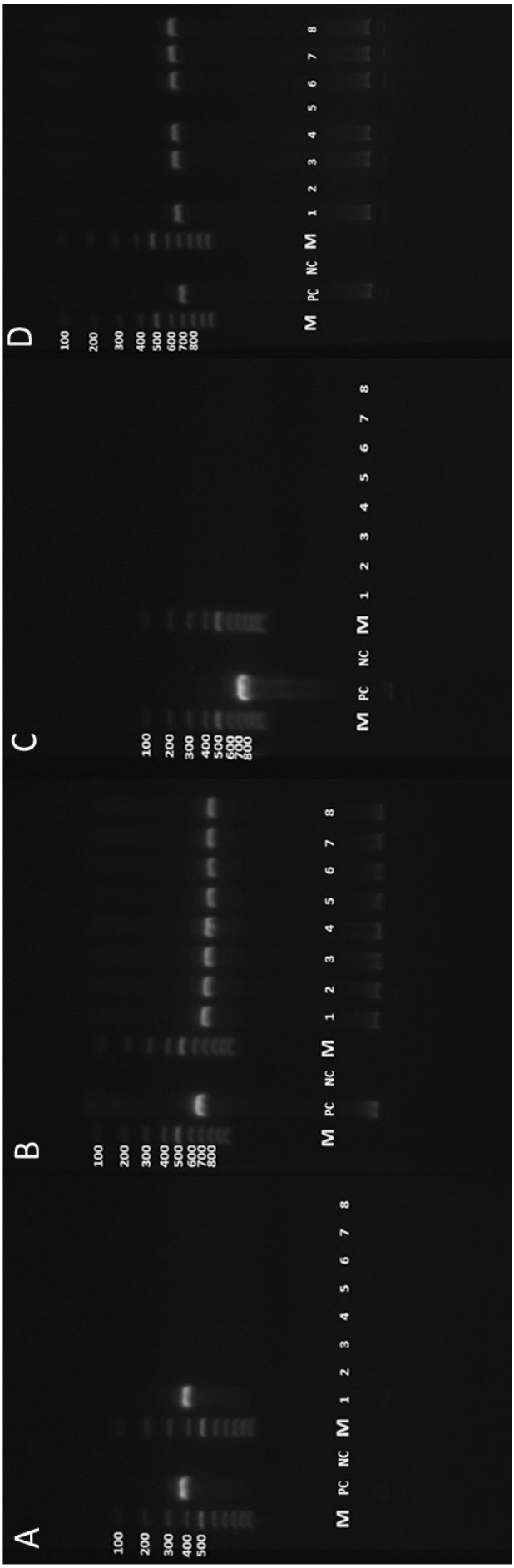

Fig 2. PCR gel images of honey bee viruses obtained from One-step RT-PCR. PC; Positive control, NC; Negative control, M; marker. * PCR images are provided for only eight samples. **A:** Acute Bee Paralysis Virus (ABPV), ABPV showed a 398 bp band in the hive. 1-Positive, 2-7 Negative, **B:** Black Queen Cell Virus (BQCV). BQCV showed a 700 bp band in all hives, 1-8 Positive. **C:** Chronic Bee Paralyses Virus (CBPV). CBPV did not show a band in any of the hives. **D:** Deformed Wing Virus (DWV), DWV showed a 702 bp band in eight hives, 1,3,4,6-8 Positive, 2,5 Negative.

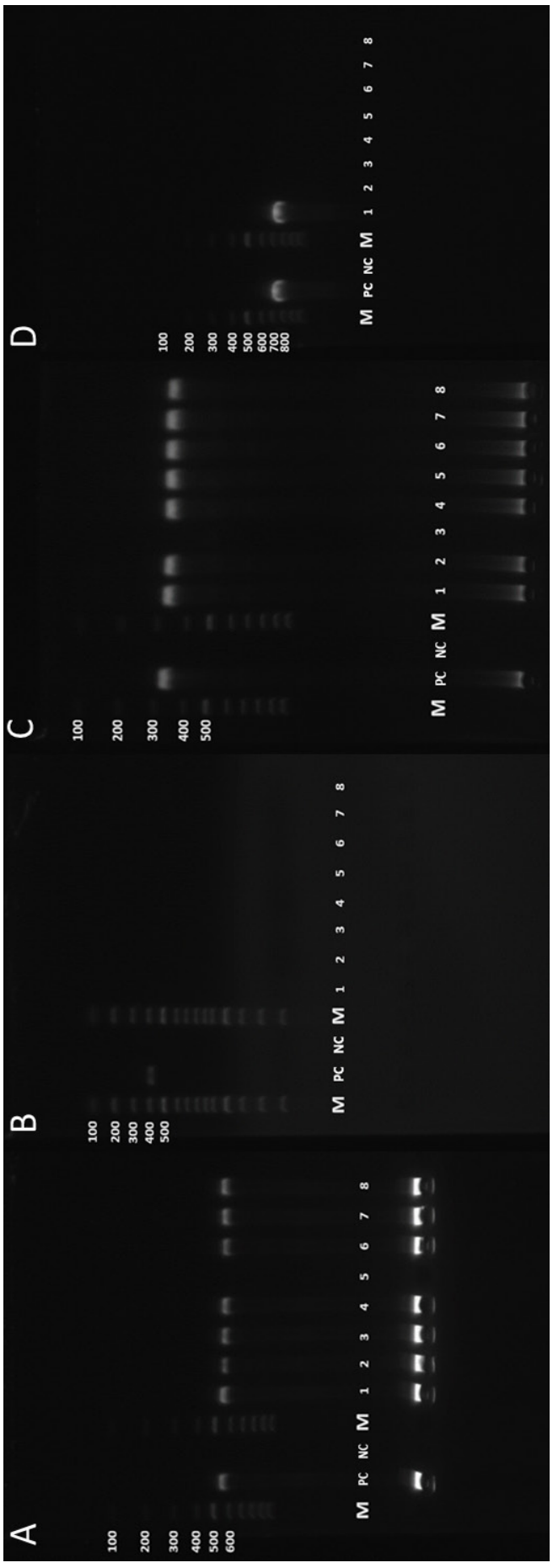

Fig 3. PCR gel images of honey bee viruses obtained from One-step RT-PCR. PC; Positive control, NC; Negative control, M; marker. *PCR images are provided for only eight samples. **A:** *Apis mellifera* Filamentous Virus (*Am*FV). *Am*FV showed a 551 bp band in ten hives, 1-4, 6-8 Positive, and 5 Negative. **B:** Kashmir Bee Virus (KBV), KBV did not show a band in any of the hives. **C:** Sacbrood Virus (SBV), SBV showed a 342 bp band in ten hives, 1,2,4-8 Positive, and 3 Negative. **D:** Israeli Acute Bee Paralysis Virus (IAPV), IAPV showed a 767 bp band in the hive, 1 Positive, 2-8 Negative.

Initially, *Am*FV was considered a weak pathogen for bees; however, some studies have suggested that this virus might weaken bees to some extent, making them more susceptible to other pathogens (Yang et al., 2022). A PCR-based study conducted in the United States (US) and Switzerland reported that *Am*FV was detected in 64% of Swiss and 100% of U.S. colony samples (Hartmann et al., 2015). No correlation was found between the presence and prevalence of *Am*FV and *Varroa destructor*, suggesting that *Varroa* is not a significant factor in the prevalence and spread of *Am*FV (Gauthier et al., 2015). In our study, the presence of *Am*FV in Ağrı province was detected, with a prevalence of 91% (Fig 3A). These findings align with previous studies reporting a high prevalence of *Am*FV in Ağrı province.

Kashmir Bee Virus is an important viral pathogen that can cause significant colony losses in honeybees. The virus is widespread in different regions of the world, and various studies have been conducted on its prevalence and effects. In Türkiye, studies by Tozkar et al. (2015), Rüstemoğlu & Sipahioğlu (2016), Çağırgan (2018), and Kalaycı et al. (2020) reported that they could not detect KBV, while Aydın (2020) identified KBV presence in 1.1% of the samples. Additionally, a study conducted in India by Ram et al. (2024) reported that KBV infection rates ranged from 5.71% to 28.33%, exemplifying the virus‘s prevalence in a country geographically close to Türkiye. In our study, similar to the findings of other researchers, KBV was not detected (Fig 3B)

Sacbrood Bee Virus is a widespread disease affecting honeybees worldwide. Prevalence studies conducted both in Türkiye and globally highlight the extent and impact of SBV. Globally, SBV has been reported to affect 15% of *Apis mellifera* colonies (Rao et al., 2015). Studies conducted in Türkiye reveal that SBV is widespread across the country. In 2017, the presence of SBV was detected in Türkiye, and phylogenetic analyses revealed that Turkish isolates formed a new genotype (Kalaycı et al., 2020). A large-scale study conducted in Türkiye found an SBV prevalence of 49.7% in samples from 400 beekeeping operations across 40 provinces (Akpınar et al., 2024a). As a result, it is evident that SBV is widespread both globally and in Türkiye and poses a significant threat to bee health. In our study, the infection rate of SBV in the samples collected from Ağrı province was relatively high, at 91% (Fig 3C).

Israeli Acute Paralysis Virus is a widespread RNA virus found in honeybee colonies worldwide. Studies have shown that this virus is particularly associated with Colony Collapse Disorder cases in the US. IAPV can affect all life stages of bees and cause systemic infection. The virus is transmitted by *Varroa destructor* mites and weakens the immune system, increasing the severity of the infection. The presence of the virus has been reported in various countries (Kukielka & Rodríguez, 2010; Formato et al., 2011; Akpınar et al., 2024a). In studies conducted in Türkiye, Tozkar et al. (2015) and Çağırgan (2018) reported no positivity. However,

Kalaycı et al. (2020) found a 6.5% prevalence, and Özkırım & Schiesser (2013) reported virus presence in 10 out of 71 beekeeping operations. In our study, the presence of IAPV was detected at a rate of 9.1% (Fig 3D). Prevalence studies related to IAPV highlight the spread of the virus between honeybees and *Varroa* mites.

Varroa destructor Virus-1 is a variant of the DWV complex and is closely associated with the *Varroa destructor* mites, which act as vectors for the virus. Studies have shown that the prevalence of VDV-1 is widespread in areas with *Varroa* mite infestations, and these infections contribute to the intensification of colony collapse in honey bee populations. In a study conducted in the UK, a high prevalence of VDV-1 was detected in honey bee populations infected with DWV-A simultaneously. These infections negatively affect honey bees' health, contributing to global bee losses (Ryabov et al., 2014). Additionally, interspecies transmission cases have been reported between honey bees and wild bees (e.g., species of wild bees like *Bombus pascuorum*), which further increases the ecological impact of the virus. Genetic analyses have shown that VDV-1 is typically found in mixed infections with DWV variants, which may influence the pathogenicity and spread of the virus. The presence and impact of VDV-1 are considered significant factors threatening bee health. In 2016, a study in 603 hives in the US found VDV-1 at a prevalence of 66.0%, making it the second most common virus in honey bees after DWV (Ryabov et al., 2017). The prevalences of DWV and VDV-1 are parallel, but there is limited information on the prevalence of VDV-1. While it was initially identified as VDV-1, it was later referred to as DWV-B or genotype B in subsequent years (Paxton et al., 2022). There is a lack of information regarding its potential role in colony losses due to viral diseases. Paxton et al. (2022) reported that DWV is one of the main factors in colony collapse, and VDV-1 has started to replace DWV in Europe. This is because VDV-1 is a recombinant form of DWV, which may further increase the severity of the disease and colony collapse. In our study, the prevalence of VDV-1 was 18.2% (Fig 4A). The lower prevalence compared to other studies is attributed to the absence of *Varroa destructor*.

Nosemosis is a disease caused by the microsporidian parasites *N. apis* and *N. ceranae*, resulting in significant losses in honey bee populations worldwide. *N. ceranae* is more widespread and pathogenic compared to *N. apis*. Research conducted in Europe has shown that *N. ceranae* infections are more common than *N. apis* infections, with mixed infections also present. This situation leads to high mortality rates, particularly in colonies (Mazur & Gajda, 2022). Studies conducted in Türkiye have reported that the prevalence of nosemosis ranges from 0% to 100% (Balkaya et al., 2016a; Akpınar et al., 2024b; Bozdeveci et al., 2024). In a study conducted in Türkiye from 2009 to 2016, the prevalence of *N. ceranae* was found to be between 26.8% and 72.6%, while *N. apis* ranged from 8.8% to 29.2%, with mixed infection rates at 18.49% (Kutlu & Kaftanoğlu, 1990; Şimşek et al., 2001; Balkaya et al., 2016b). *Nosema* infections typically peak in the spring and autumn. For instance, in a study conducted in the Ankara region of Türkiye, *Nosema* spp. infections were found to be at 15% in the summer months, with most infections concentrated in the northern and central regions. In our study, no *N. apis* was detected, while *N. ceranae* was found to be present at a rate of 27.3% (Fig 4B). In studies conducted in Türkiye, the prevalence of *Varroa* spp. was reported to range from 6.2% to 100% (Bozdeveci et al., 2024; Gürler et. al, 2025). The damage caused by varroosis stems from both the direct effects of the mite itself and the diseases caused by the viruses it vectors. *Varroa* spp. was not detected in our study, likely due to the collection of samples after parasite control

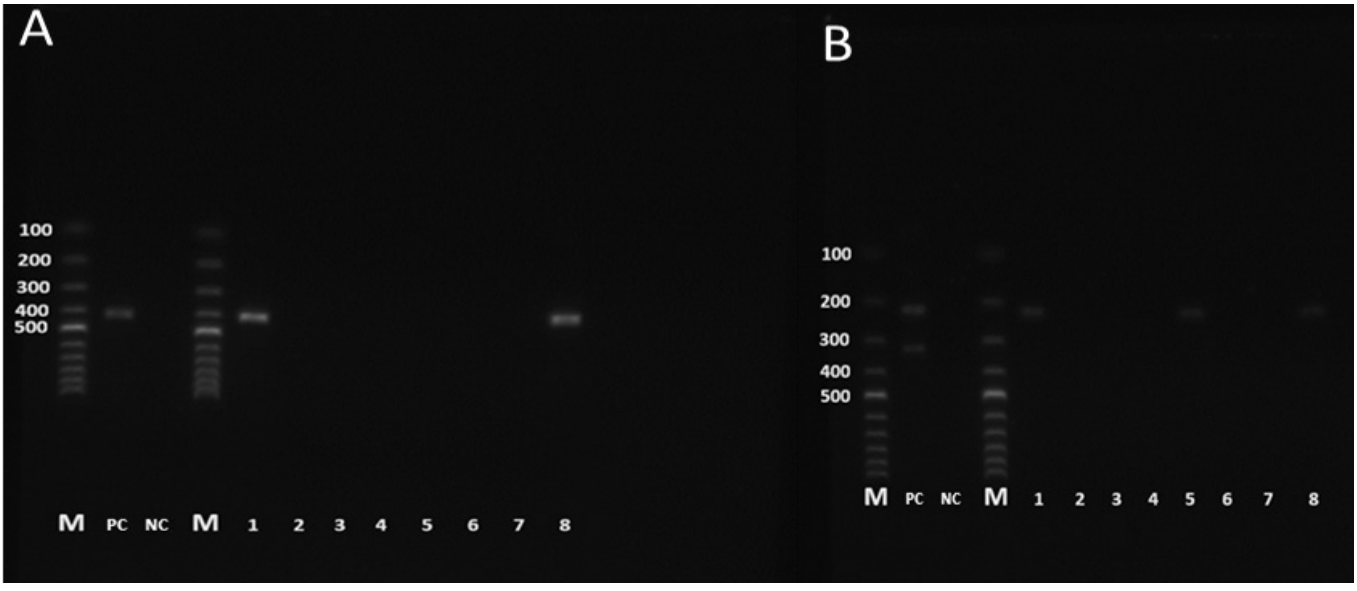

Fig 4. PCR gel images of honey bee samples obtained from One-step RT-PCR. PC; Positive control, NC; Negative control, M; marker. *PCR images are provided for only eight samples. **A:** *Varroa destructor* Virus-1 (VDV-1), VDV-1 showed a 413 bp band in 2 hives, 1,8-Positive, 2-7-Negative. **B:** *Nosema ceranae*. *Nosema ceranae* showed a 218-219 bp band in 3 hives, 1,5,8 Positive, 2-4,6,7 Negative.

measures during the summer season. It is well-known that this mite is responsible for colony losses in Türkiye and worldwide, and acts as a vector for most viral pathogens.

The analyses revealed the absence of CBPV, KBV, and AFB agents in the colonies. However, multiple infections were detected in the hives as follows: a quintuple infection (BQCV, DWV, *Am*FV, SBV, IAPV) in hive 1, a triple infection (BQCV, DWV, SBV) in hive 2, a septuple infection (ABPV, BQCV, DWV, *Am*FV, SBV, VDV-1, *Nosema*) in hive 3, a triple infection (BQCV, AmFV, SBV) in hive 4, a quintuple infection (BQCV, *Am*FV, SBV, VDV-1, *Nosema*) in hive 5, a triple infection (BQCV, *Am*FV, SBV) in hive 6, a triple infection (BQCV, DWV, *Am*FV) in Hive 7, a quadruple infection (BQCV, DWV, *Am*FV, SBV) in hive 8, a quadruple infection (BQCV, DWV, *Am*FV, SBV) in Hive 9, a quintuple infection (BQCV, DWV, *Am*FV, SBV, *Nosema*) in hive 10, and a quadruple infection (BQCV, DWV, *Am*FV, SBV) in hive 11. The distribution of diseases by hives is presented in Fig 5, while the number of hives affected by each disease is shown in Fig 6.

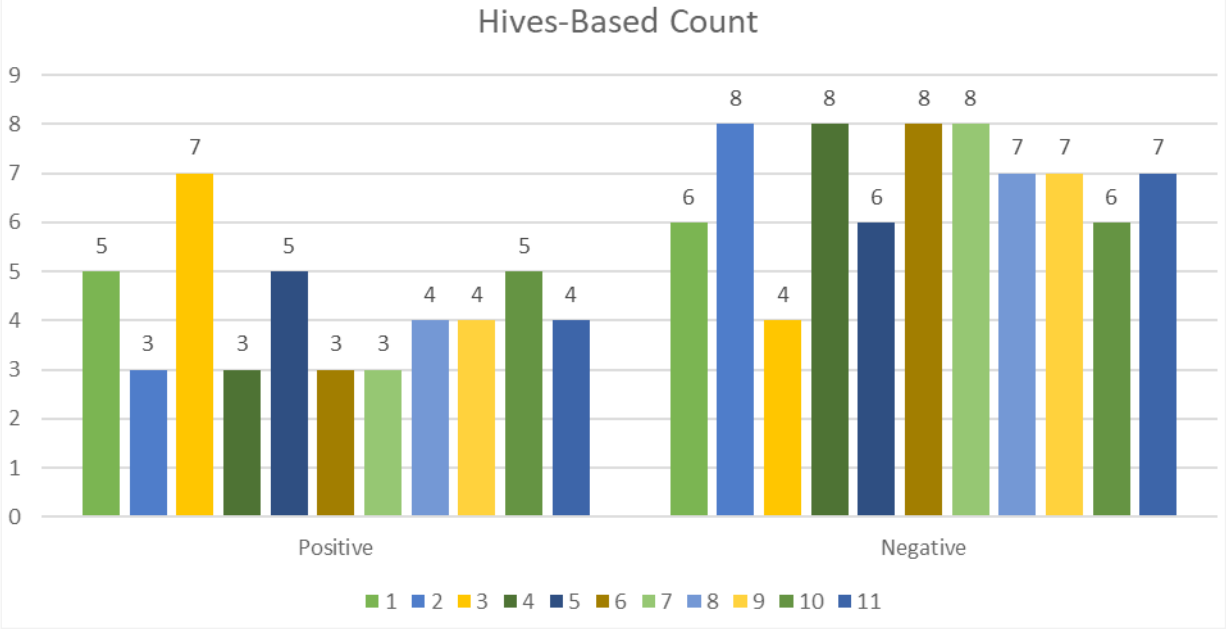

Fig 5. The number of diseases observed in hives.

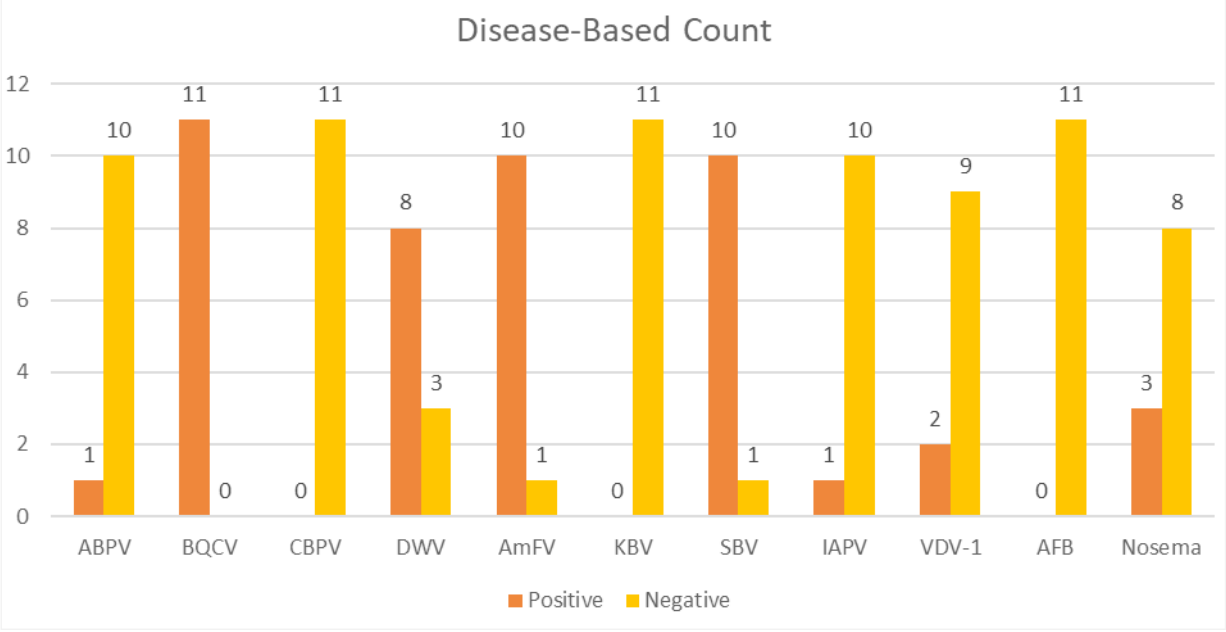

Fig 6. The number of diseases by hives.

Conclusion

This study has revealed the prevalence and interactions of bacterial, viral, and parasitic pathogens in honey bee colonies in Ağrı Province, Türkiye. The analyses detected no presence of *P. larvae* (AFB), CBPV, or KBV. However, multiple infections with different combinations were identified at the colony level. Notably, BQCV was the most common viral pathogen, with a prevalence of 100%. *Am*FV and SBV were detected at a rate of 90.9%, and DWV was found in 72.7% of the samples. Additionally, VDV-1 had a prevalence of 18.2%, and both ABPV and IAPV were detected in 9.1% of the samples. Regarding parasitic diseases, *N. apis* was not detected, whereas *N. ceranae* was present in 27.3% of the samples. Furthermore, no *Varroa destructor* mites were found in any of the colonies. The absence of AFB and some other pathogens may be attributed to factors such as the lack of migratory beekeepers in the area and geographic conditions that create a more isolated environment compared to areas where other beekeepers are present. The findings provide valuable insights for developing regional beekeeping management and disease control strategies.

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