jotaf **Journal of Tekirdag Agricultural Faculty**
Tekirdağ Ziraat Fakültesi Dergisi

Aralık/December 2024, 21(5)
Başvuru/Received: 15/01/24
Kabul/Accepted: 31/08/24
DOI: 10.33462/jotaf.1419608

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of The Prevalence of Honey Bee Diseases and Parasites in Samples from Sivas Province

Sivas İli Örneklerinde Bal Arısı Hastalıkları ve Parazitlerin Yaygınlığının Belirlenmesi

Arif BOZDEVECİ[1], Rahşan AKPINAR[2], Şengül ALPAY KARAOĞLU[3*]

Abstract

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), are the most important pollinators of agricultural products and plants in the natural environment. Honeybees are an important ecosystem component due to their role in nature and agricultural production. Bacterial, fungal, viral, and parasitic factors in bee farms are among the most important causes of honey bee colony losses. Honey bee diseases (bacterial, fungal and viral) and parasites are among the most important factors limiting beekeeping development and production efficiency in Türkiye. In addition to diseases caused by bacterial and fungal agents, diseases caused by viral agents are very diverse. Viruses, especially mixed infections, cause colony losses and are the most important factors in the decline of honey bee colonies. In this study the presence and prevalence of honey bee pathogens (*Varroa destructor*, *Nosema ceranae*, *Paenibacillus larvae*, and nine viruses) in suspicious samples with colony losses were investigated in Sivas province. For this purpose, microscopic, microbiological, and molecular methods were investigated on larvae and adult bee. The results showed that the most common viral pathogens in samples from Sivas province were Deformed Wing Virus (70%), *Apis mellifera* Filamentous Virus (60%), Black Queen Cell Virus (60%), Sacbrood Virus (55%) and *Varroa destructor* virus-1 (40%), respectively. In some samples, it was observed that there was a double (17.5%), triple (30%), quadruple (22.5%), or even quintuple (17.5%) association of viral agents. The viral infection/varroa coexistence rate was determined to be 50%. It was determined that 22.5% of the samples examined contained *Nosema* spores, while 12.5% were positive for *P. larvae*. Revealing the distribution of bee diseases will help beekeepers in disease-fighting and taking measures. This study showed the presence of the *Am*FV and the *Varroa destructor* virüs-1 in the Sivas province of Türkiye for the first time.

Keywords: *Apis mellifera* L., Honey bee pathogens, Virus, *Varroa destructor*, Sivas

[1]Arif Bozdeveci, Recep Tayyip Erdogan University, Rize, Türkiye. E-mail: arif.bozdeveci@erdogan.edu.tr OrcID: 0000-0002-0729-9143
[2]Rahşan Akpınar, Samsun Veterinary Control Institute, Samsun, Türkiye. E-mail: rahsankoc23@hotmail.com OrcID: 0000-0003-0075-9247
[3]***Sorumlu Yazar/Corresponding Author:** Şengül Alpay Karaoğlu Recep Tayyip Erdogan University, Rize, Türkiye . E-mail: sengul.karaoglu@erdogan.edu.tr OrcID: 0000-0003-1047-8350

Atıf: Bozdeveci, A., Akpınar, R., Karaoğlu, Ş. A. (2024). Sivas İli örneklerinde bal arısı hastalıkları ve parazitlerin yaygınlığının belirlenmesi. *Tekirdağ Ziraat Fakültesi Dergisi,* 21(5): 1148-1160.
Citation: Bozdeveci, A., Akpınar, R., Karaoğlu, Ş. A. (2024). Determination of the prevalence of honey bee diseases and parasites in samples from Sivas Province. *Journal of Tekirdag Agricultural Faculty,* 21(5): 1148-1160.

Öz

Bal arıları, *Apis mellifera* L. (Hymenoptera: Apidae), doğal ortamdaki tarım ürünleri ve bitkilerin en önemli tozlayıcılarıdır. Bal arıları doğadaki ve tarımsal üretimdeki bu rolleri nedeniyle ekosistemin önemli bir bileşenidir. Arı çiftliklerinde bakteriyel, fungal, viral ve paraziter faktörler bal arısı koloni kayıplarının en önemli nedenleri arasında yer almaktadır. Bal arısı hastalıkları (bakteriyel, fungal ve viral) ve parazitleri Türkiye'de arıcılığın gelişmesini ve üretim verimliliğini sınırlayan en önemli faktörler arasında yer almaktadır. Bakteriyel ve fungal etkenlerin neden olduğu hastalıkların yanı sıra viral etkenlerin neden olduğu hastalıklar da oldukça çeşitlidir. Virüsler, özellikle de karışık enfeksiyonlar koloni kayıplarına neden olur ve bal arısı kolonilerinin azalmasındaki en önemli faktörlerdir. Bu çalışmada Sivas ilinde koloni kayıpları olan şüpheli örneklerde bal arısı patojenlerinin (*Nosema ceranae*, *Varroa destructor*, *Paenibacillus larvae* ve dokuz virüs) varlığı ve yaygınlığı araştırılmıştır. Bu amaçla larva ve ergin arılarda mikroskobik, mikrobiyolojik ve moleküler yöntemlerle araştırmalar yapılmıştır. Sonuçlar Sivas ilinden alınan örneklerde en sık görülen viral patojenlerin sırasıyla Deformed Wing Virus (%70), *Apis mellifera* filamentous virus (%60), Black Queen Cell Virus (%60), Sacbrood Virus (%55) ve *Varroa destructor* virus-1 (%40) olduğunu göstermiştir. Bazı örneklerde viral etkenlerin ikili (%17.5), üçlü (%30), dörtlü (%22.5) ve hatta beşli (%17.5) birlikteliği olduğu görülmüştür. Viral enfeksiyon/varroa birlikteliği oranı %50 olarak belirlenmiştir. İncelenen örneklerde *Nosema* spor varlığı %22.5, *P. larvae* pozitiflik oranı ise %12.5 olduğu belirlenmiştir. Arı hastalıkları dağılımının ortaya çıkarılması arıcılara hastalıklarla mücadele ve önlem alma konusunda yardımcı olacaktır. Bu çalışma ile Türkiye'nin Sivas ilinde ilk kez *Am*FV ve *Varroa destructor*-1 virüsünün varlığını gösterilmiştir.

Anahtar Kelimeler: *Apis mellifera* L., Bal arısı patojenleri, Virus, *Varroa destructor*, Sivas

1. Introduction

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), are the most important pollinators of agricultural products and plants in the natural environment. Worker bees, which constitute most of the honey bee population, produce a wide variety of products with high economic value, such as honey, pollen, propolis, royal jelly, bee venom, and beeswax. However, parasites and diseases seen in honey bees negatively affect the development and progress of beekeeping activities. Many pathogens and parasites that affect honey bees also have a worldwide distribution. These pathogens (bacteria, fungi, and viruses) and parasites can be found alone or in groups (Steinhauer et al., 2018; Beaurepaire et al., 2020; Nanetti et al., 2021). Nosemosis and varroosis, common and harmful infectious diseases in honey bee and larvae, play an important role in low honey yield (Salkova and Gurgulova, 2022). Varroosis is one of the diseases that directly or indirectly causes bee loss in beekeeping. Varroosis is so common that it has been reported in almost every part of the world today (Doğanay and Aydın, 2017). *Varroa destructor* causes loss of development by sucking hemolymph in the larval and pupal stages of the honey bee. In addition to causing an average 7% weight loss in the pupa, varroa can act as a biological vector for many viruses and transmit viral agents horizontally and vertically. Nosemosis is one of the most important and common diseases of beekeeping, seen in the digestive system of adult honey bees and causing both colony losses and low honey yield. According to new studies, Nosemosis disease is of fungal origin and is caused by two species, *Nosema apis*, and *N. ceranae*, which belong to the *Nosema* genus in Microspora (Grupe and Quandt, 2020). Many factors play a role in the spread of nosemosis, such as climatic conditions, inadequate care conditions, diseases such as amoebiasis, and insufficient nutritional supplements during the winter (Mayack and Hakanoğlu, 2022). American Foulbrood (AFB) disease is a contagious infection that causes severe losses to the larvae of the honey bee *Apis mellifera* L. and other *Apis* species. *Paenibacillus larvae*, the causative agent of AFB, is a Gram-positive, dangerous, and contagious bacterium whose spores are highly resistant to adverse environmental conditions (Pernal and Clay, 2013). The spore form germinates in honey bee larvae and causes disease. The spore form, which is pathogenic for larvae, does not cause pathogenicity in adult bees. The spore form is highly resistant to environmental conditions, heat, and chemicals. AFB disease occurs after germinating spores in the midgut due to hatched larvae consuming food contaminated with bacterial spores, leading to septicemia (Chioveanu et al., 2004).

One of the problems in beekeeping is symptomatic and asymptomatic infections caused by viral disease. Bee viruses can be transmitted to bees horizontally, vertically, or via vectors (Beaurepaire et al., 2020). *Varroa destructor* is a biological or mechanical vector for many bee viruses and is accepted as an ectoparasite of honey bees in vector-mediated transmission (Yañez et al., 2020). It is known that there is a close mutualistic relationship, especially between the Deformed Wing Virus (DWV) and *Varroa* mite, and even for a long time, the clinical symptoms of DWV were mistakenly associated with *Varroa destructor* (Gebremedhn et al., 2020). Bee viruses can cause wing deformations, loss of body hair, paralysis, tremors, developmental disorders in larvae, the short lifespan of bees, or death in different life forms in honey bees. While most viruses known to exist in honeybees, showing symptomatic or asymptomatic symptoms, carry RNA genetic material, a small portion carry genetic material in DNA. The research on viral infections in honey bees has focused chiefly on RNA viruses (Beaurepaire et al., 2020). Acute Bee Paralysis Virus (ABPV), *Varroa destructor* virus-1 (VDV1), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), Sacbrood Virus (SBV), and Israeli Acute Paralysis Virus (IAPV) are some of the most studied honeybee RNA viruses (Beaurepaire et al., 2020). *Apis mellifera* Filamentous Virus (*Am*FV) is a double-stranded DNA virus that infects honey bees. *Am*FV is thought to be the most common and least harmful bee virus (Bailey, 1982).

The study aimed to identify the presence and distribution of parasitic and disease-causing pathogens in honeycomb, adult bees, and larvae samples taken from suspicious beehives in different districts of Sivas province. The study determined the distribution of honey bee disease pathogens and parasites and the viral factor-induced multiple infection rates.

2. Materials and Methods

2.1. Collection of suspicious samples and microscopic diagnosis of parasitic diseases

The study was conducted with suspected samples from Sivas province, where bee enterprises are practiced

(*Figure 1*). Suspicious samples were taken from the hives in the bee enterprises. Samples from different hives (honeycomb and adult bees) were examined for *Varroa destructor* positivity and *Nosema ceranae* positivity in adult bees. *V. destructor* identification was carried out according to the instructions described in the OIE Terrestrial Manual (OIE, 2021). Morphological identification of *V. destructor* was performed by detection with the alcohol washing method (Oliver, 2020). Both larval and adult bee samples were examined to investigate the presence of varroosis in the hives. All pupae, larvae, and honeybee samples collected from each hive were examined separately, and all containers were placed at -20°C overnight, and the surviving bees were allowed to die or dorm. The container with bees was poured into a petri dish, and 50-500 adult bees were examined under the stereomicroscope for *Varroa* spp., presence. The spaces between the abdominal segments and under the wings of all bees were especially checked. *V. destructor* samples were first examined morphologically. All positive samples were stored at -20°C (Doğanay and Aydın, 2017; OIE, 2021). The abdomens of 20 bees were separated and crushed in 3 mL of SF (serum physiologic) to diagnose nosemosis. For direct examination, approximately 3 drops of the resulting suspension were taken and placed on a microscope slide, a coverslip was applied, and the spores were examined under a light microscope (400X magnification). Samples found positive for *Nosema* spores were photographed (Tel et al., 2021).

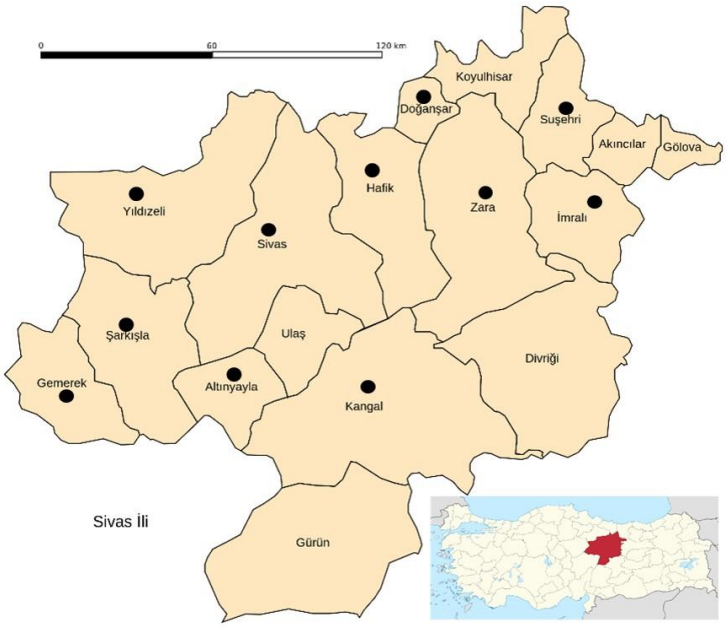

Figure 1. Districts where suspicious samples were collected in Sivas province

2.2. Investigation of American Foulbrood disease by culture method

In the diagnosis of AFB, 3-5 honey bee larvae selected from each comb were suspended in 9 ml of PBS (phosphate-buffered salt solution). Approximately 15 adult bee samples were transferred to a cryotube in 3 ml PBS and disrupted in a homogenizer (Bead Ruptor Elite, Bead Mill Homogenizer, USA). All prepared solutions were heated in a water bath at 80°C for 10 minutes to eliminate vegetative bacteria. The basic honeycomb sample weighing 1.5 grams was dissolved in 10 ml chloroform. Two millilitres of this solution were mixed with six millilitres of physiological saline. Following this, a direct inoculation of 100 microliters solution was carried out. For the isolation of *Paenibacillus larvae*, Brain Heart Infusion agar (BHIA, Thiamine added), Colombia sheep blood agar (CSA 5% sterile defibrinated sheep blood, Nalidixic acid/Pipedimic acid added) and MYPGP agar (Mueller-Hinton broth, yeast extract, K_2HPO_4, glucose, sodium pyruvate, agar and nalidixic acid/pipedimic acid added) were used. All cultured media were incubated at 37°C in an incubator with 5-7% CO_2 for 3-5 days. Petri dishes on which no microorganism growth was observed were incubated under the same conditions for one week. Gram and nigrosin staining were performed and examined under a light microscope to identify the observed microorganism colonies. Additionally, catalase testing was performed on bacterial cultures for diagnosis (OIE, 2018).

2.3. Total Nucleic Acid Extraction and One-step RT-PCR Analysis

Adult bees and larvae taken from each hive were homogenized separately. Fifteen larvae samples from the same hive were transferred to 7 mL cryotubes, and 3 mL of PBS (Sigma, 806544, USA) was added. Larval and adult bee samples were homogenized in an automatic homogenization device (Bead Mill Homogenizer SKU 19-042E, OMNI International, USA). After homogenization, the samples were centrifuged at 4000 rpm at 4 °C for 15 min, and the supernatants were collected. The supernatant was used for RNA extraction. RNA extraction was performed using a commercial kit (High Pure Viral Nucleic Acid Kit, Roche, Germany) according to the company's protocol.

RNA extracts obtained from larval and adult bee samples were tested separately with One-step RT-PCR for nine viruses that are thought to cause honey bee diseases. The list of primers used for each disease is given in *Table 1*. Reaction mixtures and thermal conditions were applied the same for one-step RT-PCR testing in all viral diseases. The one-step RT-PCR method specified by Chen et al. (2006) was used for this purpose. A commercial kit (RT-PCR Kit, QIAGEN, Germany) was used for the one-step RT-PCR assay. The reaction mixture was prepared by adding 12.5 µL of RT-PCR Master Mix, 0.8 µL of each of 10 pmol of forward and reverse primers, 0.25 µL of RT Mix, and 5.65 µL of ultrapure water. By adding 5 µL of the suspicious RNA sample, the total reaction volume became 25 µL. The reaction mixtures were transferred to the Thermal Cycler device (T100 Thermal Cycler, BIO-RAD, Singapore). Thermal conditions were applied in the Thermal Cycler, so RT and PCR steps were performed as one. It was kept at 50 °C for 30 minutes to convert the RNAs into cDNA and then at 95 °C for 15 minutes to inactivate the reverse transcriptase enzyme. Following this process, 60 seconds at 94 °C for pre-denaturation, 60 seconds at 55 °C for annealing, and 60 seconds at 72 °C for extension were applied in 40 repetitions. Finally, the reaction was terminated by a final extension at 72 °C for 10 minutes. After the PCR reaction, PCR samples were run on a 2% agarose gel containing ethidium bromide for 45 minutes at 75 Volts and visualized under UV light. Positive virus DNA samples and accession numbers used in the study, respectively, ABPV (OP504103), BQCV (OK345070), DWV (OP504101), *Am*FV (OP642035), CBPV (EU122229), KBV (OP504105), SBV (OP504108), IAPV (OP504110), and VDV1 (OP504112). These positive virus DNAs were used as positive controls in PCR.

Table 1. Primers used in this study

Viruses *	Primer sequences	(bp)	References
ABPV	ABPV-F: 5'- CATATTGGCGAGCCACTATG -3' ABPV-R: 5'-- CCACTCCACACAACTATCG3'	398	(Benjeddou et al., 2001)
IAPV	IAPV-F: 5'-CGATGAACAACGGAAGGTTT-3' IAPV-R: 5'-ATCGGCTAAGGGGTTTGTTT-3'	767	(Palacios et al., 2008)
KBV	KBV-F: 5'-GATGAACGTCGACCTATTGA-3' KBV-R: 5 -TGTGGGTTGGCTATGAGTCA-3'	415	(Stoltz et al., 1995)
BQCV	BQCV-F: 5'-CTTTATCGAGGAGGAGTTCGAGT-3' BQCV-R: 5'-GCAATAGATAAAGTGAGCCCTCC-3'	536	(Sguazza et al., 2013)
DWV	DWV-F: 5'-TGGTCAATTACAAGCTACTTGG -3' DWV-R: 5'-TAGTTGGACCAGTAGCACTCAT -3'	269	(Sguazza et al., 2013)
SBV	SBV- F: 5'-CGTAATTGCGGAGTGGAAAAGATT-3' SBV- R: 5'-AGATTCCTTCGAGGGGTACCCTCATC-3'	342	(Sguazza et al., 2013)
CBPV	CBPV-F: 5'- AACCTGCCTCAACACAGGCAAC-3' CBPV-R: 5'- ACATCTCTTCTTCGGTGTCAGC-3'	774	(Sguazza et al., 2013)
*Am*FV-112	AmFV-F:5'-CAGAGAATTCGGTTTTTGTGAGTG -3' AmFV-R:5'-CATGGTGGCCAAGTCTTGCT-3'	551	(Gauthier et al., 2015)
VDV1	VDV-1 F:5'- GCCCTGTTCAAGAACATG-3' VDV-1 R:5'-CTTTTCTAATTCAACTTCACC-3'	430	(Gauthier et al., 2011)

* 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-112: *Apis mellifera* Filamentous Virus, VDV1: *Varroa destructor* Virus-1

3. Results and Discussion

3.1. Determination of Varroosis, Nosemosis, and American Foulbrood

Türkiye has a rich plant diversity due to its geographical structure and climatic conditions, and beekeeping activities are carried out in many regions. Sivas province is among the provinces with the highest honey production in Turkey due to its climate and flora (Yüzbaşıoğlu, 2022). In Sivas province, the beekeeping activity lasts shorter than in other regions due to the extended winter season, and the flowering periods of the plants differ according to the regions. Although temperature conditions shorten the duration of beekeeping activities, non-sweltering summer temperatures create favorable conditions for this activity (Koç et al., 2020). Diseases encountered in beekeeping, such as viruses, bacteria, *Nosema*, and *Varroa*, affect adult honey bees and their larvae, causing low honey productivity, colony losses, and serious economic losses globally (Bordin et al., 2022).

Larval honeycomb and adult bee samples sent to the Samsun Veterinary Control Institute from Sivas province between 2020 and 2023 were examined. The research was carried out in the Sivas center and eight districts. The study investigated the presence and spread of *N. ceranae*, *P. larvae* spores and *V. destructor* in honey bees and suspected larvae. The results showed that *N. ceranae* spores were present in the preparations, while *V. destructor* was present in the bees, as shown in *Figure 2*. The study found that *Varroa* mite was quite common in the samples examined, with a 50% prevalence rate, whereas the presence of *Nosema* spores was observed to be less frequent, with a prevalence rate of 22.5%.

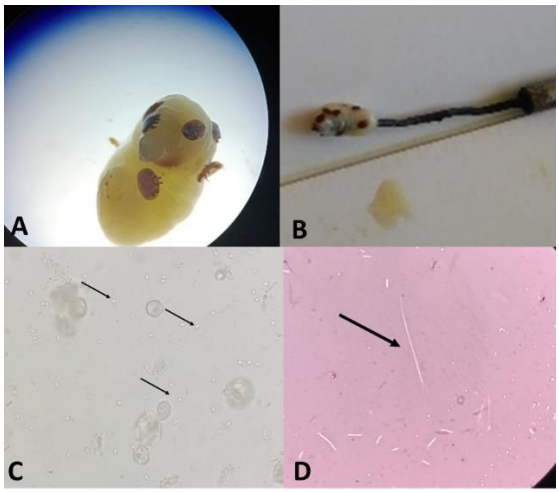

Figure 2. A-B; V. destructor on honey bee larvae, C: Nosema ceranae spores, D: Paenibacillus larvae nigrosin staining zigzag positive image

In a study conducted in Bulgaria in 2020-2021, *Varroa destructor* was investigated in honey bee samples, which were examined by light microscopy. The results revealed that 32.43% of the bees were infected with *V. destructor* (Salkova and Gurgulova, 2022). In the study by Yilmaz et al. (2018), where they examined the frequency of *V. destructor* in hives in the autumn period in the Eastern Black Sea region, the highest incidence of *V. destructor* was observed in Rize province (47.7%), while the lowest incidence was reported to be in Bayburt province (20%). Köseman et al. (2017) reported that varroosis is the most common honey bee disease in Malatya (47.8%). Karapınar et al. (2018) reported that the suspicious samples from Van province that were examined morphologically, were found to be *V. destructor*, the prevalence 89%. In our study, *V. destructor* prevalence was determined to be 50% in microscopic and morphological examination of samples from Sivas province. The discrepancies observed in studies conducted in different regions of Türkiye are attributed to the variation in climate and seasonal timings. Determining the prevalence of *Varroa* mite is crucial as it is a vector for bee viruses, acting as an ectoparasite. *V. destructor* is one of the reasons for the morphological damage it causes to adult bees and larvae, as well as the spread of disease agents in hives. In a study conducted in Bulgaria, it was observed that 25.40% of the honey bee samples were contaminated with *N. ceranae* spores. It was determined that 32.43% of the Bulgarian samples were infected with both *Varroa* mite and *Nosema* spores. Negative samples had the lowest value, with 9.74% (Salkova and Gurgulova, 2022). In a study conducted in the South Marmara region, it was reported that none of the colonies sampled were infected with *N. apis*, and *N. ceranae*

was found in 64.3% of all sampled colonies (Mayack and Hakanoğlu, 2022). In a study conducted in Central Anatolia and Mediterranean regions, the rate of *N. ceranae* was reported as 46% (Avcı et al., 2022). In a survey conducted in the Muğla provinces, only the *N. ceranae* species was detected, while the prevalence of nosemosis was reported as 71.53% (Kartal et al., 2021). It is thought that the high disease intensity in Siirt, Şanlıurfa (43.8%) (Tel et al., 2021), Muğla (71.53%) (Kartal et al., 2021), and Ordu (44%) (Yaman et al., 2015) provinces may be due to climate differences and bee population density. According to Özgör et al. (2015), it was reported that temperature and humidity values directly affect *Nosema* species and density in apiaries. The spores of *N. ceranae* increased where these two factors increased, and the density of *Nosema apis* increased where these two factors decreased. The reason for the difference in disease prevalence between regions may be the differences in climatic conditions. In our study, the nosemosis infection rate was found as 22.5%. Our result was not as high as the prevalence in Muğla and Ordu provinces. The reason for that Sivas province has cold and harsh winters, and there is abundant snowfall in the winter months. In fungal infections, humidity and other conditions may also affect nosemosis intensity. Although the long winter season in Sivas province shortens the duration of beekeeping activities, the summer temperature values create suitable conditions for this activity.

In our study, *P. larvae*-positive samples were determined by cultural analysis of suspicious samples from Sivas province. The samples sent to investigate the positivity of American Foulbrood (AFB) disease agent *Paenibacillus larvae* were analyzed. Clinical observation revealed that the larvae had concave and punctured lids, and the color of the larvae changed from beige to brown. In the matchstick test, when a needle was inserted into the larval remains and removed from the cell, it was observed that the larvae elongated in threads, which is known as AFB symptoms. Microscopically, a spiralling structure typical of AFB was observed in the nigrosin staining preparation (*Figure 2*). In culture examination, small, regular, sometimes rough, and beige-colored *P. larvae* colonies were observed on MYPGP agar medium. *P. larvae* could not be detected in any of the basic comb samples received. In the cultural analysis of larval combs and adult bees, a 12.5% spread was determined (*Table 2*). It was determined that the samples with positive AFB culture came from the centre, Yıldızeli, and Kangal districts. Among the samples examined in the Northwest Pakistan region, the prevalence of AFB-positive samples was in the Kohat region, with the highest positivity rate of 39%, followed by Bannu at 37% and Karak region at 34.5% (Anjum et al., 2015). Şık et al. (2022) reported 30.7% *P. larvae* positivity in their study of suspected AFB samples from Ankara Etlik Veterinary Control Central Research Institute between 2015 and 2020. In a study conducted in the South Marmara region, AFB was prevalent in 31.3% (Mayack and Hakanoğlu, 2022). Another study conducted in the Central and Eastern Black Sea region reported a prevalence of 4.38% for AFB (Akpınar et al., 2023). In our study, it was observed that *P. larvae* positivity was less than the results in other studies (Mayack and Hakanoğlu, 2022; Şık et al., 2023; Akpınar et al., 2023).

3.2. Determination of viral agents by One-step RT-PCR method

Between 2020 and 2023, suspicious larval honeycomb and adult bee samples in Sivas province were sent to the Samsun Veterinary Control Institute to be examined for the presence of the virus. For this purpose, the larval and adult bee samples were homogenized and used for RNA isolation. The resulting RNA material was converted into cDNA to use in RT-PCR. PCR products resulting from RT-PCR were visualized on a 2% gel. The presence of PCR products of different sizes obtained for nine viruses was evaluated as positive (*Table 1, Figure 3*).

Tentcheva et al. (2004) reported that the virus prevalence was higher in adult bees than in larvae. Chronic Bee Paralysis Virus (CBPV) prevalence was 28% in adult bees but could not be detected in larvae. In a study conducted in Croatia in 2014, the frequency of CBPV was reported to be very low at 9.75% (Gajger et al., 2014). Other European survey studies observed that the prevalence of CBPV was relatively low. Gümüşova et al. (2010), reported a study in the Black Sea region and a prevalence of 25% of CBPV, while Çağırgan and Yazıcı (2021), conducted a study in the Aegean Region and reported a prevalence of 1.8%. The results showed that the virus with the highest prevalence was Deformed Wing Virus (DWV) (19.8%), followed by Black Queen Cell Virus (BQCV) (18%), Acute Bee Paralysis Virus (ABPV) (3.6%), and Sacbrood Virus (SBV) (2.7%), respectively. Consistent with the study conducted in the Marmara region, it was reported that CPBV could not be detected (Mayack and Hakanoğlu, 2022). It was determined that CBPV, one of the nine viruses investigated in the suspicious samples in our study, could not be detected in any sample. Similar to our study's result, it was reported that CPBV could not be detected in the study conducted in the Marmara region (Mayack and Hakanoğlu,

2022). Although the values varied in studies conducted on the prevalence of CBPV, it was observed that the results were consistent with the results in our study. In the study conducted in Amasya and Elazığ provinces, the spread of three viruses, DWV (23.81%), ABPV (12.93%), and BQCV (10.20%), was revealed (Aydın and Oksal, 2023). In Bingöl province, SBV and BQCV infection rates were reported as 7.03% and 11.70%, respectively, but ABPV infection was not detected in any of the apiaries (Güller and Kurt, 2022). Avcı et al. (2022) showed that 90% of worker bees were infected with at least one virus. BQCV (90%) had the highest prevalence, followed by DWV and ABPV prevalence of 84% and 62%, respectively. In our study, the SBV and BQCV infection rates were relatively high in our samples from Sivas province, at 55% and 60%, respectively, while the ABPV infection rate was low at 7.5%.

The presence of honey bee viruses in different climatic regions of Argentina was evaluated and compared. In the study, it was reported that Kashmir Bee Virus (KBV) and Israeli Acute Paralysis Virus (IAPV) could not be detected, and five other viruses had different prevalence: DWV (35%), ABPV (21.5%), BQCV (8.0%), CBPV (2.2%) and SBV (1.1%). Approximately 25% of sampled colonies were found to have double and triple viral associations (Molineri et al., 2017). Other studies reported the presence of KBV in France and Denmark (Tentcheva et al., 2004), but KBV could not be detected in Austria, Croatia, and Hungary (Gajger et al., 2014). According to Tozkar et al. (2015), KBV was not detected in their studies conducted in Türkiye. Similarly, Çağırgan and Yazıcı (2021) reported that neither KBV nor IAPV were detected in larvae and honey bee samples collected from the Aegean region. In the study conducted in the Marmara region, the KBV rate was reported as 2.4% (Mayack and Hakanoğlu, 2022). The result in our study was determined as KBV 2.5% and IAPV 10%. When the studies were compared in general, the prevalence of KBV and IAPV was very low or could not be detected at all.

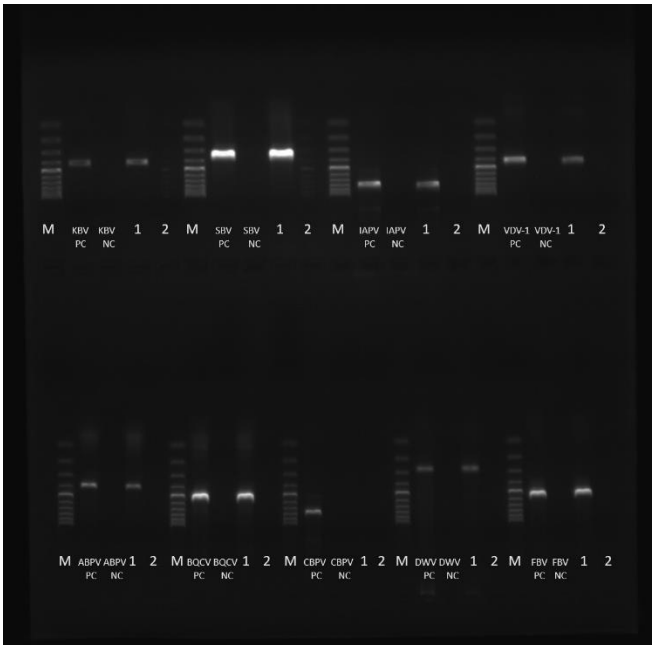

Figure 3. PCR gel images of viruses in One-step RT-PCR

PC; Positive control, NC; Negative control, M; marker, Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralyses Virus (CBPV), Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), Sacbrood Virus (SBV), Israeli Acute Paralysis Virus (IAPV), Apis mellifera Filamentous Virus (FV), Varroa destructor Virus-1 (VDV1)

The number of studies revealing the existence and spread of viral diseases of honey bees in Türkiye has increased rapidly in recent years. DWV and the closely related *Varroa destructor* virus-1 (VDV1) are the most common honeybee viruses. VDV1 was known to cause high rates of overwintering colony losses in Europe but was unknown in the USA. After developing new sequencing technology, VDV1 was identified in honeybee pupae in the USA. In 2016, VDV1 was found in 66.0% of 603 beehives in the USA; this makes VDV1 the second most common virus after DWV, found in 89.4% of colonies (Ryabov et al., 2017). DWV and VDV1 are related, but there is little information about the prevalence of VDV1. While it was known as VDV1 when first

identified, it was named DWV-B, or genotype B in the following years (Paxton et al., 2022). There is a knowledge gap about this virus, considering its potential role in the colony loss rates of virus diseases. Paxton et al. (2022) reported that DWV is one of the main factors in colony collapse, and VDV1 has started to replace the DWV virus in Europe. Since VDV1 is a recombinant form of DWV, it may further increase the severity of the disease and colony collapse. In our study, the prevalence of DWV and VDV1 in Sivas province was determined to be 70% and 40%, respectively. It is noteworthy that DWV prevalence is relatively high in suspected samples, while VDV1 prevalence is close to *Varroa* prevalence (50%). Considering that *V. destructor* is accepted as an ectoparasite vector for DWV and VDV1 (Paxton et al., 2022), it is an inevitable result that the prevalence values are similar.

Table 2. Percentage of positivity in suspicious samples from Sivas province by district

Locality	Sample	ABPV	BQCV	CPBV	DWV	KBV	SBV	IAPV	VDV1	*Am*FV	Var	Nos	AFB
Center	10	0	5	0	6	0	5	1	4	6	4	0	2
Yıldızeli	6	2	2	0	6	1	3	0	3	3	4	2	2
Zara	6	0	3	0	3	0	2	0	6	5	2	0	0
Gemerek	5	0	4	0	5	0	4	0	1	2	4	2	0
Kangal	4	0	4	0	1	0	3	1	1	2	2	3	1
İmranlı	3	0	3	0	2	0	3	1	0	2	0	1	0
Doğanşar	2	1	2	0	2	0	1	0	1	1	2	0	0
Altınyayla	1	0	0	0	1	0	0	0	0	0	1	0	0
Hafik	1	0	1	0	1	0	0	0	0	1	1	1	0
Şarkışla	1	0	0	0	1	0	1	0	0	1	0	0	0
Suşehri	1	0	0	0	0	0	0	1	0	1	0	0	0
Total	40	3	24	0	28	1	22	4	16	24	20	9	5
%	100	7,5	60	0	70	2,5	55	10	40	60	50	22,5	12.5

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, VDV1; Varroa destructor Virüs-1; AmFV: Apis mellifera Filamentous Virus, AFB; American Foulbrood, Nos; Nosema ceranae, Var; Varroa destructor

Apis mellifera filamentous virus (*Am*FV) is a double-stranded DNA virus that infects honey bees. The prevalence of *Am*FV in apiaries was found to vary between 10% and 80% in China (Hou et al., 2017), and that prevalence was 61% (19/31) in eight provinces of Argentina (Quintana et al., 2021). Although *Am*FV was considered a weak pathogen for honey bees, it has been proposed that it may weaken the bees to a certain degree, making them more susceptible to other pathogens (Yang et al., 2022). In a study comparing the prevalence of *Am*FV in the USA and Switzerland using PCR analysis, it was reported to be detected in 64% of Swiss colonies and 100% of US colonies (Hartmann et al., 2015). The presence and prevalence of *Am*FV were not associated with *V. destructor* in honey bee colonies, suggesting that *Varroa* is not an important factor in *Am*FV prevalence and transmission (Gauthier et al., 2015). In our study, the presence of *Am*FV was detected in all districts sampled in Sivas province (except Altınyayla), and its prevalence was determined to be 60%. *Am*FV prevalence (60%) was one of the two most common viruses after the DWV virus (70%). Similar to previous studies (Quintana et al., 2021), the prevalence of *Am*FV in Sivas province was determined to be high. In a study in Germany, the most common viruses found were BQCV (84%) and DFW-B (35%).

Honey bees in asymptomatic colonies were infected with an average of two different viruses, and simultaneous infection with four to six viruses was common (14%) (D'Alvise et al., 2019). In the multiple infection rates of honey bees in beekeeping enterprises in the Burdur region, the percentage of samples that were not infected at all was only 6.4% (Usta and Yildirim, 2022). In comparison, the share of positive samples infected with one virus was 38.7%, the rate of positive samples infected with two viruses was 29%, and the percentage of virus-infected samples with three viruses was 25.8%. As a result of our study, it was determined that the viruses were sometimes found alone and sometimes in multiple mixtures (double, triple, or quintuple). In our study, the percentage of samples with no viral infection was 7.5%, while the percentage of samples infected with a single virus was 5%. The percentages of double, triple, quadruple, and quintuple viral infections resulting in multiple viral infections were 17.5%, 30%, 22.5%, and 17.5%, respectively. These results were similar to those of other studies (D'Alvise et al., 2019; Usta and Yildirim, 2022)

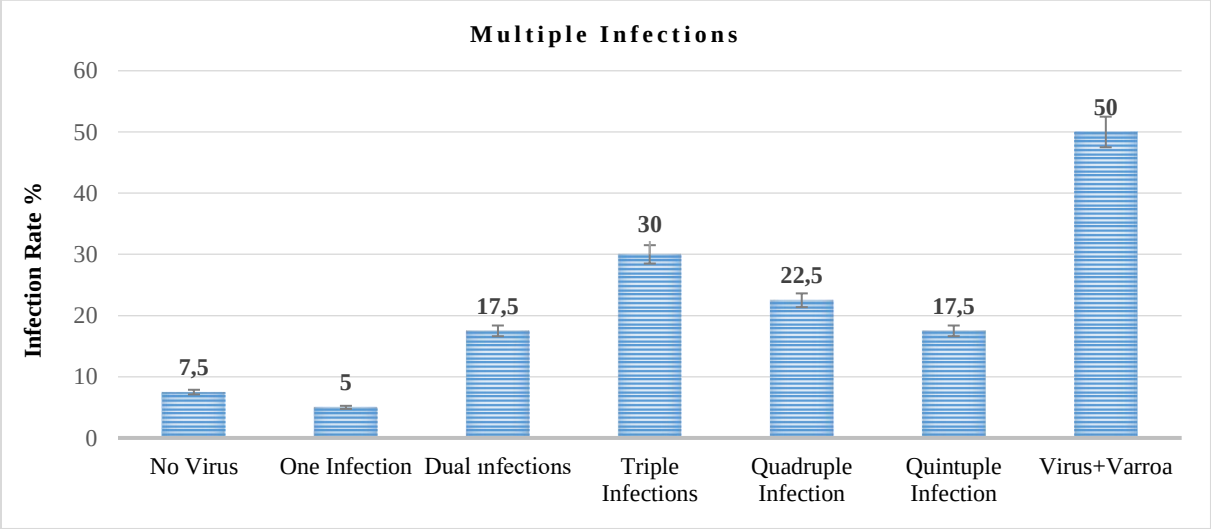

Figure 4. The multiple viral infection rates in suspicious samples from Sivas province

4. Conclusions

In conclusion, according to the study data, it has been concluded that *V. destructor* and *N. ceranae* are prevalent and dominant species in Sivas province, as in many other regions of Türkiye. Gaining insight into the dynamics of honeybee bacterial and virus infections can aid in mitigating their harmful impact on colonies. Developing a strategy to tackle *Varroa* mites and viral infections can help beekeepers prevent colony losses. Therefore, creating prevention and control measures for honey bee diseases and parasites that pose a global threat is crucial.

Acknowledgment

This work supported no grants from any funding agency or industry.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Karaoglu, S. A., Akpınar, R., Bozdeveci, A.; Design: Bozdeveci, A.; Data Collection or Processing: Akpınar, R., Bozdeveci, A.; Literature Search: Akpınar, R., Bozdeveci, A.; Writing, Review and Editing: Karaoglu, S. A., Akpınar, R., Bozdeveci, A.

References

Akpınar, R., Bozdeveci, A. and Çelik, S. (2023). Diagnosis of American Foulbrood in honey bees and honeycomb in the Central and Eastern Black Sea region. *Fırat University Veterinary Journal of Health Sciences*, 37(3): 245-250.

Anjum, S. I, Shah, A. H, Azim, M. K., Yousuf, M. J, Khan, S. and Khan, S. N. (2015). Prevalence of American Foulbrood disease of honeybee in North-West Pakistan. *Biotechnology & Biotechnological Equipment*, 29(4): 659-665.

Avcı, O., Oz, M. E. and Dogan M. (2022). Silent threat in honey bee colonies: infection dynamics and molecular epidemiological assessment of black queen cell virus in Turkey. *Archives of Virology*, 167: 1499–1508.

Aydın, S. and Oksal, H. (2023). Prevalence and molecular characterization of deformed wing, acute bee paralysis and black queen cell viruses ınfecting honey bees and varroa mites. *Journal of Apicultural Science*, 67(1):73-86.

Bailey, L. (1982). Viruses of honeybees. *Bee World*, 63(4): 165-173.

Beaurepaire, A., Piot, N., Doublet, V., Antunez, K., Campbell, E., Chantawannakul, P., Chejanovsky, N., Gajda, A., Heerman, M., Panziera, D., Smagghe, G., Yañez, O., de Miranda, J. R. and Dalmon, A. (2020). Diversity and global distribution of viruses of the western honey bee. *Apis mellifera*. *Insects*, 11(4): 239. https://doi.org/10.3390/insects11040239

Benjeddou, M., Leat, N., Allsopp, M. and Davison, S. (2001). Detection of Acute Bee Paralysis Virus and Black Queen Cell Virus from honeybees by reverse transcriptase PCR. *Applied and Environmental Microbiology*, 67(5): 2384–2387.

Bordin, F., Zulian, L., Granato, A., Caldon, M., Colamonico, R., Toson, M., Trevisan, L., Biasion, L. and Mutinelli, F. (2022). Presence of known and emerging honey bee pathogens in apiaries of Veneto Region (Northeast of Italy) during Spring 2020 and 2021. *Applied Science*, 12(4):2134.

Çağırgan, A. and Yazıcı, Z. (2021). The prevalance of seven crucial honeybee virüses using Multiplex RT-PCR and their phylogenetic analysis, *Turkish Journal of Veterinary & Animal Sciences*, 45(1): 44-55.

Chen, Y. P., Pettis, J. S., Collins, A. and Feldlaufer, M. F. (2006). Prevalence and transmission of honeybee viruses. *Applied and Environmental Microbiology*, 72(1): 606-611.

Chioveanu, G., Ionescu, D. and Mardare, A. (2004). Control of Nosemosis-treatment with Protofil. *Apiacta*, 39:31–38.

D'Alvise, P., Seeburger, V., Gihring, K., Kieboom, M. and Hasselmann, M. (2019). Seasonal dynamics and co-occurrence patterns of honey bee pathogens revealed by high-throughput RT-qPCR analysis. *Ecology and Evolution*, 9(18): 10241–10252.

Doğanay, A. and Aydın, L. (2017). Honey Bee, Breeding, Products, Health. 1st edition. Bursa Dora Yayınevi, Bursa, Türkiye. (In Turkish)

Gajger, I., Kolodziejek, J., Bakonyi, T. and Nowotny, N. (2014). Prevalence and distribution patterns of seven different honeybee viruses in diseased colonies: a case study from Croatia. *Apidologie*, 45: 701-706.

Gauthier, L., Ravallec, M., Tournaire, M., Cousserans, F., Bergoin, M., Dainat, B. and de Miranda, J. R. (2011). Viruses associated with ovarian degeneration in *Apis mellifera* L. queens. *PLoS ONE*, 6: e0016217.

Gauthier, L., Cornman, S., Hartmann, U., Cousserans, F., Evans, J. D., de Miranda, J. R. and Neumann, P. (2015). The *Apis mellifera* Filamentous Virus Genome. *Viruses*, 7(7): 3798–3815. https://doi.org/10.3390/v7072798

Gebremedhn, H., Deboutte, W., Schoonvaere, K., Demaeght, P., De Smet, L., Amssalu, B., Matthijnssens, J. and de Graaf, D. C. (2020). Metagenomic approach with the NetoVIR enrichment protocol reveals virus diversity within Ethiopian honey bees (*Apis mellifera simensis*). *Viruses*, 12: 1218.

Grupe, A. C II. and Quandt C.A. (2020). A growing pandemic: A review of Nosema parasites in globally distributed domesticated and native bees. *PLOS Pathogens*, 16(6): e1008580. https://doi.org/10.1371/journal.ppat.1008580

Güller, A. and Kurt, Z. (2022). Occurrence and molecular phylogeny of economically relevant viruses infecting honey bees (*Apis mellifera* L.) of Bingöl Province, Turkey. *Journal of Apicultural Science*, 66(1): 85-96.

Gümüşova, S., Albayrak, H., Yazıcı, Z. and Kurt, M. (2010). Prevalence of three honey bee viruses in Turkey. *Veterinarski Arhiv*, 80(6): 779-785.

Hartmann, U., Forsgren, E., Charrière, J. D., Neumann, P. and Gauthier L. (2015). Dynamics of *Apis mellifera* Filamentous Virus (*Am*FV) infections in honey bees and relationships with other parasites. *Viruses*, 7: 2654–2667.

Hou, C., Li, B., Deng, S., Chu, Y. and Diao, Q. (2017). Diagnosis and distribution of the *Apis mellifera* Filamentous Virus (*Am*FV) in honey bees (*Apis mellifera*) in China. *Insectes Sociaux*, 64(4): 597–603.

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

Kartal, S., İvgin Tunca, R., Özgül, O., Karabağ, K. and Koç, H. (2021). Microscopic and molecular detection of *Nosema* sp. in the Southwest Aegean Region. *Uludag Bee Journal*, 21(1): 8-20.

Koç, H., Ergün, A. and Kartal, F. (2020). Problems and solution suggestions of honey producers in Sivas province. *Journal of International Social Science Education*, 6(2): 327-362.

Köseman, A., Şeker, İ., Karlıdağ, S. and Aygen, S. (2017). Beekeeping Activities II: The evaluaton of beekeeping activities in terms of beekeeper preferences, production quality and bee diseases in Malatya. *Journal of Tekirdag Agricultural Faculty*, 14(2):54-63.

Mayack, C. and Hakanoğlu, H. (2022). Honey Bee pathogen prevalence and interactions within the Marmara region of Turkey. *Veterinary Sciences*, 9(10): 573.

Molineri, A. I., Pacini, A., Giacobino, A., Bulacio-Cagnolo, N., Aignasse, A., Zago, L., Fondevila, N., Ferrufino, C., Merke, J., Orellano, E., Bertozzi, E., Pietronave, H. and Signorini, M. L. (2017). Prevalence of honey bee (*Apis mellifera*) viruses in temperate and subtropical regions from Argentina. *Revista Argentina de Microbiología* 49(2): 166-173.

Nanetti, A., Bortolotti, L. and Cilia, G. (2021). Pathogens spillover from honey bees to other Arthropods. *Pathogens*, 10(8): 1044. https://doi.org/10.3390/pathogens10081044

OIE (2018). Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 3.2.2. American foulbrood of honey bees (infection of honey bees with *Paenibacillus larvae*: 719-735.

OIE (2021). Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 3.2.7. Varroosis of honey bees (infestation of honey bees with *Varroa* spp.), (version adapted in 2021): 1-14.

Oliver, R. (2020). Refining the mite wash: Part 3 dislodgement, precipitation, and separation. *American Bee Journal*, 160: 1013.

Özgör, E., Güzerin, E. and Keskin, N. (2015). Determination and comparison of *Nosema apis* and *Nosema ceranae* in terms of geographic and climatic factors. *Hacettepe Journal of Biology and Chemistry*, 43(1): 9-15.

Palacios, G., Hui, J., Quan, P. L., Kalkstein, A., Honkavuori, K. S., Bussetti, A. V., Conlan, S., Evans, J., Chen, Y. P., vanEngelsdorp, D., Efrat, H., Pettis, J., Cox-Foster, D., Holmes, E. C., Briese, T. and Lipkin, W.I. (2008). Genetic analysis of Israel Acute Paralysis Virus: distinct clusters are circulating in the United States. *Journal of Virology*, 82 (13): 620917.

Paxton, R. J., Schäfer, M. O., Nazzi, F., Zanni, V., Annoscia, D., Marroni, F., Bigot, D., Laws-Quinn, E. R., Panziera, D., Jenkins, C. and Shafiey, H. (2022). Epidemiology of a major honey bee pathogen, deformed wing virus: potential worldwide replacement of genotype A by genotype B. *International Journal for Parasitology: Parasites and Wildlife* 18: 157–171. https://doi.org/10.1016/j.ijppaw.2022.04.013

Pernal, S. F. and Clay, H. (2013). American Foulbrood. Honey Bee Diseases and Pests. 3rd ed. Beaverlodge, Alberta, Canada: Canadian Association of Professional Apiculturists; 5-10.

Quintana, S., Brasesco, C., Porrini, L. P., Di Gerónimo, V., Eguaras, M. J. and Maggi, M. (2021). First molecular detection of *Apis mellifera* filamentous virus (*Am*FV) in honey bees (*Apis mellifera*) in Argentina. *Journal of Apicultural Research*, 60(1):111-114.

Ryabov, E. V., Childers, A. K., Chen, Y., Madella, S., Nessa, A., van Engelsdorp, D. and Evans, J. (2017). Recent spread of *Varroa destructor* virus-1, a honey bee pathogen, in the United States. *Scientific Reports*, 7(1): 17447. https://doi.org/10.1038/s41598-017-17802-3

Salkova, D. and Gurgulova, K. (2022). Detection of *Varroa destructor* mite and *Nosema* spp. in bee samples from Bulgaria. *Bee Studies*, 14 (1): 21-26.

Sguazza, G. H., Reynaldi, F. J., Galosi, C. M. and Pecoraro, M.R. (2013). Simultaneous detection of bee viruses by multiplex PCR. *Journal of Virological Methods*, 194(1-2): 102–106. https://doi.org/10.1016/j.jviromet.2013.08.003

Şık, Z., Atıcı, E.G., Altıntaş, Ö., Elitok, Y. and Şen, S. (2022). Frequency of American Foulbrood in honeybees: 2015-2020 Data of the Veterinary Control Central Research Institute in Turkey. *Etlik Veteriner Mikrobiyoloji Dergisi*, 33(2):15-20.

Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P. and Chauzat, M. P. (2018). Drivers of colony losses. *Current Opinion in Insect Science*, 26: 142–148.

Stoltz, D., Shen, X. R., Boggis, C. and Sisson, G. (1995). Molecular diagnosis of Kashmir bee virus infection. *Journal of Apicultural Research*, 34(3): 153-160.

Tel, O. Y., Ötkün, S., Güllü Yücetepe, A. G., Gürbilek, S. E. and Keskin, O. (2021). Molecular identification of nosemosis agents in honeybees found in Siirt and Sanliurfa. *Turkish Journal of Agricultural and Natural Sciences*, 8(4): 995-1000.

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

Tozkar, C. Ö., Kence, M., Kence, A., Huang, Q. and Evans, J. D. (2015). Metatranscriptomic analyses of honey bee colonies. *Frontiers in Genetics*, 19(6): 100. https://doi.org/10.3389/fgene.2015.00100

Usta, A. and Yildirim, 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 Üniversitesi Veteriner Fakültesi Dergisi*, 69(3): 303-311.

Yaman, M., Yarılgaç, Ş., Güner, B. G. and Ertürk, Ö. (2015). Presence of nosemosis in honeybees (*Apis mellifera*) in Ordu province. *Turkish Journal of Parasitology*, 39: 47-51.

Yañez, O., Piot, N., Dalmon, A., de Miranda, J. R., Chantawannakul, P., Panziera, D., Amiri, E., Smagghe, G., Schroeder, D. and Chejanovsky, N. (2020). Bee Viruses: Routes of infection in Hymenoptera. *Frontiers in Microbiology*, 11: 943. https://doi.org/10.3389/fmicb.2020.00943

Yang, D., Wang, J., Wang, X., Deng, F., Diao, Q., Wang, M., Hu, Z. and Hou, C. (2022). Genomics and proteomics of *Apis mellifera* filamentous virus isolated from honeybees in China. *Virologica Sinica*, 37(4): 483–490. https://doi.org/10.1016/j.virs.2022.02.007

Yilmaz, F., Öztürk, S., Kuvanci, A., Kayaboynu, Ü., Karataş, Ü., Kaya, S., Derebaşi, E. and Buldağ, M. (2018). Epidemiology of Nosema *apis* and Nosema *ceranae* in Eastern Black Sea Region. *Bee Studies*, 10(2): 34-44.

Yüzbaşioğlu, R. (2022). Honey consumption of individuals and reasons for preference Zara honey that a local product (Sivas Provincial Center District). *Journal of Tekirdag Agricultural Faculty*, 19(1) :16-27. https://doi.org/10.33462/jotaf.863430