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Research Article

Molecular detection of deformed wing virus, black queen cell virus in honey bees in balıkesir province

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ABSTRACT

Viral infections are among the risk factors affecting the health of honey bees, which are economically and ecologically important insects. These infections cause large-scale colony losses. The primary threat to the well-being of western honey bees *(Apis mellifera)* is the ectoparasitic mite *Varroa destructor*, mainly because of its role as a transmitter of viruses. This study aimed to investigate the presence of deformed wing virus (DWV) and black queen cell virus (BQCV) infections, which cause significant colony losses in honey bees, using the reverse transcriptase polymerase chain reaction (RT-PCR) method. For this purpose, adult bees were taken from 50 hives by random sampling in three different regions of Balıkesir. As a result of the analysis of the samples, the genome positivity of DWV and BQCV infections was determined to be 86% (43/50) and 24% (12/50), respectively. In the study's bee colonies, multiple infections with both viruses were found at a rate of 18% (9/50). The results obtained revealed that *Varroa* was not effectively controlled in the colonies, especially in the last month of spring, and accordingly, that viral diseases may occur due to *Varroa* infestation in the winter months.

INTRODUCTION

Honey bee farming plays an important role throughout the world in terms of the sustainability of biodiversity and agricultural production. Bees serve as essential pollinators with significant agronomic, environmental, and commercial value (Khalifa et al., 2021). Honey bees (*Apis mellifera* L.) are very important economically due to their pollinating role, which helps balance the ecosystem, bee products (honey, beeswax, royal jelly, pollen, propolis) and the use of these products in apitherapy (Kalayci et al., 2020). Multiple factors may affect bee health, whether alone or in combination (Woodford et al., 2023). Factors that threaten the health of the honey bee colony include viruses, parasites, bacteria, fungi, pesticides and poor nutrition (Usta & Yıldırım, 2020). Viruses reduce the productivity of a colony by causing anomalies in organs and disorders in the immune system. Transmission of viruses in honey bees occurs horizontally or vertically. Vertical transmission occurs when viruses pass from the queen bee to the egg or from the drone to the semen. Horizontal transmission occurs between bees directly (through consumption of feces and contaminated food, cleaning activities, etc.) or through mechanical or biological vectors (de Miranda & Fries, 2008). Problems with beekeeper management or parasites, such as *Varroa destructor*, that lower host immunity or serve as a vector for various viruses can result in morbidities (Smeele et al., 2023).

Since the first honey bee virus was detected in bees in 1913, 26 bee viruses have been identified (Usta & Yıldırım, 2020). The most important viral infections that threaten honey bee colonies include deformed wing virus (DWV) and black queen cell virus (BQCV).

DWV (Iflavirus aladeformis) is a member of the Iflavirus genus within the *Iflaviridae* family. It was initially discovered in Japan and subsequently detected extensively worldwide. The virion, which has a positive-strand RNA genome, is approximately 30 nm in diameter, non-enveloped and has an icosahedral symmetric structure (Lanzi et al., 2006; ICTV, 2022). The virus has a large RNA genome. While DWV follows an asymptomatic course in healthy colonies, it causes individual deaths as well as a decrease in the colony population in weaker colonies. It has been reported that stress factors, insecticides, malnutrition and *Varroa* infestation are among the predisposing factors for infection (Nazzi & Pennacchio, 2018). DWV causes clinical findings including characteristic wing deformities such as shrunken and wrinkled wings, paralysis, abdominal distension, discoloration and rapid death in honey bees (Lanzi et al., 2006).

BQCV (Triatovirus nigereginacellulae) is the second most common infection in honey bees and was first isolated from dead queen larvae and cell-sealed pupae. The causative agent is a non-enveloped, icosahedral symmetrical, single-stranded RNA virus belonging to the Triatovirus genus of the *Dicistroviridae* family (Leat et al., 2000; ICTV, 2022). Thickening and a pale yellow color in the cell wall of larvae and pupae, death in queen bee larvae, diarrhea in adult bees, edema in the intestines and enlarged abdomen are signs of infection. BQCV infection generally occurs subclinically in worker bees. It has been determined that the *Nosema apis* parasite transmits

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the infection, and it is thought that *V. destructor* may also play a role in transmission (Karapinar et al., 2018).

The *Varroa* genus, which includes several species, is the cause of varroosis, a disease that affects honey bees. Some of these species are well-known, while others are overlooked and may pose a threat to apiculture. The primary species belonging to the *Varroa* genus include *V. jacobsoni*, *V. rindereri*, *V. underwoodi* and *V. destructor* (Hua et al., 2023). *V. destructor* is a severe danger to apiculture. With the exception of Australia, this mite is found all over the world, causing direct losses as well as transferring infections (such as DWV) that cause overwinter mortality. *V. destructor* is also a carrier for a number of viruses, particularly Kashmir bee virus (KBV), Sacbrood virus (SBV), acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), and DWV (Shojaei et al., 2023).

This study used reverse transcriptase polymerase chain reaction (RT-PCR) to assess the incidence of DWV and BQCV infections, which can lead to considerable colony losses, in honey bee colonies in Balıkesir province.

MATERIALS and METHODS

Samples for the study were collected from beekeeping hives in various districts of Balıkesir province in February 2024. Sampling was carried out in the center of Balıkesir (Kabakdere) and two districts in the south (Sındırgı and Bigadiç). There were 65 bee colonies in the first apiary, 50 in the second apiary and 60 in the third apiary. Adult honey bee samples were taken randomly from 50 colonies: 25 in Kabakdere, 15 in Sındırgı, and 10 in Bigadiç. A total of 30 adult honey bees from the outermost frame of each hive were taken into sterile containers. The samples were stored at -18□ C until analyzed. *Varroa* agents were observed by inspection in all the sampled hives. The honey bee samples were stored in the laboratory for one day to ensure that the bees were unable to move.

Viral RNA Extraction

In order to extract viral RNA, 10–30 bees were taken from the same hive and processed as a single sample. The homogenate was then prepared by crushing the samples in a mortar and diluting them with phosphate-buffered saline (PBS). After the prepared homogenate was centrifuged at 4000 rpm for 10 minutes, the resulting supernatant was used for viral RNA extraction. Viral nucleic acid isolation (Viral RNA+DNA Preparation Kit, Jena Bioscience, Germany) was used to extract viral RNA in compliance with the kit's instructions.

Complementary DNA (cDNA) Synthesis and Reverse Transcriptase Polymerase Chain Reaction (RT- PCR)

A complementary DNA kit (First Strand cDNA synthesis kit, Thermo Fisher Scientific, Germany) was used to convert RNA into complementary DNA before the PCR stage. For cDNA synthesis of each sample, after preparing the first mixture, which included 3 µL of sterile distilled water, 0.5 µL of random hexamer primer and 3 µL of RNA, the tubes were put in the thermal cycler. The tubes were held at 70°C for five minutes before being put on ice. In the second stage, the tubes holding the first mixture were filled with the second mixture, which contained 2.0 µL of 5x reaction buffer, 1.0 µL of 10 mM dNTP mix, and 0.5 µL of M-MuLV reverse transcriptase, for a total of 3.5 µL. The tubes were then incubated at 48 □ C for 45 minutes.

For DWV in the RT-PCR reaction forward DWV1 5'-TTTGCAAGATGCTGTATGTGG-3' and reverse DWV2 primer pair were used (Gülmez et al., 2009). For BQCV in the RT-PCR reaction forward 5'-TGGTCAGCTCCCACTACCTTAAAC-3' and 5'-GCAACAAGAAGAAACGTAAACCAC-3' primer pair were used (Benjeddou et al., 2001). A total of 30 µl PCR master mix was prepared for both PCR amplification processes. The amount of DNA it contained was 3 µl, 75 mM Tris-HCl (pH 8.8), 20 mM $NH_4(SO_4)_2$, 1.5 mM of $MgCl_2$, primers 10 pmol, 0.2 mM of dNTP. Taq DNA polymerase was prepared as 0.5 U (MBI, Fermentas, Lithuania). Controls available in the Department of Virology were used as positive controls. For DWV, following a 6-minute denaturation phase at 95°C, 30 seconds at 95°C, 60 seconds at 54°C, and 30 seconds at 72°C were applied, and this heat program was repeated for 35 cycles. Finally, a 7-minute, 68°C extension was used to finish the procedure. The heat program for BQCV was performed 40 times, with 30 seconds at 95°C, 60 seconds at 55°C, and 120 seconds at 68°C applied after a 2-minute denaturation phase at 95°C. A final 7-minute extension at 68°C brought the procedure to a completion. A 100 bp DNA marker was used for evaluation, and the amplified DNA products were detected in the gel imaging device under UV light after being dyed with safe view dye in 1.5% agarose gel.

Varroa agents were observed by inspection in all sampled hives. The honey bee samples were stored in the laboratory at -18°C for one day to ensure that the bees were unable to move.

RESULTS

The study samples were collected from beekeeping hives in various parts of Balıkesir province. When the study results were examined, DWV was found in all studied bee colonies. While 43 of the 50 (86%) apiaries sampled were determined to be positive for DWV, 12 (24%) were determined positive for BQCV (Table 1). In the study's bee colonies, multiple infections with both viruses were found at a rate of 18% (9/50). Three separate apiary samplings were conducted: one from the Central province (Kabakdere), one from Sındırgı, and one from the Bigadiç. DWV was detected as 88% (22/25) in the first apiary, 86.6% (13/15) in the second apiary, and 80% (8/10) in the third apiary. BQCV was detected as 28% (7/25) in the first apiary, 13.3% (2/15) in the second apiary, 3% (3/10) in the third apiary. The regions from where the samples were taken are shown in Figure 1.

In the study, DWV was detected using RT-PCR with primers for the particular polyprotein gene, and a 395 bp region was amplified (Figure 2). The presence of BQCV was tested using RT-PCR primers for the particular structural polyprotein gene, which amplified a 700 bp region (Figure 23). The *Varroa* agent on the live bee is shown in Figure 4.

Table 1. DWV, BQCV RT-PCR positivity rates according to the sampled regions.

Code	Sampling location	Number of hives examined	Number of hives in the apiary	DWV	BQCV	DWV BQCV
1	Central province (Kabakdere)	25	65	22 (88%)	7 (28%)	6 (24%)
2	Sındırgı	15	50	13 (86.6%)	2 (13.3%)	1 (6.6%)
3	Bigadiç	10	60	8 (80%)	3 (3%)	2 (20%)
Total	50		175	43 (86%)	12 (24%)	9 (18%)

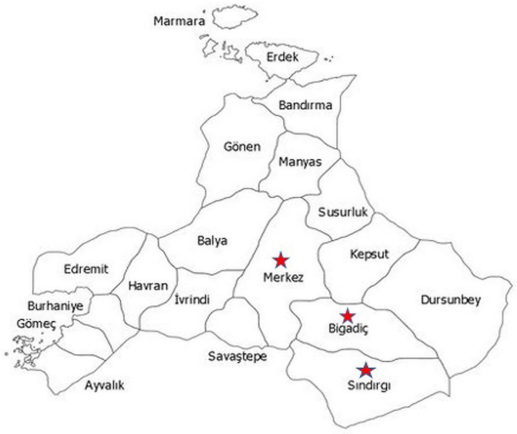

Figure 1. Distribution of beekeeping regions sampled in Balıkesir province

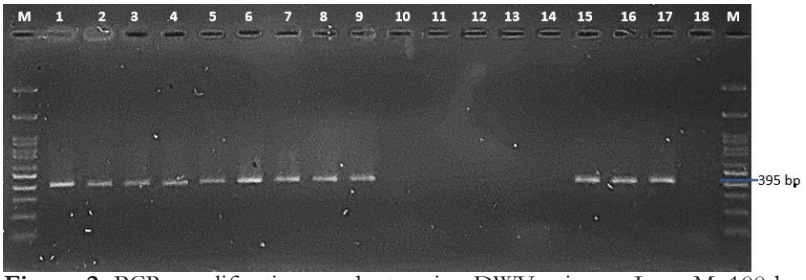

Figure 2. PCR amplification products using DWV primers. Lane M: 100-bp DNA ladder marker (Fermentas); lanes 1-9, 15,16: positive amplification PCR products, lanes 10-14: negative amplification PCR products.); lane 17: positive control; lane 18: negative control (distilled water)

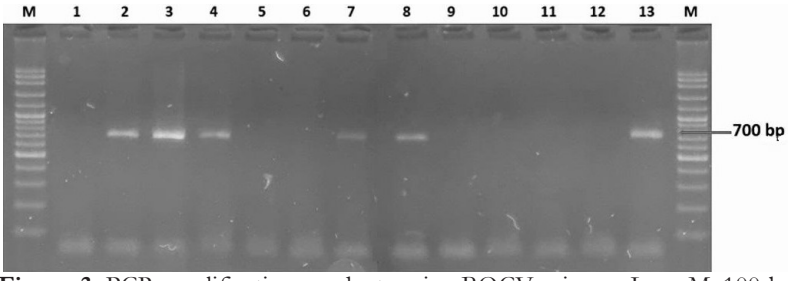

Figure 3. PCR amplification products using BQCV primers. Lane M: 100-bp DNA ladder marker (Fermentas); lane 1: negative control (distilled water), lane 2: positive control; lanes 3,4,7,8,13: positive amplification PCR products; lanes 5,6,9-12: negative amplification PCR products

Figure 4. *Varroa* agent on the live bee shown with a yellow arrow (original)

DISCUSSION

Beekeeping plays a significant economic role in all countries worldwide. Considering Türkiye's rich environment of plant flora and fauna, the results of the present study are due to a lack of effective hive and colony management, as well as insufficient measures taken to combat parasitic, bacterial, and viral infections. Recently, a number of studies have revealed the high rates of colony collapse in honey bees, which has significant implications for the conservation of biodiversity (Tang et al., 2023).

DWV is one of the most common honey bee viruses in the world. In studies conducted in different parts of the world, the prevalence of infection has been reported as 97%, 91%, 95%, 72% and 97% in Austria, Croatia, Hungary, Denmark and France, respectively (Amiri et al., 2015; Berényi et al., 2006; Forgách et al., 2008; Tentcheva et al., 2004; Tlak Gajger et al., 2014). BQCV has been reported at very different rates in in different regions of the world, with a prevalence of 91%, 86%, 65% and 81% in Uruguay, France, Australia and Chile, respectively (Antúnez et al., 2006; Roberts et al., 2017; Rodríguez et al., 2012; Tentcheva et al., 2004).

Numerous studies on viral infections of honey bees have been carried out in various regions of Türkiye. The virus was widely detected in all bee farms sampled in seven different districts of Hatay province (Muz & Muz, 2009). The presence of DWV in Ordu was detected by RT-PCR using primers for the specific polyprotein gene (Gülmez et al., 2009). Studies conducted in our country generally aim to investigate more than one viral agent in honey bees. DWV and BQCV infections are among the most common viral infections. In a study investigating three different viruses in honey bees in the Black Sea region, the presence of chronic bee paralysis virus (CBPV) was determined as 25%, the presence of BQCV was determined as 21.42%, while acute bee paralysis virus (ABPV) was not detected (Gumusova et al., 2010). In a study investigating four different viruses in Van province, DWV positivity was found to be 69.23% and BQCV positivity was 88.46%; ABPV and CBPV were not detected in the hives sampled in the same study (Karapinar et al., 2018). In a study in which DWV, BQCV and ABPV infections in different bee breeds were studied using the RT-PCR method in random sampling from 31 hives in 15 bee farms located in very different regions, the prevalence of these diseases was determined as 74.19%, 25.81% and 74.19%, respectively (Usta & Yildirim, 2022). In research on DWV, ABPV and BQCV in Burdur province, DWV and ABPV were found in 13 of the 15 apiaries studied, while BQCV was detected in 8 of 11 apiaries (Cagirgan et al., 2020). In a study investigating the main factors causing colony losses, the prevalence of DWV, ABPV, BQCV, SBV, CBPV and IAPV was determined as 44.7%, 35.5%, 28.9%, 22.3%, 18.4% and 6.5%, respectively (Kalayci et al., 2020). In a study investigating apiaries located in many provinces in the Aegean region, 111 hives were sampled and DWV, BQCV, ABPV, SBV, CBPV positivity was found to be 19.8%, 18%, 3.6%, 2.7% and 1.8%, respectively (Çağirgan & Yazici, 2021). In the present study, DWV positivity was determined as 86% (43/50) and BQCV positivity was determined as 24% (12/50).

Viral bee diseases reduce bee productivity, causing a direct impact on bee colony losses and thus the yield of bee products. In colonies affected by DWV, wingless bees are observed and the fertility of the queen bee decreases. Furthermore, weak bee colonies spread infections and lead to transmission of disease inside the colony. DWV can be transmitted through semen and eggs, both fertile and unfertile, and transmitted vertically to subsequent generations (de Miranda & Fries, 2008). In this study, it was observed that the viral genome positivity rate was quite high in the hives sampled. Winter conditions can be more difficult for honey bees in temperate climates, resulting in winter losses in honey bee colonies that cannot survive the season. While the reason for these losses may be other natural causes, viral infections and *Varroa* infestation have been documented to exacerbate this situation (Francis et al., 2013). In the present study, these viral infections, especially

DWV, were detected quite frequently. It is thought that the fact that the sampling was carried out in winter contributed to this result.

Studies have shown that BQCV epidemiology is related to the *Nosema* parasite. It has been revealed that the presence of the virus increases with the intensification of *Nosema* infestation, especially in the summer months when the temperature increases (Berényi et al., 2006; Oguz et al., 2017; Tentcheva et al., 2004). From this information, it is possible that one of the reasons why the density of BQCV was lower than that of DWV in the present study was that the sampling was conducted during the winter.

Results obtained from field data have demonstrated that the *Varroa* mite is a very effective vector for DWV and haven shown that when the virus changes host, it becomes more virulent in bees infected with *V. destructor*. The degree of virus concentration in infected colonies is positively correlated with *Varroa* infestation, and it has been found that the *Varroa* mite is not only a carrier but also an activator of viral replication in honey bees. Furthermore, parasitism lowers bee immunity, which promotes virus proliferation (Chen et al., 2005). *Varroa* recently moved to the western honeybee (*A. mellifera*) from its natural host, *A. cerana*. The host-parasite relationship in *A. mellifera* is not yet balanced. Nonetheless, it may be expected that a steady coexistence between the parasite and host will develop over time (Fanelli & Tizzani, 2020). The parasite directly harms the colony, resulting in fewer swarms, which has a financial impact on beekeepers. Furthermore, colonies with parasitic mite syndrome may exhibit scattered brood, crawling, and crippled bees. The spillover to wild pollinators raises further concerns. The life period of the mite varies based on humidity and temperature, ranging from a few days to several months. Clinical signs typically appear in the later stages of an infestation, with the peak of the infestation occurring later in the season (Gela et al., 2023). It has been noted that the main reason why varroosis is so common in our country is that migratory beekeeping is conducted in an uncontrolled manner and beekeepers are unaware of the mite's transmission, biology and control (Gülmez et al., 2009). The same results were obtained from the present study, and it seems that the current measures taken to fight *Varroa* mites are not sufficient.

DWV and IAPV, in particular, have an effect on learning and memory, causing foragers to lose orientation and wander to surrounding colonies. The process allows both viruses and mites to disperse (Li et al., 2013). In the light of this information, combating DWV, which is known to have a significant effect and is widely detected in bee colonies, is of vital importance in the beekeeping sector. It is necessary to effectively fight against infections to prevent colony losses and ensure the continued health of the beekeeping industry.

CONCLUSION

Viral agents that cause infection in honey bees pose a substantial threat because they are difficult to diagnose, have no treatment, and are more easily contagious. Due to the lack of proper *Varroa* control, serious bee colony losses are experienced both in Türkiye and worldwide.

Preventing or minimizing unexplained colony losses, especially in beehives, is essential for the future and continuation of the beekeeping industry. Balıkesir province is currently experiencing an increase in the number of honey bee farms due to its favorable temperature and geography. In the present study, updated data on DWV and BQCV in the Balıkesir region were obtained, and it is thought that this will form the basis for new research on viral infections in honey bees in the region. In order to prevent colony losses, ensure the continuance of bee breeding and bee products, detailed studies on bee viruses need to be carried out in order to determine the causes of viral infections in bees and to obtain more information about the epidemiology, genetics and pathogenesis of infections.

DECLARATIONS

Ethics Approval

It has been reported that the Approval of the Local Ethics Committee for Animal Experiments is not required for the study, in accordance with the provisions of Article 4.1 (d) of the 'Regulation on Working Procedures and Principles of Animal Committees' published in the Official Gazette No. 28914 on 15.02.2014.

Conflict of Interest

The authors declared that there is no conflict of interest.

Consent for Publication

Not applicable.

Competing Interest

The authors declare that they have no competing interests.

Author contribution

Idea, concept and design: ZK, MÖ

Data collection and analysis: MÖ, ZK

Drafting of the manuscript: ZK, MÖ

Critical review: ZK, MÖ

Data Availability

Not applicable.

Acknowledgments

Not applicable.

REFERENCES

Amiri, E., Meixner, M., Nielsen, S. L., & Kryger, P. (2015). Four categories of viral infection describe the health status of honey bee colonies. PLoS ONE, 10(10), 10–12. https://doi.org/10.1371/journal.pone.0140272

Antúnez, K., D'Alessandro, B., Corbella, E., Ramallo, G., & Zunino, P. (2006). Honeybee viruses in Uruguay. Journal of Invertebrate Pathology, 93(1), 67–70. https://doi.org/10.1016/j.jip.2006.05.009

Benjeddou, M., Leat, N., Allsopp, M., & 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. https://

doi.org/10.1128/AEM.67.5.2384-2387.2001

Berényi, O., Bakonyi, T., Derakhshifar, I., Köglberger, H., & Nowotny, N. (2006). Occurrence of six honeybee viruses in diseased Austrian apiaries. Applied and Environmental Microbiology, 72(4), 2414–2420. https://doi.org/10.1128/AEM.72.4.2414-2420.2006

Çağirgan, A. A., & Yazici, Z. (2021). The prevalence of seven crucial honeybee viruses using multiplex RT-PCR and their phylogenetic analysis. Turkish Journal of Veterinary and Animal Sciences, 45(1), 44–55. https://doi.org/10.3906/VET-2004-139

Cagirgan, A. A., Yildirim, Y., & Usta, A. (2020). Phylogenetic analysis of deformed wing virus, black queen cell virus and acute bee paralysis viruses in Turkish honeybee colonies. Medycyna Weterynaryjna, 76(8), 480–484. https://doi.org/10.21521/mw.6437

Chen, Y., Pettis, J. S., & Feldlaufer, M. F. (2005). Detection of multiple viruses in queens of the honey bee Apis mellifera L. Journal of Invertebrate Pathology, 90(2), 118–121. https://doi.org/10.1016/j.jip.2005.08.005

de Miranda, J. R., & Fries, I. (2008). Venereal and vertical transmission of deformed wing virus in honeybees (Apis mellifera L.). Journal of Invertebrate Pathology, 98(2), 184–189. https://doi.org/10.1016/j.jip.2008.02.004

Fanelli, A., & Tizzani, P. (2020). Spatial and temporal analysis of varroosis from 2005 to 2018. Research in Veterinary Science, 131(January), 215–221. https://doi.org/10.1016/j.rvsc.2020.04.017

Forgách, P., Bakonyi, T., Tapaszti, Z., Nowotny, N., & Rusvai, M. (2008). Prevalence of pathogenic bee viruses in Hungarian apiaries: Situation before joining the European Union. Journal of Invertebrate Pathology, 98(2), 235–238. https://doi.org/10.1016/j.jip.2007.11.002

Francis, R. M., Nielsen, S. L., & Kryger, P. (2013). Varroa-Virus Interaction in Collapsing Honey Bee Colonies. PLoS ONE, 8(3). https://doi.org/10.1371/journal.pone.0057540

Gela, A., Atickem, A., Bezabeh, A., Woldehawariat, Y., & Gebresilassie, A. (2023). Insights into varroa mite (Varroa destructor) infestation levels in local honeybee (Apis mellifera) colonies of Ethiopia. Journal of Applied Entomology, 147(9), 798–808. https://doi.org/10.1111/jen.13171

Gülmez, Y., Bursali, A., & Tekin, Ş. (2009). First molecular detection and characterization of deformed wing virus (DWV) in honeybees (Apis mellifera L.) and mite (Varroa destructor) in Turkey. African Journal of Biotechnology, 8(16), 3698–3702.

Gumusova, S. O., Albayrak, H., Kurt, M., & Yazici, Z. (2010). Prevalence of three honey bee viruses in Turkey. Veterinarski Arhiv, 80(6), 779–785.

Hua, T., Chantawannakul, P., Tsai, C. L., & Yeh, W. Bin. (2023). Genetic Profile of the Parasitic Varroan Mite Varroa destructor (Arachnida: Mesostigmata: Varroidae) in Taiwan: a New Taiwanese Haplotype Intermediate Between the Highly Virulent Russian and Less Virulent Japanese Types Identified in the Honey Bee Host Ap. Zoological Studies, 62, 1–12. https://doi.org/10.6620/ZS.2023.62-11

ICTV. (2022). International Commitee on Taxonomy of Viruses. Available at https://ictv.global/taxonomy/. Date of Access: 24.04.2024

Kalayci, G., Cagirgan, A. A., Kaplan, M., Pekmez, K., Beyazit, A., Ozkan, B., Yesiloz, H., & Arslan, F. (2020). The role of viral and parasitic pathogens affected by colony losses in Turkish apiaries. Kafkas Universitesi Veteriner Fakultesi Dergisi, 26(5), 671–677. https://doi.org/10.9775/kvfd.2020.24154

Karapinar, Z., O□ uz, B., Dinçer, E., & Öztürk, 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. https://doi.org/10.21521/mw.5990

Khalifa, S. A. M., Elshafiey, E. H., Shetaia, A. A., El-Wahed, A. A. A., Algethami, A. F., Musharraf, S. G., Alajmi, M. F., Zhao, C., Masry, S. H. D., Abdel-Daim, M. M., Halabi, M. F., Kai, G., Al Naggar, Y., Bishr, M., Diab, M. A. M., & El-Seedi, H. R. (2021). Overview of bee pollination and its economic value for crop production. Insects, 12(8), 1–23. https://doi.org/10.3390/insects12080688

Lanzi, G., de Miranda, J. R., Boniotti, M. B., Cameron, C. E., Lavazza, A., Capucci, L., Camazine, S. M., & Rossi, C. (2006). Molecular and Biological Characterization of Deformed Wing Virus of Honeybees (Apis mellifera L.) . Journal of Virology, 80(10), 4998–5009. https://doi.org/10.1128/jvi.80.10.4998-5009.2006

Leat, N., Ball, B., Govan, V., & Davison, S. (2000). Analysis of the complete genome sequence of black queen-cell virus, a picorna-like virus of honey bees. Journal of General Virology, 81(8), 2111–2119. https://doi.org/10.1099/0022-1317-81-8-2111

Li, Z., Chen, Y., Zhang, S., Chen, S., Li, W., Yan, L., Shi, L., Wu, L., Sohr, A., & Su, S. (2013). Viral Infection Affects Sucrose Responsiveness and Homing Ability of Forager Honey Bees, Apis mellifera L. PLoS ONE, 8(10), 1–10. https://doi.org/10.1371/journal.pone.0077354

Muz, D., & Muz, M. N. (2009). Survey of the occurrence of deformed wing virus and multiple parasites of queens (Apis mellifera L.) in apiaries with collapsed colonies in Hatay, Turkey. Journal of Apicultural Research, 48(3), 204–208. https://doi.org/10.3896/IBRA.1.48.3.09

Nazzi, F., & Pennacchio, F. (2018). Honey bee antiviral immune barriers as affected by multiple stress factors: A novel paradigm to interpret colony health decline and collapse. Viruses, 10(4). https://doi.org/10.3390/v10040159

Oguz, B., Karapinar, Z., Dinçer, E., & Deĝer, M. S. (2017). Molecular detection of Nosema spp. and black queen-cell virus in honeybees in Van Province, Turkey. Turkish Journal of Veterinary and Animal Sciences, 41(2), 221–227. https://

doi.org/10.3906/vet-1604-92

Roberts, J. M. K., Anderson, D. L., & Durr, P. A. (2017). Absence of deformed wing virus and Varroa destructor in Australia provides unique perspectives on honeybee viral landscapes and colony losses. Scientific Reports, 7, 6925. https://doi.org/10.1038/s41598-017-07290-w

Rodríguez, M., Vargas, M., Gerding, M., Navarro, H., & Antúnez, K. (2012). Viral infection and Nosema ceranae in honey bees (Apis mellifera) in Chile Viral infection and Nosema ceranae in honey bees (Apis mellifera) in Chile. Journal of apicultural research, 51, 285-287. https://doi.org/10.3896/IBRA.1.51.3.12

Shojaei, A., Nourian, A., Khanjani, M., & Mahmoodi, P. (2023). The first molecular characterization of Lake Sinai virus in honey bees (Apis mellifera) and Varroa destructor mites in Iran. Journal of Apicultural Research, 62(5), 1176–1182. https://doi.org/10.1080/00218839.2021.1921467

Smeele, Z. E., Baty, J. W., & Lester, P. J. (2023). Effects of Deformed Wing Virus-Targeting dsRNA on Viral Loads in Bees Parasitised and Non-Parasitised by Varroa destructor. Viruses, 15(11). https://doi.org/10.3390/v15112259

Tang, J., Ji, C., Shi, W., Su, S., Xue, Y., Xu, J., Chen, X., Zhao, Y., & Chen, C. (2023). Survey Results of Honey Bee Colony Losses in Winter in China (2009–2021). Insects, 14(6), 1–18. https://doi.org/10.3390/insects14060554

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., & 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. https://doi.org/10.1128/AEM.70.12.7185-7191.2004

Tlak Gajger, I., Kolodziejek, J., Bakonyi, T., & Nowotny, N. (2014). Prevalence and distribution patterns of seven different honeybee viruses in diseased colonies: a case study from Croatia. Apidologie, 45(6), 701–706. https://doi.org/10.1007/s13592-014-0287-0

Usta, A., & 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 Universitesi Veteriner Fakultesi Dergisi, 69(3), 303–311. https://doi.org/10.33988/auvfd.824882

Usta, A., & Yıldırım, Y. (2020). Bal Arılarının Viral Hastalıkları. Cukurova University, Agriculture Faculty, 1(35), 57–66. https://doi.org/10.36846/cjafs.2020.18

Woodford, L., Sharpe, G., Highet, F., & Evans, D. J. (2023). All together now: Geographically coordinated miticide treatment benefits honey bee health. Journal of Applied Ecology, 60(5), 790–802. https://doi.org/10.1111/1365-2664.14367