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Investigation of the Relationship Between *Nosema* spp. and Black Queen Cell Virus in Honey Bees in Türkiye

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Abstract

Significant losses in honey bee colonies have been reported worldwide, largely due to pathogens affecting bee health. Among these, *Nosema* species and Black queen cell virus (BQCV) are particularly prominent. This study aimed to determine the prevalence of *Nosema* spp. and BQCV in honey bees, investigate the genetic diversity of BQCV, and assess *Nosema* spp. and BQCV potential association. Adult honey bee samples were collected from 62 randomly selected apiaries in Hatay Province, Türkiye. *Nosema* spp. were detected and identified at the species level using a multiplex PCR assay, while BQCV was detected by a one-step real-time RT-PCR assay. BQCV was found in 21.0% of the apiaries, and *Nosema ceranae* infection in 48.4%. Co-infection occurred in 8.1% of the apiaries. No significant association was observed between *N. ceranae* and BQCV positivity. However, shared use of beekeeping equipment among hives was significantly associated with both pathogens. Phylogenetic analysis of the BQCV helicase gene revealed that isolates identified in this study clustered with Asian-origin sequences, suggesting the circulation of different BQCV genotypes in Türkiye. These findings provide insight into pathogen prevalence and co-occurrence in honey bee colonies. Further studies are warranted to characterize BQCV genotypes and to explore the relationship between *Nosema* spp. and BQCV in Türkiye in greater detail.

Introduction

Honey bees, which play a critical role in the sustainability of terrestrial ecosystems, have experienced substantial colony losses and sudden die-offs worldwide in recent years (Aizen & Harder, 2009). These declines in bee populations are widely attributed to the combined effects of multiple abiotic and biotic factors. Among the principal abiotic stressors are habitat destruction, intensive pesticide use, and climate change (Barnett et al., 2007; Pettis et al., 2012). In contrast, pathogens and parasites, including bacteria, fungi, viruses, protozoa, and arthropods, represent the major biotic factors contributing to colony losses (Abdi et al., 2023). Common diseases and pests affecting honey bees include varroosis, American foulbrood, chalkbrood, nosemosis, and various viral infections. These agents adversely affect bee development during the larval and/or adult stages, ultimately compromising colony health and survival (Abdi et al., 2023; Aydın et al., 2017).

Due to its prevalence and adverse effects on colony health, nosemosis holds a particular significance among honey bee diseases. This disease is caused by the microsporidian species *Nosema apis* (*N. apis*, currently classified as *Vairimorpha apis*) and *Nosema ceranae* (*N. ceranae*, currently classified as *Vairimorpha ceranae*). Targeting primarily the digestive system of adult bees, nosemosis is contagious and can lead to colony losses, making it economically important (Galajda et al., 2021; Whitaker et al., 2011). The clinical manifestations of nosemosis vary depending on the causative species. Infections with *N. apis* typically present with diarrhea, which is readily observable as fecal streaks on the frames, combs, and outer walls of the hive (Galajda et al., 2021). In contrast, *N. ceranae* has been reported to be more pathogenic than *N. apis* (Higes

et al., 2007; Mayack & Naug, 2009). Infections caused by this species often progress without obvious clinical symptoms, potentially leading to sudden colony collapse (Galajda et al., 2021; Higes et al., 2008). *Nosema* infections primarily occur through the ingestion of spores in contaminated food. Additionally, spores can be transmitted during trophallaxis or through the bees' hygienic behaviors (Galajda et al., 2021).

Viral infections in honey bees also have significant adverse effects on colony health and productivity. Most viruses that infect honey bees are reported to belong to the RNA virus families *Dicistroviridae* and *Iflaviridae* (Tantillo et al., 2015). Among these, black queen cell virus (BQCV, also known as *Triatovirus nigereginacellulae*), a member of the genus *Triatovirus* within the *Dicistroviridae* family and possessing a linear single-stranded positive-sense RNA genome, is considered one of the most widespread viruses affecting honey bees worldwide (Spurny et al., 2017). Although BQCV has been primarily associated with the death of queen bee larvae, it has also been reported to infect pupae and adult bees (Tentcheva et al., 2004). The main clinical signs of BQCV infection include the death of queen larvae, diarrhea in adult bees, and the thickening and pale yellow discoloration of larval and pupal cell walls (Leat et al., 2000). Transmission of BQCV occurs primarily via the fecal–oral route (Al Naggar & Paxton, 2020).

Three viral infections are associated with *Nosema* species: Bee virus Y (YV), Filamentous virus (FV), and BQCV (Galajda et al., 2021). Among these, BQCV is considered the most significant threat to honey bees (Bailey et al., 1983). *Nosema* infections are thought to facilitate BQCV infection by damaging midgut epithelial cells (Al Naggar & Paxton, 2020). Conversely, BQCV is known to exacerbate the course of *Nosema* infections in adult bees, often without causing obvious clinical symptoms (Bailey, 1975; Bailey et al., 1983). Co-infections with *N. apis* and BQCV have been reported to result in more severe clinical outcomes than single infections (Galajda et al., 2021). Similarly, synergistic interactions between *N. ceranae* and BQCV have been observed in worker bees, significantly reducing their survival rates (Gajda et al., 2021). BQCV prevalence tends to increase during spring and summer, coinciding with peak *Nosema* spp. infections, and co-infections of BQCV and *Nosema* spp. can substantially reduce host survival (Bailey et al., 1983; Doublet et al., 2015). However, some studies have reported no statistically significant relationship between BQCV and *Nosema* spp. infections, such as research conducted in Italy (Bordin et al., 2022). Additionally, experimental studies found no evidence of a synergistic effect between *N. ceranae* and BQCV in adult male and female honey bees (Retschnig et al., 2014).

Based on these findings, the nature of the relationship between *Nosema* spp. and BQCV remains unclear. Therefore, the present study aimed to determine the prevalence of *Nosema* spp. and BQCV in honey bees, to assess the genetic diversity of BQCV, and to evaluate the potential relationship between *Nosema* spp. and BQCV.

Materials and Methods

Sample collection

This study was conducted in Hatay province, one of the major beekeeping centers in Türkiye, between 2022 and 2023 (Fig 1). To determine the number of apiaries to be sampled, a 90% confidence level, a 10% margin of error, and an expected prevalence of 50% were assumed; based on these criteria, it was determined that sampling 62 apiaries would be sufficient. A list of apiaries was obtained from the Hatay Beekeepers' Association, and 62 apiaries were randomly selected from this list using the randomization tool in Microsoft Excel (Microsoft Corporation, USA). From each selected apiary, a total of 100 adult worker bees, either alive or dead, were randomly collected. The samples were kept on ice and transferred to the laboratory as soon as possible, where they were stored at -85 ºC until analysis.

Questionnaire survey

A semi-structured questionnaire was administered to the owners of the sampled apiaries. The questionnaire included items aimed at collecting data on the type of beekeeping practiced (migratory or stationary), the honey bee breed

Fig 1. Map showing the location of Hatay province, highlighted by a red star.

reared in the apiary (native (Hatay honeybee, ecotype of *Apis mellifera anatoliaca*; Kandemir et al., 2000; Palmer et al., 2000) or other honey bee breeds), the number of hives (≥100 or <100), and the shared use of beekeeping equipment among different hives within the same apiary (yes or no).

Extraction of nucleic acids

The collected honey bee samples were pooled by apiary in phosphate-buffered saline (PBS), with each pool containing 100 bees. Following homogenization with a tissue homogenizer (TissueRuptor, Qiagen, Germany), the resulting homogenates were centrifuged at 16,000 g for 3 minutes. Total nucleic acids were extracted from the supernatants of the homogenates using a commercial kit (QIAamp Cador Pathogen Mini Kit, Qiagen, Germany). The obtained extracts (which had a 50 µl elution volume per sample) were stored at −85 °C until molecular analyses were performed.

Detection of Nosema spp. using multiplex polymerase chain reaction (PCR)

To detect *N. apis* and *N. ceranae* species, multiplex PCR analysis was performed using species-specific primer pairs targeting the 16S rRNA gene region (Table 1), as described by Martín-Hernández et al. (2007). PCR reactions were prepared in a total volume of 25 µl, consisting of 12.5 µl Taq 2× Master Mix (Biolabs, New England, USA), 0.2 µl of each primer, 3 µl of DNA, and 8.7 µl of PCR-grade water. The amplification protocol included an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min (Ütük et al., 2010). The resulting PCR products were electrophoresed on 1.5% agarose gels, stained with SafeView™ Classic, and visualized under a UV transilluminator to assess the presence of target bands. Previously molecularly confirmed *N. apis* and *N. ceranae* DNA samples were used as positive controls, while sterile nuclease-free water served as the negative control in the multiplex PCR analyses.

Detection of Black queen cell virus using one-step real-time reverse transcription PCR (RT-PCR)

To detect BQCV in the samples, primer and probe sets described by Chantawannakul et al. (2006) were used (Table 1). PCR reactions were prepared in a total volume of 25 µl, consisting of 4 µl master mix (5x One-step Probe Mix, Solis Biodyne, Tartu, Estonia), 0.5 µl one-step mix (40x One-step SOLIScript Mix, Solis Biodyne, Tartu, Estonia), 320 nM of each primer, 200 nM of probe, 8.8 µl of PCR-grade water, and 5 µl of extracted RNA. One-step real-time RT-PCR was performed using a LightCycler 2.0 real-time PCR instrument (Roche, Germany). The amplification conditions consisted of reverse transcription at 45 °C for 10 minutes, initial activation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds. Sterile nuclease-free water was used as a negative control in all analyses.

Nucleotide sequence and phylogenetic analyses

Samples that tested positive for BQCV by one-step real-time RT-PCR were re-analyzed using one-step RT-PCR with primer pairs (Table 1) reported by Tapaszti et al. (2009). Reactions were prepared in a total volume of 50 µl, containing 25 µl buffer (2X RT-PCR Buffer, Ag-Path-ID one-step RT-PCR kit, Applied Biosystems, Thermo Fisher, Hillsboro, USA), 500 nM of each primer, 4 µl enzyme mix (Ag-Path-ID one-step RT-PCR kit, Applied Biosystems, Thermo Fisher, Hillsboro, USA), 11 µl of PCR-grade water, and 5 µl of extracted RNA. The amplification conditions included reverse transcription at 60 °C for 15 minutes, initial activation at 98 °C for 30 seconds, followed by 35 cycles of 98 °C for 10 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds, with a final extension at 72 °C for 2 minutes. The PCR products were electrophoresed on agarose gels containing GelRed and

Table 1. Primers used for the detection of *Nosema apis* (*N. apis*) and *Nosema ceranae* (*N. ceranae*) in multiplex PCR, and primers and probe used for the detection of Black queen cell virus (BQCV) in one-step RT-PCR and real-time RT-PCR assays.

Primer/Probe	Sequence (5'-3')	Concentration in a reaction	Product size (bp)	Specificity
218MITOC-F	CGGCGACGATGTGATATGAAAATATTAA	0.4 µM	218-219	*N. ceranae*
218MITOC-R	CCCGGTCATTCTCAAACAAAAAACCG	0.4 µM		
321APIS-F	GGGGGCATGTCTTTGACGTACTATGTA	0.4 µM	321	*N. apis*
321APIS-R	GGGGGGCGTTTAAAATGTGAAACAACTATG	0.4 µM		
BQCV-F[a]	TGA GAG CTG CAG AAC AAG AG	0.5 µM	514	BQCV
BQCV-R[a]	TCC ATG GCG ACA GTT ACA TC	0.5 µM		
BQCV8195-F[b]	GGTGCGGGAGATGATATGGA	0.32 µM	70	BQCV
BQCV8265-R[b]	GCCGTCTGAGATGCATGAATAC	0.32 µM		
BQCV8217-P[b]	FAM-TTTCCATCTTTATCGGTACGCCGCC-TAMRA	0.2 µM		

[a]Primers used in one-step RT-PCR for the detection BQCV, [b]Primers and probe used in one-step real-time RT-PCR for the detection BQCV.

visualized under a UV transilluminator. Samples yielding strong bands in the one-step RT-PCR were purified from the gel using a commercial purification kit (QIAquick PCR Purification Kit, Qiagen, Hilden, Germany) and sequenced by an external service provider (BM Laboratory, Ankara, Türkiye) using the Sanger technique. Only samples yielding strong, specific amplicons in the conventional one-step RT-PCR assay were selected for sequencing, as samples with low viral load detected by real-time RT-PCR did not produce sufficient amplification for reliable sequencing.

The obtained sequence data were analyzed using BioEdit software (version 7.0.5.3) and compared with other BQCV isolates available in GenBank. A phylogenetic tree was constructed using both the sequences from this study and those retrieved from GenBank. Phylogenetic analysis was performed using MEGA software (version 11.0) with the neighbor-joining (NJ) method and a bootstrap value of 1,000.

Statistical analyses

The collected data were statistically analyzed using SPSS (version 22; IBM, Armonk, NY, USA). The univariable analysis was performed using Fisher's exact test, and variables with p-values < 0.2 were included in a logistic regression model for the multivariable analysis to evaluate potential risk factors for *N. ceranae* and BQCV infection. A p-value of ≤ 0.05 was considered statistically significant.

Results

Detection of Nosema spp.

Nosema ceranae was detected in 48.4% (30/62) of the sampled apiaries, while *N. apis* was not detected (Table 2). Univariable analysis showed that the type of beekeeping, the breed of honey bee, and sharing beekeeping equipment among different hives within the same apiary had a higher probability of being positive for *N. ceranae* (Table 3). However, in the subsequent multivariable logistic regression analysis, only sharing beekeeping equipment among different hives was found to be a significant risk factor for *N. ceranae* infection (OR: 5.5; 95% CI: 1.1-27.9; P: 0.04) (Table 3).

Detection of Black Queen Cell Virus

Black queen cell virus was detected in 21.0% (13/62) of the examined apiaries. Among the 13 BQCV-positive apiaries, co-infection with *N. ceranae* was observed in 5 of them, corresponding to an 8.1% (5/62) prevalence across all apiaries (Table 2). Univariable analysis showed that the type of beekeeping, the honey bee breed, and the number of hives in apiaries were not significantly associated with BQCV infection (Table 4). However, univariable and multivariable logistic regression analyses showed that sharing beekeeping equipment among different hives was a significant risk factor for BQCV infection (OR: 1.3; 95% CI: 1.1-1.6; p = 0.05) (Table 4).

Table 2. Distribution of positivity rates of Black queen cell virus (BQCV), *Nosema ceranae* (*N. ceranae*), and co-infection according to districts.

District	Number of apiaries (n)	BQCV n (%)[a]	*N. ceranae* n (%)[a]	Co-infection n (%)[a]
Antakya	14	2 (3.2%)	5 (8.1%)	1 (1.6%)
Arsuz	7	7 (11.3%)	1 (1.6%)	1 (1.6%)
Dörtyol	30	2 (3.2%)	18 (29.0%)	1 (1.6%)
Kırıkhan	4	1 (1.6%)	1 (1.6%)	1 (1.6%)
Samandağ	7	1 (1.6%)	5 (8.1%)	1 (1.6%)
Total	62	13 (21.0%)	30 (48.4%)	5 (8.1%)

[a]The percentages were determined by taking into account the total number of apiaries (n = 62).

Table 3. Results of logistic regression analyses for risk factors associated with *Nosema ceranae* (*N. ceranae*) detection.

Variable	Category	Number of apiaries	*N. ceranae* positive	Univariable	Multivariable		
				p value	OR	95% CI	p value
Type of beekeeping	Migratory	46	25	0.15	2.6	0.8-8.7	0.12
	Stationary	16	5	Reference			
Breed of honey bee	Native	45	25	0.09	3.0	0.9-9.9	0.07
	Other	17	5	Reference			
Number of hives	≥100	55	28	0.43	N/A	N/A	N/A
	<100	7	2	Reference			
Sharing beekeeping equipment	Yes	51	28	0.04	5.5	1.1-27.9	0.04
	No	11	2	Reference			

OR: Odds Ratio; CI: Confidence Interval.

Table 4. Results of logistic regression analyses for risk factors associated with Black queen cell virus (BQCV) detection.

Variable	Category	Number of apiaries	BQCV positive	Univariable	Multivariable		
				p value	OR	95% CI	P value
Type of beekeeping	Migratory	46	11	0.48	N/A	N/A	N/A
	Stationary	16	2	Reference			
Honey bee breed	Native	45	11	0.49	N/A	N/A	N/A
	Other	17	2	Reference			
Number of hives	≥100	55	13	0.33	N/A	N/A	N/A
	<100	7	0	Reference			
Sharing beekeeping equipment	Yes	51	13	0.03	1.3	1.1-1.6	0.05
	No	11	0	Reference			

OR: Odds Ratio; CI: Confidence Interval.

Genetic variability of Black queen cell virus isolates

Three of the 13 BQCV-positive samples were submitted for sequence analysis. Three samples were chosen for sequencing due to their strong bands in a one-step RT-PCR assay and their suitability for sequencing. Sequence analysis of the partial helicase gene region of BQCV revealed 100% nucleotide homology among the three isolates in this study. As a result, the sequence data from one of these isolates (accession number PX677346) were submitted to GenBank. The BQCV isolates identified in this study exhibited nucleotide homology ranging from 41.6% to 97.2% with BQCV isolates from different countries. The lowest nucleotide homology was observed with isolates from the United States (PP830638 and PP830639), while the highest homology was found with an isolate from Uzbekistan (OR912392).

A phylogenetic tree was constructed from 259-bp nucleotide sequences of the BQCV helicase gene region. Sequences that were 100% identical were excluded from this analysis. The phylogenetic tree based on the partial helicase coding region was divided into five main groups. The BQCV isolate characterized in this study (TR_Hatay-54) clustered together with isolates from China, South Korea, and Uzbekistan (Fig 2). The South African reference isolate and

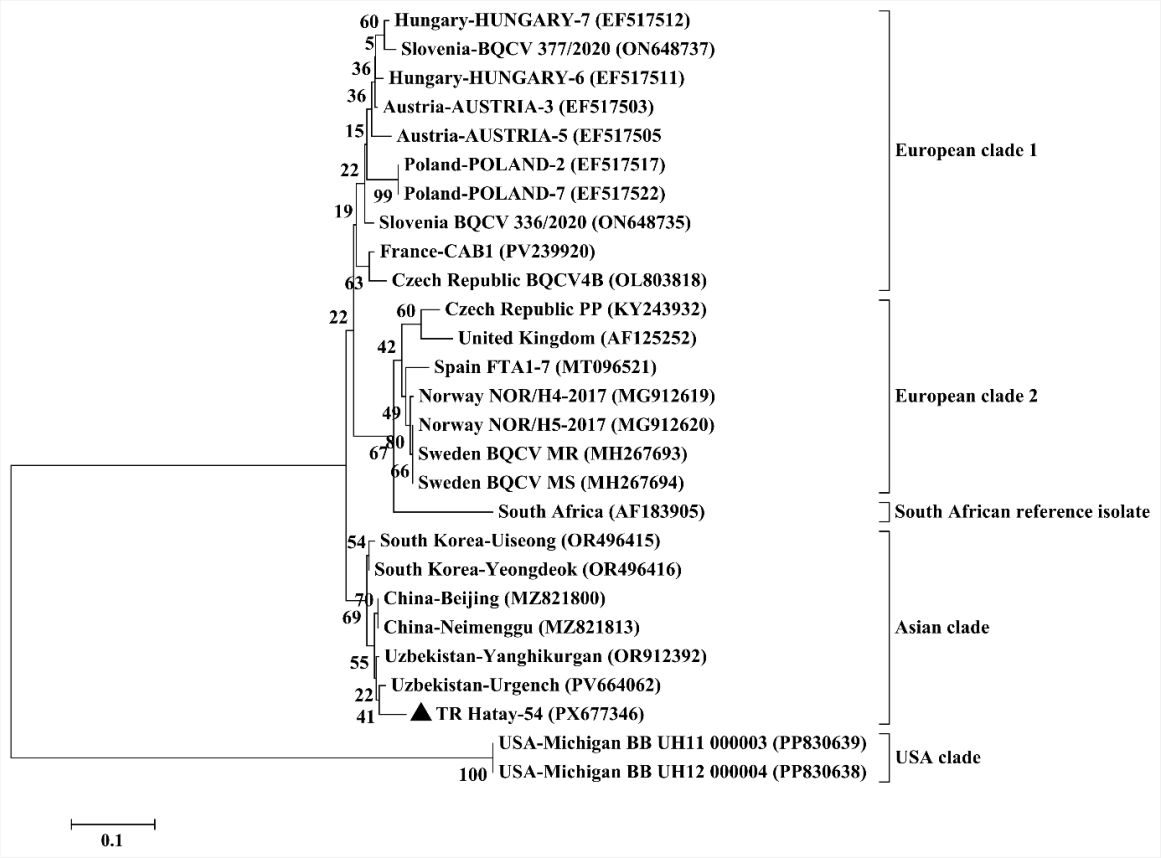

Fig 2. Phylogenetic relationship of Black queen cell virus between the field isolate from this study and isolates from different countries. The sequence of field isolates from this study is marked by a black triangle (▲).

European isolates diverged from the main group, with the European isolates forming two distinct clusters (Europe clade 1 and Europe clade 2) (Fig 2).

Discussion

Black queen cell virus is one of the major viruses, widely distributed worldwide, and is capable of causing lethal infections in honey bee queen larvae and pupae (Bailey & Woods, 1977). Previous studies have reported a synergistic association between *Nosema* spp. and BQCV, with increased honey bee mortality observed in apiaries where both agents are present (Bailey et al., 1983; Berényi et al., 2006). This synergistic effect has been suggested to be linked to the suppression of the host immune response and disruptions in energy metabolism associated with *Nosema* spp. infection (Doublet et al., 2015; Mendoza et al., 2014). Accordingly, the present study aimed not only to determine the prevalence of *Nosema* spp. and BQCV in honey bees, but also to evaluate the genetic diversity of BQCV and to elucidate the relationship between *Nosema* spp. and BQCV.

In this study, 13 samples were identified as BQCV-positive using the one-step real-time RT-PCR method, whereas only three samples produced strong bands with the conventional one-step RT-PCR assay. This discrepancy can be attributed to the higher sensitivity of real-time RT-PCR, which enables detection of samples with lower viral loads and is reported to be approximately 1,000 times more sensitive than conventional RT-PCR (Harju et al., 2005; Chantawannakul et al., 2006).

The majority of studies conducted in European countries have demonstrated a marked increase in the prevalence of *N. ceranae* infections, indicating that this species is progressively replacing *N. apis*. This trend suggests that *N. ceranae* is becoming an increasingly significant threat to European apiculture and underscores the need for close monitoring of its spread (Galajda et al., 2021). Indeed, in Bulgaria, 52.8% of reported nosemosis cases were entirely caused by *N. ceranae* (Shumkova et al., 2018). Similarly, a nationwide study conducted in Hungary revealed exceptionally high *Nosema* spp. infection rates ranging from 95% to 98%, with the vast majority of cases attributed to *N. ceranae* (Csáki et al., 2015).

Recent studies conducted in Türkiye likewise indicate that *N. ceranae* has become the dominant species and that nosemosis should be regarded as a widespread and significant honey bee disease across the country (Akpınar et al., 2024). Epidemiological investigations carried out over the past 15 years to assess the prevalence of *Nosema* infections in honey bees in Türkiye have reported *N. ceranae* prevalence rates ranging from 3.5% to 100% (Ivgin Tunca et al., 2016; Mayack & Hakanoğlu, 2022; Whitaker et al., 2011; Zerek et al., 2022). In the present study conducted in Hatay province, the detection of *N. ceranae* in 48.4% of honey bee apiaries, coupled with the absence of *N. apis*, is consistent with these epidemiological findings and further supports the continued regional predominance of *N. ceranae*.

In the present study, the prevalence of BQCV in honey bee apiaries in Hatay province was 21%. This rate is consistent with the BQCV prevalence values reported from different regions of Türkiye. Previous studies conducted in Türkiye have reported BQCV detection rates ranging from 21.42% to 90% (Avci et al., 2022; Cagirgan et al., 2020; Okur Gumusova et al., 2010; Oguz et al., 2017; Usta & Yıldırım, 2022). Moreover, BQCV prevalence has been reported as 23% in France (Tentcheva et al., 2004), 25.6% in Iran (Moharrami & Modirrousta, 2018), 29.3% in Croatia (Tlak Gajger et al., 2014), 32.6% in Brazil (Chagas et al., 2020), 65% in Australia (Roberts et al., 2017), 66% in Mexico (Ramos-Cuellar et al., 2024), and 80% in Serbia (Milićević et al., 2018). These findings indicate that BQCV is widely distributed across different geographical regions, with considerable variation in prevalence rates.

The variability in reported BQCV prevalence among different countries and regions may be attributed to several factors, including the type of beekeeping practice (migratory or stationary), the developmental stage of the sampled bees (larvae, pupae, or adults), the sampling method and timing, as well as the number of colonies examined. The relatively low BQCV prevalence detected in the present study may be associated with the exclusive sampling of adult bees and the random selection of apiaries. In studies reporting higher prevalence rates, it is noteworthy that sampling strategies often included apiaries experiencing severe colony losses and incorporated larval and pupal samples in addition to adult bees.

The literature reports a positive correlation between BQCV and *N. apis* as well as *N. ceranae*, indicating a tendency for these pathogens to occur concurrently (Avci et al., 2022; Bailey et al., 1983; Berényi et al., 2006; Mendoza et al., 2014; Oguz et al., 2017). In a study conducted in Austria, the infection rate of *N. apis* was 78%, and BQCV was detected in 75% of the same samples (Berényi et al., 2006). Furthermore, increased honey bee mortality has been reported when BQCV and *N. apis* were detected simultaneously (Benjeddou et al., 2002). Consistent with previous findings, co-infection with BQCV and *N. ceranae* was identified in five apiaries in the present study (Bailey et al., 1983; Bordin et al., 2022; Chagas et al., 2020).

In contrast, no statistically significant association was detected between the presence of *N. ceranae* and BQCV positivity in the present study ($p = 0.53$). Similarly, a study conducted in Italy between 2020 and 2021 reported no significant relationship between BQCV and *N. ceranae* (Bordin et al., 2022). An experimental study by Retschnig et al. (2014) also demonstrated the absence of a strong association between *N. ceranae* and BQCV in both male and female honey bees (Retschnig et al., 2014). The discrepancies among studies may be attributable to variations in host genetics, environmental conditions, and immune responses (Alaux et al., 2010; Dussaubat et al., 2012; Retschnig et al., 2014).

In the present study, a statistically significant association was identified between both *N. ceranae* and BQCV positivity and the shared use of beekeeping equipment among different hives. This finding clearly underscores the critical role of hygienic practices in maintaining colony health. The shared use of equipment facilitates the mechanical transmission of fungal, bacterial, and viral agents between colonies, thereby accelerating the spread of infections. Considering the environmental resilience of *Nosema* spores and the potential for RNA viruses such as BQCV to be passively transmitted via contaminated materials, non-compliance with hygiene measures may be a common risk factor for multiple pathogen groups (Schittny et al., 2020; Soklič & Gregorc, 2016). These findings support the notion that limiting equipment sharing between hives is a fundamental preventive measure to reduce the mechanical dissemination of infectious pathogens among colonies.

Sequence analyses revealed that all BQCV isolates identified in this study originated from apiaries in Hatay province, and analysis of the helicase gene showed 100% nucleotide homology among the three field isolates. This finding is consistent with previous reports indicating that BQCV genotypes tend to be most similar within the same geographic region (Tapaszti et al., 2009). A phylogenetic tree constructed from helicase gene sequences demonstrated that the BQCV field isolate characterized in this study clustered with isolates from China, South Korea, and Uzbekistan (Fig 2). These isolates were clearly distinct from the South African reference genotype, as well as from European and U.S.A. isolates. Notably, the results obtained here do not align with previous studies reporting a close relationship between Turkish and European BQCV isolates (Avci et al., 2022; Muz & Muz, 2018). This discrepancy is not unexpected, as queen-rearing colonies are imported into Türkiye from various countries, creating the possibility of introducing different genotypes. Additionally, the BQCV helicase gene is reported to exhibit high mutation rates and provide opportunities for recombination. Genetic recombination among virus genotypes may result in the same viral strain appearing in different clusters within a phylogenetic tree (Tapaszti et al., 2009).

The lack of whole-genome sequencing of BQCV isolates is a limitation of this study. The phylogenetic analysis in this study was carried out on only a small fragment of the helicase gene sequences (259 bp), and it's possible that the BQCV genome may display distinct variations through whole-genome sequencing.

Conclusions

In this study, both *N. ceranae* and BQCV were detected in honey bees as single infections and co-infections. Although further studies are needed to elucidate the distribution and prevalence of *Nosema* spp. and BQCV across Türkiye, the findings of the present study suggest that both agents may be associated with reductions in honey bee populations in Hatay province. Additionally, unlike some previous studies conducted in Türkiye, no statistically significant association was observed between the presence of *N. ceranae* and BQCV positivity. Nevertheless, the significant association between the shared use of beekeeping equipment among hives and positivity for both *N. ceranae* and BQCV highlights the critical role of hygienic practices in maintaining colony health. Future studies encompassing broader geographic regions, detailed investigations of the relationship between *Nosema* spp. and BQCV, and genomic analyses of BQCV field isolates will provide valuable insights into the epidemiology of BQCV infections in Türkiye.

Supplementary information

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Conflict of Interest: The authors declare no potential conflicts of interest.

Ethics approval: The study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (No. 2025/03-01).

Availability of data and materials: The datasets and materials used in this study are available from the corresponding authors upon reasonable request.

Authors' Contribution

A.Z.: Conceptualization, methodology, investigation, formal analysis, writing-original draft, writing-review and editing.
İ.E.: Methodology, formal analysis, writing-review and editing.
M.Ş.: Conceptualization, methodology, investigation, formal analysis, writing-original draft, writing - review and editing.
All authors reviewed and approved the final version of the article.

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