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RESEARCH ARTICLE

The investigation of Israil acute bee paralysis virus, sacbrood virus, kashmir bee virus and chronic bee paralysis virus in honeybees (Apis mellifera)

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Bal arılarında (Apis mellifera) İsrail akut arı felci virusu, sacbrood virusu, kaşmir arı felci virusu ve kronik arı felci virusunun araştırılması

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Öz

Amaç: Bu araştırmada, Burdur yöresinde halk elinde bulunan kovanlıklarda İsrail akut arı felci virusu (IAPV), Sacbrood virus (SBV), Kaşmir arı felci virusu ve Kronik arı felci virusunun (CBPV) Reverz Transcriptase-Polymerase Chain Reaction (RT-PCR) yöntemiyle varlıklarının/yaygınlıklarının araştırılması amaçlanmıştır.

Gereç ve Yöntem: Araştırmada, 15 arıcılık işletmesinden 30 kovan rastgele seçilerek örnekleme yapılmıştır. Numune alınan kovanların her birinden 30 ergin arı toplanarak, havuz oluşturulmuştur. Örnekler homojenize edildikten sonra total nükleik asit ekstraksiyonu yapılmış, ardından spesifik primerler kullanılarak RT-PCR yöntemiyle söz konusu viruslara ait nükleik asit varlıkları araştırıldı.

Bulgular: Araştırmamızda 15 arıcılık işletmesinden alınan 30 örneğin RT-PCR yöntemi ile analiz edilmesi sonucunda 5 arıcılık işletmesinden alınan 10 numunede (%33.3) SBV, iki arıcılık işletmesinden alınan 4 numunede (%13.3) ise KBPV tespit edilmiştir. IAPV ve KBV hiçbir kovanlıkta tespit edilmemiştir. Yapılan değerlendirmelerde 2 işletmeden alınan 4 örneğin hem SBV hem de CBPV ile enfekte olduğu, yani bu kovanlarda miksenfeksiyonun bulunduğu belirlenmiştir.

Öneri: Söz konusu hastalıkların hem Burdur bölgesinde hem de Türkiye'nin diğer bölgelerinde arı yetiştiriciliği bakımından önemli olduğu ve enfeksiyonların önlenmesi amacı ile ciddi tedbirlerin alınması gerektiği görülmektedir. Arılarda birçok viral etkenin yayılmasında kilit rol oynayan Varroa ile mücadele edilmeli, arı immun direnci arttırılmalı ve viral etkenlerin bulaşmasına engel olmak için gerekli hijyen ve sanitasyon kurallarına uyulmalıdır. Ayrıca bundan sonra yapılacak bal arısı virusları ile alakalı çalışmaların daha geniş alanları ve popülasyonları kapsayacak şekilde ve viral filogenilerin ortaya konulacak şekilde planlanmasının faydalı olacağı kanısına varılmıştır.

Anahtar kelimeler: İsrail akut arı felci virusu, kaşmir arı felci virusu, kronik arı felci virusu, Reverz Transcriptase-Polymerase Chain Reaction (RT-PCR), sacbrood virus

Abstract

Aim: This study aims to investigate the presence and prevalence of Israel acute bee paralysis virus (IAPV), Sacbrood virus (SBV), Kashmir bee virus (KBV) and Chronic bee paralysis virus (CBPV) by Reverz Transcriptase-Polymerase Chain Reaction (RT-PCR) method in apiaries around Burdur region.

Materials and Methods: During the study, 30 beehives from 15 apiaries were selected randomly and sampling procedures were carried out. 30 adult bees were collected from each of the sampled hives to form a pool. After the samples were homogenized, total nucleic acid extraction was performed and then viral nucleic acid was searched by RT-PCR method using specific primers.

Results: In this study, in consequence of analyzing 30 samples from 15 apiaries by RT-PCR method, SBV was detected in 10 samples obtained from 5 apiaries (33.3%) and CBPV was found in 4 samples from 2 apiaries (13.3.%). IAPV and KBV were not detected. As a result of evaluations, 4 samples from 2 apiaries were considered to have been infected with both SBV and CBPV, which means a mixed infection occurred in these hives.

Conclusion: It is clear that the so-called diseases were regarded important in terms of beekeeping in not only Burdur region but also in the other regions of Turkey and serious precautions should be taken to prevent infections. Struggling with bee parasites that play the key role in transmission viral agents and enhancing the immune resistance of bees and obeying the rules of hygiene and sanitation should not be ignored in protecting bee health. Besides, the future studies on honeybee viruses should be planned to include wider areas and populations and to reveal viral phylogenies.

Keywords: Israil acute bee paralysis virus, kashmir bee virus, chronic bee paralysis virus, Reverz Transcriptase-Polymerase Chain Reaction (RT-PCR), sacbrood virus.

Introduction

Honeybees are necessary for bio-diversity and pollination of plant species. Contribution of insect-based pollination to food sector is estimated to be around 153 billion dollars (Gallai et al 2009). In addition, apiculture products are commonly used in many fields such as food, cosmetics, health and chemistry sectors (Doğanay and Girisken 2017).

Nearly 26 bee virus types are known to appear (Usta, 2020), however this amount is estimated to increase due to developments in new diagnostic methods (Levin et al 2017, Levin et al. 2019). A great majority of bee viruses is single-stranded RNA viruses with positive polarity belonging to Dicistroviridae and Iflaviridae families (McMenamin and Flenniken McMenamin and Flenniken, 2018). Amongst the most important honeybee viruses included in studies, IAPV, ABPV and KBV are classified within Dicistroviridae family while SBV is within Iflaviridae family (Chen and Siede 2007). Chronic bee paralysis virus has not yet been classified by International Committee on Taxonomy of Viruses (ICTV) however, it is stated that it shows similarities with RNA1-ORF3 Nodaviridae and Tombusviridae families (Oliver et al 2008).

IAPV first appeared in Israel in 2004 because of colony losses (Maori et al 2007). The virus is genetically close to KBV and ABPV; besides they have similar features such as ways of transmition, life cycles in the first host and being mortality in high titers (de Miranda et al 2010). The agent might be detected in all biological stages of the bee as egg, larvae, pupae and adults. An important factor in transmission of bees with many viral agents, Varroa destructor, also plays a role in trasmitted of IAPV (Chen et al 2014). The presence of this agent in our country was previously reported (Ozkırım and Schiesser 2013).

KBV was first isolated from Apis cerena in India and is in the same virus family as IAPV and ABPV (de Miranda et al. 2010). However, it doesn't cause paralysis-like symptoms in adult bees as ABPV does. In an experimental study, when a few virus particles were injected into both adult bees and pupas, the virus proliferated rapidly and caused death in three days (Bailey et al 1979). The presence of this disease has not been reported in Turkey so far.

SBV is the first identified bee virus (White 1917). It can be seen in larva and adult bees, but especially two-days larvae are quite sensitive to the disease (Ball and Bailey 1997). Infected larvae begin to turn from white to yellow and fail to pupate while ecdysial fluid rich in SBV accumulates beneath their unshed skin, forming the sac. The virus displays a subclinical progress without causing clinical symptoms in bees (Chen and Siede 2007). The molecular characterization of the virus that was previously reported in Turkey was also revealed (Kalayci et al 2019).

Another infectious and contagious bee disease, CBPV, is named as 'hairless black syndrome' or 'little black' due to its black and hairless clinical appearance (Bailey et al 1963). The disease is classified as type 1 syndrome and type 2 syndrome according to its symptoms. In type 1 syndrome, bees unable to fly but crawl in front of the hive, on the ground and up plant stems. Bees inside the hive gather at the uppermost part of the frame. In type 2 syndrome, bees can fly but they become almost hairless, black and shiny. Inability to fly and death occurs in a few days (Ribiere et al 2010). In studies carried out in Turkey, presence of chronic bee paralysis has been reported (Gumusova et al 2010, Rustemoglu 2015, Cagirgan 2018).

Turkey is one of the most prominent countries in the world in terms amounts of bee colony (Doganay and Girisken 2017). Colony losses whose reason is unknown is observed in Turkey as well as it is in the world. Viruses are known to be one of the most etiological agents of colony losses (Tozkar et al 2015, Muz and Muz 2017, Kalayci et al 2019).

The aim of this study to reveal the molecular presence and prevalence of IAPV, SBV, KBV and CBPV around Burdur region in the south of Turkey.

Material and Methods

In this study, samplings were carried out within Burdur region study area between the dates June 2019 and September 2019 in order to reveal the presence of viral bee diseases such as IAPV, SBV, KBV and CBPV. 30 samples were taken from 15 apiaries located around Burdur and its villages (Karaçal, Kumruca, Akyaka, Yazıköy, Çentik, Gökçebağ) and brought into lab under cold chain.

A pool consisting of 30 adult bees from each apiaries was homogenized with 5mL Eagle's minimum essential medium (Sigma, United Kingdom). Then, it was centrifuged at 4°C for 30 min at 5000 rpm.

Following the centrifugation of the homogenate, 200 µl was taken from the obtained supernatant for RNA extraction. Total nucleic acid extraction was carried out by using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany) according the manufacturer's instructions. The extracted RNA was kept at 80°C until testing.

The obtained RNA was directly used for RT-PCR amplification. The specific primers used for amplification are shown in Table-1. Xpert One-Step RT-PCR Kit (Grisp Research Solutions, Porto, Portugal) was used for amplification. The reaction was performed in a reaction volume of 25 µl and primer

Table 1. Primers used for detection in this study

Viruses	Primer pairs	Amplified fragments (bp)	Reference
IAPV-F	*CGAACTTGGTGACTTGAAG*	110 bp	Cox-Foster et al., 2007
IAPV-R	*GCATCAGTCGTCTTCCAGG*		
SBV-F	*CGTAATTGCGGAGTGGAAAGATT*	342 bp	Sguazza et al.,2013
SBV-R	*AGATTCCTTCGAGGGTACCTCATC*		
AIV-F	*GGTGCCCTATTTAGGGTGAGGA*	641 bp	Reynaldi et al., 2013
KBV-R	*TGCACGGGAAGTATAAATAATTCT*		
CBPV-F	*AACCTGCCTCAACACAGGCAAC*	774 bp	Squazza et al.,2013
CBPV-R	*ACATCTCTTCTTCGGTGTCAGCC*		

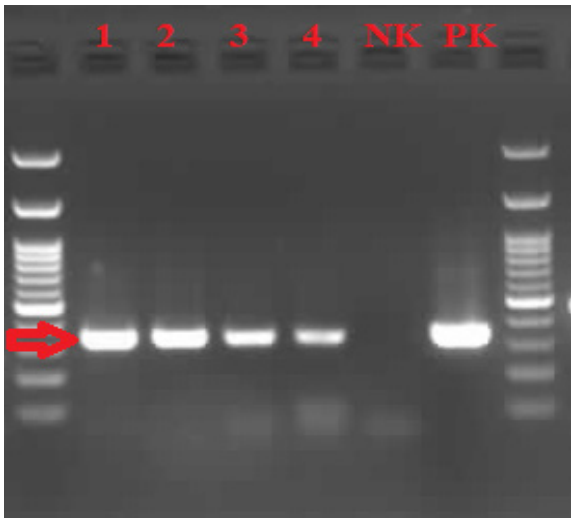

Figure 1. RT-PCR results for sacbrood virus (342 bp) analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. 100–1000 bp DNA ladder (Molecular marker). 1, 2, 3, 4: Samples, NC: Negative Control, PC: PositiveControl

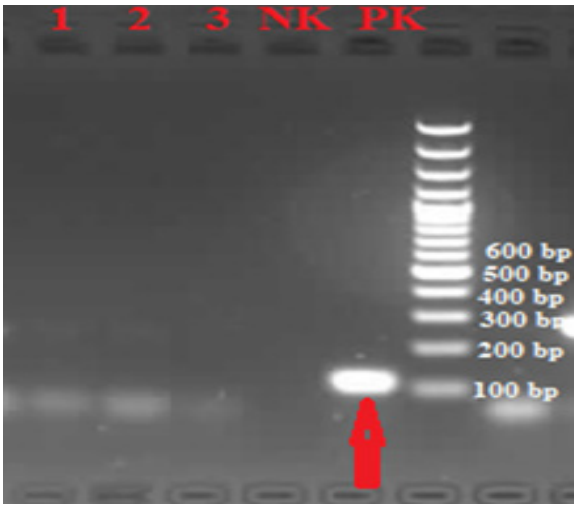

Figure 3. RT-PCR results for Israel acute bee paralysis virus (110 bp) analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. 100–1000 bp DNA ladder (Molecular marker). 1, 2, 3: Samples, NC: Negative Control, PC: Positive Control

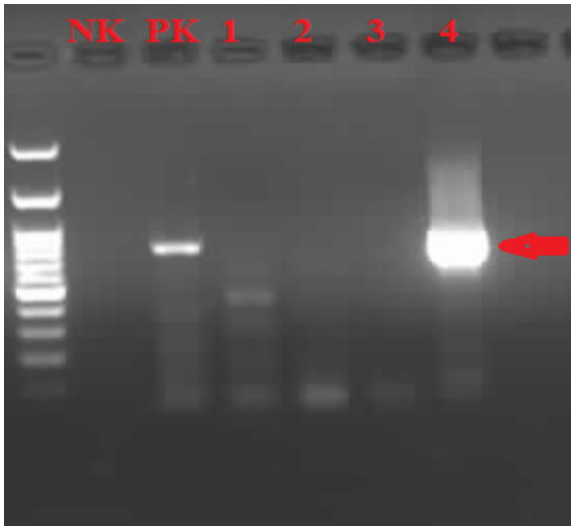

Figure 2. RT-PCR results for chronic bee paralysis virus (774 bp) analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. 100–1000 bp DNA ladder (Molecular marker). 1, 2, 3, 4: Samples, NC: Negative Control, PC: Positive Control

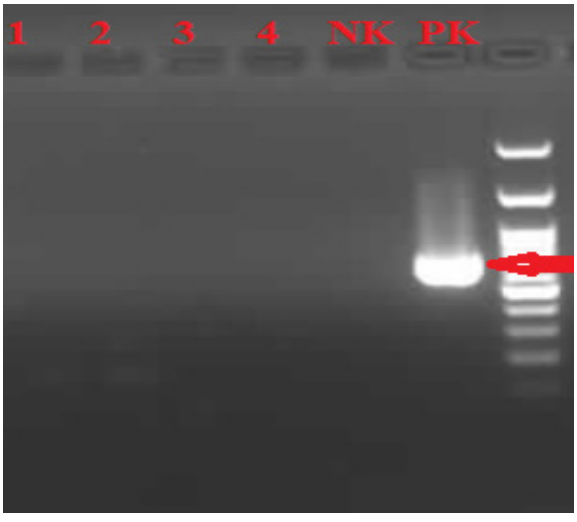

Figure 4. RT-PCR results for kashmir bee virus (641 bp) analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. 100–1000 bp DNA ladder (Molecular marker). 1, 2, 3, 4: Samples, NK: Negative Control, PC: Positive Control

final concentration as 0.4 mM.
The thermal cycle profile was as follows: 45 °C for 15 min for reverse transcription , predenaturation and inhibition of reverse transcriptase at 95°C for 3 min; followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 56 °C for 10 s, and extension at 72 °C for 30 s, with final elongation at 72 °C for 10 min.

PCR products were run on 1.5% agarose gel, which was stained with ethidium bromide in TAE buffer and RT-PCR gel images were evaluated by UV Transilluminator device.

Results

10 out of the 30 examined samples (33.3%) were detected as SBV positive. 342 bp amplified by SBV specific primers can be seen in Figure 1. Positive detected 10 samples were reported to have been collected from 5 different apiaries. SBV could not be detected in 10 other apiaries where samplings were carried out. CBPV was found in 4 out of 30 samples (13.3.%). These CBPV positive samples belonged to 2 apiaries. 774 bp was amplified by using CBPV specific primers (Figure 2). In our study, IAPV and KBV could not be detected. (Figure 3 and Figure 4)

During our data analysis, in one colonies where CBPV was positive, SBV was detected simultaneously. Mixed infection was detected in this colony.

Discussion

Apiculture is highly important not only ecologically but economically as well. When considered in this sense, it has been an agricultural field continuing since ancient times. The fact that it is an alternative means of living for families that are not dependent on land and that have no or insufficient land is one of the most crucial reasons. Turkey is one of the most ideal countries for apicultural activities with its suitable ecological conditions and rich plant flora and is considered among the prominent arbiter ones in apiculture and honey production in the world using this potential. It is highly important to maintain the health of honeybees and fight with their diseases to save and improve this feature. It is a must to prevent unknown colony losses and decrease it to the lowest level especially in bee hives.

Various factors as well as viruses play a role for losses in bee colonies. In studies, the rate of simultaneous infection of bee populations in which colony extinctions are observed with viral and parasitary factors has been higher than the rate of other pathogens being together (Cox et al 2007, Muz and Muz 2017).

Since the detection of the first bee virus (White 1907), the ecological field of bee viruses has remarkably expanded and been a topic of research (Tentcheva et al 2004, Berenyi et al 2006, Nielsen et al 2008, Baker and Schroeder 2008, Gajger et al 2014, Kalayci et al 2019). In various studies around European countries, SBV infection was found in high prevalence. In the study by Tentcheva et al (2004) in France, they detected SBV positivity rate as 86% for adult bees in hives and 80% for pupae. In the same study, in Varroa samples taken from hives, SBV positivity rate was found as 45%. In the study by Nielsen et al. (2008) in Denmark, they reported that SBV was the most viral factor in their hives and SBV was detected in 78 of 96 hives from which they obtained samples. In their study in England in bee colonies using RT-PCR method, Baker and Schroeder (2008) found SBV positivity rate as 1.4%, Gajger et al. (2014) in Croatia as 40.24%, Berenyi et al. (2006) in Austria 49%, Robert et al. (2017) as 35% and Sguazza et al (2013) in Argentina as 13.6%.

In studies in our country, Rustemoğlu (2015) found SBV prevalence as 12.2% around Hakkari region and Cagirgan (2018) as 2.7% around Aegean region in his thesis work. In our study, SBV positivity rate was detected as 33.3% (30/10) in bee colonies. Even though this rate was parallel to those found in various studies of many other countries, it was higher than previously reported positive ones in Turkey. The reason for this condition was believed that samplings were carried out during summer and autumn months depending on seasonal sensitivity of Sacbrood virus, that Burdur region is located on the route of migratory bees and is the housing place for them and that common Varroa infestation was found in colonies (Tentcheva et al 2004).
In a study by Tentcheva et al. (2004) on adult bees in France, chronic bee paralysis disease, in which behavioral and physiological changes occur together in bees, was proved to display prevalence at a rate of 28%. In the same study, CBPV presence could not be found in larvae and pupa frequencies in hives. In the study carried out in Austria, CBPV was detected in 10% of the colonies while KBV did not occur in any of the samples (Berenyi et al 2006). In Denmark, Nielsen et al (2008) found CBPV positivity in 4 hives out of 96 (4.17%). In Croatia, Gajger et al (2014) reported that 9.75% of bee hives were infected by CBPV. In a study performed in Argentina, prevalence of CBPV was stated as 12.3% (Sguazza et al 2013).

In the studies on CBPV in Turkey, presence of this disease at various rates has been revealed. Gumusova et al (2010) detected a positivity of CBPV at a rate of 25% by RT-PCR analysis they applied on honeybee samples that they collected from different provinces of Black Sea region. In another study (Rustemoglu 2015), CBPV presence was found in 8.8% of the samples taken from 90 apiaries.

Cagirgan (2018) detected a CBPV positivity at a rate of 1.8% in honeybee colonies in Aegean region. In our study, CBPV

prevalence was found as 13.3% (4/30). This rate compromises with a great deal of research data found in Turkey and around the world.

In our study, 4 samples proved to be CBPV positive were also SBV positive. These samples with mixed-infections were taken from two apiaries and these apiaries were far from each other in terms of their geographical localizations (distance between 50 km).

Within our study, other searched honeybee viruses, KBV and IAPV, were not seen in bee colonies of apiaries. In worldwide studies on these two factors, reports were at different rates. In France, Tentcheva et al (2004) stated KBV prevalence as 17% for adult bees, 6% for pupae and 5% for Varroa samples taken from colonies. In Denmark, Nielsen et al (2008) detected KBV in only one hive out of 96. About IAPV, positivity was stated at a rate of 45.9% in Argentina (Sguazza et al 2013) and 21% in Australia (Roberts et al 2017). In the study carried out in Croatia in 2014 (Gajger et al 2014), neither KBV nor IAPV could not be detected. KBV wasn't seen in Austria and Hungary, either (Berenyi et al 2006, Forgach et al 2008) Thus, KBV was considered exotic for Europe. (Berenyi et al 2006) In Turkey, in the studies by Rustemoglu (2015) and Cagirgan (2018) at different dates, KBV and IAPV infections were not detected. Tozkar et al (2015) could not find KBV presence in their study. Only Ozkirim and Schiesser (2013) reported IAPV in 15 samples out of 71 they collected from 20 provinces. The results of studies on KBV and IAPV in our country and around the world were parallel to those of this study. The fact that both infections were not detected in many studies shows that they are not common in bee colonies.

In our study, that clinical symptoms were seen in some determined hives and not in the others proves that viral factors occur in these colonies persistently. When we consider the transmission cycles of viral factors, CBPV and SBV infections reveal a risk of spreading around the country since Burdur region is located on the route of migratory bees and is their temporary housing place.

Presence of healthy bee colonies in our country and around the world is highly important both for human health and natural life. One of the most important criteria to achieve this is to breed bee colonies free from viral agents. Therefore, correct and swift diagnosis and protective precautions are crucial in struggling with these infections.

Conclusion

As a result, when we consider the data of this study in which a common presence of SBV and CBPV infections was revealed around the region where the study was carried out, it is clear that the so-called diseases were regarded important in terms of beekeeping in not only Burdur region but also in the other regions of Turkey and serious precautions should be taken to prevent infections. Struggling with bee parasites that play the key role in transmission viral agents and enhancing the immune resistance of bees and obeying the rules of hygiene and sanitation should not be ignored in protecting bee health. Especially in small family apiaries where organized apiculture is not performed, these topics bear more importance.

Besides, the future studies on honeybee viruses should be planned to include wider areas and populations and to reveal viral phylogenies.

Conflict of Interest

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