YYÜ TAR BİL DERG
(YYU J AGR SCI)
2016, 26(2): 174-182

Geliş Tarihi (Received): 03.11.2015
Kabul Tarihi (Accepted): 12.03.2016

Araştırma Makalesi/*Research Article (Original Paper)*

Occurrence and Molecular Characterization of Acute Bee Paralysis Virus (ABPV) in Honeybee (*Apis mellifera*) Colonies in Hakkari Province

Mustafa RÜSTEMOĞLU[1*], Hikmet Murat SİPAHİOĞLU[2]

[1] Department of Plant Protection, Faculty of Agriculture, Sirnak University, Sirnak, TURKEY
[2]Department of Plant Protection, Faculty of Agriculture, Inonu University, Malatya, TURKEY
*e-mail: mustafa.rustemoglu@gmail.com

Abstract: A study was carried out during May-August 2014 to investigate the occurrence and prevalence of *Acute bee paralysis virus* (ABPV) in 90 different apiaries located in Hakkari province (Turkey). To detect the viral nucleic acids, an RT-PCR assay has been implemented for a sensitive and specific diagnosis of ABPV, by using a pair of primer specifically designed to coat protein gene of ABPV, amplifying a 476 bp nucleotide fragment. ABPV was found to occur in 2 samples from two distinct locations among 90 apiaries indicating that ABPV is not widespread in Hakkari province. To understand the molecular relationship between the isolates and the strains of the same virus, the amplified ABPV fragments cloned and sequenced and the sequence was compared with the fragments of ABPV sequences from different geographic origin of the world. The phylogenetic analysis revealed nucleotide identity rates between 85 and 97 %. The partial CP gene of ABPV sequence deposited in the GenBank database with the accession number of KP259311.

Key words: Survey, *Acute bee paralysis virus*, Hakkari province, molecular characterization

Hakkari İli Bal Arısı (Apis Mellifera L.) Kolonilerindeki Acute Bee Paralysis Virus (ABPV)'ünün Varlığının Saptanması ve Moleküler Karakterizasyonu

Özet: Hakkari ilinde 2014 yılı Mayıs ve Ağustos ayları arasında 90 farklı arılıkta yürütülen sürvey çalışmalarında *Acute bee paralysis virus* (ABPV)'ünün varlığı ve yaygınlığı araştırılmıştır. Viral nükleik asidi hassas ve güvenilir biçimde teşhis edebilmek için ABPV'nin genomunda yer alan kılıf protein genine spesifik olarak dizayn edilen ve yaklaşık 476 bç uzunluğunda DNA fragmenti üreten bir çift primer RT-PCR testlerinde kullanılmıştır. Sürvey gerçekleştirilen toplam 90 arılığın sadece iki tanesinde APBV infeksiyonu tespit edilmiş ve virüsün Hakkari ili bal arısı kolonilerinde yaygın olmadığı belirlenmiştir. Tespit edilen ABPV'nin dünyada bilinen diğer izolat ve ırklar ile olan genetik akrabalığını araştırmak için viral genomunda çoğaltılan fragmentler moleküler klonlama yöntemi ile klonlanmış ve DNA dizilemesi gerçekleştirilmiştir. Dizilemesi gerçekleştirilen izolat dünyanın farklı coğrafik bölgelerinde tespit edilen APBV'lere ait diziler ile karşılaştırılmıştır. Yürütülen Filogenetik analizler sonucunda Hakkari ilinde tespit edilen APBV izolatının nükleotid dizisinin, Genbankasına kayıtlı dünyadaki diğer izolatlar ile % 85 ile 97 arasında benzerlik gösterdiği tespit edilmiştir. APBV'nin kısmi kılıf protein geni dizisi Gen Bankasına KP259311 ulaşım numarası ile kaydedilmiştir.

Anahtar kelimeler: Survey, *Acute bee paralysis virus*, Hakkari, moleküler karakterizasyon

Introduction

As of 2012 Turkey, with a production of 89,162 tons, is the second biggest honey producer in the world after China. Hakkari province is one of the exclusive and traditional honey production sites of Turkey with a production of approx. 1,300 tons (TÜİK 2013). Honeybees are influenced by variety of pests and pathogens (Morse and Flottum 1997; Schmid-Hempel 1995), such as the microsporidia *Nosema ceranae* and *Nosema apis*, mites *V. destructor* and *Acarapis woodi*, the bacteria *Paenibacillus larvae* and

Melissococcus plutonius, fungi *Pericystis apis* and RNA viruses. The presence of RNA viruses, in addition to other pathogens, may be one of possible causes of honeybee mortality (Antunez et al. 2012).

To date, 24 viruses have been reported to infect the honey bee. Among them, the most studied are positive-sense single-stranded RNA viruses (de Miranda et al. 2013). It has been considered vital to predict and control them by monitoring the global prevalence and spread of these viruses (Freiberg et al. 2012). There are very limited numbers of reports about viral diseases of honeybee in Turkey. These are *Deformed wing virus* (Gülmez et al. 2009), *Chronic bee paralysis virus*, *Black queen cell virus* (Gümüşova et al. 2010) and *Israeli acute paralysis virus* (Özkırım and Schiesser 2013).

ABPV is a common infective viral agent of bees which is frequently detected in apparently healthy colonies. It has been presumed that this virus plays a role in cases of sudden collapse of honeybees (*Apis mellifera* L.), one of the most serious problems that beekeepers struggle periodically worldwide (Bekesi, et al. 1999; Nordstrom et al. 1999; Antunez et al. 2012).

ABPV was first discovered as an inapparent infection of adult bees (Bailey et al. 1963) and it accumulates in the brain and hypopharyngeal glands of honeybee (Bailey and Milne 1969). ABPV can also be detected facilely in feces (Bailey and Gibes 1964; de Miranda et al. 2010) including several oral transmission routes involving adults, larvae, cannibalized brood, contaminated food and/or feces (Chen and Siede 2007). No organs and part of honeybee was found to be invariably free of virus (Bailey and Gibes 1964). ABPV has a single-stranded, positive-sense, polyadenylated RNA genome comprising 9,491 nucleotides (Bakonyi et al. 2002) and it is a member of the family Dicistroviridae (de Miranda et al. 2010). Its genome contains two open reading frames (ORFs). ORF1 encodes the nonstructural proteins (RNA-dependent RNA polymerase, helicase and protease) transcribed, while ORF2 encodes the three major structural proteins and a minor protein together in a capsid polyprotein (Govan et al. 2000).

ABPV has been considered to be a common infective agent of honey bees from Asia (Haddad et al. 2008; Sanpa and Chantawannakul 2009), America (Hung et al. 1996; Antunez et al. 2006; Weinstein-Teixeira et al. 2008), Europe (Faucon et al. 1992; Bakonyi et al. 2002; Tentcheva et al. 2004; Berenyi et al. 2006; Grabensteiner et al. 2007; Nielsen et al. 2008; Bacandritsos et al. 2010), Africa (Benjeddou et al. 2001).

Viral diseases may cause drastic changes in infected honey bees. The early detection of infectious viruses may prevent considerable economic losses. In the present study a large-scale survey was performed in Hakkari province, one of traditional and major honey bee producer, in order to investigate the aetiology and incidence of the causal agent of *Acute bee paralysis virus* in bee hives. We report on the ABPV frequency and distribution using an RT-PCR (Reverse transcriptase-polymerase chain reaction) screening of 90 apiaries. Furthermore, Turkish ABPV isolate originating from Hakkari province was characterized and compared with published sequences of other ABPV available in the GenBank/EMBL databases.

Materials and Methods

Survey and sample collection

During the surveys, as many different areas as possible were visited in Hakkari province. When any kind of symptoms similar to those caused by bee viruses were suspected, samples were collected. Live bee (*A. mellifera*) samples from colonies suffering with symptoms of paralysis and sudden collapse were randomly collected from each of 90 apiaries (a total of 270 samples) located in central Hakkari and its three districts: Yüksekova, Şemdinli, Çukurca (Turkey) (Fig. 1). Honey bees were collected from May to August in 2014. Samples were returned in cool chain and stored at -20 ºC until analyzed.

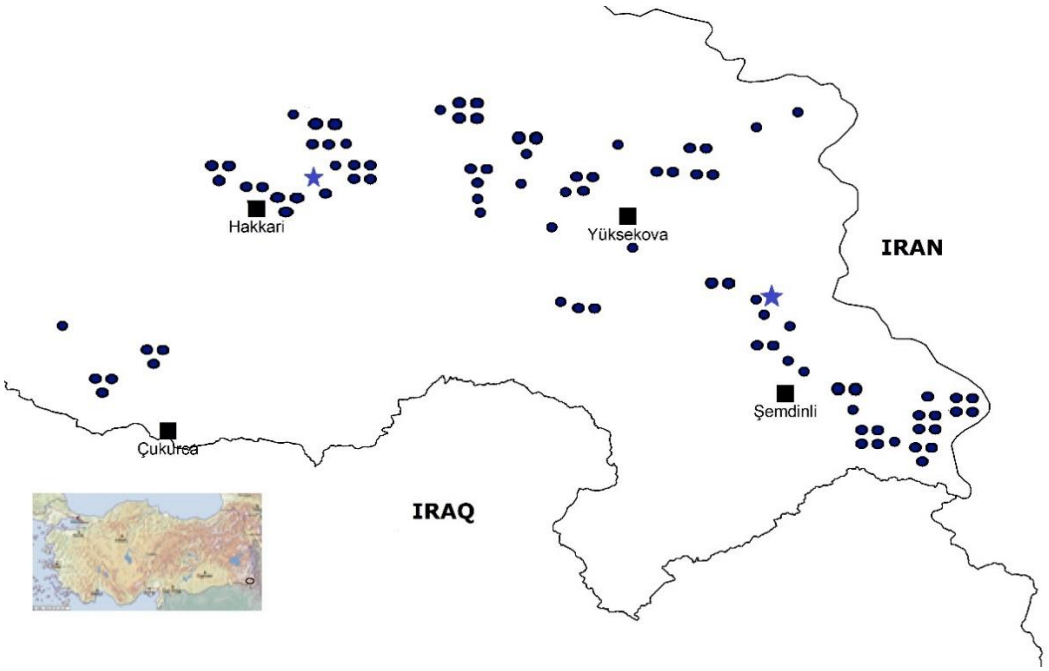

Fig. 1. Sampling sites in three districts of Hakkari province in South Eastern Turkey. Stars represent approximate locations of infected apiaries included in the survey.

RNA extraction

RNA preparations were made with a modified silica-capture procedure (Foissac et al. 2001). For each apiary three honey bees were extracted in a sterile mortar. Total RNAs were stored at – 20 °C until use.

Primer design and RT-PCR of CP gene

For the detection of ABPV we designed genome specific primers based on the published complete genome sequences at GenBank (accession number: AF486073.2) amplifying the 476 bp fragments of CP gene (ABPV-F 5'-GTATGGAAGTGGGCTGAGGA-3', ABPV-R 5'-CGCGGTACTAAAAAGCTACGA-3'). The reverse transcription was carried out with purified RNA using RevertAid First Strand cDNA kit as per the manufacturer's protocol (Vilnius, Thermo-Fermentas-Lithuania). The PCR reaction was carried out with a final volume of 25 µl. The mixture contained 2,5 µl of 10× reaction buffer (200 mM Tris–HCl pH: 8.4, 500 mM KCl), 1 µl of cDNA, , 0,5 µl of dNTPs (20 mM each), 1,5 µl of $MgCl_2$ (25 mM), 0,5 µL of each primer (100 pmol), 0.2 µL of Taq DNA polymerase, and 18,3 µl of nuclease free water. The following thermal cycling scheme was used to amplify the partial CP gene by RT-PCR: 2 min at 94 °C, 35 cycles of 60 seconds at 94 °C, 30 s at 57 °C, and 45 s at 72 °C followed by a final incubation of 10 min at 72 °C. Amplification products were separated by 1.5 % agarose gel and visualized after staining with ethidium bromide (Sambrook et al. 1989).

Molecular cloning and sequencing of CP gene

One isolate which was identified in central part of Hakkari was cloned and sequenced. Amplified fragments were separated on 1% agarose gel and recovered using a GeneJET Gel Extraction Kit (Thermo Scientific) according to manufacturer instructions. The purified DNA fragments were ligated into the pGEM®-T Easy vector (Promega). The constructed plasmid was used to transform Escherichia coli *JM 109 competent cells to ampicillin resistance by electroporation (BioRad, USA). The transformants harboring the DNA of ABPV-Hakkari isolate selected by blue-white selection on X-gal medium plate and screened as positive clones by colony PCR. Two clones containing the CP gene of APBV-Hakkari isolate were selected for propagation. One clone, named ABPV-Hakkari-1, was chosen for DNA sequencing. The cDNA clones were sequenced by automated DNA sequencer at Refgen Research and Biotechnology Company (Turkey).*

Phylogenetic analysis

ABPV sequences used for comparisons were retrieved from the Genbank database. The unreleased sequence of Turkish ABPV isolate was also included in the analysis. For sequence similarity and phylogenetic analysis multiple sequence alignments and phylogenetic reconstructions were performed using CLC Main Workbench software. We included the black queen cell virus (BQCV) coat protein sequence as out group sequence. The robustness of these phylogenies was inferred by 1000 bootstrap replication by using maximum likelihood method.

Results

Development of an RT-PCR assay for ABPV

A primer pair specific for ABPV was designed from the GenBank sequence of AY053373.2. We designed a primer pair which targeted the CP gene. Data bank searches produced no further sequences homologous to the oligonucleotide pair. The primer pair ABPV-F and ABPV-R generated a 476 base pair long product.

Field survey

The numbers of apiaries during the surveys in 2014 are summarized in Table I. A total of 270 honeybee samples representing 90 apiaries originated from Hakkari province, were tested by RT-PCR for the presence of the ABPV, one of the most important honeybee viruses.

Table I. Frequencies and the rate of ABPV infections in 90 apiaries located Hakkari province and its district

Location	Apiaries surveyed	Number of infection	Infection rate (%)
Central Hakkari	25	1	4
Yüksekova	29	0	--
Şemdinli	29	1	3.4
Çukurca	7	0	--
Total	90	2	2.2

Two apiaries tested positive for ABPV using specific primers for the coat protein (CP) gene. The infection level with ABPV was 2.2 % in the study.

Phylogenetic analysis of the cloned sequence

The PCR products from one of these samples were cloned and sequenced and confirmed to be greater than 96 % identical, within the range of world variants of ABPV. Fig. 2 shows the phylogenetic relationships of the Turkish isolate, along with several confirmed ABPV samples from databases and the one out group virus.

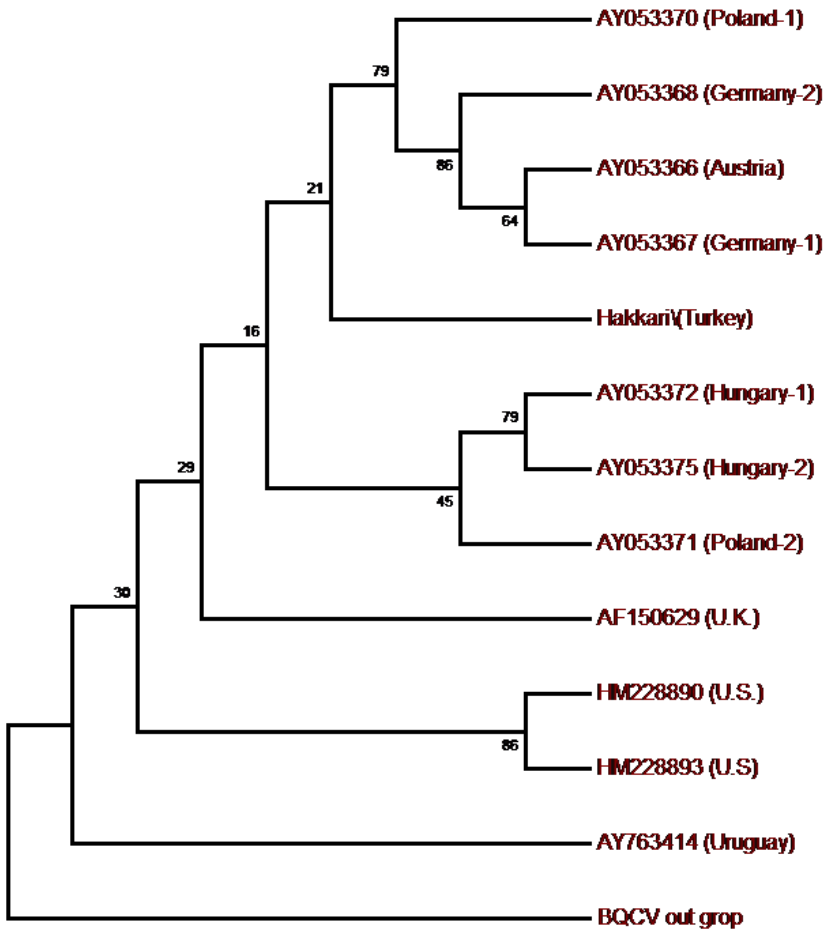

Fig. 2. Phylogenetic relationship of ABPV-Hakkari isolate capsid protein based on the RNA sequences using the Neighbor-Joining method. Bootstrap values are shown above branches.

By using the CP coding sequences and the maximum likelihood method the phylogeny of Turkish and the world APBV isolates was constructed (Fig. 2). Since many sequences of APBV isolates from different countries are available in GenBank, the CP gene was selected for the analysis. The phylogenetic analysis showed that the Turkish isolate of ABPV was closely related with to a cluster including Polish (95 - 96%), Hungarian (96 - 97%), Austrian (95%), and German (94 96%) isolates (Fig. 2).

The Turkish ABPV sequences were more closely related to the ABPV sample from Europe (94 -97%), but differed from the United States (93%) and Uruguay (85%) isolates showing the trends of geographic separation. Fig. 3 shows nucleotide sequence identities of the CP region of ABPV isolates on 476 bp CP gene.

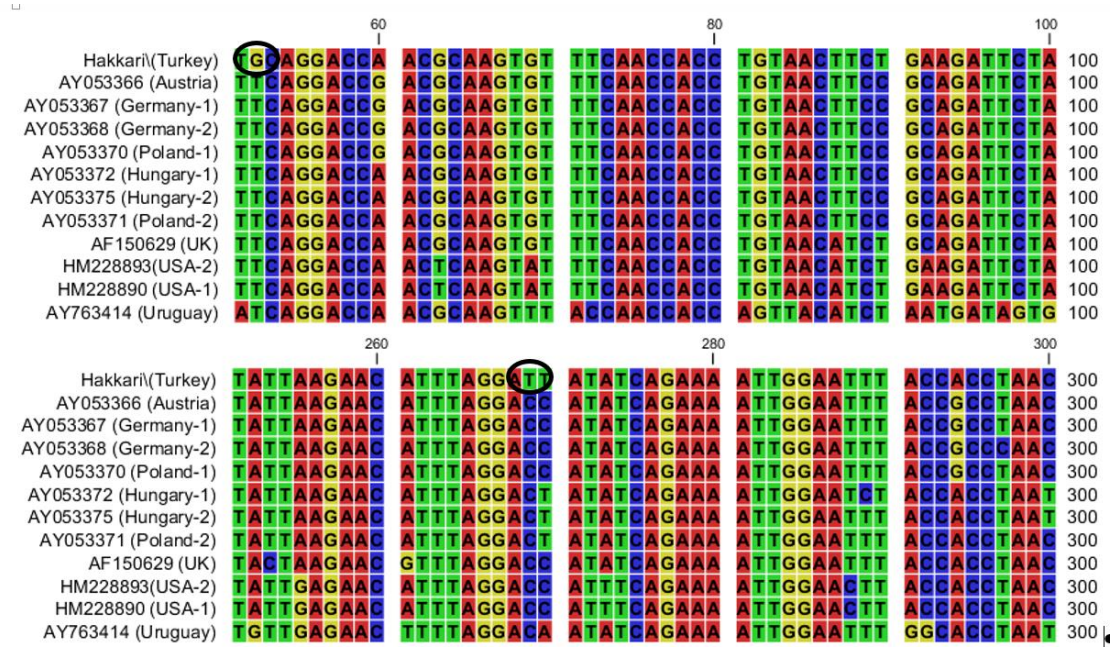

Fig. 3. Sequence alignment of ABPV-Hakkari isolate with available world isolates based Circles indicate the point mutations determined on Turkish isolate

We identified that the ABPV-Hakkari-1 CP sequence had a substitution of Guanine with Thymine (T) at position of 52 and Thymine (T) with Cytosine(C) at position of 269 sequence. These point mutations differenced the ABPV-Hakkari sequence from the same nucleotide positions of other ABPV sequences. It was clearly confirmed by bidirectional sequencing that the presence of nucleotide substitution on both sequences at the corresponding sites of DNA molecule.

Discussion

The present survey is the largest single study of the distribution of ABPV infections in bee colonies in Hakkari. An extremely low level of ABPV infection was detected in the surveyed apiaries. The present results indicate that ABPV is not widespread in Hakkari province and suggests that the two infections may have originated from the different presumably infected sources. The low incidence of infection of bees probably resulted from high mountains constitute the several ecozones for bees in the surveyed areas, restricting the flying of bees. The overall infection level with ABPV was only 2.2 %. This is an enormous advantage for the honey industry of the region. The present study is in correlation with the study carried out by Todd et al. (2007). The workers did not found APBV in New Zealand honey bee colonies. On the other hand, by comparison with reports from other countries that record the ABPV infection of 29 % in England (Baker and Schroeder 2008), 27.1 % in Brazil (Weinstein-Teixeira et al. 2008), 20 % in Northern Thailand (Sanpa and Chantawannakul 2009), 9% in Uruguay (Antunez et al. 2006), 58 % in France (Tentcheva at al. 2004), 12.2 % in Hungary (Bakonyi et al. 2002), it is plausible to conclude that the cleanest bee colonies are in Hakkari. In Turkey, ABPV was first reported by Francis and Kryger in 2012. This is the first report of the occurrence of ABPV in Hakkari province and is not consistent with its worldwide distribution, which may result from global trade of *A. mellifera* queens among many countries (Morimoto et al. 2012).

It has been reported that the first requirement for the control of such diseases is the proper diagnostic method for causative viruses. Because of its high sensitivity, RT-PCR method has been considered one of the most commonly used molecular method in diagnostic laboratories (Chen 2010).

By using genome specific primers an approximately 476 bp partial CP product was detected sensitively in adult worker bee samples belonging to different apiaries (Table I.). One of the ABPV infected apiary was located in central Hakkari and the other one was found in Şemdinli district having a distance of each other at least 90 kilometers. Since ABPV was detected in only two apiaries, it was not possible to establish a causal link between the mortality of honeybee colonies periodically seen in Hakkari. Siede et al. (2008)

were found a significant relationship between winter mortality and the total ABPV burden of a colony and indicated that ABPV prevalence was significantly linked with winter mortality. The RT-PCR assays described in this study proved to be sensitive and reliable methods for the detection ABPV isolates from various bee sources. The assays may provide an appropriate tool for further investigations.

A cloned RT-PCR product was sequenced in both directions. The sequences were identified as ABPV sequences by a BLAST search in databases. The sequenced nucleic acids were aligned with the CP gene of ABPV sequences deposited in the GeneBank revealed a 97% homology. The cloned and sequenced RT-PCR products displayed minor heterogeneity. Parsimony analyses of the sequences show the Turkish ABPV isolate to be most closely related to the Polish, German, Hungarian and Austrian ABPV isolates (Fig. 3). Sequence analysis of ABPV-Hakkari revealed that it contains dual point mutations, demonstrated by bidirectional sequencing.

This survey has demonstrated that a very low number of bees are infected with ABPV. Prior to this study, no data were available for the presence and incidence of ABPV in appraise of Hakkari province. In the course of surveys it has been noted that the beekeepers complained of colony collapses resulting significant economic loses. Discussion with beekeepers indicated that they had very little knowledge concerning virus diseases of bee and their spread. Further studies are needed to determine the incidence and prevalence of ABPV and the other bee viruses and possible vectors in Turkey and their contribution to the colony losses.

Conclusions

The virus status of Hakkari's (Turkey) honey bee colonies infected with ABPV was studied in the year of 2014. The presence of ABPV was found during the study, with an infection level of 2.2 %, demonstrating an extremely low level of ABPV infections. The genome of the Hakkari's ABPV isolate was partially sequenced. Phylogenetic analyses showed this isolate to be unique and most closely related to the Hungarian (96 - 97%), Polish (95 - 96%), Austrian (95%), and German (94 96%) isolates.

Acknowledgements

The study is a part of PHd study and supported by a grant from the Research Fund of Yuzuncu Yil University (Project no: 2014-FBE-D030)

References

Antunez K, D'Alessandro B, Corbella E, Ramallo G, Zunino P (2006) Honey bee viruses in Uruguay. Journal of Invertebrate Pathology. 93: 67–70. DOI: 10.1016/j.jip.2006.05.009.

Antunez K, Andio M, Garrido-Bailon E, Botias C, Zunino P, Martinez-Salvador A, Mzrin-Hernandez R, Higes M (2012) Low prevalence of honeybee viruses in Spain during 2006 and 2007. Research in Veterinary Science 93: 1441–1445. DOI: 10.1016/j.rvsc.2012.03.006.

Bailey L, Milne RG (1969) The multiplication regions and interaction of acute and chronic bee paralysis viruses in adult honeybees. Journal of General Virology 4: 9–14.

Bailey L, Gibbs AJ (1964) Acute infection of bees with paralysis virus. Journal of insect pathology 6: 395–407.

Bailey L, Gibbs AJ, Woods RD (1963) Two viruses from adult honey bees (*Apis mellifera* L.). Virology 21: 390–395.

Baker AC, Schroeder DC (2008) Occurrence and genetic analysis of picorna-like viruses infecting worker bees of *Apis mellifera* L. populations in Devon, South West England. Journal of Invertebrate Pathology 98: 239–242. DOI: 10.1016/j.jip.2008.02.010.

Bakonyi T, Grabensteiner E, Kolodziejek J, Rusvai M, Topolska G, Ritter W, Nowotny N (2002) Phylogenetic analysis of acute bee paralysis virus strains. Applied and Environmental Microbiology 68: 6446–6450. DOI: 10.1128/AEM.68.12.6446–6450.2002.

Bacandritsos N, Granato A, Budge G, Papanastasiou I, Roinioti E, Caldon M, Gallina A, Mutinelli F, Falcaro C (2010) Sudden deaths and colony population decline in Greek honey bee colonies. Journal of Invertebrate Pathology 105: 335-340. DOI: 10.1016/j.jip.2010.08.004.

Bekesi L, Ball BV, Dobos-Kovacs M, Bakonyı T, Rusvaı M (1999) Occurrence of Acute Paralysıs Vırus of The Honey Bee (Apis mellıfera) in a Hungarıan Apıary Infested wıth The Parasıtıc Mıte *Varroa Jacobsoni*. Acta Veterinaria Hungarica 47(3): 319–324.

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: 2384–2387. DOI: 10.1128/AEM.67.5.2384±2387.2001.

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

Chen J (2010) Molecular Detection of Cucumber Mosaic Virus and Other RNA Viruses Based on New Techniques. Zhejiang University Press. Hangzhou and Springer-Verlag. Berlin. Heidelberg.

Chen Y, Siede R (2007) Honey bee viruses. Advances in Virus Research 70: 33–80.

De Miranda JR, Bailey L, Ball BV, Blanchard P, Budge G, Chejanovsky N, Chen YP, Gauthıer L, Genersch E, De Graaf D, Rıbıère M, Ryabov E, De Smet L, Van Der Steen JJM (2013) Standard methods for virus research in *Apis mellifera*. In: Dietemann V, Ellis JD, Neumann P (eds). Volume II: The COLOSS BEEBOOK, standard methods for Apis mellifera pest and pathogen research: Journal of Apicultural Research 52(4): DOI: 10.3896/IBRA.1.52.4.22.

De Miranda JR, Cordoni G, Budge G (2010) The Acute bee paralysis virus–Kashmir bee virus-Israeli acute paralysis virus complex. Journal of Invertebrate Pathology 103: 30–47. DOI: 10.1016/j.jip.2009.06.014.

Faucon JP, Vitu C, Russo P, Vignomi M (1992) Diagnosis of acute bee paralysis: application to the epidemiology of honey bee viral diseases in France in 1990. Apidologie 23: 139–146.

Foissac X, Svanella-Dumas L, Dulucq MJ (2001) Polyvalent detection of fruit tree tricho, capillo and foveaviruses by nested RT-PCR using degenerated and inosine containing primers (PDO RT-PCR). Acta Horticulturae 550: 37-43. DOI: 10.17660/ActaHortic.2001.550.2

Francis RM, Kryger P (2012) Single assay detection of *Acute bee paralysis virus*, *Kashmir bee virus*.and *Israeli acute paralysis virus.* Journal of Apicultural Science 56(1): 137-146. DOI: 10.2478/v10289-012-0014-x.

Freiberg M, De Jong D, Message D, Cox-Foster D (2012) First report of sacbrood virus in honey bee (*Apis mellifera*) colonies in Brazil. Genetics and Molecular Research 11: 3310-3314. DOI: 10.4238/2012.September.12.14.

Grabensteiner E, Bakonyi T, Ritter W, Pechhacker H, Nowotny N (2007) Development of a multiplex RT-PCR for the simultaneous detection of three viruses of the honeybee (*Apis mellifera* L.): *Acute bee paralysis virus, Black queen cell virus* and *Sacbrood virus*. Journal of Invertebrate Pathology 94: 222–225. DOI: 10.1016/j.jip.2006.11.006.

Govan VA, Leat N, Allsopp M, Davison S (2000) Analysis of the complete genome sequence of acute bee paralysis virus shows that it belongs to the novel group of insect-infecting RNA viruses. Virology 277: 457–463.DOI: 10.1006/viro.2000.0616.

Gülmez Y, Bursalı A, Tekin Ş (2009) First molecular detection and characterization of Deformed Wing Virus (DWV) in honeybee (*Apis melifera*) in Turkey. African Journal of Biotechnology 8: 3698-3702.

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

Haddad N, Brake M, Migdadi H, de Miranda JR (2008) First detection of honey bee viruses in Jordan by RT- PCR. Jordan Journal of Agricultural Sciences 4(3): 242-247.

Hung ACF, Shimanuki H, Knox DA (1996) Inapparent infection of acute bee paralysis virus and Kashmir bee virus in the US honey bees. American Bee Journal 136: 874– 876.

Morimoto T, Kojima Y, Yoshiyama M, Kimura K, Yang B, Kadowaki T (2012) Molecular Identification of *Chronic bee paralysis virus* Infection in *Apis mellifera* Colonies in Japan. Viruses 4: 1093-1103. DOI: 10.3390/v4071093

Morse RA, Flottum K (1997) Honey Bee Pests, Predators and Diseases. 3rd ed., Peacock Press. Ohio. USA.

Nielsen SL, Nicolaisen M, Kryger P (2008) Incidence of Acute bee paralysis virus, Black queen cell virus, Chronic bee paralysis virus, Deformed wing virus, Kashmir bee virus and Sacbrood virus in honey bees (*Apis mellifera*) in Denmark. Apidologie 39: 310–314.DOI: 10.1051/apido:2007021.

Nordstrom S, Fries I, Aarhus A, Hansen H, Korpela S 1999. Virus infections in Nordic honey bee colonies with no, low or severe *Varroa jacobsoni* infestation. Apidologie 30: 475–484.

Özkırım A, Schiesser A (2013) Israeli acut paralysis virus (IAPV) in Turkish bees. Journal of Apicultural Research 52: 56-57.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A laboratory manual. 2nd ed., Cold Sprig Harbor Laboratory Press. New York.

Sanpa S, Chantawannakul P (2009) Survey of six bee viruses using RT-PCR in Northern Thailand. Journal of Invertebrate Pathology 100: 116–119. DOI: 10.1016/j.jip.2008.11.010.

Schmid-Hempel P. (1995) Parasites and Social Insects. Apidologie 26 (3): 255-271.

Siede R, Konig M, Buchler R, Failing K, Thiel H-J (2008). A real-time PCR based survey on acute bee paralysis virus in German bee colonies. Apidologie 39: 650–661. DIO: 10.1051/apido:2008044.

Tentcheva D, Gauthier L, Zappulla N, Dainat B, Cousserans F, Colin ME, 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: 7185–7191. DOI: 10.1128/AEM.70.12.7185–7191.2004.

Todd HJ, De Miranda JR, Ball VB (2007) Incidence and molecular characterization of viruses found in dying New Zealand honey bee (Apis mellifera) colonies infested with Varroa destructor. Apidologie 38: 354–367. DOI: 10.1051/apido:2007021

TÜİK (2014) Türkiye İstatistik Kurumu (TÜİK)/ Veritabanları/ Hayvancılık İstatislikleri. http://tuikapp.tuik.gov.tr/hayvancilikapp/hayvancilik.zul. Ankara (Turkey). (Access date: 30.12.2013).

Weinstein-Teixeira E, Chen YP, Message D, Pettis J, Evans JD (2008) Virus infections in Brazilian honey bees. Journal of Invertebrate Pathology 99: 117–119. DOI: 10.1016/j.jip.2008.03.014