Original Article

Evaluation of Molecular Epidemiology of IAPV in Several Regions of Iran

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How to cite this article: Moharrami M, Modirrousta H, Bagheri M. Evaluation of Molecular Epidemiology of IAPV in Several Regions of Iran. Archives of Razi Institute. 2023;78(5):1572-78.

DOI: 10.32592/ARI.2023.78.5.1572

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Article Info:
Received: 30 January 2023
Accepted: 03 March 2023
Published: 31 October 2023

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ABSTRACT

In this study, the Israeli acute paralysis virus (IAPV), a single-stranded RNA virus, was investigated in honey bee colonies, which had a history of mortality, population decline, and parasitic diseases. Samples (adult honey bees) were collected from 328 apiaries from three provinces (Tehran, Alborz, and Mazandaran) of Iran to detect IAPV. After sample preparation, RNA was extracted and cDNA was synthesized to perform the reverse transcription polymerase chain reaction (RT-PCR) method using a PCR primer pair, and a 185 bp fragment was amplified. The results showed that out of 328 samples, 103 (31.4%) samples were positive, which were from Mazandaran (14.33%), Tehran (8.84%), and Alborz (8.23%) provinces. Subsequently, some of the positive samples were sequenced and a phylogenetic tree was drawn. The phylogenetic tree showed that the virus isolates were divided into two distinct groups, including one group that had a high similarity to the European acute bee paralysis virus (ABPV) and one group that had a high similarity to the Kashmir bee virus. In addition, the sequences of the samples in three regions were separated in a node from the strains of ABPV from Eastern Europe. Since the length of the branch between the Iranian sequences and the different strains of ABPV from Eastern Europe was short, it can be assumed that the sequences from Iran have a common ancestor with the mentioned strains of ABPV from Eastern Europe.

Keywords: Honey bee, IAPV, Phylogenetic tree, RT-PCR

1. Introduction

Honey bees are the most valuable pollinators of agricultural products and are considered one of the most important components in agricultural ecosystems [1]. They play a key role in pollination and production of about 15-30% of food worldwide. They also increase the quality and yield of agricultural products [2].

RNA viruses are the most diverse and widespread pathogens [3, 4]. They play an important role in the current high losses of pollinators. Many studies have focused on the epidemiology of honey bee viruses (*Apis mellifera*) at the colony level. To date, more than 18 different honey bee viruses have been described, most of which cause non-obvious infections in bees [5]. The infections are caused by mites, bacteria, viruses, or environmental pollution [6].

A virus associated with severe losses in honey bees was detected in Israel, causing major damage to the honey industry. The bees showed similar symptoms to the acute bee paralysis virus (ABPV), which was named Israeli acute paralysis virus (IAPV) [7]. After the deformed wing virus and black queen cell virus, IAPV is the third most common virus causing infections in honey bees [8]. Preliminary studies on healthy colonies and colony collapse disorder (CCD) colonies in the United States showed a significant association between CCD and IAPV [7, 9]. *Varroa destructor,* as a vector of IAPV, plays a key role in the horizontal transmission of this virus [8]. IAPV infection causes more changes in gene expression in adult bees than in larvae. These changes are four times more in adult honey bees than in larvae. Genes involved in host immunity are significantly expressed in infected adult honey bees. This suggests that IAPV infection leads to an active immune response in adult honey bees [8]. In Israel, IAPV was isolated from dead honey bees, and the characterization of the virus showed that there was a high similarity between this virus and the Kashmir bee virus and ABPV [7]. There are also several reports characterizing IAPV in other countries [10-13]. Moreover, the results of studies show that IAPV causes a permanent infection at all growth stages and in different honey bee species. It also causes systemic infection via two horizontal and vertical vectors [8].

The direct diagnosis of viral infections in honey bees using the reverse transcription polymerase chain reaction (RT-PCR) method can solve the diagnostic problems of viral infections in honey bees. This is because this method does not rely on antisera. Moreover, the final diagnosis can be supported by genetic identification [14]. This study aimed to detect and differentiate IAPV in honey bee hives in different regions of Iran using the RT-PCR method.

2. Materials and Methods

2.1. Data collection and sample preparation

A random procedure was conducted to select 328 apiaries from Tehran (n=121), Alborz (n=80), and Mazandaran (n=127) provinces in Iran during 2017-2019. From each apiary, 50 adult honey bees were collected as one sample and all samples were stored at -20°C until further use.

2.2. RNA extraction and cDNA synthesize

After preparing samples as described by Berenyi et al. (2006) [15], RNA extraction was performed using an RNA extraction kit (Jena Bioscience, Germany) according to the manufacturer's instructions. Subsequently, cDNA was synthesized using the cDNA synthesis kit (Bioneer, Germany). For this purpose, a mixture of 1 µg total RNA and 1 µL oligo (dT) primer (100 µM) was incubated at 65°C for 5 min. Afterward, in a final volume of 20 µL, including 1 µL RNase inhibitor (20 U/mL), 4 µL 5X reaction buffer, 2 µL dNTP (10 mM), and 1 µL M-MuLV RT enzyme (200 U/µL), and 10 µL pure nuclease-free water, the reaction was stopped. The reaction was performed at 42°C for 60 min, followed by a 5-min incubation period at 65°C using

an iCycler thermal cycler (Bio-Rad Laboratories; Hercules, CA, USA).

2.3. Polymerase chain reaction

For PCR, a primer pair (forward: 5'-AGGTGCCCTATTTAGGGTGAGGA-3' and reverse: 5'- CGAAGCGAGTTCCGTATTGTGTAC-3') was used according to Francisco et al. (2011) to amplify a 185 bp fragment of IAPV. PCR was performed using AccuPower® PCR Premix Kit (Bioneer, South Korea) as follows: 13 μL RNase-free water, 5 μL cDNA, and 1 μL of each primer (10 μM) in the final 20 μL volume of the reaction. PCR conditions consisted of initial denaturation at 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 25 s. The final extension was at 72°C for 5 min, and PCR products were detected by 1% agarose gel electrophoresis.

2.4. Sequencing and phylogenetic tree

PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Germany) according to the manufacturer's instructions. For each region, 5 replicates of each purified PCR product were sequenced by Bioneer (South Korea). Using the neighbor-joining method, a phylogenetic tree was constructed from 185 bp fragments of the IAPV sequences using the program MEGAX 4.0, and bootstrap support was estimated from 1,000 replicates.

3. Results

The PCR products of the 185 bp fragment of the standard IAPV as the positive control, the negative control, and the collected unknown samples from three provinces were viewed on a 1% agarose gel (Fig. 1). The results showed that out of 328 samples, 103 (31.4%) samples were positive, which were from Mazandaran (14.33%), Tehran (8.84%), and Alborz (8.23%) provinces.

The sequencing results of the IAPV samples showed that an amino acid deletion was present. In addition, three amino acid deletion regions were detected in 15 sequences from this study, which were classified in the same group as five APV sequences in NCBI. Another 5 sequences from this study, included in a separate group, had two amino acid deletion regions. In addition, there were other amino acid differences between this study and other groups (Fig. 2), suggesting that they were distinct from other similar pathogenic honey bee viruses. The phylogenetic trees for the sample sequences are shown in Fig. 3.

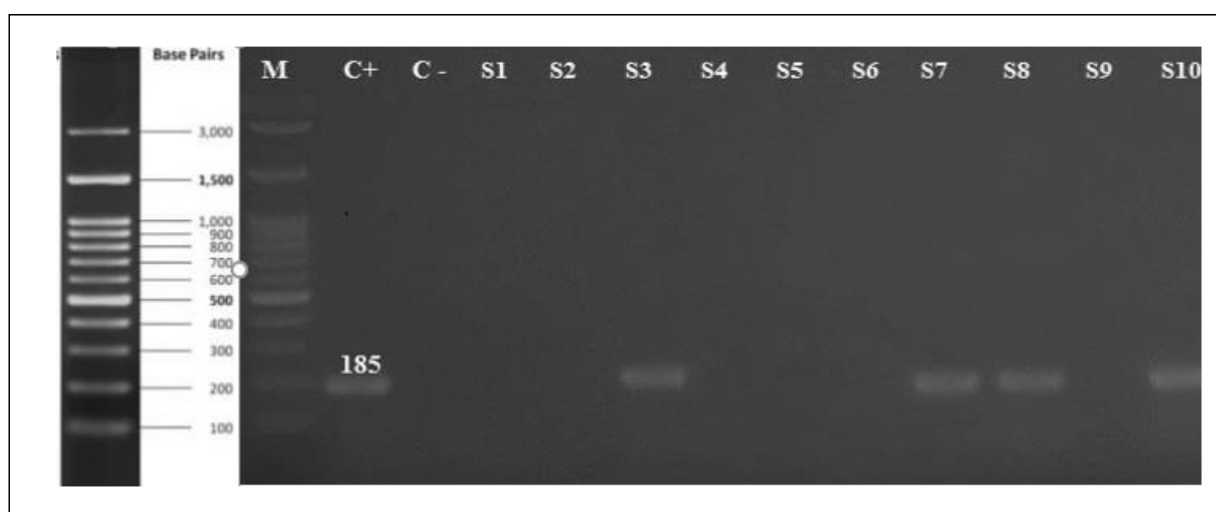

Figure. 1. The PCR products of 185 bp fragment of standard IAPV as positive control, negative control and collected unknown samples from 3 provinces on 1% agarose gel. Samples 3,7,8 and 10 were positive

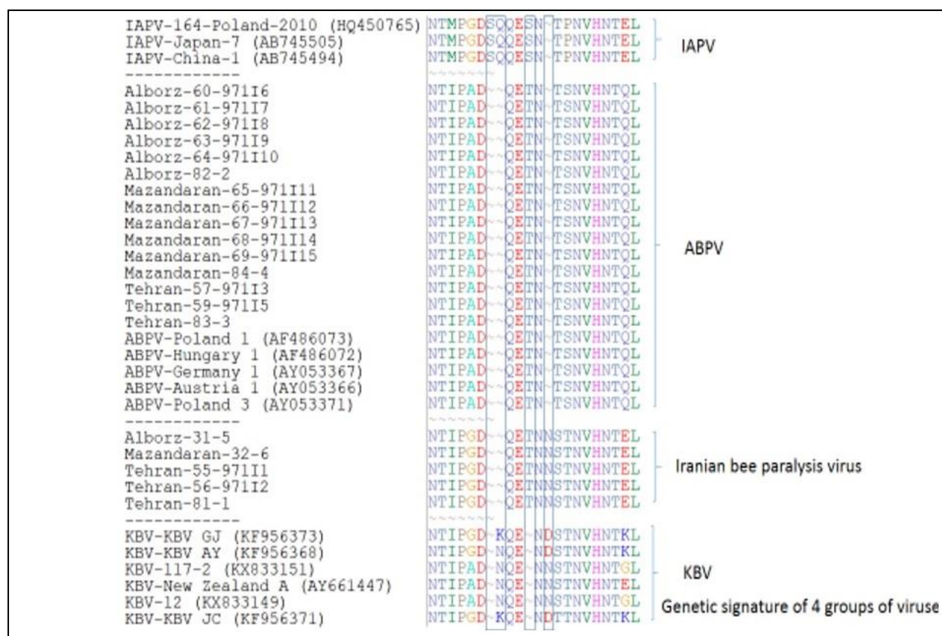

Figure. 2. The amino acid differences of the research and other similar honey bee pathogenic viruses

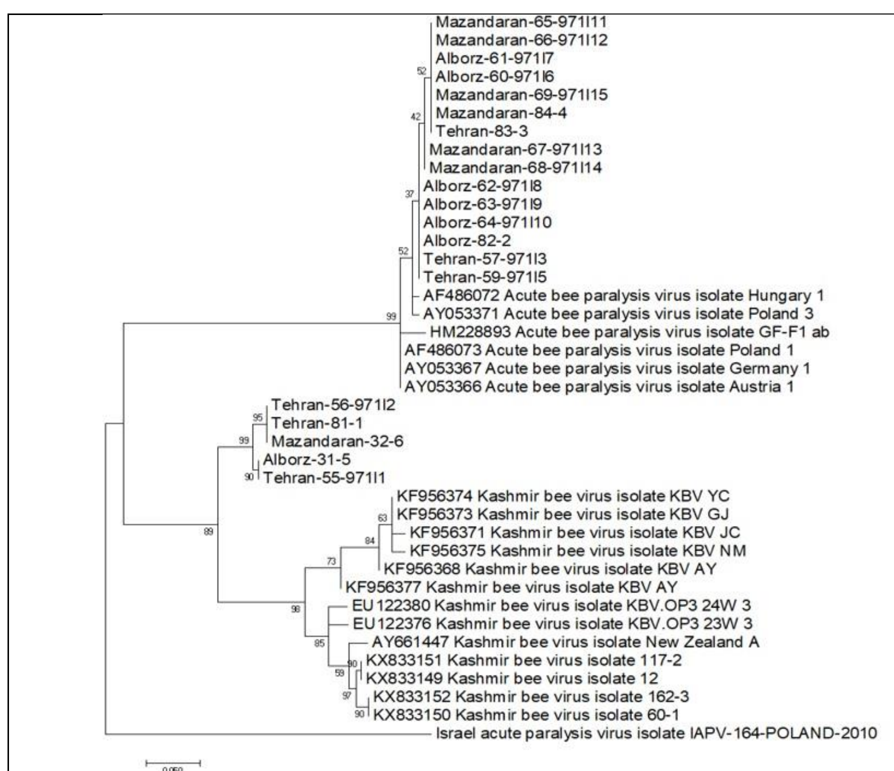

Figure. 3. Phylogenetic tree and evolutionary relationships of nucleotide sequences of 185 bp fragment of IAPV

4. Discussion

Chen et al. (2014) reported that there is a positive relationship between the virus and the *Varroa* population in honey bee hives, and the only way to prevent virus infections is to reduce the *Varroa*

population in the hive [8]. In 2006, CCD was observed in hives in the United States, resulting in a sudden and unusual decline in the number of worker honey bees, with no evidence of disease, starvation, or reproductive failure [16]. CCD is caused by a combination of several factors, including pathogens, parasites, pesticides, malnutrition, environmental stress, low genetic diversity, and beekeeper migration patterns. There is evidence that interactions between pathogens and neurotoxic pesticides have a synergistic effect on honey bee mortality, resulting in colony decline [17, 18]. Damage to the honey bee trachea due to IAPV infection has been reported to cause paralysis, indicating a direct causal relationship between paralysis and tissue tropism. This finding has provided new insights into understanding the underlying mechanism of paralysis symptoms in honey bees following viral infection and has implications for viral disease prevention and specific therapeutics in practice [19, 20].

The use of the RT-PCR method as a sensitive, rapid, and specific method for diagnosing IAPV infection showed that the spread of the virus in different regions can be influenced by geographical conditions as well as by the density of bee colonies. The population density of bee colonies in different areas provides a great opportunity for colonies to come into close contact with each other or even intermingle, which is considered one of the important factors for the spread or not spread of infectious agents. IAPV virus is associated with the loss of bee colonies. The findings of studies have demonstrated that honey bees become infected with IAPV after exposure to the *Varroa* mite which carries the virus. This issue suggests that the *Varroa* mite is a mediator in the horizontal transmission of the virus [8]. Blanchard et al. (2008) [10] examined 35 hives suffering from population decline and mortality and identified IAPV in 5 (15.2%) hives using the RT-PCR method. The phylogenetic tree showed that the strains were divided into two groups, and the strains isolated from France were in group A with some strains from America and Australia [10]. Truong et al. (2019) [21] developed a new detection method based on multiple detection sites within the IAPV genome and ultrafast real-time quantitative PCR. The results confirmed that it was a useful tool for rapid identification of IAPV in hives [21].

To the best of our knowledge, no study has been dedicated to investigating the presence of the IAPV virus in honey bee colonies in Iran so far. Therefore, this study was the first molecular investigation for the identification of IAPV in Iranian hives in 2017-2019. It has been reported that paralysis symptoms or trembling was actually to mitigate tachypnea induced by IAPV infection due to the impairment of honey bee tracheae, revealing a direct causal relationship between paralysis symptoms and tissue tropism [22]. These results provide new insights into understanding the underlying mechanism of honey bee paralysis symptoms following viral infection and have implications for viral disease prevention and specific therapeutics in the field.

The homology between the sequenced samples and the reported IAPV sequences in NCBI ranged from 83% to 97%. The results showed that there are many amino acid differences between this research and other groups, suggesting that they are distinct from other similar pathogenic viruses of honey bees. The phylogenetic tree showed that the different samples from different regions were divided into two different groups. One group showed a high similarity to the APVs of the European honey bee and the second group showed a high similarity to the viruses of the Kashmir honey bee. In other words, the results of this study indicated that the sequences of some samples from the studied regions were separated from the other samples of the studied regions, which could be due to mutation in this sequence and divergence from other samples. It is noteworthy that no significant homology was detected between the studied cases and IAPV.

The phylogenetic tree shows that the sequences of the samples were separated into three regions in one node from the ABPV strains from Eastern Europe (Poland and Hungary). Therefore, based on the short length of the branch between the Iranian sequences and some ABPV strains from Eastern Europe, it can be concluded that these sequences from Iran have a common ancestor with the mentioned ABPV strains from Eastern Europe and can be derived from the same species.

In conclusion, the phylogenetic tree shows that the sequences from Iran share a common ancestor with the BPV strains from Eastern Europe mentioned above. However, with regard to amino acid differences, the complete sequence of the samples is required to propose a new species or subspecies for these sequences. In addition, our results suggest that to maintain honey bee colonies (migratory or fixed colonies) honey bee viruses should be regularly controlled and monitored. In this way, any diseases caused by these viruses, as well as emerging disorders, such as CCD, could be predicted and their spread in honey bee colonies prevented.

Authors' Contribution

Study concept and design: M. M., H. M. M. and M. B.
Acquisition of data: H. M. M.
Analysis and interpretation of data: M. M. and H. M. M.
Drafting of the manuscript: M. B.
Critical revision of the manuscript for important intellectual content: M. B., M. M. and H. M. M.
Statistical analysis: H. M. M.
Administrative, technical, and material support: M. M., H. M. M. and M. B

Ethics

The procedures were approved by the ethics committee of the Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Calderone NW. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992-2009. PLoS One. 2012;7: e37235.
2. Shah KS. Localization of Deformed Wing Virus (DWV) in the Brains of Apis mellifera (European Honey Bees). Honor's Theses, Bucknell University. 2009;19.
3. Mihara T, Koyano H, Hingamp P, Grimsley, N, Goto, S, Ogata, H. Taxon richness of megaviridae exceeds those of bacteria and archaea in the ocean. Microbes Environ. 2018; 33:162–171.
4. Shi M, Lin X, Dm Tian JH, Chen LJ, Chen X, Li CX, Qin XC, Li J, Cao JP, Eden JS, et al. Redefining the invertebrate RNA virosphere. Nat Cell Biol. 2016; 540:539–543.
5. Castelli L, Genchi García ML, Dalmon A, Arredondo D, Antúnez K, Invernizzi C, Reynaldi FJ, Le Conte Y, Beaurepaire A. Intra-Colonial Viral Infections in Western Honey Bees (Apis Mellifera). Microorganisms. 2021;9(5):1087.
6. Brødsgaard CJ, Ritter W, Hansen H, Brødsgaard HF. Interactions among *Varroa* jacobsoni mites, acute paralysis virus, and *Paenibacillus larvae* larvae and their influence on mortality of larval honeybees in vitro. Apidologie. 2000; 31:543-554.
7. Maori E, Lavi S, Mozes-Koch R, Gantman Y, Peretz Y, Edelbaum O, Tanne E, Sela I. Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra-and inter-species recombination. J Gen Virol. 2007; 88:3428-3438.
8. Chen YP, Pettis JS, Corona M, Chen WP, Li CJ, Spivak M, and et al. Israeli acute paralysis virus: epidemiology, pathogenesis and implications for honey bee health. PLOS Pathog. 2014;10 :7
9. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, et al. Ametagenomic survey of microbes in honey bee colony collapse disorder. Science. 2007; 318:283-287.
10. Blanchard P, Schurr F, Celle O, Cougoule N, Drajnudel P, Thiéry R, Faucon JP, Ribière M. First detection of Israeli acute paralysis virus (IAPV) in France, a dicistro virus affecting honeybees (Apis mellifera). J Invertebr Pathol. 2008;99: 348-50.
11. Yan X, Liu XJ, Chen JH, Zhang Y, Han RC. First detection of Israel acute paralysis virus (IAPV) in Apis mellifera (Hymenoptera: Apidae) in China. Sociobiology. 2009; 95-100.
12. Reynaldi FJ, Sguazza GH, Tizzano MA, Fuentealba N, Galosi CM, Pecoraro MR. First report of Israeli acute paralysis virus in asymptomatic hives of Argentina. Revista Argentina de Microbiología. 2011; 43:84-86.
13. Mayra C, Alejandro RC, Alma I, Gerardo PO, Carlos H. Detection of Israeli Acute Paralysis Virus (IAPV) and Apis Apis mellifera Filamentous Virus (AmFV) in Honey Bees in Mexico. J Apic Sci. 2018;62(1):141-144.
14. Johnson KN, Christian PD. Molecular characterization of Drosophila C virus isolates. J Invertebr Pathol. 1999;73: 248-254.
15. Berenyi O, Bakonyi T, Derakhshifar I, Koglberger H, Nowotny N. Occurrence of six honeybee's viruses in diseased Austrian apiaries. Appl Environ Microbiol. 2006;2414-2420.
16. VanEngelsdorp D, Underwood R, Caron D, Hayes Jr J. An estimate of managed colony losses in the winter of 2006–2007: A report commissioned by the apiary inspectors of America. Am Bee J. 2007;147: 599–603.
17. Pettis JS, Lichtenberg EM, Andree M, Stitzinger J, Rose R, vanEngelsdorp D. Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen *Nosema ceranae*. PLoS One. 2013;8: e70182.
18. Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J,3Aurelie Baldy,1Luc P. Belzunces2and Yves Le Conte1 Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environ Microbiol. 2010; 12:774-782.
19. Deng Y, Yang S, Zhao H, Diao Q, Hou C. IAPV-Induced Paralytic Symptoms Associated with Tachypnea via Impaired Tracheal System Function. Int J Mol Sci. 2021; 22(18):10078.
20. Bhatia S, Baral SS, Vega Melendez C, Amiri E, Rueppell O. Comparing Survival of Israeli Acute Paralysis Virus Infection among Stocks of U.S. Honey Bees. Insects. 2021;12(1):60.
21. Truong AT, Kim B, Kim S, Kim M, Kim J, Kim S, Yoon B. Rapid detection of Israeli acute paralysis virus using multi-point ultra-rapid real-time PCR (UR-qPCR). J Apic Res. 2019; 58:746–53.
22. Wang H, Meeus I, Piot N, Smagghe G. Systemic Israeli acute paralysis virus (IAPV) infection in bumblebees (Bombus terrestris) through feeding and injection, J Invertebr Pathol. 2018; 151:158-164.