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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

FIRST MOLECULAR DETECTION OF THE ACUTE BEE PARALYSES VIRUS (ABPV) OF THE HONEY BEE (*Apis mellifera* L.) IN AZERBAIJAN

Azerbaycan'da Bal Arısı (*Apis mellifera* L.) Akut Arı Felci Virüsünün (ABPV) İlk Moleküler Tespiti

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ABSTRACT

Almost all regions of Azerbaijan are engaged in beekeeping. There is a significant demand for bee products. In recent years, there has been an increase in the number of bee deaths in the country, and cases such as bees not returning home. While monitoring the beehives in the areas where we conducted the research, cases such as the loss of the ability to fly and tremors were observed in the bees. As a result of PCR analysis, the ABPV virus was detected for the first time in the materials collected from the North-East and South-East regions of Azerbaijan. The highest prevalence was recorded in Guba region of the North-East zone of the country (62.5%) and Astara region of the South-East zone (75%). In the North-East zone, the least infection was found in the samples taken from Shabran region, and in the South-East zone, in Masalli. The study revealed that almost 40% of ABPV cases were concentrated in the eastern part of the country.

Keywords: *Apis mellifera*, Viral disease, ABPV, Molecular detection, Azerbaijan

ÖZ

Azerbaycan'ın hemen hemen tüm bölgelerinde arıcılık yapılmaktadır. Arı ürünlerine talep oldukça fazla olmaktadır. Ülkemizde son yıllarda arı ölümlerinde artış olamkta ve arıların geri dönmemesi gibi durumlar yaşanmaktadır. Araştırmayı yürüttüğümüz bölgelerdeki arı kovanlarını izlerken arılarda uçma yeteneğini kaybetme, titreme gibi durumlar gözlenmiştir. Azerbaycan'ın Kuzey-Doğu ve Güneydoğu bölgelerinden toplanan materyallerde PCR analizi sonucunda ilk kez ABPV virüsüne rastlanmıştır. Ülkenin Kuzeydoğu bölgesinde en yüksek yaygınlık oranı Guba bölgesinde (%62,5) ve Güneydoğu bölgesinde Astara bölgesinde (%75) kaydedilmiştir. Kuzeydoğu bölgesinde en az enfeksiyon Şabran bölgesinden alınan örneklerde, Güneydoğu bölgesinde ise Masallı bölgesinden alınan örneklerde tespit edilmiştir. Genel olarak ABPV hastalığının yaklaşık %40'ının ülkenin Doğu kesiminde yayıldığı belirlendi." değiştirirseniz memnun olurum.

Anahtar kelimeler: *Apis mellifera*, Viral hastalık, ABPV, Moleküler tanı, Azerbaycan

GENİŞLETİLMİŞ ÖZET

Azerbaycan'ın son istatistiksel verileri ve yapılan bilimsel çalışmalar, özellikle viral hastalıklar nedeniyle arıların kitlesel ölümlerinde artış olduğunu göstermektedir. Özellikle, bugüne kadar Azerbaycan'da ABPV (Acute Bee Paralysis Virus) hastalığının varlığına ilişkin herhangi bir bilimsel çalışmanın yapılmamış olması dikkat çekicidir. **Materyal ve Metot**: Örnekler, Azerbaycan'ın güneydoğu bölgesinde yer alan Lankaran-Astara ekonomik bölgesinin Jalilabad, Masalli, Yardimli, Lankaran, Lerik ve Astara ilçelerinden; kuzeydoğu

bölgesinde yer alan Guba-Khachmaz ekonomik bölgesinin Shabran ve Guba ilçelerinden toplanmıştır.
Azerbaycanın güneydoğu bölgesindeki 48 arılıktan ve kuzeydoğu bölgesindeki 16 arılıktan toplamda 282 arı kovanından örnek alınmıştır. Her kovandan alınan 15 yetişkin arı örneği 7 mL'lik kriyotüplere aktarılmış ve her tüpe 3 mL fosfat tamponlu salin (PBS; SIGMA, 806544-500ML, ABD) eklenmiştir. Örnekler daha sonra otomatik homojenizör (Bead Ruptor Elite, Bead Mill Homogenizer; SKU 19-042E, OMNI International, ABD) kullanılarak homojenleştirilmiş ve PCR analizine kadar −20 °C'de saklanmıştır. Elde edilen RNA'lar, yapısal poliprotein genini hedef alan ve 900 bp'lik fragmanın amplifikasyonunu sağlayan RT-PCR kullanılmıştır.

Bulgular: ABPV virüsü, Güneydoğu bölgesinde Masalli ilinde alınan örneklerin %12,5'inde, Yardimli ilinde %25'inde, Lankaran ilinde %62,5'inde, Lerik ilinde %37,5'inde ve Astara ilinde %75'inde tespit edilmiştir. Kuzeydoğu bölgesinde, ABPV virüsü Şabran ilinde alınan örneklerin %12,5'inde ve Guba ilinde alınan örneklerin %62,5'inde tespit edilmiştir. Sonuçlara göre, ABPV, Kuzeydoğu bölgesindeki 16 arı kovanından 6'sında (70 kovandan 36'sında) ve Güneydoğu bölgesindeki 48 arı kovanından 17'sinde (212 kovandan 84'ünde) tespit edildi. En yüksek enfeksiyon oranı Astara bölgesinde gözlemlenirken, Jalilabad bölgesinde hiçbir enfeksiyon tespit edilmemiştir. Karşılaştırmalı olarak, Masalli ve Shabran bölgeleri en düşük enfeksiyon oranlarını göstermiştir (Tablo 2). Bu çalışmada, ABPV'nin varlığı, 900 bp'lik bir fragmanı amplifiye eden yapısal poliprotein genini hedef alan RT-PCR ile doğrulanmıştır.

Sonuç: Bilgimiz dahilinde, bu çalışma Azerbaycan'da Akut Arı Felci Virüsü'nün (ABPV) varlığını doğrulayan ilk araştırmadır. Bulgularımız, ABPV'nin Güneydoğu ve Kuzeydoğu bölgelerinde, özellikle Rusya ve İran sınırındaki bölgelerde daha yaygın olduğunu göstermektedir. Buna karşılık, Jalilabad gibi ulusal sınırlardan daha uzak bölgelerden toplanan örneklerde virüs bulunmazken, Shabran ve Masalli gibi bölgelerde sadece düşük enfeksiyon oranları görülmüştür.

Azerbaycan'da ABPV'nin yaygınlığını daha iyi karakterize etmek için daha kapsamlı çalışmalara ihtiyaç duyulmaktadır. Özellikle Karabağ, Doğu Zangazur, Aran, Nahçıvan, Küçük ve Büyük Kafkasya gibi diğer önemli bölgelerden arıların örneklemesi ve moleküler analizi yapılmalıdır. Ayrıca, filogenetik bir ağaç oluşturulması, bu bölgelerde dolaşan ABPV suşlarının genetik ilişkileri ve kökenleri hakkında değerli bilgiler sağlayacaktır.

INTRODUCTION

Beekeeping is a well-developed field in Azerbaijan, with numerous apiaries established across every region of the country. Honey bee products are sold both domestically and internationally. The productivity of honey production largely depends on the health of the bees. However, in recent years, a significant increase in bee mortality has been observed. Several factors contribute to this issue, with unfavorable weather conditions and the spread of diseases being among the primary causes.

Bees commonly suffer from diseases causing tremors, bristle loss, and flight issues, mainly linked to viral infections in honey bees (de Miranda et al. 2010). Acute Bee Paralysis Virus, Israeli Acute Paralysis Virus, and Chronic Bee Paralysis Virus cause these signs in infected bees (Bailey and Ball 1991, Chen and Siede 2007).

Acute Bee Paralysis Virus (ABPV), newly named as *Aparavirus apisacutum*, is an RNA virus belonging to the genus Aparavirus in the family Dicistroviridae (ICTV, 2023). It was first identified in 1963 during research on Chronic Bee Paralysis Virus (CBPV) (Bailey et al. 1963).

ABPV is transmitted from infected to healthy bees at the larval, pupal, and adult stages. Transmission can occur through asymptomatic worker bees or via the *Varroa destructor* mite, which serves as a major vector (Moore et al. 2015). Varroa mites are estimated to be responsible for 50–80% of ABPV transmission in bee populations (Ball 1985, Chejanovsky 2010).

Infected bees exhibit symptoms such as wing fluttering, loss of flight ability, crawling behavior, failure to return to the hive, and the accumulation of dead bees around the hive (Bailey et al. 1963, Chejanovsky 2010, De Miranda et al. 2010).

In recent years, Azerbaijan has experienced a significant increase in mass bee mortalities, as reflected in statistical data. One of the primary causes identified is the prevalence of viral infections,

which has been confirmed by several studies (Khanbekova et al. 2013). Notably, however, no scientific research has yet been conducted specifically on the presence of Acute Bee Paralysis Virus (ABPV) within Azerbaijan. Therefore, the aim of this study is to conduct the first molecular identification and assess the infection rate of ABPV in the South-Eastern and North-Eastern regions of the country.

MATERIAL AND METHODS

Samples were collected from the Jalilabad, Masalli, Yardimli, Lankaran, Lerik, and Astara districts of the Lankaran-Astara economic region (South-Eastern Azerbaijan), as well as from the Shabran and Guba districts of the Guba-Khachmaz economic region (North-Eastern Azerbaijan). Samples were collected from a total of 64 apiaries and 282 bee hives located in the mentioned regions to detect the presence of Acute Bee Paralysis Virus (ABPV). From each hives, a sample consisting of 15 adult bees was collected and brought to the laboratory for PCR analysis.

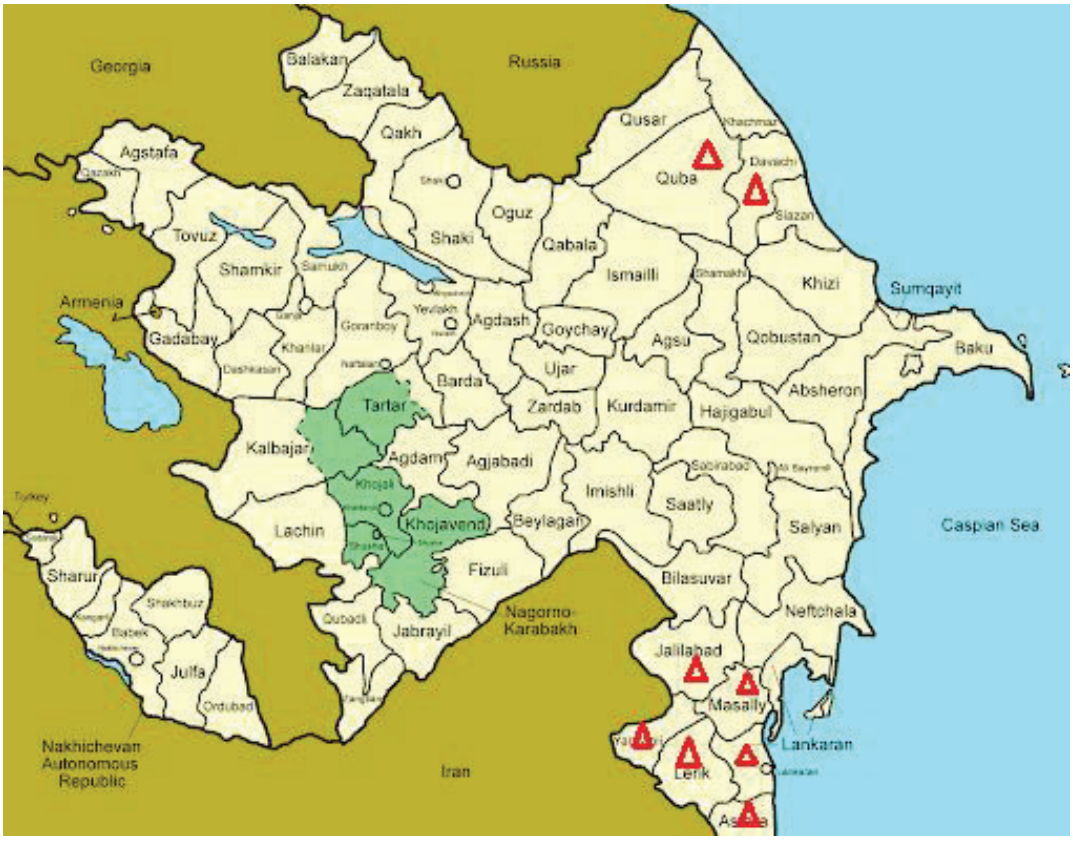

Figure 1. The regions that samples were taken.

The samples were placed in thermal bags containing ice packs immediately after collection and transported to the laboratory. Upon arrival, all materials were stored at –20 °C until they were processed for PCR analysis.

Homogenization, Total Nucleic Acid Extraction, and One-step RT-PCR analysis of Virus

Fifteen adult honey bees from each hive were pooled and transferred into 7 mL cryotubes. To each tube, 3 mL of phosphate-buffered saline (PBS; SIGMA, 806544-500ML, USA) was added. The samples were then homogenized using an automatic

homogenization device (Bead Ruptor Elite, Bead Mill Homogenizer; SKU 19-042E, OMNI International, USA).

Following homogenization, the samples were centrifuged at 4000 rpm for 15 minutes at +4 °C. RNA was extracted from the resulting supernatants using a commercial RNA extraction kit (High Pure Viral Nucleic Acid Kit; REF: 11858874001, Roche, Germany), following the manufacturer's instructions. Surplus homogenized material was stored at –20 °C. Extracted RNA samples were also stored at –20 °C until further analysis.

The extracted RNAs were subjected to one-step reverse transcription polymerase chain reaction (one-step RT-PCR) for the detection of Acute Bee Paralysis Virus (ABPV). The primers used for ABPV detection are listed in Table 1. The reaction components and thermal cycling conditions were based on previously published protocols (Benjeddou et al., 2001; Cox-Foster et al., 2007; Sguazza et al., 2013; Stoltz et al., 1995), and followed the method described by Chen et al. (Chen et al. 2006). A commercial one-step RT-PCR kit (QIAGEN, Germany) was used for the assay. The reaction mixture (25 µL total volume) consisted of 12.5 µL of RT-PCR Master Mix, 0.8 µL of each forward and reverse primer (10 pmol), 0.25 µL of RT Mix, 5.65 µL of nuclease-free water, and 5 µL of RNA template. Amplification was performed in a thermal cycler (T100 Thermal Cycler, BIO-RAD, Singapore) under the following conditions: reverse transcription at 50 °C for 30 minutes, followed by initial denaturation at 95 °C for 15 minutes. This was followed by 40 amplification cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 60 seconds, and extension at 72 °C for 60 seconds. A final extension step was carried out at 72 °C for 10 minutes (Table 1).

Table 1. Primers used in the diagnosis of ABPV disease.

Virus	Primers	Amplicon (bp)
ABPV	ABPV-F: 5'-TTATGTGTCCAGAGACTGTATCCA-3'	900 bp
	ABPV-R: 5'-GCTCCTATTGCTCGGTTTTTCGGT-3'	

The PCR amplicons were run in TAE buffer with 1% agarose gel containing ethidium bromide and using a UV transilluminator. The results were evaluated based on the expected amplicon sizes for each virus.

A separate mixture is prepared for ABPV viral disease and the appropriate Primers reported are added to this mixture. The obtained PCR products were carried out by 2% agarose gel electrophoresis and the products were visualized under UV. The RNA bands given below for ABPV viral disease run in Gel using Positive and Negative Controls are examined under an ultraviolet transilluminator. While evaluating the test result, Acute Bee Paralysis Virus 900 bp also give bands, the results are considered positive. The Negative Control should give no bands.

Positive virus samples used in this study were obtained from the Samsun Veterinary Control Institute. The GenBank accession number for the ABPV-positive control was OP504103.

RESULTS

The study materials were collected from apiaries located in the Guba-Khachmaz economic region (North-Eastern Azerbaijan) and the Lankaran-Astara economic region (South-Eastern Azerbaijan). During the investigation, ABPV was not detected in any samples collected from the Jalilabad district of the South-Eastern zone. However, the prevalence of ABPV in other districts of the South-Eastern zone was as follows: 12.5% in Masalli, 25% in Yardimli, 62.5% in Lankaran, 37.5% in Lerik, and 75% in Astara. In the North-Eastern zone, ABPV was detected in 12.5% of samples from Shabran and 62.5% of samples from Guba (Table 2).

Table 2. Prevalence of ABPV in Eastern of Azerbaijan

Location	Number of apiaries examined	Number of hives examined in apiaries	Number of positive samples (hives)	Number of positive simples (apiaries)
Jalilabad	8	20	0	0 (0%)
Masalli	8	37	5	1 (12.5%)
Yardimli	8	40	10	2 (25%)
Lankaran	8	60	38	5 (62.5%)
Lerik	8	25	9	3 (37.5%)
Astara	8	30	22	6 (75%)
Shabran	8	15	2	1 (12.5%)
Guba	8	55	34	5 (62.5%)
Total	64	282	120	23 (~40%)

According to the results, ABPV was detected in 6 of 16 apiaries (36 of 70 hives) in the Northeast zone and in 17 of 48 apiaries (84 of 212 hives) in the Southeast zone. The highest infection rate was observed in the Astara district, while no infections were detected in the Jalilabad district. Compared to the other regions, Masalli and Shabran showed the lowest infection rates (Table 2).

In this study, the presence of ABPV was confirmed by RT-PCR targeting the structural polyprotein gene, which amplified a 900 bp fragment (Figure 2).

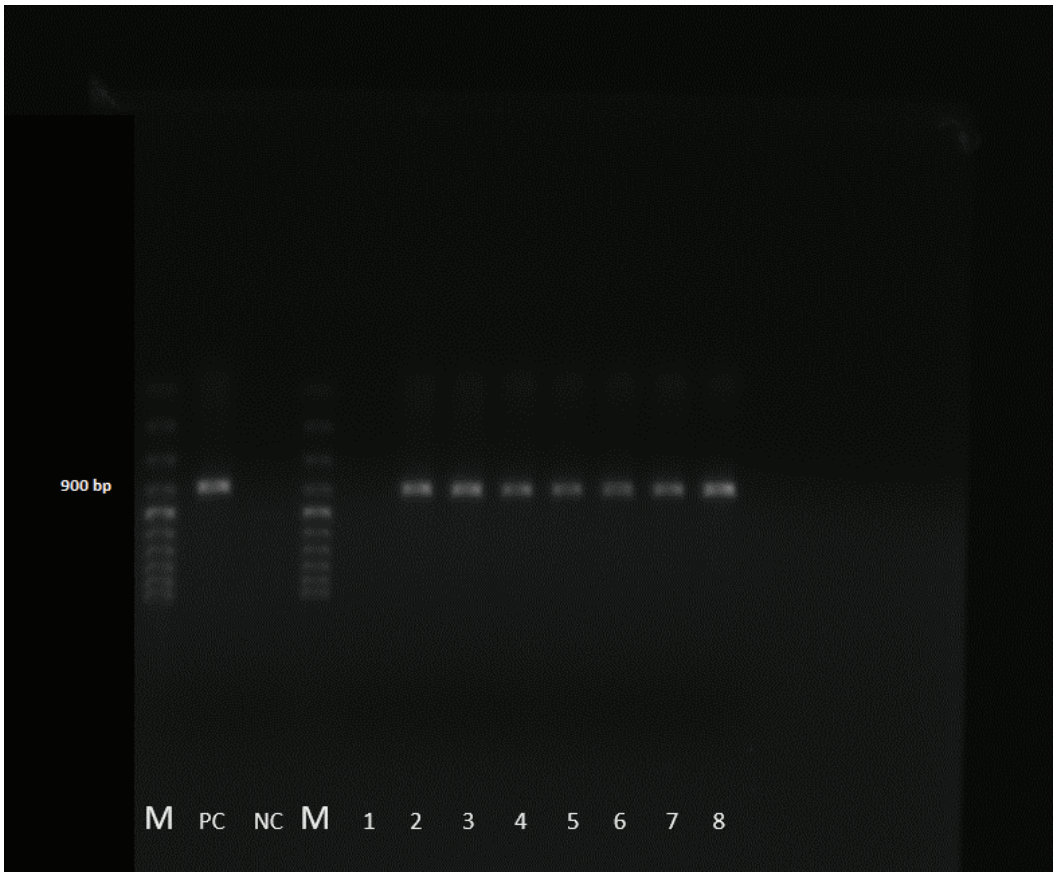

Figure 2. M: Marker PC: Positive controls of ABPV, NC: Negative controls of ABPV 1: Jalilabad 2: Masalli, 3: Yardimli, 4: Lankaran, 5: Lerik, 6: Astara, 7: Shabran, 8: Guba.

DISCUSSION

Bees and their products are indispensable to humanity. Honeybees are found almost worldwide, with the exception of Antarctica. They play a crucial role in biodiversity conservation by contributing significantly to pollination (Gallai et al. 2009).

Acute Bee Paralysis Virus (ABPV) has been detected in both honeybee larvae and adults, with infections particularly prevalent during the summer months when bee populations are at their highest (Bailey et al. 1981). ABPV has been reported worldwide, with prevalence rates of 79.78% in apiaries in Slovenia, 54.5% in Brazil, and between 73% and 80% in Germany (Nogueira et al. 2024, Siede et al., 2008, Šimenc et al. 2021).

In neighboring regions of Azerbaijan, varying prevalence rates have been documented. Kalashnikov et al. (2013) reported a 13.3% prevalence of ABPV in samples collected from the Udmurtia region of the Russian Federation, located northeast of Azerbaijan. A broader study across 18 regions of Russia—including Arkhangelsk, Belgorod, Voronezh, Kirov, Leningrad, Moscow, and others—also noted that ABPV causes significant losses in beehives (Zinatullina et al. 2018). In our study, a 62.5% prevalence of ABPV was observed in the Guba region, which borders Russia.

In the southern bordering regions of Iran—Mazandaran, Khorasan, and Kurdistan—the prevalence of ABPV was reported as 3.37% during research conducted between 2015 and 2016 (Ghorani et al. 2017). Notably, in the Astara district of Azerbaijan, which shares a land border with Iran, our research detected a 75% prevalence rate.

In Türkiye, the spread of ABPV varies by region: 25% in the Black Sea region (Gumusova et al. 2010), 3.6% in the Aegean region (Çağırgan 2018), 2.2% in the South-East region (Rüstemoğlu 2015), and up to 74% in the Anatolian region (Usta and Yildirim 2022).

Based on these findings, it can be concluded that ABPV prevalence is notably higher in Azerbaijan's border regions adjacent to Russia and Iran.

Conclusion

To the best of our knowledge, this study represents the first investigation confirming the presence of Acute Bee Paralysis Virus (ABPV) in Azerbaijan. Our findings indicate a higher prevalence of ABPV in the South-Eastern and North-Eastern zones, particularly in regions bordering Russia and Iran. In contrast, samples collected from regions located further from the national borders, such as Jalilabad, showed no presence of the virus, while areas like Shabran and Masalli exhibited only low infection rates.

To better understand the distribution and potential transboundary spread of ABPV within Azerbaijan and neighboring countries, more extensive and comprehensive studies are needed. In particular, sampling and molecular analysis of bees from other key regions—such as Karabakh, Eastern Zangazur, Aran, Nakhchivan, and the Lesser and Greater Caucasus—should be undertaken. Additionally, constructing a phylogenetic tree would provide valuable insights into the genetic relationships and origins of ABPV strains circulating in these areas.

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Conflict of Interest: There is no potential conflict of interest.

Ethics approval: There is no animal or human study requires ethics permission.

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Data Availability: Not applicable

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