TURKISH JOURNAL OF VETERINARY RESEARCH
TJVR 2017;1(1):20-23
Published online at www.dergipark.gov.tr/tjvr
Research Article

Analysis of COI Gene Region of *Varroa destructor* in Honey Bees (*Apis mellifera*) in Province of Siirt

Adnan AYAN[1], Osman Selcuk ALDEMIR[2], Zeliha SELAMOGLU[3]

[1]Department of Genetics, School of Veterinary Medicine, University of Yuzuncu Yil, Van, Turkey
[2]Department of Parasitology, School of Veterinary Medicine, University of Adnan Menderes, Aydın, Turkey
[3]Department of Biotechnology, School of Arts and Science, University of Omer Halisdemir, Niğde, Turkey

Received: 11.11.2017 Accepted: 01.12.2017

ABSTRACT

Objectives: *Varroa destructor* is the most damaging ectoparasite to the beekeeping economy. The mite has different haplotypes. It is aimed to determine which haplotype is present by examining the cytochrome c oxidase subunit 1 (COI) gene region of V. destructor found in honey bees in Siirt region.

Materials and Methods: Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) methods were applied in the analysis of the COI gene region of V. destructor in Siirt region. To do this, V. destructor samples were collected from 387 enterprises in the Siirt region. DNA extraction followed the PZR. Subsequently, 1.5% agarose gel images were obtained by electrophoresis. The PCR products were then subjected to XhoI and SacI restriction enzymes and 2% agarose gel images were obtained. 38 of the samples (10%) were sent to a private enterprise for sequencing. The obtained sequences were blasted and compared with the corresponding reference sequences in GenBank.

Results: According to the results of PZR and RFLP obtained from the 387 V. destructor samples in the study towards the COI gen region, all of the samples were found to be Korean haplotypes and Japanese haplotypes were not found in any of 387 samples. At the same time, it was also confirmed that the 38 sequenced samples were Korean haplotypes.

Discussion: The results obtained from this study are significant in terms of forming a groundwork for future studies.

Keywords: PCR, Siirt, *Varroa destructor*

INTRODUCTION

Varroa destructor, the biggest problems of beekeeping all over the world, is an ectoparasite of Apis mellifera (Rosenkranz et al., 2010). It is also a vector that carries various infectious diseases such as Kashmir Bee Virus, Deformed Wing Virus, Acute Bee Paralysis Virus in A. mellifera colonies (Tentcheva et al., 2006; Strapazzon et al., 2009). Considering economic losses, bee has an important place among the causes of the disease. It causes winter losses in honey bees, and body deformation, decrease in colony growth rate, decrease in nectar and pollen collection capacity, decrease in bee flight activity, advanced infestations, serious economic losses and colony infections in adult bees. In advanced infestations, the colony disappears, and serious economic losses occur in beekeeping (Marcangeli et al., 1992; Duay et al., 2003; Amdam et al., 2004; Garedew et al., 2004). The disease is widespread throughout the world except in the Hawaiian Islands, Australia, New Zealand, and some regions of Africa (Anderson and Trueman, 2000). Recent studies have shown that significant

Corresponding author: *Adnan AYAN* ***E-mail***: *adnanayan@yyu.edu.tr*

changes occurred in V. destructor Japanese haplotypes and Korean haplotypes (Strapazzon et al., 2009; Guerra et al., 2010).

There are no studies on the cytochrome c oxidase subunit 1 (COI) gene region of V. destructor in the Siirt region of Turkey. Therefore, it is aimed in this study to examine the COI gene region of 387 V. destructor samples collected from Siirt region.

MATERIALS AND METHODS

In this study; 387 adult female *Varroa destructors* were collected among queen bees, male bees and worker bees from the barrels of beekeeping enterprises in Pervari, Siirt province and Şirvan in Siirt region. Collected samples were brought to the laboratory. V. destructor samples brought to the laboratory were DNA extracted using the Qiagen DNeasy Blood & Tissue Kit as described in the kit protocol. COXF [5'GG(A/G)GG(A/T)GA(C/T)CC(A/T)ATT(C/T)T(A/T)TATCAAC3'] and COXRa [5'GG(A/T)GACCTGT(A/TA(A/T)AATAGCAAATAC3'] primers used by Strapazzon et al. in their study (2009) for the Polymerase Chain Reaction (PCR) were also used in our study.

In a total of 50 µL reaction, 5 µL 10X PCR Buffer, 3 mM $MgCl_2$, DNase/RNase-Free Distilled Water, 0.3 µM of each primer, 1.25 units of Taq DNA polymerase, 1 mM dNTP and 2-25 ng of DNA sample were used. The reaction was carried out on an AB Applied Biosystems Veriti brand automated thermal cycler. The steps of the reaction were preliminary denaturation at 94 °C for 4 min, denaturation in each of the cycles at 94 °C for 1 min, annealing at 50 °C for 1.30 min, elongation at 72 °C for 1.30 min, consisted of 35 cycles and the last elongation step was carried out at 72°C for 10 minutes. Then 1.5% agarose gel was prepared. Amplified PCR products were separated on agarose gel electrophoresis. After this, images were obtained on the gel imaging device (UV transilluminator, UVP EC3 ChemiHR 410 Imaging System).

Then, the Restriction Fragment Length Polymorphism (RFLP) method was used to identify *Varroa destructor* Japanese and Korean haplotypes. *Sac*I and *Xho*I digestion enzymes used by Strapazzon et al. (2009) in their study was also used in our study. After this procedure, 2% agarose gel images were obtained. 38 (10%) of the samples were sent to a private enterprise for bidirectional sequence analysis. The obtained sequences were blasted and compared with the corresponding reference sequences in GenBank.

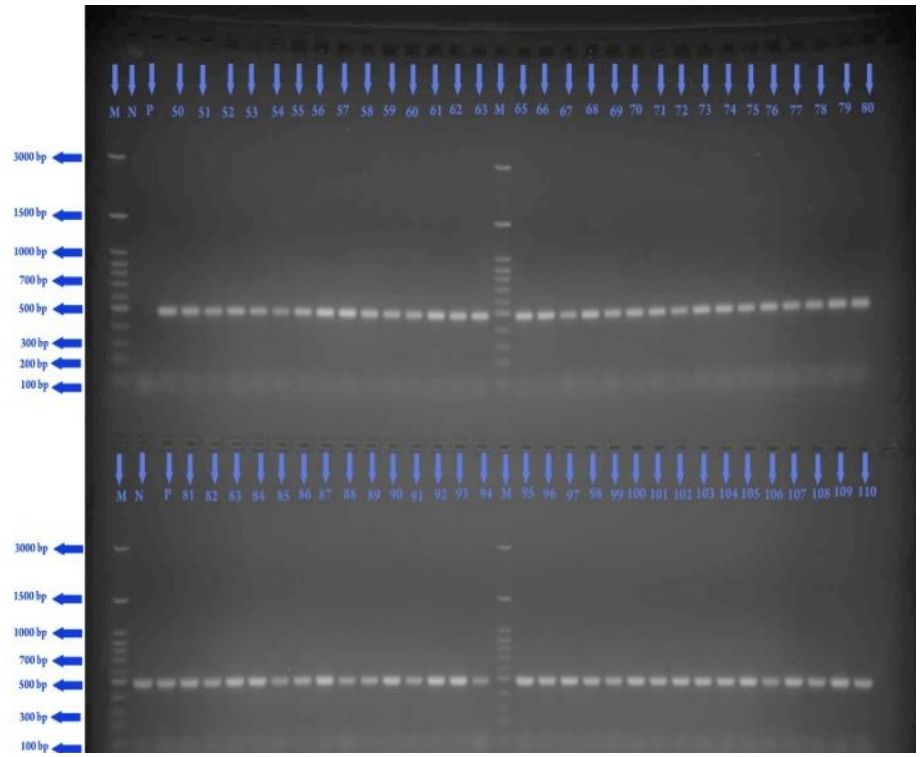

Figure 1. Varroa destructor COI gene region of mtDNA 1.5 % gel electrophoresis image of some of the samples in the PCR process (approximately 570 bp).

RESULTS

In the PCR amplification of the COI gene region of the 387 *V. destructor* samples used in the study, bands about 570 bp in size were obtained (Figure 1). *Xho*I

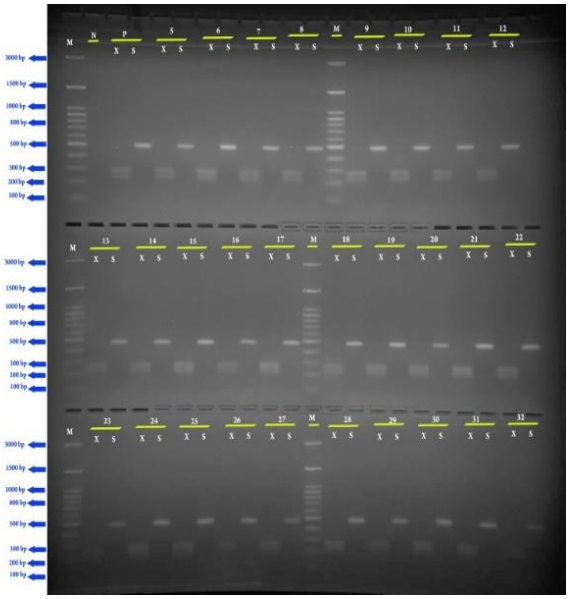

Figure 2. Varroa destructor restriction profiles of the COI region of mtDNA were digested with endonucleases SacI (S) and XhoI (X) 2% Gel electrophoresis image of the samples in the restriction fragment length polymorphism (RFLP) process.

and *Sac*I restriction enzymes were applied to all samples. The *Xho*I restriction enzyme cut only two amplified genomic DNA fragments, and two bands of 270 and 300 bp in size were obtained. However, the *Sac*I restriction enzyme did not cut. The bands obtained were specific for the *V. destructor* Korean haplotype (Figure 2). It was confirmed that the 38 sequenced samples were *V. destructor* Korean haplotypes. (Anderson & Fuchs, 1998; Genbank accession number:AF010478)

Based on these results, it was determined that the band sizes obtained in the study on the COI gene region of the 387 *V. destructor* examined and all the samples according to the results of RFLP were *V. destructor* Korean haplotype and *V. destructor* Japanese haplotypes were not observed in any of the 387 samples.

DISCUSSION

In this study, the COI gene region of 387 *V. destructor* samples collected from Siirt region was examined using molecular techniques. The presence of *Varroa destructor* Korean haplotypes identified by Anderson and Trueman (2000) was confirmed in the province of Siirt, Pervari and Şirvan districts. According to Solignac et al. (2005), the Korean haplotype invades all European and African honey bee breeds. Compared to the Japanese haplotype, it is more virulent (Anderson and Trueman, 2000). Fazier et al. (2010) reported Korean haplotype in Kenya.

In their study, Navajas et al. (2010) detected Korean haplotypes of *Varroa destructor* in Asia and some regions of Russia (Xishuanbanna, Nanchang, Seoul, Vladivostok and Hanoi), and Japanese haplotypes in the other regions of Asia (provinces of Taichung and Chiang Mai). At the same time, they found both Korean haplotypes and Japanese haplotypes in Tokyo. Rasolofoarivao et al., (2013) found that Korean haplotypes in Madagascar. Solignac et al. (2005) reported Japanese haplotypes in Japan, Taiwan, French Guyana and Chile. They also reported Korean haplotypes in France (Avignon, Alsace, Cevennes, Ardieche, Charente, Orne, Sarthe), Poland (Varsovie), England (Sheffield), Scotland (Lockerbie), Algeria, Africa (Pretoria), Chile A (Valdivia1), Chile B (Valdivia2) Chile C (Valdivia3), Chile D (La Union1), Chile E (La Union2), Chile F (Futrono1), Chile G (Futrono2), Chile H (Futrono3), Chile I (Santiago1), Chile J (Santiago2), Argentina A, Argentina B, French Guyana (Sinnamary), Mexico (Mexico A, Mexico B, Mexico C), United States of America (Pennsylvania, Michigan, Boulder Creek CA), New Zealand (Auckland), Israel (Bet Degan), Philippines (Beppu, Bico, Luzon, Cebu City), China (Beijing), Taiwan (Taichung), Japan (Yatsushiro, Machida, Noda, Yokohama, Tokyo), Nepal (Kathmandu). Strapazzon et al., (2009) reported the *V. destructor* Korean haplotype in Santa Catarina, while reported the *V. destructor* Japanese haplotype from Fernando de Noronha, Brazil

Warrit et al., (2004) reported that all the samples from Eregli, Kastamonu-İnebolu, Sinop-Erfelek, Samsun-Bafra, Ordu-Yokuşdibi, Gümüşhane-Kurtun, Bayburt and Rize-Anzer in Black Sea Region of Turkey were all Korean haplotypes.

In this study carried out in the Siirt region, the Korean haplotypes of *V. destructor* were detected in all the samples examined. But the Japanese haplotype was not detected. This shows that *V. destructor* in honey bees in Siirt region proves to be more pathogenic and will

cause serious problems in the colony. This and similar molecular studies are important in the diagnosis and isolation of parasitic agents, and developing prevention strategies against agents. It is thought that the results obtained from this study are also important in terms of forming grounds for future studies.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Akinwande KL, Badejo MA, Ogbogu SS. Incidence of the Korean haplotype of *Varroa destructor* in southwest Nigeria. *J Apic Res* 2012; 51:369-70.

Amdam GV, Hartfelder K, Norberg K, Hagen A, Omholt SW. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering? *J Econ Entomol* 2004; 97:741-747.

Anderson DL, Fuchs S. Two genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on *Apis mellifera*. *J Apic Res* 1998; 37: 69-78.

Anderson DL, Trueman JWH. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exp Appl Acarol* 2000; 24:165-189.

Duay P, De Jong D, Engels W. Weight loss in drone pupae (*Apis mellifera*) multiply infested by *Varroa destructor* mites. *Apidologi*e 2003; 34:61-65.

Fazier M, Muli E, Conklin T, et al. A scientific note on *Varroa destructor* found in East Africa; threat or opportunity? Apidologie 2010; 41:463-465

Gajic B, Radulovic Z, Stevanovic J, et al. Variability of the honey bee mite *Varroa destructor* in Serbia, based on mtDNA analysis. *Exp Appl Acarol* 2013; 61:97-105.

Garedew A, Schmolz E, Lamprecht I. The energy and nutritional demand of the parasitic life of the mite *Varroa destructor*. *Apidologie* 2004; 35:419-430.

Guerra JCV Jr, Iss MRC, Carneiro FE, Strapazzon R, Moretto G. Genetics and molecular research RAPD identification of *Varroa destructor* genotypes in Brazil and other regions of the Americas. *Genet Mol Res* 2010; 9:303-308

Marcangeli JA, Eguaras MJ, Fernandez NA. Reproduction of *Varroa jacobsoni* (Acari: Mesostigmata: Varroidae) in temperate climates of Argentina. *Apidologie* 1992; 23:57-60.

Navajas M, Anderson DL, De Guzman LI, et al. New Asian types of *Varroa destructor*: a potential new threat for world apiculture. *Apidologie* 2010; 41:181-193.

Rasolofoarivao H, Clemencet J, Ravaomanarivo LH, Razafindrazaka D, Reynaud B, Delatte H. Spread and strain determination of *Varroa destructor* (Acari: Varroidae) in Madagascar since its first report in 2010. *Exp Appl Acarol* 2013; 60:521-530

Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of *Varroa destructor*. *J Invertebr Pathol* 2010; 103:96-119.

Solignac M, Cornuet JM, Vautrin D, et al. The invasive Korea and Japan types of Varroa destructor, ectoparasitic mites of the Western honeybee (*Apis mellifera*), are two partly isolated clones. *Proc R Soc Lond B Biol Sci* 2005; 272:411-419.

Strapazzon R, Carneiro FE, Guerra Jr JCV, Moretto G. Genetic characterization of the mite Varroa destructor (Acari: Varroidae) collected from honey bees *Apis mellifera* (Hymenoptera, Apidae) in the state of Santa Catarina, Brazil. *Genet Mol R*es 2009; 8:990-997.

Tentcheva D, Gauthier L, Bagny L, et al. Comparative analysis of deformed wing virus (DWV) RNA in Apis mellifera and *Varroa destructor*. *Apidologie* 2006; 37:41-50.

Warrit N, Hagen TAR, Smith DR, Cakmak IA. Survey of *Varroa derstructor* strains on *Apis mellifera* in Turkey. *J Apic Res* 2004; 3:190-191.