Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.051

RESEARCH ARTICLE

Characterization of Infectious Laryngotracheitis Virus Isolates from Turkey by Molecular and Sequence Analysis

Kezban Can-Sahna[1], Hasan Abayli[1*], Remziye Ozbek[1], Sukru Tonbak[1] and Hakan Bulut[2]

[1]Department of Virology, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey
[2]Department of Virology, Faculty of Veterinary Medicine, Namik Kemal University, Tekirdag, Turkey
*Corresponding author: habayli@firat.edu.tr

ARTICLE HISTORY (20-006)

Received:	February 22, 2020
Revised:	April 27, 2020
Accepted:	May 12, 2020
Published online:	June 08, 2020

Key words:
ILTV
PCR-RFLP
sequence analysis
Turkey

A B S T R A C T

Infectious laryngotracheitis (ILT) is an economically important respiratory disease affecting the poultry industry worldwide. The aim of this study was to characterize the Turkish ILT virus (ILTV) isolates by sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). ILTV isolates obtained from laying hens in Turkey in 2003 and 2018 were used in this study. The isolates were analyzed for ICP4, gG, gE and TK gene regions by PCR. The amplification products were used in RFLP analysis to determine the differences among the isolates. Sequence analyses of TK and ICP4 regions were carried out and a phylogenetic tree was formed by using the Maximum Likelihood method. Nucleotide identity values were then calculated among five isolates and other strains/isolates in Genbank. In addition, about 200 amino acid sequences of the start and end regions of the ICP4 gene were compared to other strains in Genbank. PCR-RFLP analysis indicated that Turkish ILTV isolates were low-virulent. In general, the nucleotide sequence similarities of the TK and ICP4 gene regions among Turkish isolates and others was more than 95% (lower in some Egyptian and Bangladeshi strains, 41 and 45% respectively); in the amino acid sequence, it was close to 100%. As a result, PCR-RFLP results were similar in many gene regions. However, evolutionary analysis of ICP4 and TK gene regions did not yield reliable results based on geographic distribution or pathogenicity levels. For this purpose, different methods, such as Bayesian analysis or the involvement of samples from different gene regions can yield more reliable results, just like whole-genome sequences.

To Cite This Article: Can-Sahna K, Abaylı H, Ozbek R, Tonbak S and Bulut H, 2020. Characterization of Infectious Laryngotracheitis Virus Isolates from Turkey by Molecular and Sequence Analysis. Pak Vet J, 40(3): 337-342. http://dx.doi.org/10.29261/pakvetj/2020.051

INTRODUCTION

Infectious laryngotracheitis (ILT) is an economically important acute respiratory disease of poultry - especially of chickens - affecting the industry worldwide. The disease is frequently seen in densely populated poultry production areas and causes serious production losses due to reduced egg and meat yields, susceptibility to respiratory pathogens (*Escherichia coli*, *Mycoplasma*, etc.) and deaths (70% mortality in severe outbreaks [Garcia *et al*., 2013]).

There are two forms of the disease: epidemic and endemic. The epidemic form appears when ILTV has not been seen in the area it strikes; the affected birds are in poor health and have a high mortality rate. Following infection, the virus becomes persistent and constitutes the endemic form, resulting in sporadic and mild outbreaks (Guy and Garcia, 2008). Recently, the authors of several studies have shown that the source of outbreaks may be chicken embryo or tissue culture origin vaccines (Lee *et al.*, 2012; Chacon *et al*., 2015).

Gallid herpesvirus 1 (GaHV-1) or infectious laryngotracheitis virus (ILTV) is the causative agent of infectious laryngotracheitis. This agent has been described in the taxonomic classification of the *iltovirus* genus of the subfamily *Alphaherpesvirinae* in *Herpesviridae* family (McGeoch *et al*., 2000, 2006). In this subfamily, *Mardivirus*, *simplex virus*, and *Varicellovirus* genera are also classified (Davison *et al*., 2005). The linear, double-stranded DNA (dsDNA) genome of the ILTV is

approximately 150 kilobases (kb) in length. This structure consists of UL (Unique long-133 kb), US (Unique short-13 kb) and inverted repeat sequences (11 kb). It has about 80 open reading regions, and 63 of them are homologous to Herpes Simplex Virus-1 (HSV-1 [McGeoch *et al*., 2000; Thureen and Keeler, 2006]).

Since immunodominant epitopes are very close, it is not possible to distinguish ILTV strains by serological techniques, and therefore this virus is known as a single serotype. Although there are different forms of ILT, high-sequence similarities among ILTV strains complicate their molecular characterization (Guy and Garcia, 2008; Ou and Giambrone, 2012; Menendez *et al*., 2014). ILTV strains have been initially differentiated by restriction endonuclease analysis of viral DNA but routine RFLP analysis has limitations due to the need for high purity of the viral genome. Recently, the use of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) of multiple genome regions has proven to be a very useful method to search for regions of genetic diversity within the ILTV genome and to differentiate vaccine and field strains genetically. This approach has been utilized successfully in several countries to characterize circulating field strains (Oldoni *et al*., 2008). Several studies have been carried out for this purpose including those involving PCR-RFLP analysis of infected cell protein 4 (ICP4), thymidine kinase (TK), glycoprotein G (gG), and glycoprotein E (gE) gene regions in different countries (Chang *et al*., 1997; Oldoni and Garcia, 2007; Kim *et al*., 2013). In addition, some researchers have preferred gG, UL47, ICP4, and TK gene sequences in the differentiation of ILTV strains (Han and Kim, 2001; Ojkic *et al*., 2006; Chacon *et al*., 2010).

In this study, we aimed to characterize Turkish ILTV isolates using sequencing and PCR-RFLP methods. The molecular characterization of ILTV strains may be important in determining the types of infection, as this may sometimes point to the persistence and re-virulence of vaccines of chicken origin or reinfection after latent infection. Precautions for these types of infections may require the use of stable, non-virulent and/or non-latent TCO vaccines or safe, recombinant vaccines.

MATERIALS AND METHODS

Ethical statement: The study was approved with the decision number 2017/08 of the local ethics committee (Elazig Veterinary Control Institute) of animal experiments of Republic of Turkey, Ministry of Agriculture and Forestry.

Virus and cell culture: One of the isolates (Tur-Elzg-2004) used in this study was from the ILT epidemic that occurred in the laying hen flock in Elazig in October 2003. That same year, an ILTV stock isolated from primary culture cells of chicken origin (Gulacti *et al*., 2007) was kept in the Faculty of Veterinary Medicine, Firat University.

In 2018, four other isolates were obtained from tracheal swap clinical samples of different laying hens in Elazig (Tur-19-01, Tur 19-02, Tur-19-03) and Bingol (Tur-19-04) provinces. Isolation of the ILTV virus from clinical samples diagnosed in Firat University Veterinary Faculty Diagnostic Laboratory was performed in primary cell cultures of chicken origin.

DNA extraction and Polymerase Chain Reaction (PCR): Primary culture cells infected with the ILT virus were freeze-thawed several times and then lysed. Following the instructions for use of the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany), DNA was isolated from the lysate of the infected cells. Temperature parameters and primers used in the PCR step were adapted to the references indicated in Table 1.

Restriction Fragment Length Polymorphism (RFLP): RFLP analysis was performed according to the method reported by Chacon *et al*. (2010). For this purpose, the TK gene (*Hae*III, *Sau*96I and *Nci*I, Thermo Fisher, MA, USA), the gE gene (*Dde*I), the UL47-gG gene (*Nla*IV, Thermo Fisher, MA, USA), and the ICP4 gene (*Hae*III and *Msp*I, Thermo Fisher, MA, USA) were fragmented with restriction enzymes and examined on the agarose gel.

Sequence analysis: Bidirectional sequence analysis of the amplicons obtained by PCR was carried out (Medsantek Ltd. Co. Ist., Turkey). At this stage, the ABI3730XL sanger sequencing device (Applied Biosystems, CA, USA) and the BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, CA, USA) were used. The sequence data belonging to the TK and ICP4 (parts 1 and 2) genes of the ILTV were verified through nucleotide BLAST (http://www.ncbi.nlm.nih.gov) and entered into the Genbank database. Multiple alignments including the five ILTV genomes and some the ILTV sequences available in GenBank were performed using ClustalW2. Similarly, the translated ORFs of all the genomes were analyzed. Thymidine kinase sequences present in the Genbank (except vaccines) were selected from strains/isolates from different geographical locations. Parts 1 and 2 of the ICP-4 region were examined together with some ILTV full genome sequences and vaccine sequences present in the Genbank. The phylogenetic tree was generated by using the Maximum Likelihood method and the Tamura-Nei model; Bootstrap values were calculated in 1000 replicates. Besides, the nucleotide identity values of the multiple aligned sequence data were calculated by Sequence Identity and Similarity (SIAS).

RESULTS

PCR and sequence analysis of TK and ICP4 genes: At the end of the PCR, amplicons of the size indicated in Table 1 were obtained (excluding the Tur-Elzg-2004 isolate for ICP4 long primers). Sequence data for the ICP4 fragments are stored in the Genbank (MN073043, MN073044, MN073045, MN073046, MN073047, MN073048, MN073049, MN073055, MN073056, MN057733). Sequence data for the TK gene are stored in the Genbank (MN073050, MN073051, MN073052, MN073053, MN073054). According to SIAS analysis, the nucleotide identity ratios among Turkish isolates were calculated as 91 to 100%, 96.8 to 99.8%, and 98.9 to 99.9% in the first and second parts of the ICP4 and TK genes, respectively. Similarly, the nucleotide identity ratios among the Turkish isolates and the others were calculated

Table 1: Primer sets used in the PCR stage

Primer name	Region	Nucleotide sequence	Size	Reference
TKOP F	TK	CGGGATCCATCGTATAGGCCAGCCTT	1.3 kb	(Han and Kim, 2001)
TKOP R		GCTCTAGACCACGCTCTCTCGAGTAA		
ULP47gG F	gG	TCTTGAATGACCTTGCCCCAT	2.9 kb	(Kim *et al.,* 2013)
ULP47gG R		ACTCTCGGGTGGCTACTGCTG		
ICP4 F	ICP4	AACCTGTAGAGACAGTACCGTGACCC	4.9 kb	(Chang, 1997)
ICP4 R		CCATTACTACGTGACCTACATTGAGCC		
ICP41F part. 1	ICP4	ACTGATAGCTTTTCGTACAGCACG	688 bp	(Chacon and Ferreira, 2009)
ICP41R part. 1		CATCGGGACATTCTCCAGGTAGCA		
ICP4 2F part. 2	ICP4	CTTCAGACTCCAGCTCATCTG	635 bp	(Chacon and Ferreira, 2009)
ICP4 2R part. 2		AGTCATGCGTCTATGGCGTTGAC		
gE pozitive	gE	GGCTGACCAGGATAGTGAAC	1.8 kb	(Ojkic *et al.,* 2006)
Gg negative		GGTAAGATTTCCCGATTTCTC		

Table 2: Amino acid differences encoded at the beginning and end of the ICP4 gene in some ILTV strains

Strain/isolate name	Amino acid differences							
USDA Reference strain	65	87-90	199-205	1303	1337	1339-1340	1434	1460
	S	AAQD	A-VARSL	P	W	GG	P	V
Tur-19-01	.	.	A-MARSL	L	.	RD	.	.
Tur-19-02	.	.	A-MARSL	L	.	RD	.	.
Tur-19-03	.	.	A-MARSL	L	.	RD	.	.
Tur-19-04	L	.	A-MARSL	L	.	RD	.	.
Tur-Elzg-2004	.	absent	A-MARSL	L	.	RD	.	Y
LT-Blen-vaccine	.	absent	A-MARSL	L	.	RD	.	.
TCO-IVAX vaccine	.	.	same	.	.	. D	.	.
SA2 vaccine	.	.	same	.	.	RD	L	.
LaryngoVac vaccine	.	absent	A-MARSL	L	.	RD	.	.
Poulvac vaccine	.	absent	A-MARSL	L	.	RD	.	.
TCO-TRVX vaccine	.	absent	A-MARSL	L	.	RD	.	.
CL9 Aust virulent	.	absent	A-MARSL	L	.	RD	.	.
1874C USA virulent	.	.	P-WRDLW	.	.	RD	L	.
81658 USA Virulent	.	.	same	.	.	. D	.	.
ACC78 Aust Virulent	.	absent	A-MARSL	L	.	RD	.	.
JX458823.1 China Virulent		absent	A-MARSL	L	L	RD	L	.
CSW-1 Aust virulent	.	.	AMMARSL	.	.	RD	.	.
JX458822 China Virulent	.	absent	A-MARSL	L	.	RD	.	.
KU128407.1 Russia	.	.	A-MARSL	L	.	RD	.	.

as 91 to 100%, 95 to 99.6%, and 41 to 99.9% in the first and second parts of the ICP4 and TK genes, respectively. According to this analysis, although the identity ratio of the thymidine kinase gene was generally above 95%, it showed 41 to 45% identity with some Egyptian (JX977078.1) and Bangladeshi (KC576527.1) strains, respectively.

Phylogenetic analysis of ICP4 gene fragments: The phylogenetic relationship among Australian strains (CL9; ACC78; CSW-1), Chinese strains (JX458823.1; JX458822), Russian stain (KU128407.1) and USA strains (1874C; 81658) and six vaccine strains (LT-Blen, TCO-IVAX, SA2, LaryngoVac, Poulvac, and TCO-TRVX) was showed in Fig. 1 and Fig. 2. According to the phylogenetic evolution analysis of ICP4 part 1, two groups were drawn. The first group included the USDA reference strain, SA2, 1874C, 81658, and the TCO-Ivax strain, while the others were clustered in the second group. Similarly, two groups were drawn in the evolution analysis of ICP4 part 2. In contrast, in group 1, only Turkish ILTV isolates were included, while others were clustered in group 2.

Comparison of the amino acid sequences of the ICP4 gene: The amino acid sequences of nine virulent strains (Australian strains [CL9; ACC78; CSW-1], Chinese strains [JX458823.1; JX458822], Russian stain [KU128407.1], and USA strains [1874C; 81658]) and six vaccine strains (LT-Blen, TCO-IVAX, SA2, LaryngoVac, Poulvac, and TCO-TRVX) were aligned with published amino acid sequences of ICP4 gene. Table 2 summarizes the differences in the amino acid sequences of ICP4 parts. According to these results, we found some differences between amino acid sequences of Turkish ILTV isolates and others. Turkish ILTV isolates had ten different amino acid sequences from the USDA reference strain for the ICP4 gene. Some changes in the amino acid sequence of Turkish ILTV isolates (^{201}Val → Met, ^{1303}Pro → Leu, ^{1339}Gly → Arg, ^{1340}Gly → Asp) were the same. Also, the Tur-19-04 isolate (^{65}Ser → Leu and the Tur-Elzg-2004 isolate (^{1460}Val → Tyr) had different substitutions and there was no ^{57}AADQ^{60} amino acid sequence in Tur-Elzg-2004 isolate. We also did not find a specific amino acid change responsible for the virulence between a vaccine and other strains.

Phylogenetic analysis of TK gene: A multiple sequence alignment of 42 genomes from different countries was generated and a phylogenetic tree was created (Fig. 3). After the phylogenetic analysis of the TK gene, Turkish and some American ((1874C5, JN542533.1); (USDA, JN542534.1); (81658, JN542535.1)), Peruvian (VFAR, MG775218.1), South Korean (40798/10, MH937566.1), Russian (O-strain, KU128407.1), Chinese (MH159185.1), and Egyptian (JX977077.1) ILTV strains/isolates were included in the same group, which showed very high similarity.

Comparison of the RFLP patterns of the TK, gG, gE, and ICP4 genes: For RFLP analysis of the TK gene, *Hae*III (Fig. 4, lane 1-5), *Sau*96I (Fig. 4, lane 6-10), and *Nci*I (Fig. 4; lane 11-15) were selected. For RFLP analysis

Fig. 1: Phylogenetic evolution analysis of the part 1of ICP4 using Maximum likelihood method and Tamura-Nei model. Bootstrap values calculated in 1000 replicates. The part 1 of the ICP-4 region were examined together with some ILTV full genome sequences and vaccine sequences present in the Genbank. Turkey isolates are displayed in blue.

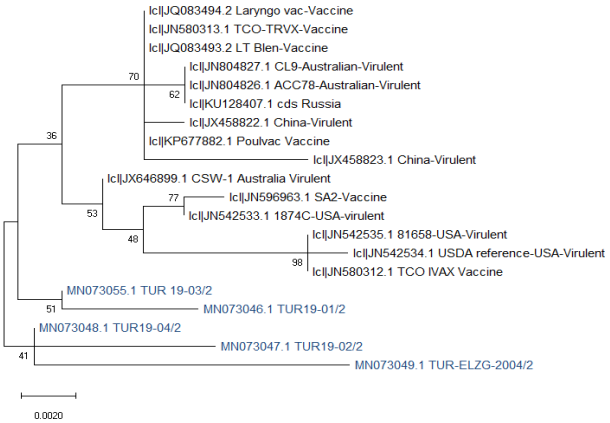

Fig. 2: Phylogenetic evolution analysis of the part 2 of ICP4 using Maximum Likelihood method and Tamura-Nei model. Bootstrap values calculated in 1000 replicates. The part 2 of the ICP-4 region were examined together with some ILTV full genome sequences and vaccine sequences present in the Genbank. Turkey isolates are displayed in blue.

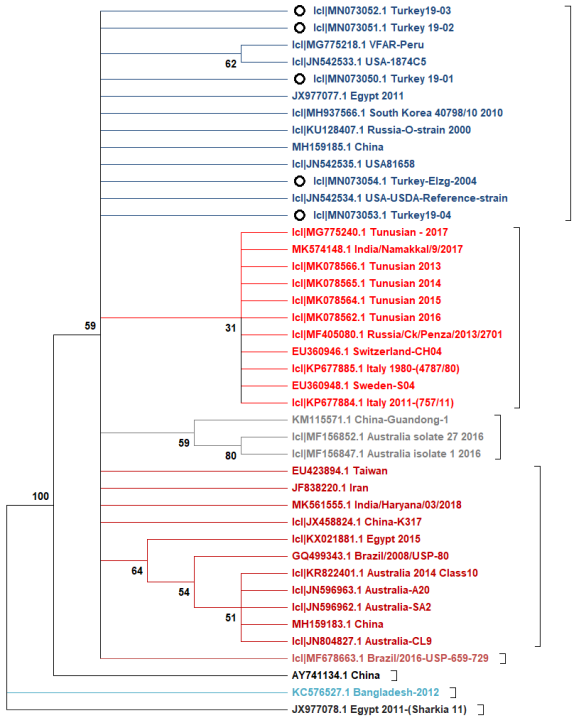

Fig. 3: Phylogenetic evolution analysis of the thymidine kinase gene of ILTV using the Maximum Likelihood method and Tamura-Nei model. Bootstrap values calculated in 1000 replicates. Thymidine kinase sequences present in the Genbank (except vaccines) were selected from strains/isolates with different geographical locations. Sequence clusters are shown in different colors.

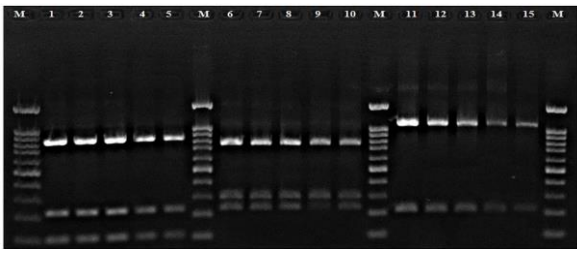

Fig. 4: RFLP analysis of the thymidine kinase gene of ILTV. Lane 1-5 HaeIII; lane 6-10; Sau96I; lane 11-15 NciI restriction enzyme. Lane 1; 6; 11 Tur 19-01 isolate, lane 2; 7; 12 Tur 19-02 isolate, lane 3; 8; 13 Tur 19-03 isolate; lane 4; 9; 14 Tur 19-04 isolate, lane 5; 10; 15 Tur-Elzg-2004 isolate M; 100 bp DNA ladder (Cleaver Scientific, England).

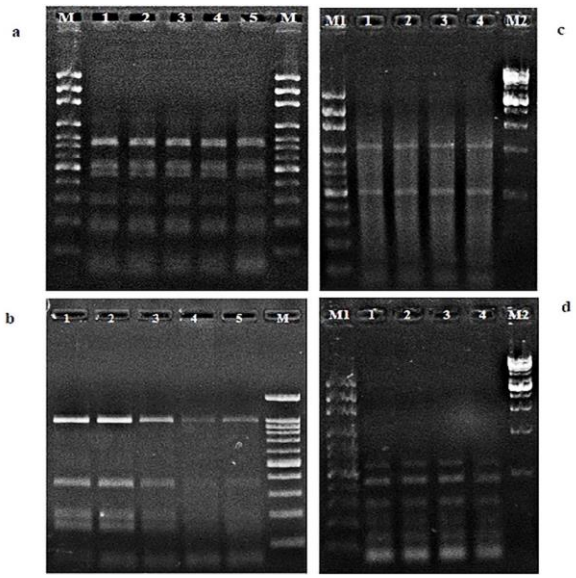

Fig. 5: RFLP analysis of gG (a; NlaIV), gE (b; DdeI) and ICP4 genes (c; MspI, d; HaeIII) of ILTV. Lane 1; Tur 19-01 isolate, lane 2; Tur 19-02 isolate, lane 3; Tur 19-03 isolate, lane 4; Tur 19-04 isolate, lane 5; Tur-Elzg-2004, M-M1; 100bp DNA ladder, M2; 1 kb DNA ladder (Vivantis, Malaysia).

of the gG, gE, and ICP4 genes were selected, *Nla*IV (Fig. 5a), *Dde*I (Fig. 5b) and *Msp*I (Fig. 5c) and *Hae*III (Fig. 5d), respectively. RFLP results showed the presence of at least one pattern recognizing restriction enzymes. There was no pattern difference between the isolates after PCR-RFLP analysis.

DISCUSSION

The laryngotracheitis virus has always been an important pathogen of concern for the modern poultry industry (Menendez *et al*., 2014). Although biosecurity is the basis for ILT control, vaccination is used to control the disease worldwide (Dufour-Zavala, 2008; Chin *et al*., 2009).

In Turkey, the ILT outbreak among laying hen flocks was first reported in 2003 in Elazig. The affected flocks by this epidemic exhibited severe symptoms (Gulacti *et*

al., 2007). Then, in 2013, 2014, 2016, and 2018, some poultry farms in Central and Eastern Anatolia in Turkey had outbreaks resulting in mild infections (Menendez *et al*., 2014; Aras *et al*., 2018; Kaya and Akan, 2018).

In this study, we aimed to characterize Turkish ILTV isolates using sequencing and PCR-RFLP methods. There was no pattern difference among the isolates based on the results of PCR-RFLP analysis. In our literature search, we found reports indicating that low virulence laryngotracheitis virus strains exhibit patterns similar to those of the current Turkish isolates. This was confirmed by similar results in multiple gene regions such as ICP4, gE, TK, and gG and the results strongly suggest that the isolates have low virulence, however, considering the report of Gulacti *et al*. (2007), the Tur-Elzg-2004 isolate which was isolated from the ILT epidemic in 2003 would be expected to have high virulence. There may be a number of reasons for this, one of which may be the increase of virulence by horizontal transmission. A second possible reason is the presence of mixed infections (low-high virulent, or secondary infections with other respiratory agents), and finally, host susceptibility. In order to determine the exact level of virulence, it is probably best to conduct experimental infections. The other four isolates (TUR-19-01, TUR-19-02, TUR-19-03, and TUR-19-04) were found to have low virulence as predicted (low mortality ratio). Although the main factor for mild infections is live vaccines since Turkey is restricting the use of live vaccines it is unlikely in this case (Garcia and Zavala, 2019). Recombinant vaccines have been frequently used in Turkey since 2014, but there is no information about vaccination before then. Therefore, an attenuated vaccine strain could not be studied in RFLP analysis.

Kirkpatrick *et al*. (2006) stated that PCR-RFLP for gG and TK genes should be used as the primary screening technique in determining the relationship between disease-related ILTV strains and vaccine strains, but combinations with ICP18.5 and ICP4 are recommended for comprehensive identification. In the same study, PCR-RFLP analysis showed that most of the ILTV Australian isolates were different from the vaccine strains (SA2 and A20). Only Q1-95, Q1-96, and Q1-100 isolates showed similar patterns with vaccine strains. These findings may reveal that existing isolates have resulted from increasing the virulence of the vaccine.

In a study conducted by Oldoni *et al*. (2008) 46 samples were collected from five poultry production centers in the USA between 2006 and 2007; these were analyzed by PCR-RFLP. Accordingly, American isolates were included in the group III-IV-V-VI. According to the same analysis, 63% (group III-IV-V) of the collected samples were similar to CEO and TCO, while 4% were both field and vaccine strains (virus mixtures). This shows that PCR-RFLP analysis with TK, ICP4, and gG genes is sufficient for differentiating most of the field isolates from the vaccine strain. However, only 3 isolates were distinguished from the CEO vaccine strain with the difference in gM.

Although RFLP results give some predictions about the virulence of the virus, it is difficult to see this in the sequence-evolution analysis. The reason for this is the high sequence similarity due to the fact that mutations are less common in alphaherpesviruses (Thiry *et al*., 2005). There is no definite limit between virulent and vaccine strains on the phylogenetic tree formed according to the Maximum Likelihood method of the TK and ICP4 (partials) genes. Virulent and vaccine strains are very similar to each other and there is no specific amino acid change among the strains. Additionally, the ICP4 and TK genes may not be suitable sites for sequence analysis. For example, Ojkic *et al*. (2006) preferred sequence analysis of the UL47 and gE regions with the ILT vaccine and in wild strain discrimination. When the clusters in the phylogenetic tree are examined, the geographic distribution of the strains containing the clusters cannot be fully correlated. Other methods, such as Bayesian analysis, may provide more reliable information about the evolution of the virus. Alternatively, evolutionary analysis including mutation and recombination can be considered by increasing the number of complete genome sequence data points of the ILT virus in the Genbank.

Conclusions: The characterization study for four different genes of the ILTV isolates was performed for the first time in Turkey and they were found to be low virulent. Further studies were needed using more isolates.

Acknowledgements: This work was supported by the project number VF18.07 by the Scientific Research Projects Coordination Unit of Firat University.

Authors contribution: KCS, HB; Conceived and designed study. HA, RO; Executed the experiments. ST, HB; Analyzed the data. HA, KCS; Prepared the manuscript.

REFERENCES

Aras Z, Yavuz O and Golen GS, 2018. Occurrence of Infectious laryngotracheitis outbreaks in commercial layer hens detected by ELISA. J Immunoassay Immunochem 39:190-5.

Chacón JL and Ferreira AJP, 2009. Differentiation of field isolates and vaccine strains of infectious laryngotracheitis virus by DNA sequencing. Vaccine 27:6731-8.

Chacón JL, Núñez LFN, Vejarano MP, *et al*., 2015. Persistence and spreading of field and vaccine strains of infectious laryngotracheitis virus (ILTV) in vaccinated and unvaccinated geographic regions, in Brazil. Trop Anim Health Prod 47:1101-8.

Chacon LJ, Mizuma MY and Ferreira AJP, 2010. Characterization by restriction fragment length polymorphism and sequence analysis of field and vaccine strains of infectious laryngotracheitis virus involved in severe outbreaks. Avian Pathol 39:425-33.

Chang PC, Lee YL, Shien JH, *et al*., 1997. Rapid differentiation of vaccine strains and field isolates of infectious laryngotracheitis virus by restriction fragment length polymorphism of PCR products. J Virol Methods 66:179-86.

Chin RP, García M, Corsiglia C, *et al*., 2009. Intervention strategies for laryngotracheitis: impact of extended downtime and enhanced biosecurity auditing. Avian Dis 53:574-7.

Davison AJ, Eberle R, Hayward GS, *et al*., 2005. In: Virus Taxonomy: Eight Report of the International Committee on Taxonomy of Viruses (Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, eds): Academic Press, San Diego pp:193-212.

Dufour-Zavala L, 2008. Epizootiology of infectious laryngotracheitis and presentation of an industry control program. Avian Dis 52:1-7.

Garcia M, Spatz S and Guy JS, 2013. Infectious laryngotracheitis. In: Diseases of Poultry (Swayne DE, ed): John Wiley & Sons Inc., Iowa, pp:161-79.

Garcia, M and Zavala G, 2019. Commercial vaccines and vaccination strategies against infectious laryngotracheitis: what we have learned and knowledge gaps that remain. Avian Dis 63:325-34.

Gulacti I, Bulut H, Eroksuz Y, *et al*., 2007. Outbreak of clinical infectious laryngotracheitis in Turkey. Vet Rec 160:554-5.

Guy JS and García M, 2008. Laryngotracheitis. In: Disease of Poultry (Saif YM, Fadly AM, Glisson JR, McDougald LL, Nolan LK, Swayne DE, eds): Blackwell Publishing, Ames, pp:137-52.

Han MG and Kim SJ, 2001. Analysis of Korean strain of infectious laryngotracheitis virus by nucleotide sequences and restriction fragment length polymorphism. Vet Microbiol 83:321-31.

Kaya I and Akan M, 2018. First report of avian infectious laryngotracheitis infection in broiler breeders in Turkey, Ankara Üniversitesi Veteriner Fakültesi Dergisi, 65:331-4.

Kim HR, Kang MS, Kim MJ, *et al*., 2013. Restriction fragment length polymorphism analysis of multiple genome regions of Korean isolates of infectious laryngotracheitis virus collected from chickens. Poult Sci 92:2053-8.

Kirkpatrick NC, Mahmoudian A, O'Rourke D, *et al.,* 2006. Differentiation of infectious laryngotracheitis virus isolates by restriction fragment length polymorphic analysis of polymerase chain reaction products amplified from multiple genes. Avian Dis 50:28-34.

Lee SW, Markham PF, Coppo MJ, *et al*., 2012. Attenuated vaccines can recombine to form virulent field viruses. Science 337:188.

McGeoch DJ, Dolan A and Ralph AC, 2000. Toward a comprehensive phylogeny for mammalian and avian herpesviruses. J Virol 74:10401-6.

McGeoch DJ, Rixon FJ and Davison AJ, 2006. Topics in herpesvirus genomics and evolution. Virus Res 117:90-104.

Menendez KR, Garcia M, Spatz SJ, *et al*., 2014. Molecular epidemiology of infectious laryngotracheitis: a review. Avian Pathol 43:108-17.

Ojkic D, Swinton J, Vallieres M, *et al*., 2006. Characterization of field isolates of infectious laryngotracheitis virus from Ontario. Avian Pathol 35:286-92.

Oldoni I and Garcia M, 2007. Characterization of infectious laryngotracheitis virus isolates from the Unites States by polymerase chain reaction and restriction fragment length polymorphism of multiple genome regions. Avian Pathol 36:167-76.

Oldoni I, Rodríguez-Avila A, Riblet S, *et al.,* 2008. Characterization of infectious laryngotracheitis virus (ILTV) isolates from commercial poultry by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Avian Dis 52:59-63.

Ou SC and Giambrone JJ, 2012. Infectious laryngotracheitis virus in chickens. World J Virol 1:142-9.

Thiry E, Meurens F, Muylkens B, *et al*., 2005. Recombination in alphaherpesviruses. Rev Med Virol 15:89-103.

Thureen DR and Keeler CL, 2006. Psittacid herpesvirus 1 and infectious laryngotracheitis virus: Comparative genome sequence analysis of two avian alphaherpesviruses. J Virol 80:7863-72.