



Circulation of Recently Reported Sub-genotype VII.1.1 of Newcastle Disease Virus in Commercial and Backyard Chicken in north of Iran

Saeed Seifi^{1*}, Mojtaba Khosravi²

¹ Department of Clinical Sciences, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

² Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

Abstract

BACKGROUND: Newcastle disease virus (NDV) majorly infects the poultry, and despite high rates of vaccination, it is still circulating in different geographical regions. Due to the high mortality rate, the economic loss of Newcastle disease (ND) is enormous.

OBJECTIVES: The molecular characterization of NDV isolates from chicken farms in Northern Iran, during 2017-2018, was the main goal of this study.

METHODS: We isolated and characterized five NDVs from commercial broiler and backyard chicken farms during severe disease outbreak. The partial coding sequence of fusion (F) genes of isolates was determined and compared with those of other published NDVs.

RESULTS: Phylogenetic analysis revealed that all of the isolates were grouped into sub-genotype VII.1.1 (formerly known as VIII). All isolates carried multi-basic amino acid residues at the cleavage site of fusion protein, typical of virulent strains. Studied NDV isolates had high homology with the prevalent genotype NDV strains that currently circulate in China and Republic of Korea (96.94%).

CONCLUSIONS: Our results suggested that NDV sub-genotype VII.1.1 (VIII), circulating among chicken farms, may be a dominant sub-genotype. Considering the genetic variation between the used vaccine strains (B1, LaSota, and Clone 30, all belonging to genotype II) and circulating NDVs, it is recommended that a contemporary homologous virus should be developed as the vaccine strain to avert the outbreaks of genotype VII viruses.

KEYWORDS: Chicken, F gene, Newcastle disease virus, Outbreak, Phylogenetic analysis

Correspondence

Saeed Seifi, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

Tel: +98 (011) 44271057, Fax: +98 (011) 44271054, Email: saeedseifi57@gmail.com

Received: 2020-09-06

Accepted: 2020-11-25

Copyright © 2021. This is an open-access article distributed under the terms of the Creative Commons Attribution- 4.0 International License which permits Share, copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, even commercially.

How to Cite This Article

Seifi, S., Khosravi, M., A. (2021). Circulation of Recently Reported Sub-genotype VII.1.1 of Newcastle Disease Virus in Commercial and Backyard Chicken in north of Iran. *Iranian Journal of Veterinary Medicine*, 15(1), 1-15.

Introduction

Newcastle disease (ND) is a highly contagious viral disease of most avian species caused by the virulent strains of Newcastle disease virus (NDV). Etiologically, NDV (avian paramyxovirus type-1) is a member of the Avulavirus genus of subfamily Paramyxovirinae (Afonso *et al.*, 2016). Pathogenicity of the virus amongst avian species is variable. Poultry are most susceptible to NDV and severity of the disease may vary from mild infection with no apparent clinical signs to highly pathogenic form with 100% mortality (Alexander, 2003). NDV genome is about 15.2 kb in length which codes for six major proteins including nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large RNA polymerase (L) (Wang *et al.*, 2017). HN and F are glycoproteins that allow the attachment and fusion of the virus to the host cells for initiating an NDV infection (Xiao *et al.*, 2012). The F protein is usually chosen for comparative analysis and molecular determination of NDV, because it is more likely to show genetic variation compared to other internal nucleocapsid genes (Cattoli *et al.*, 2010). NDV strains are classified into two distinct classes (class I and class II) within a single serotype, according to the phylogenetic analysis (Czeglédi, 2006). These two classes can then be further classified into genotypes. Class I viruses contain a single genotype, whereas class II viruses contain 21 (I–XXI) genotypes (Dimitrov *et al.*, 2019). Class I viruses are frequently isolated from wild birds and live poultry markets and are generally avirulent (Kim *et al.*, 2007; Zhu *et al.*, 2014), whereas class II viruses have been responsible for the spread of ND. Among class II NDVs, genotype VII is one of the most prevalent genotypes circulating worldwide. Based on the updated classification and nomenclature of NDV, previously identified NDV VII genotype was divided into sub-genotypes VII.1.1 and VII.1.2. The sub-genotype VII.1.1 combines the former

sub-genotypes VIIb, VIId, VIIe, VIIj, and VIII. The former sub-genotype VIIf was classified as sub-genotype VII.1.2. Sub-genotypes VIIa, VIIh, VIIi, and VIIk were merged into a single sub-genotype, namely VII.2 (Dimitrov *et al.*, 2019). Based on the clinical signs and severity of the disease, NDV strains are classified into four main pathotypes: asymptomatic enteric (avirulence), lentogenic (low-virulence), mesogenic (intermediate-virulence), and velogenic (high-virulence). Velogenic strains can be further categorized into two types: viscerotropic and neurotropic (James, 2017).

Notwithstanding virulent forms of ND controlled by biosecurity protocols and vaccination, outbreaks still occur and velogenic strains are endemic in the poultry flocks of Iran, which induce a large scale economic losses (Hosseini, *et al.*, 2014; Mehrabanpour *et al.*, 2014). Despite routine vaccination, a combination of killed and live vaccines as a controlling practice, there have been several reports on its occurrence in commercial and non-commercial poultry farms in recent years (Hosseini *et al.*, 2014; Sabouri *et al.*, 2016).

Poultry production is one the most important agro-industries in the north part of Iran. Mazandaran province, with 30% of total production, is the most important producer of day-old broiler chicks in Iran (IRNA, 2019). During the last decade, monitoring the NDVs suggested different genotypes of NDVs have been responsible for ND outbreak in Iran. In the current study, we aimed to define the most prevalent NDV genotypes and sub-genotypes circulating in commercial broiler and backyard chicken farms located in Mazandaran province, Iran.

Materials and Methods

Study Design

NDV develops a broad range of clinical manifestations such as respiratory signs, nervous signs, and greenish watery diarrhea. In this survey, we only focused on rural and commercial

meat-type chicken farms with a history of neurological symptoms. All samples were prepared from private poultry clinics in north of Iran during 2017-2018. Only fresh carcasses were considered. Collectively, twenty-five samples (sixteen samples from broiler farms and nine samples from backyard farms) compatible with our desired criteria were chosen for further analysis.

Sample Preparation

The whole brain of affected chickens was extracted under a Class II (laminar flow) biological safety cabinet and transferred to a sterile 15-mL falcon tube. Tissues were halogenated in phosphate buffered saline (PBS), pH 7.4, containing antibiotics of penicillin (2000 U/mL) and streptomycin (2 mg/mL), and filtered with a 0.22 µm syringe filter. Then, the filtered liquid was divided into two aliquots for RNA extraction and virus amplification, and kept at -70°C (Alexander, 2012).

Virus Propagation

To prepare virus, about 200 µL of each sample was inoculated in the allantoic cavity of 8-day old embryonated eggs and incubated at 37°C for seven days and candled twice daily.

For each sample inoculation, 3 eggs were selected. Any death after 24 hours was recorded and subjected to further analysis. Briefly, the allantoic fluid was collected by a sterile syringe carefully and stored in a sterile 15 mL falcon tube and stored at -70°C. All positive samples in HA testing were subjected to three passages in embryonated egg.

RNA Extraction and Reverse Transcriptase Polymerase Chain Reaction

Viral RNA was extracted and purified from infectious allantoic fluid and brain halogenated homogenized tissues using Viral Gene-spin™ Viral DNA/RNA Extraction Kit according to the manufacturer's instructions (iNtRON, South Korea). Then, cDNA was synthesized using Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) and Random 6-mers (Yektatajhez, cDNA Synthesis Kit, Iran). To define the NDV positive genome, PCR reaction was performed with primers A and B, which contains important structures such as the cleavage site (Kant *et al.*, 1997). Afterwards, coding sequence of F protein, which includes heads and stacking elements of protein was amplified by one set primer described by Qin *et al.* (2008). The information and annealing degree of all primer sets are illustrated in [Table 1](#)

Table 1. Sequence, annealing temperature, and position of primers used in this study

Primer	Sequence	Annealing Temperature	PCR product size (bp)
A	5'-TTGATGGCAGGCCTCTTGC-3'	52	352
B	5'-GGAGGATGTTGGCAGCATT-3'		
C	5'-ATGGGCTCCAAACCTTCTAC-3'	60	1600
D	5'-TTGTAGTGGCTCTCATC-3'		

Sequencing and Phylogenetic Analysis

To have more accurate and comprehensive information about F protein, the complete extracellular domain of protein was sequenced. The nucleotide sequences of virus isolates were investigated with Sequencher program (version

5.4.6; DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI, USA). Sequence alignment and blast analysis were done by an online webpage. The phylogenetic tree was illustrated with MEGA7 (MEGA, version

7) and the evolutionary history was inferred using the Neighbor Joining (data not shown). Maximum likelihood methods with standard errors were also calculated based on 500 bootstrap replicates (Kumar *et al.*, 2016; Tamura *et al.*, 2004).

Accession numbers

The sequence of F protein of NDV isolates (chicken/Iran/CR5/2017, chicken/Iran/CI1/2017, chicken/Iran/CI6/2017, chicken/Iran/CI3/2017, and chicken/Iran/CI9/2018) were submitted to GenBank under the accession numbers MK659694, MK659696, MK659698, MK659699, and MK659695, respectively.

Results

Nucleotide Sequencing and BLAST Analysis of Partial Fusion Gene

Altogether, out of twelve viral isolates from brain samples of RT-PCR-positive, five isolates (four from commercial and one from backyard farms) were randomly subjected to nucleotide sequencing as representative sequences. Nucleotide (nt) and amino acid (aa) comparisons of all studied isolates ranged from 98.85-99.75% and 99.23-99.53%, respectively. BLAST results of F gene revealed high percent of homology between NDV strains CK/KR/KR 005/00 (KY404087), GO/CH/LN15/15 (MF581297), and CK/UA/Lugansk/03 (KU710279) isolated from the Far East and Europe.

Phylogenetic Analysis

Coding region of the extracellular domain of F gene of the isolates were aligned with the corresponding region of the F gene from 291 NDV strains belonging to nineteen genotypes, downloaded from the GenBank and subjected to genetic analysis (data not shown). In the phylogenetic tree, our isolates were clustered with the strain under genotype VII (Figure 1). To confirm our preliminary results, the phylogenetic relationship was assessed using at least 4 strains for each sub-genotype VII (according to

the criteria proposed by Diel *et al.*, 2012). Our results showed that all the isolates belonged to sub-genotype VII.1.1 (VIII) in a well-supported cluster (60% bootstrap value) and displayed 98.03-100% homology in the nucleotide level (Figure 2)

Notes: The number of base substitutions per site from averaging the overall sequence pairs between groups is shown. Standard error estimates are shown above the diagonal and were obtained by a bootstrap procedure (500 replicates). Analyses were conducted using the maximum composite likelihood model. The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). The analysis involved 175 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 639 positions in the final data set. Evolutionary analyses were conducted in the MEGA7. The average evolutionary distance of NDV isolates obtained in the present study with other sub-genotypes from genotype VII are shown in bold (n: number of isolates from each sub-genotype).

Analysis of the Fusion Protein

Deduced amino acid sequence of the cleavage site motif, putative neutralizing epitopes, profile of glycosylation sites and cysteine amino acid residues of studied isolates, and other Iranian NDV isolates of F protein were investigated to find any possible mutations within them (Table 3).

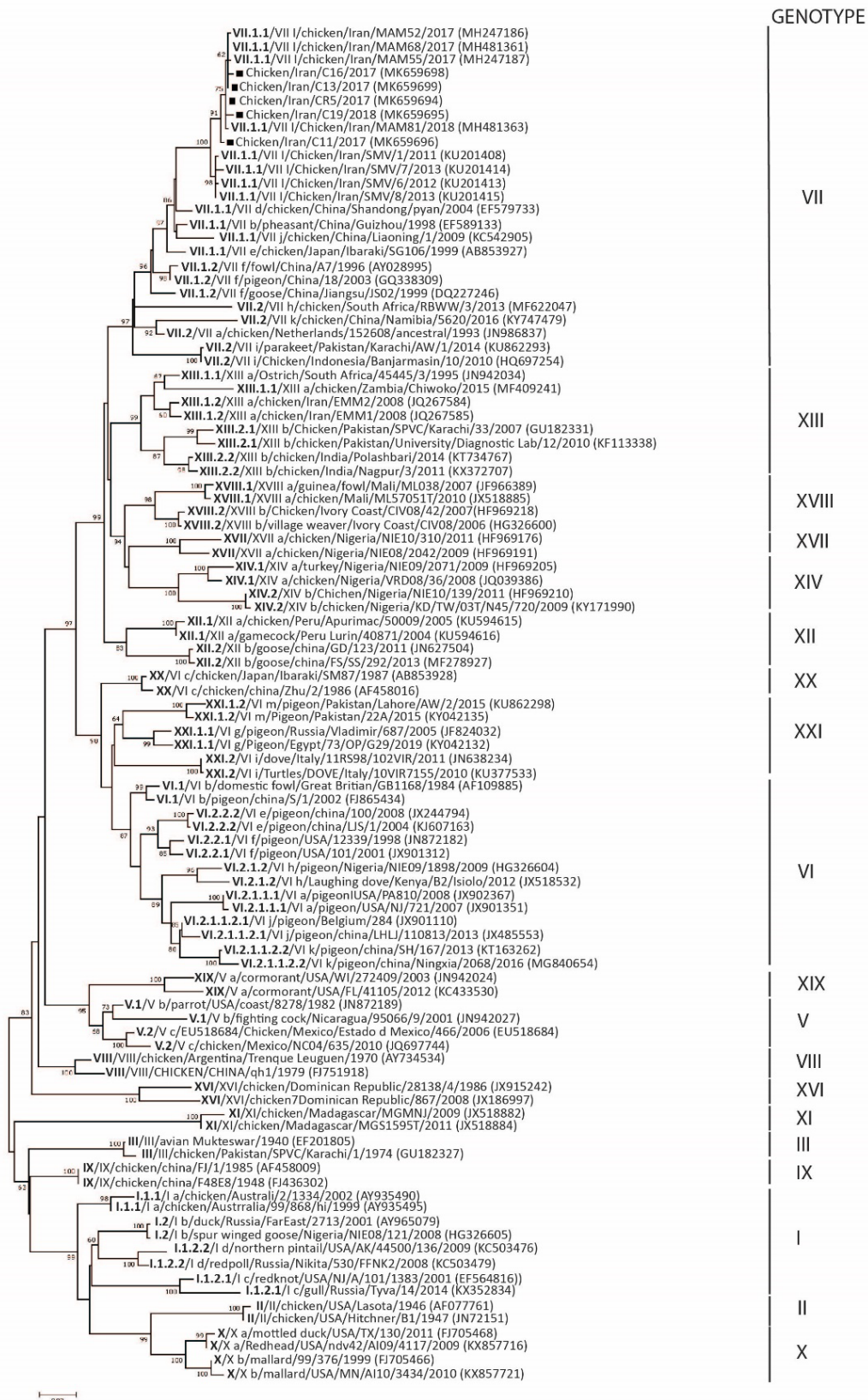


Figure 1. Phylogenetic tree of NDV strains based on the nucleotide sequence (42–1604) of the F gene. The tree was constructed using maximum likelihood method based on the Kimura 2-parameter model with MEGA7 program (Kimura, 1980). The analysis involved 121 nucleotide sequences and according to the criteria proposed by Dimitrov *et al.* (2019). Bootstrap >60% was applied for defining node. Black circle indicates the studied isolate.

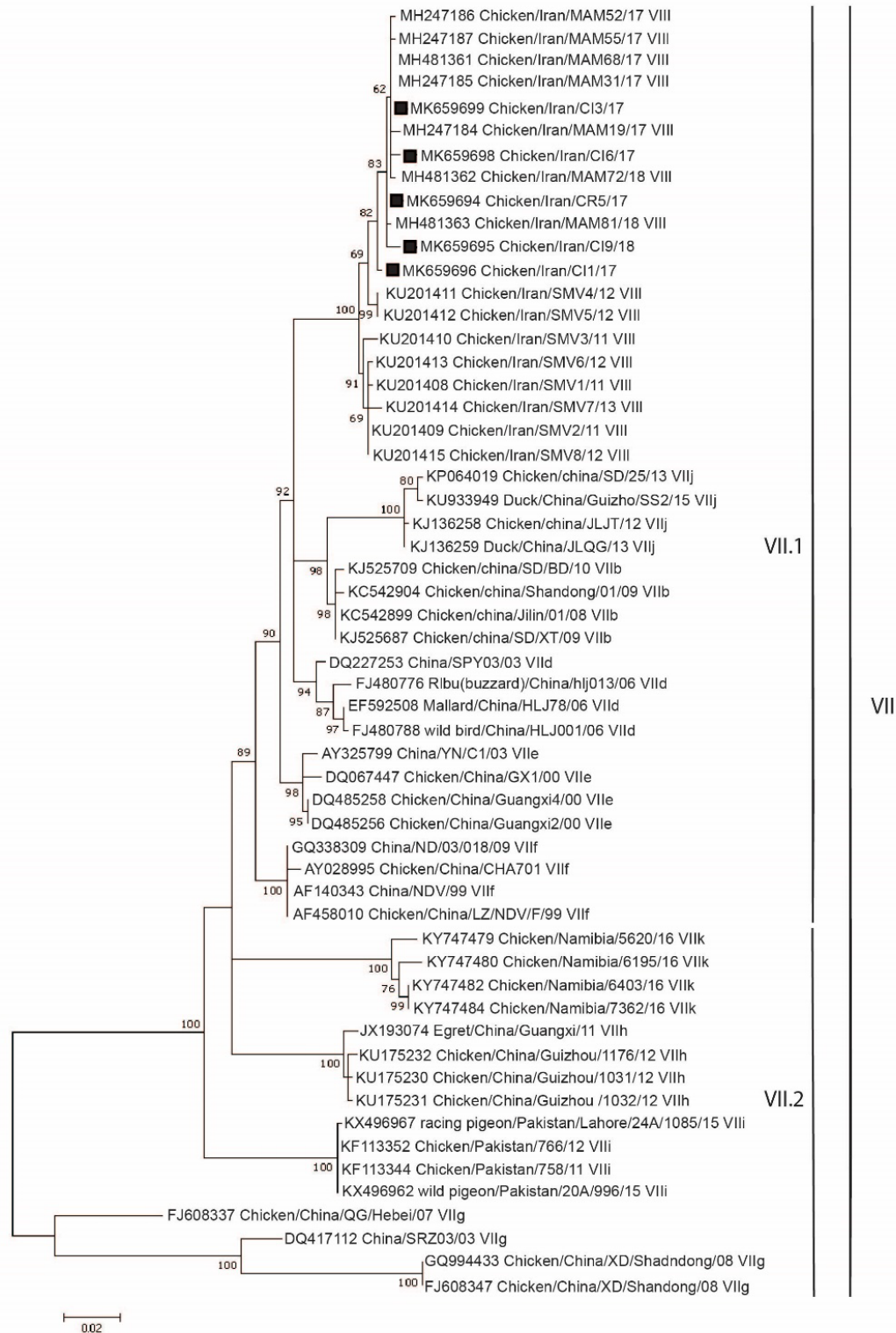


Figure 2. Phylogenetic relationships of the nucleotide sequences of VII genotype of NDV isolates based on sequences of F gene. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). Tree construction was done using the Neighbor Joining method with the maximum composite likelihood substitution model (500 replicates) in MEGA7. The viruses obtained in this study are marked with squares.

Table 2. Estimates of evolutionary diversity among sub-genotypes of genotype VII based on partial coding sequences of the F gene

	VIII	VIIId	VIIe	VIIIf	VIIg	VIIi	VIIj	VIIk	VIIh	VIIb	Our isolates
VIII (n:16)		0.0063	0.0072	0.0091	0.0175	0.0126	0.0086	0.0134	0.0114	0.0077	0.0023
VIIId (n:36)	0.0403		0.0044	0.0068	0.0153	0.0113	0.0063	0.0118	0.0102	0.0050	0.0068
VIIe (n:13)	0.0445	0.0258		0.0062	0.0156	0.0107	0.0073	0.0110	0.0095	0.0058	0.0077
VIIIf (n:5)	0.0565	0.0370	0.0280		0.0153	0.0108	0.0089	0.0108	0.0098	0.0077	0.0095
VIIg (n:5)	0.1859	0.1633	0.1628	0.1570		0.0181	0.0163	0.0167	0.0164	0.0161	0.0178
VIIi (n:30)	0.0951	0.0812	0.0725	0.0691	0.1817		0.0129	0.0132	0.0112	0.0118	0.0131
VIIj (n:23)	0.0614	0.0431	0.0464	0.0572	0.1760	0.1001		0.0125	0.0116	0.0047	0.0090
VIIk (n:5)	0.1061	0.0926	0.0811	0.0766	0.1764	0.0982	0.1022		0.0114	0.0117	0.0137
VIIh (n:14)	0.0871	0.0721	0.0641	0.0588	0.1737	0.0811	0.0869	0.0900		0.0110	0.0117
VIIb (n:28)	0.0474	0.0278	0.0312	0.0427	0.1681	0.0842	0.0274	0.0906	0.0752		0.0081
Our isolates	0.0098	0.0422	0.0467	0.0580	0.1880	0.0972	0.0635	0.1069	0.0886	0.0439	

Table 3. Comparison of studied isolates and other Iranian NDV isolates

Isolate	Genotype	Cleavage site 112RRQ KRF117	Cysteine residue	Glycosylation site	Neutralizing epitopes							
					72	74	75	78	79	157-171	343	
Studied isolates	VI II	. . . K . .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471	D	E	A	K	S	SI- AATN EAVH EV- TNG	L	
CK/IR/ Iran- EMM/1(J Q267585) 2008	XI II d	. . . K . .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TDG	L	
Ck/Iran- EMM/3 (JQ26758 3) 2009	XI II d	. . . K . .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TDG	L	

Isolate	Genotype	Cleavage site 112RRQ KRF117	Cysteine residue	Glycosylation site	Neutralizing epitopes						
					72	74	75	78	79	157-171	343
Ck/IR/NR-6 (KC161981) 2010	XI II d	. . .R . .	76,199,338,347,362,370,394,399,401,424,523	85,191,366,47,471	D	E	A	K	A	SI-AATN EAVH EV-TDG	L
CK/Iran-EMM/5 (JQ267581) 2011	XI II d	. . .K .	76,199,338,347,362,370,394,399,401,424,523	85,191,366,47,471, 541	D	E	A	K	A	SI-AATN EAVH EV-TDG	L
CK/IR/S MV-3 (KU201410) 2011	VI II	. . .K .	76,199,338,347,362-370,394,399,401,424,523	85,191,366,47,471,541	D	E	A	K	A	SI-AATN EAVH EV-TNG	L
CK/IR/SMV-2 (KU201409) 2011	VI II	. . .K .	76,199,338,347,362,370,394,399,401,424,523	85,191,366,47,471,541	D	E	A	K	A	SI-AATN EAVH EV-TNG	L
CK/IR/NR_98 (JX129809) 2012	VI Ij	. . .K .	76,199,338,347,362-370,394,399,401,424,523	85,191,366,47,471	D	E	A	K	A	SI-AATN EAVH EV-TNG	L
CK/IR/SMV-5 (KU201412) 2012	VI II	. . .K .	76,199,338,347,362,370,394,399,401,424,523	85,191,366,47,471,541	D	E	A	K	A	SI-AATN EAVH EV-TNG	L
CK/IR/NR_87 (JX129801) 2012	VI Ij	. . .K .	76,199,338,347,362,370,394,399,401,424,523,536	85,191,366,47,471	D	E	A	K	A	SI-AATN EAVH EV-TNG	L
CK/IR/SMV-8 (KU201415) 2013	VI II	. . .K .	76,199,338,347,362,370,394,399,401,424,523	85,191,366,47,471,541	D	E	A	K	A	SI-AATN EAVH EV-TNG	L

Isolate	Genotype	Cleavage site 112RRQ KRF117	Cysteine residue	Glycosylation site	Neutralizing epitopes						
					72	74	75	78	79	157-171	343
CK/IR/ 1392k (KJ17699 6) 2013	VI Ij	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/NR -10 (KC16198 4) 2014	XI II d	. . .R. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471	D	E	A	K	A	SI- AATN EAVH EV- TDG	L
CK/IR/ MSH-1 (MG5198 55) 2015	VI Id	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/ MSH-2 (MG5198 56) 2015	VI Id	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/ MSH-3 (MG5198 57) 2015	VI Id	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/ Maz15 (KY20574 2) 2015	VI Ij	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/ Behshahr (KX26835 1) 2015	VI Ij	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/M AM68 (MH4813 61) 2017	VI II	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L

Isolate	Genotype	Cleavage site 112RRQ KRF117	Cysteine residue	Glycosylation site	Neutralizing epitopes						
					72	74	75	78	79	157-171	343
CK/IR/M AM52 (MH2471 86) 2017	VI II	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L
CK/IR/M AM55 (MH2471 87) 2017	VI II	. . .K. .	76,199,338,347,362,370,39 4,399,401,424,523	85,191,366,4 47,471,541	D	E	A	K	A	SI- AATN EAVH EV- TNG	L

All the isolates carried multi-basic amino acid residues at the cleavage site of fusion protein, at positions 115 and 116 plus a phenylalanine at position 117, typical of virulent strains (OIE, 2012). The comparative analysis of amino acid sequence at neutralizing epitopes exhibited that mostly all studied NDVs had conserved amino acid compared to other Iranian NDV isolates. Exceptionally, our studied NDVs contained unique amino acid S (Serine) at residue 79, whereas all previously reported isolates from Iran had amino acid A (Alanine) in this corresponding residue. Furthermore, patterns of potential glycosylation sites and cysteine amino acid residues were conserved in all NDVs.

Discussion

In the current study, we aimed to characterize NDVs that are responsible for neurological symptoms during ND outbreaks in commercial and rural chicken farms in northern Iran. All affected farms had neurological symptoms, and commercial farms had been routinely vaccinated against NDV. During the study period, 48% of farms (12 out of 25) were detected positive for NDV by RT-PCR. Randomly, five isolates were chosen for nucleotide sequencing and virus genotyping.

BLAST search and multiple alignments showed that all of the present isolates had a high nucleotide identity (96%) with viruses reported from Republic of Korea, China, Bulgaria, and Ukraine (Liu *et al.* 2019). Annually migratory birds begin their migration from Siberia and Central Asia in autumn. The birds fly across the Caspian Sea to spend winter in wetlands of northern areas of Iran (Galbraith *et al.*, 2014). Close contact of backyard chickens with the migratory birds may pose the risk of transmitting new sub-genotypes of NDV. It seems the potential ability of migratory birds in virus dissemination to long distance areas is of great significance.

In the present study, the phylogenetic analysis revealed that all five studied isolates were closely related to each other and belonged to sub-genotype VII.1.1 (VIII) within genotype VII. Genotype VII viruses originated from the Far East in the 1980s and had spread to other regions such as Asia, Europe, South Africa, and South America in the 1990s. According to the reviewed amino acid motifs and pathotyping in a recent study, all genotype VII viruses were velogenic (Miller *et al.*, 2015). Now, genotype VII viruses are mostly prevalent in Newcastle disease outbreak in Asia and Middle East (Radwan *et al.*, 2013). Historically, genotype VII

has been reported in Iran since the middle of the 1990s (Ebrahimi *et al.*, 2012; Samadi *et al.*, 2014). Later, the genotype analysis revealed the presence of VIIb, VIIc, VIIg, VIIi, VIIj, and VIII sub-genotypes (Hosseini *et al.*, 2014; Esmaelizad *et al.*, 2017; Jabbarifakhar *et al.*, 2018; Ghalyanchi langeroudi *et al.*, 2018; Sabouri *et al.*, 2018; Molouki *et al.*, 2019). Prior studies have proposed sub-genotype VIII viruses were driven from sub-genotype VIIc and circulated in Iran for at least one decade (Sabouri *et al.*, 2018; Molouki *et al.*, 2019). Regarding phylogenetic analysis, our isolates had the highest genetic relationship with strains Ck/IR/MAM81/2018, Ck/IR/MAM72/2018, and Ck/IR/MAM19/2017 that have been recently isolated in Boushehr, Isfahan, and West Azerbaijan, respectively (Molouki *et al.* 2019).

Evolutionary diversity presented a close distance with sub-genotype VIII. Furthermore, they presented high levels of nucleotide and deduced amino acid (97-99%) homologies with other members of sub-genotype VIII that were reported from commercial and backyard poultry of Iran by Molouki *et al.* in 2019 (data not shown). Regarding the abovementioned, the hypothesis that viruses may derive from the same ancestor is reinforced. On the other hand, these viruses were isolated from different geographical regions that were relatively far from each other. These pieces of evidence might indicate sub-genotype VIII is currently spreading all over Iran.

As shown in [Table 3](#), all studied isolates shared similar virulent cleavage site motif (¹¹²RRQKRF¹¹⁷) like other reported NDV genotype VII, especially the recent sub-genotype VIII, indicating the circulation of velogenic virus in the country. In addition, the presence of the phenylalanine (F) residue at position 117 was described as a possible contributor to the neurological effects (Collins *et al.*, 1993). As previously mentioned, studied farms had neurological symptoms. [Figure 2](#) shows a close

relationship between our isolates and some recently reported isolates from Iran with Chinese isolates. Trade and economic exchanges between Iran and China have developed in the last decade. Agricultural imports from China, therefore, may have contributed to the transmission of the virus.

Traditionally, Iranian poultry industry has applied a combination of genotype II (i.e., B1, LaSota, and Clone 30) and genotype I (V4) vaccine strains to prepare an adequate immune coverage against ND. Despite this extensive vaccination program, ND still remains endemic throughout Iran and is concerned as a continual major threat to both rural and commercial poultry flocks. According to the results, the F gene nucleotide homology between studied viruses and those abovementioned for vaccine strains ranged between 82.97 to 83.88%. Furthermore, studied viruses shared high and less sequence identity with LaSota and B1 strains, respectively (data not shown). From the view of antigenic variation, studied viruses displayed conserved neutralizing epitopes compared to other studied NDV genotype VII and vaccine strains. But, only one exception was observed. Studied viruses contained unique amino acid S (Serine) at residue 79 compared to others. This variation might have been a possible reason of low efficiency of NDV vaccines. Regardless of antigenic variation, other factors, including cold chain failure and poor vaccination procedures (poor quality of vaccine, interference of maternal antibody, inadequate vaccine dose), can lead to insufficient immunity and vaccination failure.

Conclusion

In summary, it can be concluded that sub-genotype VIII is to be a problem in poultry industry in northern Iran. Furthermore, phylogenetic analysis of the F sequence revealed that the isolated virulent strain is genetically distant from the lentogenic LaSota

and B1 strains. Therefore, it seems a contemporary homologous virus should be applied as the vaccine strain to prevent outbreaks of genotype VII virus.

Acknowledgments

This research has been supported by a research grant from the Amol University of Special Modern Technologies, Amol, Iran.

References

- Afonso, C.L., Amarasinghe, G.K., Bányai, K., Bào, Y., Basler, C.F., Bavari, S. (2016). Taxonomy of the order Mononegavirales: update 2016. *Arch Virol*, 161, 2351-2360. [DOI:10.1007/s00705-016-2880-1] [PMID] [PMCID]
- Alexander, D.J. (2003). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Saif, Y.M., Swayne, D.E., Fadly, A.M. (Eds.). *Diseases of poultry*. 11th ed. Iowa State University Press, Ames, IA, 63-69.
- Alexander, D.J. (2012). *Newcastle disease*, In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Paris). http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.14_NEWCASTLE_DIS.pdf.
- Cattoli, G., Fusaro, A., Monne, I., Molia, S., Le Menach, A., Maregeya, B., Nchare, A., Bangana, I., Maina, A.G., Koffi, J.N., Thiam, H., Bezeid, O.E., Salviato, A., Nisi, R., Terregino, C., Capua, I. (2010). Emergence of a new genetic lineage of Newcastle disease virus in West and Central Africa-Implications for diagnosis and control. *Vet Microbiol*, 142(3-4), 168-176. [DOI:10.1016/j.vetmic.2009.09.063] [PMID]
- Collins, M.S., Bashiruddin, J.B., Alexander, D.J. (1993). Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Arch Virol*, 128(3-4), 363-370. [DOI:10.1007/BF01309446] [PMID]
- Czeglédi, A., Ujvári, D., Somogyi, E., Wehmann, E., Werner, O., Lomniczi, B. (2006). Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Res*, 120(1-2), 36-48. [DOI:10.1016/j.virusres.2005.11.009] [PMID]
- Diel, G., da Silva, L.H.A., Liu, H., Wang, Z., Miller, P.J., Afonso, C.L. (2012). Genetic diversity of avian paramyxovirus type 1: Proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. *Infect Genet Evol*, 12(8), 1770-1779. [DOI:10.1016/j.meegid.2012.07.012] [PMID]
- Dimitrov, K. M., Abolnik, C., Afonso, C. L., Albina, E., Bahl, J., Berg, M., ... & Wong, F. Y. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infect Genet Evol*, 74, [DOI:10.1016/j.meegid.2019.103917] [PMID] [PMCID]
- Ebrahimi, M.M., Shahsavandi, S., Moazenijula, G., Shamsara, M. (2012). Phylogeny and evolution of Newcastle disease virus genotypes isolated in Asia during 2008–2011. *Virus Genes*, 45, 63–68. [DOI:10.1007/s11262-012-0738-5] [PMID]
- Esmaelizad, M., Mayahi, V., Pashaei, M., Goudarzi, H. (2017). Identification of novel Newcastle disease virus subgenotype VII-(j) based on the fusion protein. *Arch Virol*, 162(4), 971-978. [DOI:10.1007/s00705-016-3189-9] [PMID]
- Galbraith, C.A., Jones, T., Kirby, J., Mundkur, T. (2014). *A Review of Migratory Bird Flyways and Priorities for Management*. Bonn, Germany: UNEP / CMS Secretariat.

Conflict of Interest

The authors declare that they have no conflict of interest.

- Ghalyanchilangeroudi, A., Hosseini, H., Jabbarifakhr, M., Fallah Mehrabadi, M.H., Najafi, H., Ghafouri, S.A., Mousavi, F.S., Ziafati, Z., and Modiri, A. (2018). Emergence of a virulent genotype VIIi of Newcastle disease virus in Iran. *Avian Pathol*, 47, 509–519. [DOI:10.1080/03079457.2018.1495313] [PMID]
- Hosseini, H., Ghalyanchi Langeroudi, A., Torabi, R. (2014). Molecular characterization and phylogenetic study of Newcastle disease viruses isolated in Iran, 2010-2012. *Avian Dis*, 58(3), 373-376. [DOI:10.1637/10743-120713-Reg.1] [PMID]
- Islamic Republic News Agency (IRNA). www.irna.ir/news/83505446/
- Jabbarifakhar, M., Mousavi, F.S., Ziafati, Z., Rezaee, H., Hosseini, H., Fallah, M.H., Esmaelzadeh Dizaji, R., Ghalyanchilangeroudi, A. (2018). Emergence of genotype VIIg of velogenic Newcastle disease virus in Iran, 2018: The First Report. *Iran J Virol*, 12(1), 40-46.
- James, N. (2017). *Fanner's Veterinary Virology*: Elsevier Academic Press.
- Kant, A., Koch, G., Van Roozelaar, D.J., Balk, F., Ter Huurne, A. (1997). Differentiation of virulent and non-virulent strains of Newcastle disease virus within 24 hours by polymerase chain reaction. *Avian Pathol*, 26, 837-849. [DOI:10.1080/03079459708419257] [PMID]
- Kim, L.M., King, D.J., Curry, P.E., Suarez, D.L., Swayne, D.E., Stallknecht, D.E., Slemmons, R.D., Pedersen, J.C., Senne, D.A., Winker, K., Afonso, C.L. (2007). Phylogenetic diversity among low-virulence Newcastle disease viruses from waterfowl and shorebirds and comparison of genotype distributions to those of poultry-origin isolates. *J Virol*, 81(22), 12641–12653. [DOI:10.1128/JVI.00843-07] [PMID] [PMCID]
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*, 16(2), 111-120. [DOI:10.1007/BF01731581] [PMID]
- Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7 Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol*, 33(7), 1870-1874. [DOI:10.1093/molbev/msw054] [PMID]
- Liu, Y., Sun, C., Chi, M., Wen, H., Zhao, L., Song, Y., Liu, N., Wang, Z. (2019). Genetic characterization and phylogenetic analysis of Newcastle disease virus from China. *Infect Genet Evol*, 75, 103958. [DOI:10.1016/j.meegid.2019.103958] [PMID]
- Mayahi, V., Esmaelizad, M. (2017). Molecular evolution and epidemiological links study of Newcastle disease virus isolates from 1995 to 2016 in Iran. *Arch Virol*, 162(12), 3727-3743. [DOI:10.1007/s00705-017-3536-5] [PMID]
- Mehrabanpour, M.J., Khoobyar, S., Rahimian, A., Nazari, M.B., Keshtkar, M.R. (2014). Phylogenetic characterization of the fusion genes of the Newcastle disease viruses isolated in Fars province poultry farms during 2009–2011. *Vet Res Forum*, 5(3), 187–191.
- Miller, P.J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S.F. Wajid, A., Bibi, T., Ahmad Khan, T., Yaqub, T., Setiyaningsih, S., Afonso, C.L. (2015). Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infect Genet Evol*, 29, 216-229. [DOI:10.1016/j.meegid.2014.10.032] [PMID]
- Molouki, A., Mehrabadi, M., Bashashati, M., Akhijahani, M.M., Lim, S.H.E, Hajloo, S.A. (2019). NDV sub-genotype VII(L) is currently circulating in commercial broiler farms of Iran, 2017–2018. *Trop Anim Health Prod*, 51(5), 1247-1252. [DOI:10.1007/s11250-019-01817-1] [PMID]
- OIE. (2012) *Newcastle disease*. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 1-20.
- Qin, Z.M., Tan, L.T., Xu, H.Y., Ma, B.C., Wang, Y.L., Yuan, X.Y., Liu, W.J. (2008). Pathotypical characterization and molecular epidemiology of Newcastle disease virus isolates from different hosts in China from 1996 to 2005. *J Clin Microbiol*, 46(2), 601-611. [DOI:10.1128/JCM.01356-07] [PMID] [PMCID]
- Radwan, M., Darwish, S., El-Sabagh, I., El-Sanousi, A.A., Shalaby, M.A. (2013). Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. *Virus Genes*, 47(2), 311-316. [DOI:10.1007/s11262-013-0950-y] [PMID]

- Sabouri, F., Vasfi Marandi, M., Karimi, V., Malekan, M., Bashashati, M. (2016). Genetic analysis of avian paramyxovirus type I strains isolated from backyard poultry in Iran. *Turk J Vet Anim Sci*, 40, 750-756. [[DOI:10.3906/vet-1601-117](https://doi.org/10.3906/vet-1601-117)]
- Sabouri, F., Vasfi Marandi, M., Bashashati, M. (2018). Characterization of a novel VIII sub-genotype of Newcastle disease virus circulating in Iran. *Avian Pathol*, 47(1), 90-99. [[DOI:10.1080/03079457.2017.1376735](https://doi.org/10.1080/03079457.2017.1376735)] [[PMID](#)]
- Samadi, S., Kianizadeh, M., Fathi Najaf, M., Mousavi Nasab, S.D., Hosseinnia Davatgar, A.M., Royae, A., Pilvar, P. (2014). Molecular characterization and phylogenetic study of velogenic New-castle disease virus isolates in Iran. *Virus Genes*, 48(2), 290-295. [[DOI:10.1007/s11262-013-1015-y](https://doi.org/10.1007/s11262-013-1015-y)] [[PMID](#)]
- Tamura, K., Nei, M., Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci USA*, 101(30), 11030-11035. [[DOI:10.1073/pnas.0404206101](https://doi.org/10.1073/pnas.0404206101)] [[PMID](#)] [[PMCID](#)]
- Wang, Y., Yu, W., Huo, N., Wang, W., Guo, Y., Wei, Q. (2017). Comprehensive analysis of amino acid sequence diversity at the F protein cleavage site of Newcastle disease virus in fusogenic activity. *PloS One*, 12, e0183923. [[DOI:10.1371/journal.pone.0183923](https://doi.org/10.1371/journal.pone.0183923)] [[PMID](#)] [[PMCID](#)]
- Xiao, S., Nayak, B., Samuel, A., Paldurai, A., Kanabagattebasavarajappa, M., Prajitno, T.Y., Bharoto, E.E., Collins, P.L., Samal, S.K. (2012). Generation by reverse genetics of an effective, stable, live-attenuated Newcastle disease virus vaccine based on a currently circulating, highly virulent Indonesian strain. *PloS One*, 7(12), e52751. [[DOI:10.1371/journal.pone.0052751](https://doi.org/10.1371/journal.pone.0052751)] [[PMID](#)] [[PMCID](#)]
- Zhu, J., Xu, H., Liu, J., Zhao, Z., Hu, S., Wang, X., Liu, X. (2014). Surveillance of avirulent Newcastle disease viruses at live bird markets in Eastern China during 2008-2012 reveals a new sub-genotype of class I virus. *Virol J*, 11, 211. [[DOI:10.1186/s12985-014-0211-2](https://doi.org/10.1186/s12985-014-0211-2)] [[PMID](#)] [[PMCID](#)]

چرخش تحت ژنوتیپ (VIII) VII.1.1 اخیرا گزارش شده و ویروس بیماری نیوکاسل در ماکیان صنعتی و بومی شمال ایران

سعید سیفی^{۱*}، مجتبی خسروی^۲

^۱ گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه تخصصی فناوری‌های نوین آمل، آمل، ایران

^۲ گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه تخصصی فناوری‌های نوین آمل، آمل، ایران

(دریافت مقاله: ۱۶ شهریور ماه ۱۳۹۹، پذیرش نهایی: ۰۵ دی ماه ۱۳۹۹)

زمینه مطالعه: ویروس بیماری نیوکاسل یک عامل بیماری‌زای مهم در پرندگان است و علیرغم واکسیناسیون گسترده، هنوز در مناطق جغرافیایی مختلف چرخش می‌کند. به دلیل مرگ و میر بالا، خسارات اقتصادی ناشی از بیماری نیوکاسل، بسیار زیاد است.

هدف: هدف از این مطالعه تعیین خصوصیات مولکولی جدایه‌های ویروس بیماری نیوکاسل در مزارع پرورش مرغ در شمال ایران، طی سالهای ۲۰۱۸-۲۰۱۷ بود.

روش کار: در زمان شیوع بیماری، پنج جدایه ویروس نیوکاسل از مزارع ماکیان گوشتی صنعتی و بومی جداسازی و شناسایی شدند. توالی بخشی از ژن‌های فیوژن جدایه‌ها تعیین شد و با سایر توالی‌های منتشر شده ویروس نیوکاسل مقایسه شد.

نتایج: تجزیه و تحلیل فیلوژنتیک نشان داد که همه جدایه‌ها در تحت ژنوتیپ (VIII) VII.1.1 قرار دارند. در همه جدایه‌ها الگوی مولتی بازیک محل شکاف پروتئین F شبیه سویه‌های حاد نیوکاسل وجود داشت. جدایه‌های مطالعه شده شباهت زیادی (۹۶.۹۴٪) با سویه‌های نیوکاسل شایع در چین و جمهوری کره داشتند.

نتیجه‌گیری نهایی: نتایج مطالعه پیشنهاد می‌کند که تحت ژنوتیپ (VIII) VII.1.1 ویروس بیماری نیوکاسل در مزارع پرورش طیور منطقه در حال چرخش است و ممکن است تحت ژنوتیپ غالب باشد. با توجه به اختلاف ژنتیکی میان واکنش‌های مورد استفاده علیه بیماری (B1، لاسوتا و کلون ۳۰ که همگی متعلق به ژنوتیپ II هستند) و ویروس‌های نیوکاسل در حال چرخش در منطقه، توصیه می‌شود که برای جلوگیری از شیوع ویروس‌های ژنوتیپ (VIII) VII.1.1 نیوکاسل از ویروس‌های همولوگ برای تولید واکنش استفاده شود.

واژه‌های کلیدی: ماکیان، ژن F، ویروس بیماری نیوکاسل، شیوع، آنالیز فیلوژنی