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Case Report

Emergence of Genotype VIIg of Velogenic Newcastle Disease Virus in Iran, 2018: The First Report

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Abstract

Newcastle disease virus (NDV) is a prototype member of avian paramyxovirus serotype 1 (APMV-1), which causes severe and contagious disease in the commercial poultry and wild birds. In this study, we report the results of phylogenetic analyses of recent NDVs isolated from Markazi province of Iran during a recent outbreak in the commercial broiler with respiratory signs and digestive system lesions. Phylogenetic analysis revealed that isolate was clustered within class II NDV, in sub-genotype VII-g. This NDV isolates shared high homology with the prevalent genotype NDV strains that circulate in China and Taiwan (95.39%-84.89%). Overall, our results confirmed the presence of genotype VII-g in Iran (The first report) and indicated that different genotypes of NDV could circulate simultaneously among poultry (VII-d & VII-i). The phylogenetic characterization of these isolates helps to characterize the evolution of NDV and may help with the development of vaccines specific to our regional necessities.

Keywords: Newcastle disease; Iran; Phylogenetic analysis; Genotyping, Genotype VII-g.

Introduction

Newcastle disease (ND) is one of the most contagious and devastating diseases to poultry in the world and was originally detected in Java Indonesia and Newcastle- 'On-Tyne, England (1). The causative agent of NDV is enveloped and belongs to the genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae. The viral RNA genome is negative sense, nonsegmented, single-stranded and encodes six major virus proteins, which F protein is considered to play a vital role in the virulence of NDV strains. The virulence of NDV depends upon the amino acid sequence at the cleavage site of the F protein (2, 3). It is widely accepted that cleavage of the F protein precursor (F0) is the primary determinant of NDV virulence. Virulent (velogenic or mesogenic) NDV strains contain a multi-basic amino acid-rich region (112R/K-R-X-R/K-R-F117) in the cleavage site whereas avirulent (lentogenic) strains possess (112G/E-K/R-X-G/E-R-L117) motif cleaved by secreted trypsin-like protease into active F protein, which consists of disulfide-linked F1 and F2 polypeptides (4). Based on the complete genomic length and phylogenetic relationship, NDV strains are divided into class I and class

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II. Class, I NDV strains mainly consist of avirulent strains and are mostly isolated from wild birds. On the other hand, class II includes both virulent and avirulent strains isolated from both domestic and wild birds. NDV is classified based on nucleotide sequence of the F gene into 19 genotypes, class I has single, and class II includes 18 genotypes (I-XVIII)(5). Different genotypes of NDV have been circulating worldwide (6). Genotypes VII and VIII were responsible for ND outbreaks in Asia, including Pakistan, and in Europe since 1984 or earlier (7, 8).

There were four panzootic caused by NDV different genotypes in the world since the 1920s, and the genotype VII viruses were responsible for the fourth panzootic began southeast Asia in 1985, which continues today, has spread from Asia to Africa, Europe and has even been detected in South America (6, 9-11). In Iran, first NDV outbreak was reported in the year 1926. Virulent NDV is endemic in Iran and in the past few decades, implementation of extensive vaccination programs in commercial poultry farms, and to some extent in small rural poultry farms, has reduced the number of epizootic outbreaks of ND in Iran (12). Sporadic cases of ND, however, are reported every year. In recent years, the frequent occurrence of ND outbreaks in vaccinated flocks with high hemagglutination inhibition (HI) titer antibody levels raises questions about the causative agent (12, 13). In the present report, we detected and identified the new velogenic strain of NDV in commercial broiler chicken in Iran and finally doing phylogenetic analysis on it.

Methods

In the present study, an outbreak with respiratory signs and digestive system lesions were recorded in Markazi province during the September 2017. Trachea tissue samples were collected from broilers flocks and transferred with ice to the virology laboratory of veterinary medicine faculty of Tehran University.

The NDV positive samples were collected from both ailing and dead birds reported with pathological signs and symptoms. NDV isolates were recovered by inoculating homogenized trachea tissue samples into specific pathogen-free 9-days-old SPF embryonated chicken eggs following standard procedure.

Final confirmation of the positive lung samples was done by haemagglutination assay (HA) and stored at -80 °C for further use.

Total RNA extraction from trachea samples was carried out through Qiagen RNeasy Mini extraction kit (Valencia, CA, USA) followed the standard protocol.

RNA was stored at -70°C until RT-PCR was performed. RNA reverse-transcribed to cDNA with random hexamer and RevertAidTM M-MuLV Reverse Transcriptase (Fermentas, Canada) according to the manufacturer's protocols. cDNA was amplified using Kant's primer sets (A: 5'-TTG ATG GCA GGC CTC TTG C-3' B: 5'-GGA GGA TGT TGG CAG CAT T-3') (14).

The PCR condition was 94◦C for 2 minutes denaturation, and 35 cycles of 94◦C for10 seconds, 58◦C for 20 seconds (annealing), and 72◦C for 20 seconds, followed by 70◦C for 10 minutes final extension. The product was purified and cleaned using AccuPrep® PCR Purification kit (Bioneer Co, South Korea).

For sequencing, the sample was sent to Source BioScience Company (UK). Percent nucleotide identity, sequence editing and prediction of amino acid sequences, and primary alignments were carried out using CLC sequence viewer (Ver. 6.0.2). Nucleotide analysis was performed by Molecular Evolutionary Genetics Analysis, version 7.01 (MEGA 7) (15).

Multiple nucleotides and amino acid sequence alignment for the studied gene were performed using Clustal W model.

Phylogenetic trees were drawn from amino acid based on the partial segment of F gene segments using minimum evolution analysis with neighbor joining.

The neighbor-joining algorithm was implemented with the Kimura2 parameter model (16). 1000 bootstrap trees were created to evaluate the reliability of the ancestral location.

Results and Discussion

After working on the sequences, from the pathotype prediction based on the cleavage site of the F protein, all the isolates were placed into the velogenic group with the motif 112RRQKRF 117. A phylogenetic tree (Fig. 1) was constructed based on the nucleotide sequences of the F gene hypervariable region in all field isolates of NDV and the corresponding regions of the other NDV strains retrieved from GenBank. A total two field isolates isolated in recent outbreaks were classified into genotype VII-g. These isolates shared high homology with the prevalent genotype NDV strains that circulate in China (XD/Shandong/08 China genotype VII(g) (GQ 994433.1)), Japan ((Miyadera) F0 genotype III (M18456.1)) and Taiwan (CH-A7/96 genotype _VII(f)(AY028995.1)) (95.39%-84.89%). The data have been shown in table 1.

New genotypes of NDV are continuously described worldwide. To date, 18 class II genotypes of NDV have been

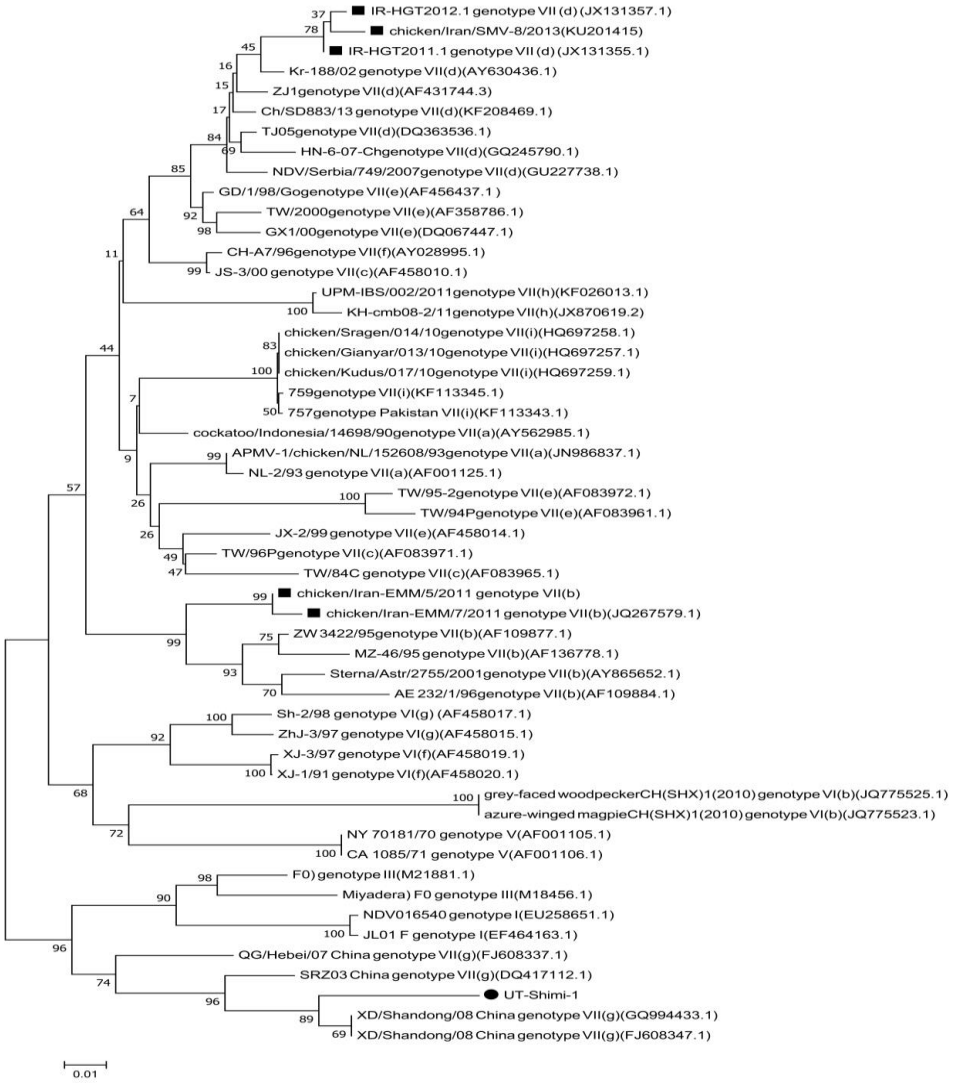

Fig. 1. Nucleotide acid-base phylogenetic relationships of fusion gene of Newcastle disease virus detected from Iran. The phylogenetic tree was generated using neighboring joining model with MEGA (version 7.0.14). Numbers below branches indicate bootstrap value from 1000 replicates, bootstrap values. Horizontal distances are proportional to the minimum number of nucleic acid differences required to join nodes. The vertical lines are for spacing branches and labels. The analysis was based on complete open reading frames of all gene segments. The scale bar represents the distance unit between sequence pairs. The virus genome characterized in this report is indicated as black circle ● . Iranian NDV are indicated black square circle ■.

Table 1. Percent identity of partial nucleotide sequences of the Fusion glycoprotein genes of new subgenotype VII-g Newcastle disease viruses (NDV) to those of NDV reference strains.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	XD/Shandong/08_China_genotype_VII(g)(FJ608347.1)															
2	UT-Shimi-1	95.39														
3	IR-HGT2012.1_genotype_VII_(d)_(JX131357.1) --iran	77.01	78.53													
4	chicken/Sragen/014/10genotype_VII(i)(HQ697258.1) --indonesia	86.44	80.31	91.48												
5	UPM-IBS/002/2011genotype_VII(h)(KF026013.1)-- Malaysia	86.58	81.89	89.43	92.32											
6	QG/Hebei/07_China_genotype_VII(g)(FJ608337.1)	93.97	84.16	81.03	89.77	89.00										
7	CH-A7/96genotype_VII(f)(AY028995.1)	89.24	84.89	92.85	92.94	93.22	91.29									
8	APMV-1/chicken/NL/152608/93genotype_VII(a)(JN986837.1)	87.28	83.25	93.13	94.68	93.94	89.76	94.48								
9	TW/95-2genotype_VII(e)(AF083972.1)	73.63	80.34	91.95	90.27	86.52	79.91	91.49	92.04							
10	F0)_genotype_III(M21881.1)	86.03	84.83	84.57	87.33	86.83	89.28	87.87	87.15	84.44						
11	Miyadera)_F0_genotype_III(M18456.1)	84.88	86.98	81.75	86.07	85.80	88.07	86.78	86.44	82.73	95.47					
12	NDV016540_genotype_I(EU258651.1)--china	86.38	84.16	81.03	86.40	84.96	92.55	85.64	85.68	79.16	93.06	91.84				
13	ZhJ-3/97_genotype_VI(g)(AF458015.1)--china2003	84.74	82.57	86.12	89.53	88.29	87.47	90.05	89.87	85.22	88.72	87.33	87.26			
14	CA_1085/71_genotype_V(AF001106.1)	80.24	78.74	85.55	86.92	84.36	82.81	88.64	88.55	85.79	87.84	85.53	83.53	91.12		
15	chicken/Iran-EMM/5/2011_genotype_VII(b)	86.46	84.01	89.34	91.76	90.99	88.52	92.11	92.78	88.40	87.20	86.72	86.12	89.93	88.93	

described, and most diversity was identified in genotype VII viruses which are undertaking ND outbreak in the Middle East and Asia. Genotype VII viruses likely originated from the Far East in the 1990s and finally spread to another continent such as Asia, Europe, South Africa and South America. According to the reviewed amino acid motifs and pathotyping, all genotype VII viruses are velogenic (17). Among class II, highly diverse genotype VII was separated into eight sub-genotypes (VII a-h). VII-a isolates had been identified in Poultry in Western Europe in the 1990s, but likely they had Indonesian ancestors from 1980s (18). Viruses of VII-b had frequently been isolated from poultry in China during 1998-2014 (19-23) and other Asian countries such as Vietnam in 2007 and Israel in 211-2014 (24). Viruses recovered from chickens and pigeons from China and Taiwan during 1996-2000 belonged to VII-c and VII-d sub-genotypes (11). VII-d viruses rapidly spread all over the world and became one of the most prevalent sub-genotypes circulating since the first of 21st century. Also, this sub-genotype is dominant in Iran (12). Isolates from this sub-genotypes have been identified from poultry in China in 1998-2013 (20-23, 25, 26), South Korea (2000-2005) (27, 28), Colombia (2006-2010) (24, 29), Israel, South Africa, Ukraine and Venezuela (2004-2009) (10, 24, 30). Viruses of VII-e were identified in China and other Asian countries such as Japan, Taiwan, and Vietnam from chickens and domestic waterfowl during 1997–2014 (11, 21, 25, 31-34). Viruses of VII-f had been isolated from domestic poultry and pigeons in China during 1996–2008 (9, 11, 21, 25). Viruses of VII-g likely all represent recombinant strains (5). This sub-genotype virus was isolated from poultry in China, Nigeria, Indonesia, and Island during 2003-2010. With a few number of isolates, VII-h had been reported in Cambodia, China, Indonesia, Malaysia, Mozambique, and South Africa during 2007–2014 (17, 24, 35). VII-i was another contemporary sub-genotype recently emerged from chickens and pet birds in Indonesia, Israel, and Pakistan since 2010 (36, 37). The increasing genomic diversity of NDV poses several problems for the control and surveillance of ND. In fact, genetically distant NDV strains might have the potential for increased virulence, increased host range, or vaccine escape (38). ND is one of the most serious infections of poultry and is endemic in

Iranian poultry industries. VII-d is currently the dominant genotypes of NDV in Iran, and it is now endemic sub-genotype in this country (12, 39). Overall, our results confirm the presence of genotype VII-g in Iran (The first report), The virulence of the new isolate was confirmed by a sequence of the predicted cleavage site of the F protein, which indicated that these two isolates carried the112-RRQKRF-117 107 motif corresponding to the cleavage site of virulent NDV.

Accordance to the high percentage of identity of the present isolate (genotype VII-g) to current Chinese isolates (genotype VII-g from Shandong province in 2008 from chickens), they originated from a common ancestor. Owing to pass of flyways of migratory birds over Iran and poor hygienic measures in poultry farms of this country, the interaction between migratory birds and rural chicken might have occurred.

Consequently, recombination was took placed and commer-cial poultry have infected by the new sub-genotype VII-g (40, 41). High identity (95.39%) of nucleotide sequence of F0 proteins between studied isolate and Chinese isolates 2008, suggesting the rapid worldwide expan-sion of this new sub-genotype in poultry and further isolation of this sub-genotype viruses from the other countries such as Nigeria, Indonesia and Island suggest that viruses of genotype VII-g may have the potential to start a new panzootic (17).

This isolate didn't show a high percentage of identity with previous Iranian isolates which were assumed originated from Russian NDV (42, 43). We first time report the presence of a new NDV sub genotype VII-g circulating in poultry in Iran, which has caused enormous losses in vaccinated poultry as compared to other subgenotypes of a virulent type such as VII-d. More studies about genome sequencing of Iranian ND viruses need to measure relate-dness among NDVs from different geographic regions of the world (44).

Overall continuous and accurate monitoring of other species of birds would help to obtain an insight into the evolution of NDVs and control of panzootic viruses in future.

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