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Short Communication

Molecular Characterization and Phylogenetic Study of Newcastle Disease Viruses Isolated in Iran, 2014–2015

Kiani MH[1], Bozaorgmehrifard MH[1*], Hosseini H[2], Charkhkar S[1], Ghalyanchilangerodui A[3]

1. Department of Poultry and obstetrics, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Clinical Science, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Alborz, Iran.
3. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Abstract

Newcastle disease (ND) is a highly contagious disease that affects many species of birds and causes significant economic losses to the poultry industry worldwide and the pathogenicity of Newcastle disease virus (NDV) strains varies with different virulence. Samples were collected from chicken commercial farms in Iran during 2014–2015. ND virus was characterized (NDV) by partial sequences of fusion genome and compared with other NDV sequences. All viruses showed the amino acid sequence 112 RRQKRF117 at the C-terminus of the F2 protein and phenylalanine at the N-terminus of the F1 protein, residue 117. These amino acid sequences were identical to a known virulent motif. Based on the phylogenetic analysis the Iranian NDV isolates are closely related to the genotype VIId of class II NDV strains. The result showed that the genotype VIId was dominant and circulating among chicken farms and caused clinical disease.

Keywords: Newcastle disease virus, poultry industry, phylogenetic analysis

Introduction

Newcastle disease (ND), caused by Newcastle disease virus (NDV), is a highly contagious and devastating viral disease of most avian species that is characterized by different clinical signs that can result in significant economic loss to the poultry industry [1]. Its etiological agent is classified as a member of Avulavirus genus within the Paramyxoviridae, Paramyxovirinae, Mononegavirales and is designated avian paramyxovirus-1 (APMV-1). The NDV has a ~15 kb RNA genome that codes for six viral proteins in the order 5-NP-P-M-F-HN-L–3 [4]. Phylogenetic analysis based on genome length and sequence of the F gene has revealed that NDV strains consist of two distinct classes (I and II) within a single serotype, with class I containing nine genotypes (1–9) and class II containing 15 genetic groups including 10 previously established (I–IX, and XI) and five new genotypes (X, XII, XIII, XIV and XV) [5]. NDV strains have been classified into two major pathotypes; on the basis ofpathogenicity for chicken[2]. Conventionally, the pathogenicity of a newly identified NDV isolate or strain is assessed by methods including the intra-cerebral pathogenicity index (ICPI), the mean death time (MDT), and/or intravenous pathogenicity index (IVPI). [16]. However; the fusion protein cleavage site of NDV can be used for virulence determinantion and is suggested by the presence of multiple basic amino acids (112R/K-R-Q-K/R-R116) motif, followed by phenylalanine at position 117, which is indicative of a virulent virus. The viruses with low virulence have sequences in the same region of112G/E-K/R-Q-G/E-R116 and

[*] **Corresponding author:** Mohammad Hassan Bozaorgmehrifard , Department of Clinical Science, Faculty of Veterinary Medicine, Karaj Branch , Islamic Azad University, Alborz, Iran. email: mhbfard@yahoo.com
Tel: +98(21) 61117150, Fax: +98(21) 66933222

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followed by Leucine at position 117 [10]. Newcastle disease is endemic in Iran and we have many reports for incidence of velogneic ND in commercial farms in recent years [3, 13]. Previous studies revealed that the genotype of NDV that were circulating in commercial farms was VIId [11]. However, it needs constant monitoring to be aware of any new genotype. The aim of this study was to shed some light on the phylogenetic relationships among the NDVs circulating in Iran between 2014 and 2015.

Methods

Virus isolation. A panel of organs (brain, trachea, and cecal tonsils) was submitted to PCR Veterinary Diagnostic Laboratory (Tehran, Iran). All samples were collected from chicken flocks that had clinical signs, including respiratory, nervous, and digestive symptoms, along with high mortality rates–all the flocks had been vaccinated with killed and live NDV vaccines. A 0.2-ml aliquot of suspensions from the organs was inoculated separately into the allantoic cavity of four 9-day-old embryonated eggs for virus isolation. After incubation at 37 C for 3 days, the allantoic fluid was harvested and tested by HA assays. Samples showing hemagglutinating activity in allantoic fluids were subjected to the HI test under the recommended procedure to specify viruses further (all data available in Table 1).

Reverse-transcription PCR (RT-PCR). Reverse-transcription PCR (RT-PCR). Reverse transcription was done by using Random Hexamer with the Revert Aid first strand cDNA Synthesis Kit (Fermentas-Thermo Fisher Scientific, Burlington, ON, Canada). The partial of the F gene including the cleavage site sequence was amplified with the use of a pair of specific primers. Primer sequences are 59-TTGATGGCAGGCCTCTTGC-39 and 59-GGAGGATGTTGGCAGCATT- 39 (14). PCR was carried out in a 50-ml reaction volume consisting of 5 ml 103 PCR buffer, 1 ml 10 mM dNTPs, 1.25 ml of each primer (10 pmol/ml), 0.25 ml Taq DNA polymerase (5U/ml), 1.5 ml 50 mM MgCl2, 33.75 ml of dH2O, and 6 ml cDNA dilution, and was programmed in the following condition: 94 C for 3 min followed by 35 cycles of 95 C for 30 sec, 53 C for 30 sec, 72 C for 60 sec, and a final extension at 72 C for 15 min. The PCR products were separated by electrophoresis with the use of 1% agarose gel.

Sequencing, Phylogenetic and Sequence Analysis. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (EB). The PCR products were purified by the PCR AccuPrep® PCR Purification Kit (Bioneer Co., Korea) and purified PCR products were sequenced with PCR primers in both directions. The nucleotide sequences were checked for base calling and edited for length prior to export in FASTA format using Chromas Lite software. Sequences used for comparison or phylogenetic analysis in this study were obtained from the GenBank database. Nucleotide acids sequences of the F genes were used to study and construct the respective phylogenetic trees. A phylogenetic tree was constructed using MEGA5.0 software (Molecular Evolutionary Genetics Analysis, version 5.0) by Neighbor-Joining method (1000 replicates for bootstrap). [18]. Bootstrap values lower than 50 were omitted.

Results

RT-PCR with primers resulted in the amplification of a 380-bp product, as expected. The predicted amino acid sequences of the NDV isolates were compared. 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 seven field isolates of NDV and the corresponding regions of the other 38 NDV strains retrieved from GenBank. The field isolates and reference strains were classified as genotypes I–IX. All

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seve field isolates isolated in 2014– 2015 outbreaks were classified into genotype VII. All of them were subclassified into sub genotype VIId.

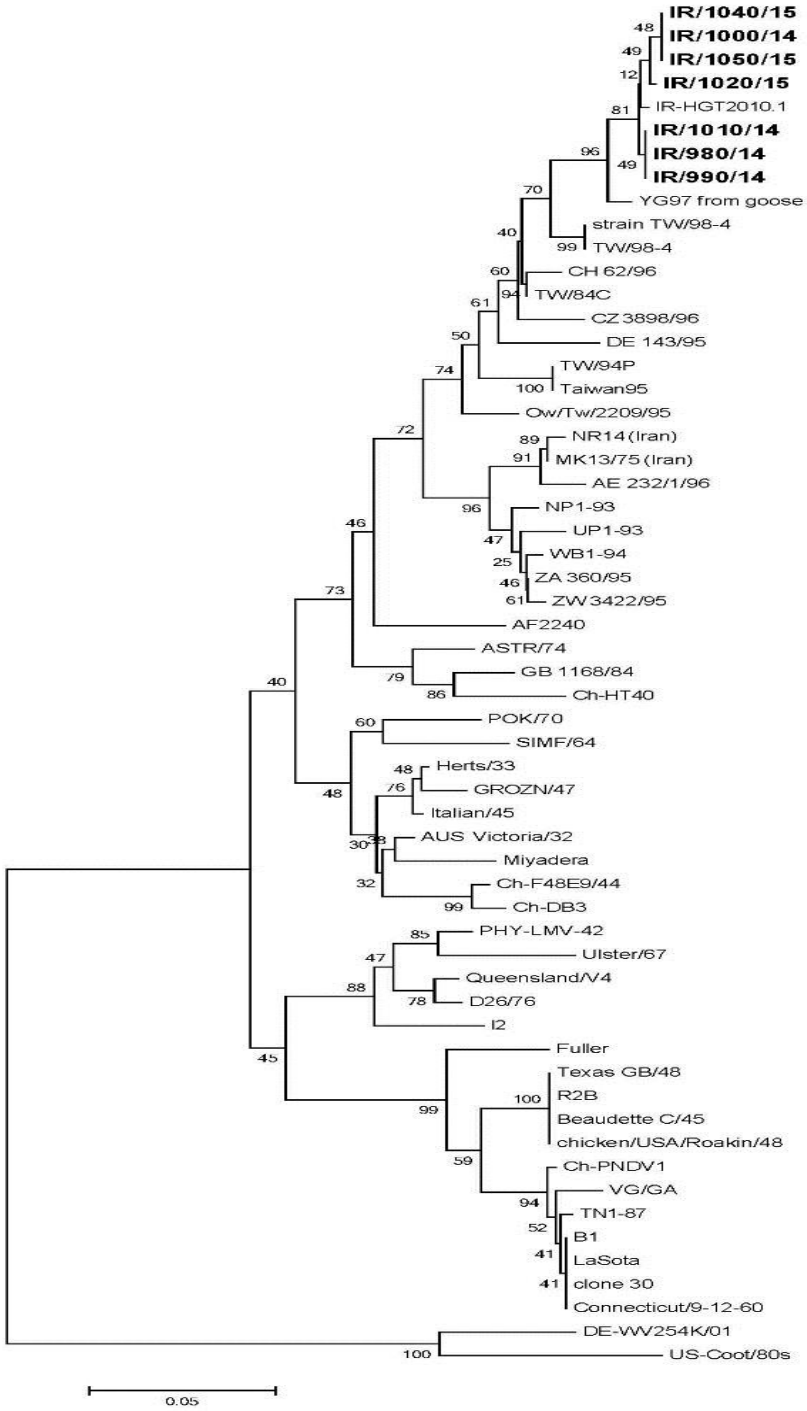

Fig. 1. Nucleic-acid–based phylogenetic relationships of fusion gene of Newcastle disease virus isolated from Iran. The phylogenetic tree was generated by the neighboring-joining model with MEGA (version 5.1 beta).

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Discussion

Newcastle disease is one of the most serious infections of poultry and is endemic in Iranian poultry industries. As vNDV is endemic in Iran, several works have been carried out on varied aspects of ND. Viral genome sequence data are undoubtedly appropriate tools for tracking the origin of ND outbreaks and monitoring the field isolates in areas in which the disease is endemic.

NDV includes two glycoproteins that are considered to be connected with the viral envelope. The HN glycoprotein is included in hemagglutinating and neuraminidase actions that have the responsibility of binding the virus to host cell receptors and destroying receptors, respectively. The fusion (F gene) is the protein responsible for fusion with the host cell membrane. It has long been thought that the 2 glycoproteins mentioned above can affect the virulence of NDV strains. Phylogenetic analyses have indicated that there are multiple velogenic genotypes circulating in Europe and Asia causing outbreaks in poultry. Among them genotype VII, representing viruses that emerged in the 1990s in the Far East were prevalent and mainly responsible for ND outbreaks in Far East, Europe, and South Africa. The genotype was divided into VIIa–VIIh subgenotypes in which VIIa–VIIe were circulating predominantly in China, Malaysia, Kazakhstan and Kyrgyzstan mainly responsible for recent outbreaks and VIIf–VIIh represent African isolates [6]. The genotype VI group contains viruses from around the world collected as early as 1978. This is a diverse group of viruses that have been phylogenetic ally characterized by many different authors into various sublineages.

Biological characterization and phylogenetic analysis the fusion (F), hemagglutinin-neuraminidase (HN) and matrix (M) genes of Newcastle disease virus isolated from outbreaks in commercial poultry and from backyard poultry flocks in Pakistan based indicated the emergence of a novel genetic group within lineage 5, distinct from isolates previously reported in the region [15]. Munir et al. (2012) in study on velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010, showed an Y526Q substitution in the HN protein, which determines neuraminidase receptor binding and fusion activity of NDV [15]. Two NDV isolated from Iranian farms are clustered in one group in genotype VIb/2, which from high homology (95.34%) with Rus/Moscow/0453/09(VIb/2) (JF824064) and Pi/Rus/Vladimir/495/04(VIb/2) (JF824029).

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