Phylogenetic study based on the phosphoprotein gene of Iranian Newcastle disease viruses (NDV) isolates, 2010 -2012

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Abstract:

BACKGROUND: Newcastle disease virus (NDV) is the causative agent of the Newcastle disease (ND), a highly contagious disease in birds that causes significant economic losses to the poultry industry worldwide. ND is endemic in Iran and outbreaks are reported regularly in commercial poultry flocks and different species of birds. **OBJECTIVES:** The current study was carried out to characterize NDV based on phosphorprotein (P) gene from recent outbreaks in Iran, 2010-2012. **METHODS:** The P gene fragment of NDV isolates of five chickens, 1 ostrich, and 1 Pigeon paramyxovirus-1 was obtained by RT-PCR and sequenced. **RESULTS:** Phylogenetic analysis of sequences revealed that chicken and ostrich NDV isolates were closely related and placed in the genotype VII and Pigeon Paramyxovirus-1 was located in the genotype V. **CONCLUSIONS:** This is the first report of Phosphoprotein gene sequences of NDV strains isolated in Iran. This study will help us to understand the epidemiology and molecular characteristics of Newcastle disease virus in Iran.

Introduction

Newcastle disease (ND), caused by Newcastle disease virus (NDV), is a highly contagious respiratory, enteric or neurological viral disease and an OIE notifiable devastating disease that is found in most avian species, especially in chickens (Miller et al., 2009).

The NDV is a member of avian paramyxovirus serotype-1 (APMV-1), which belongs to the genus Avulavirus and Paramyxoviridae family. The NDV has RNA genome (a negative-sense single stranded, 15 Kb), which codes for an RNA-directed RNA polymerase (L), hemagglutinin-neuraminidaseprotein (HN), fusion protein (F), matrixprotein (M), phosphoprotein (P), and nucleoprotein (*NP*) (Alexander et al., 1997).

All NDV isolates are classified as three pathotypes based on the disease severity: mesogenic, velogenic, and lentogenic. Genetic and antigenic analyses of NDV isolates have determined the existence of two main classes I and II, which could be divided into ten genotypes (1-10 in class I and I-X in class II): The genotypes VI and VII being further

divided into seven (VIa-VIg) and five (VIIa-VIIe) sub-genotypes respectively (Miller et al., 2010).

The phosphoprotein, or P protein, of NDV, has the critical role in replication and transcription and has multiple functions. For example, the P protein also acts as a chaperone to prevent uncontrolled encapsidation of non-viral RNA by the NP protein (Jahanshiri et al., 2005; Locke et al., 2000; Smith and Hightower, 1981). ND is endemic in Iran and we have some reports for incidence of ND in every year. In the past few decades, implementations of extensive vaccination programs in commercial poultry farms, and to some extent in small rural poultry flocks have reduced the number of epizootics outbreaks of ND in Iran(Bozorgmehri-Fard and Keyvanfar, 1979; Rezaeianzadeh et al., 2011). No data is available about the P gene of Iranian NDV isolates till now. The study is the first report on P gene characterization of Iranian NDV isolates and provides a suggestion on NDV data collection to increase future virus evolutionary analysis.

Materials and Methods

Samples: We worked on six velogenic NDV isolates and one Pigeon Paramyxovirus -1 (approved by analyses of sequences of Fusion gene) that were isolated form different outbreaks in broiler and breeder farms, ostrich and pigeon case. Ten-day-old SPF embryonated chicken eggs (Allantoic cavity) were inoculated. These eggs were incubated at 37 C for up to 2 days, embryonic death was monitored, and then allantoic fluid was collected under routine conditions. All data of mentioned isolates are available in Table 1.

RNA extraction and RT-PCR: RNA is extracted from allantoic fluid through QIAamp virus spin kit (QIAGEN, USA) and stored at -70°C. Reverse transcription was done by using random hexamer with revert aid first strand cDNA synthesis Kit (Fermentas Co, Canada) (OIE, 2012). Amplification of the P gene was carried out by PCR as described by using one pair of specific primers of phosphopotein gene (NDF: ACCAGYGGRACTGTCATHGAC, NDR: CGGACAGTGTCCYTCTCYAC).The PCR amplification was carried out in a 50 µL reaction mixture containing 0.2 lM dNTP, 0.5 mMMgCl2, 0.2 lM each primer, 19 PCR buffer and 1 U of Taq polymerase. Reactions were performed according to the following protocol: 95 ^{o}C for 5 min, followed by 35 cycles (95 ^{o}C for 45 s, 58^{o}C for 45 s, 72 ^{o}C for 1min) and a final extension step of 10 min at 72 ^{o}C.

Sequencing and bioinformatics study: The PCR products were purified with the AccuPrep® PCRPCR Purification kit (Bioneer Co, South Korea) and sent to Source BioScience Company (UK) for DNA sequencing. The primary sequence analyses and edition were carried out in the CLC sequence viewer (Ver. 6.0.2). Multiple amino acid alignments were performed on P genes representative viruses using Clustal W (MEGA5) (Tamura et al., 2011) on current study isolates and other NDV isolates from Gene bank (Table 2). Phylogenetic trees were drawn from nucleotide sequences based on the sequence of phosphprotein gene using Neibor-Joining method with MEGA5.Model with 1000 bootstrapping replications in molecular evolutionary genetics analysis (MEGA5). The structure of the NDV that were sequenced and analyzed in this study has been submitted to GeneBank under the accession numbersKF824513-KF824519 (Table 1).

Results

The partial sequences of phosphoprotein gene of six Iranian NDV isolates were amplified and sequenced. MEGA5 program were used to determine the sequence similarity and homology of the p gene among them. Results indicate that nucleotide homology among six NDVs isolates with P genes is between 99.6% and 99.9%. The percent identify based on amino acid is between 99.3%- 99.8 %. In BLAST result NDVs isolates have high nucleotide identities (97%) with JSG0210 (JF340367), chicken/TC/9/2011 (KC461214), JSD0812 (GQ849007). Pigeon Paramyxovirus-1 has 94% nucleotide identities with Pigeon paramyxovirus-1 strain AV324/96(GQ429292). A phylogenetic tree (Figure 1) was constructed based on the aminoacidemia sequences of the P genein allisolates and one and the corresponding region of the other NDV strains retrieved from Gene Bank (Table 2). All six NDV fleld isolates, isolated in 2010-2012 outbreaks, were classifled into genotype VII and one Pigeon Paramyxovirus-1 was located in the genotype V.

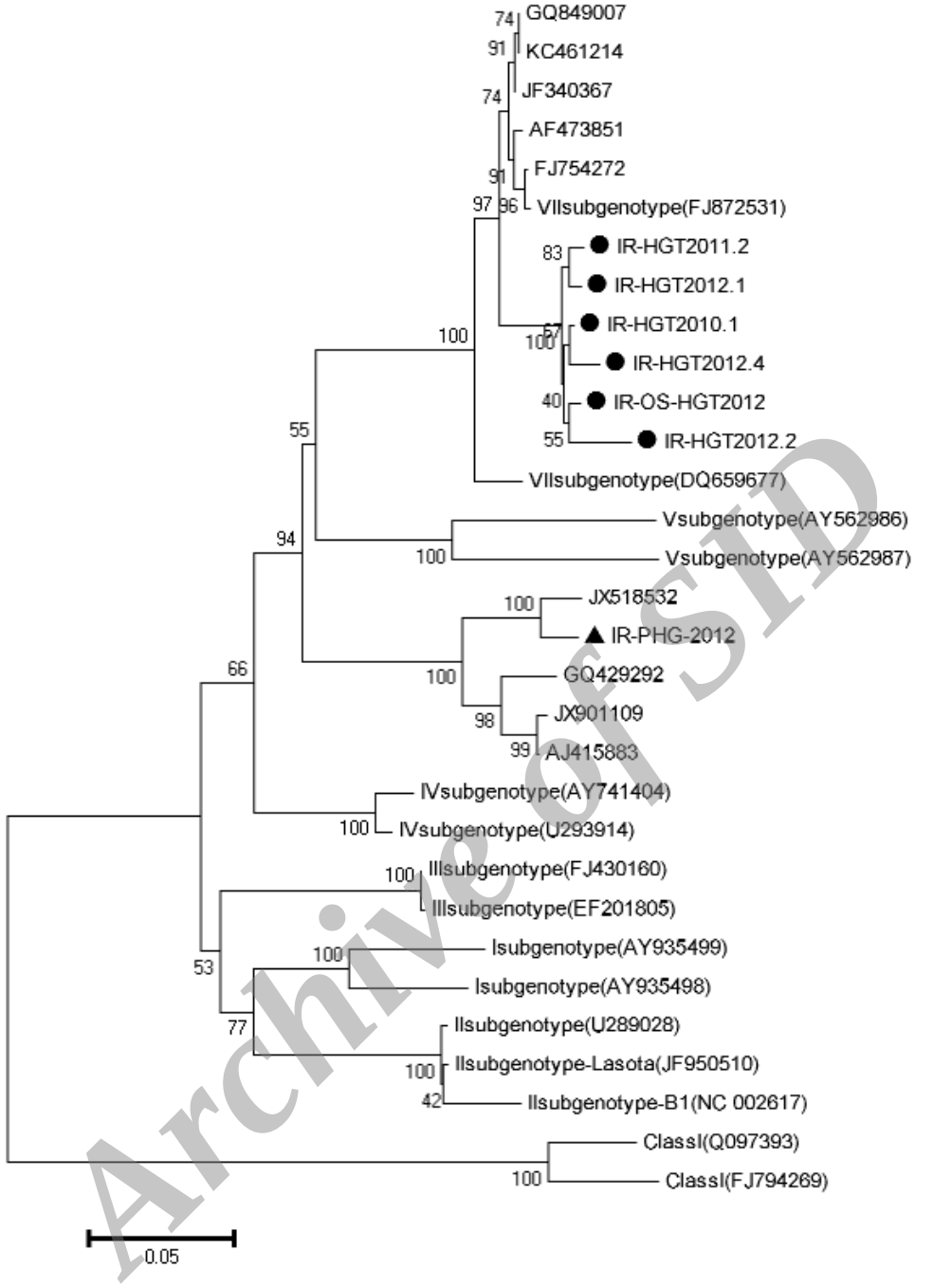

Figure 1. Nucleic acid Phylogenetic relationships of phosphoprotein gene of Newcastle disease genome isolated from broilers and pigeon, Iran. The Phylogenetic tree was generated using Neibor Joining model with MEGA (version 5.1 beta). Numbers below branches indicate bootstrap value from 1000 replicates, 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. 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 of broiler characterized in this report is indicated as Black Circle. Black triangle is indicated for pigeon isolate. The sequences obtained from Gene Bank.

Discussion

Newcastle disease (ND) has a destructive economic effect on commercial farms in Iran; thus, it is necessary to identify the ecology and transmission dynamics in order to improve disease control measures. This study was conducted to characterize

Table 1. The characteristic of Iranian Newcastle disease virus isolates (2010-2012).

No	Strain Name	Accession Number (Fusion Protein)	Accession Number (Phospho protein)	Isolation from Species	Clinical sign	Year	Province/Iran	Sample
1	IR-HGT2010.1	JX131352	KF824516	Broiler	Neurological	2010	Gilan	Brain
2	IR-HGT2011.1	JX131355	KF824517	Broiler	Neurological	2011	Mazandaran	Brain
3	IR-HGT2012.1	JX131357	KF824513	Broiler	Digestive	2012	Alborz	Cecal tonsil
4	IR-HGT2012.2	JX131358	KF824514	Broiler	Neurological	2012	Isfahan	Brain
5	IR-HGT2012.4	JX131360	KF824515	Breeder	Neurological	2012	Mazandaran	Brain
6	IR-OS-HGT2012	JX131350.1	KF824518	ostrich	Neurological	2012	Tehran	Brain
7	IR-PHG-2012	No submission	KF824519	Pigeon	Neurological	2012	Tehran	Brain

Table2. List of Newcastle disease viruses' characteristics that included in the phylogenetic.

Accession Number	Strain Name	Class	Sub-Genotype
AY935499	I-2	II	I
AY935498	99-1435	II	I
EU289028	VG/GA	II	II
NC_002617	B1	II	II
JF950510	Lasota	II	II
FJ430160	JS/9/05/Go	II	III
EF201805	Mukteswar	II	III
AY741404	Herts/33	II	IV
EU293914	Italien	II	IV
AY562986	Anhinga/U.S.(Fl)/44083/93	II	V
AY562987	Gamefowl/U.S.(CA)/211472/02	II	V
JX518532	Pigeon paramyxovirus	II	V
GQ429292	AV324/96	II	V
JX901109	PMV-1/Belgium/98-238/1998	II	V
AJ415883	Pigeon paramyxovirus-1	II	V
AY562985	Cockatoo/Indonesia/14698/90	II	VI
AJ880277	Pigeon paramyxovirus 1	II	VI
FJ872531	Muscovy duck/China(Fujian)/FP1/02	II	VII
DQ659677	NA-1(Goose)	II	VII
AF473851	SF02/Goose	II	VII
FJ754272	WF00D	II	VII
JF340367	JSG0210	II	VII
KC461214	chicken/TC/9/2011	II	VII
JSD0812	GQ849007	II	VII
DQ097393	DE-R49/99	I	--
FJ794269	NDV08-004	I	--

NDVs isolated from five chicken, one ostrich and one Pigeon paramyxovirus-1. Phylogenetic analysis of sequences revealed that chicken and ostrich origin NDV isolates were closely related and placed in the genotype VII and Pigeon Paramyxovirus-1 was located in the genotype V. It is similar to pervious results of researches of Iranians on F (Kianizadeh et al., 2002) and M gene (Langeroudi et al., 2012). Ebrahimi et al. (2012), based on F gene of Iranian NDV isolates, reported that they were representing sub-genotype VIIb (Ebrahimi et al., 2012). According to the results of our study, it is the first report that mentioned Iranian NDV strains were located in genotype VIId. Aboshah (2012), working on NDV isolates from Iranian commercial farms, been revealed that these isolates were located in VIIb subgenotype (Abdoshah, 2012). Esmaealzadeh and co-investigators showed that the 6 Iranian isolates examined share significant similarity with 2 Russian isolates, Sterna- Astr/ 2755/ 2001 and VOL95 (Esmaelizad et al., 2012).The results also indicate that genotype VII was the most prevalent isolate in Iran during the recent years. Genotype VII of NDV in Asia has been traced back to 1984 in Taiwan and to 1985 in Japan (Yang et al., 1999). According to Munir et al. (2012) the P gene was the most variable gene among the six NDV genes when compared with representatives of each genotype. Therefore, it is believed that the P gene is an evolutionary strategy of the virus that increases the coding capacity of the genome (Munir et al., 2012). It is the first report for characterization and phylogenic analysis of Pigeon Paramyxovirus-1In Iran. The data revealed that Iranian pigeon paramyxovirus-1 belongs to Genotype V beside other related pigeon Paramyxovirus-1. The presence of multiple NDV strains in Iran, the Far East and highly transmissible nature of the virus can complicate and increase the cost of attempts to prevent the spread of infection to the other parts of the world. In accordance with high occurrence of ND in pigeon population in Iran, more detailed studies should be carried out on their isolates. Finally, we suggest that researchers perform full length characterization on Iranian NDV isolates in recent years to reach more details of molecular epidemiology. In conclusion, our results reveal that NDV surveillance would be helpful to expand the understanding of NDV epidemiology in endemic regions.

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مجله طب دامی ایران، ۱۳۹۳، دوره ۸، شماره ۲، ۷۷-۷۳

مطالعهٔ شجره‌شناسی بر اساس ژن فسفوپروتئین ویروس‌های نیوکاسل جداشده در ایران، ۱۳۸۹-۱۳۹۱

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چکیده

زمینهٔ مطالعه: ویروس بیماری نیوکاسل (NDV) عامل بیماری نیوکاسل (بیماری بسیار واگیر در پرندگان) سبب واردآمدن خسارات اقتصادی قابل توجه به صنعت طیور سراسر جهان می‌گردد. بیماری نیوکاسل در ایران اندمیک است و از رخداد بیماری در طیور صنعتی و سایر پرندگان درکشور گزارش‌های متعددی داده‌شده است. **هدف:** مطالعهٔ حاضر برای توصیف خصوصیات ویروس بیماری نیوکاسل (NDV) بر اساس ژن فسفوپروتئین در واگیری اخیر این بیماری در سال‌های ۸۹ تا ۹۱ انجام شد. **روش کار:** قطعه ژن فسفوپروتئین ویروس بیماری نیوکاسل از روی جدایه‌های بدست‌آمده از پنج قطعه مرغ، یک‌شتر مرغ و یک پارامیکسوویروس تیپ ۱کبوتر با روش نسخه‌برداری معکوس- واکنش زنجیره‌های پلی‌مراز (RT-PCR) بدست‌آمد و توالی‌یابی شد. **نتایج:** تحلیل شجره‌شناسی نشان داد جدایه‌های حاصل از ماکیان و شترمرغ بسیار به یکدیگر نزدیک بودند و در ژنوتیپ هفت و جدایهٔ حاصل از پارامیکسوویروس تیپ ۱کبوتر در ژنوتیپ پنج قرار دارند. **نتیجه‌گیری نهایی:** مطالعهٔ حاضر اولین گزارش از توالی‌یابی ژن فسفوپروتئین حاصل از جدایه‌های ویروس بیماری نیوکاسل در ایران است. این مطالعه در درک همه‌گیرشناسی و خصوصیات ملکولی ویروس بیماری نیوکاسل بسیار کمک‌کننده خواهد بود.

واژه‌های کلیدی: بیماری نیوکاسل، فسفوپروتئین، مطالعهٔ شجره‌شناسی

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