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ORIGINAL ARTICLE

Phylogenetic characterization of virulent Newcastle disease viruses isolated during outbreaks in northwestern Iran in 2010

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Abstract The northwest of Iran shares long borders with three neighboring countries; therefore, it is considered one of the main entry portals of Newcastle disease virus (NDV) into the country. Ten virulent NDVs were recovered from 19 poultry farms of various prefectures in northwestern Iran during Newcastle disease outbreaks in 2010. The isolates were genotypically analyzed using an F-gene-specific reverse transcription polymerase chain reaction (RT-PCR) assay. The amplified F gene (nucleotides 189-1666) sequences of the NDV isolates were compared phylogenetically with those of previously published strains in GenBank. All of the NDV isolates belonged to genotype VIIb and were closely related to some isolates from Iran, Russia, and Sweden. Therefore, it can be postulated that these isolates evolved from previously reported strains. The velogenic viruses carried the motif ^{112}R-R-Q-K-R/F^{117} at the F_0 cleavage site and a unique substitution of ^{190}L→F which had never been reported in any NDV genotype VIIb isolate. They shared high sequence similarity with each other but were distinct from current NDV vaccines and NDV strains reported from other countries. This information is fundamental for improving the efficacy of controlling strategies and vaccine development for NDV.

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Introduction

Newcastle disease (ND) is one of the most serious and devastating diseases in the poultry industry [35]. The etiological agent, Newcastle disease virus (NDV), also known as avian paramyxovirus 1 (APMV-1), belongs to the genus *Avulavirus* within the family *Paramyxoviridae* [30]. This enveloped virus has a negative-sense, single-stranded RNA genome of approximately 15.2 kb that encodes six proteins, including nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and a large RNA-directed RNA polymerase (L) [6].

Based on conventional *in vivo* pathogenicity indices for chickens, NDV strains can be categorized into three main pathotypes: highly virulent (velogenic), intermediate (mesogenic) and low-virulent (lentogenic) [5]. This difference in pathogenicity is due to differences in the amino acid sequence of the fusion protein cleavage site (FPCS). This protein is synthesized as an inactive precursor (F_0) in non-functional state, which has to be cleaved into active F_1 and F_2 subunits for the virus to be infectious [16]. The virulent NDV (vNDV) has the motif ^{112}R/K-R-Q-R/K-R↓F^{117} in its FPCS, whereas ^{112}G/E-K/R-Q-G/E-R↓L^{117} is the consensus sequence for the cleavage site of low-virulent viruses. The presence of multiple basic amino acids in vNDV permits the virus to be cleaved by ubiquitous host proteases found in most tissues, resulting in a fatal systemic infection [38]. Conversely, the F_0 protein of low-virulence APMV-1 strains is cleaved only in cells containing unique trypsin-

like enzymes, limiting infection to mucosal tissues of the respiratory or intestinal tract of the host. Therefore, the FPCS is used as a virulence criterion along with biological virulence determinations [8].

All NDV isolates characterized to date belong to a single serotype, but significant genetic diversity among NDV isolates has been recognized [4]. Comparison of the nucleotide sequences of different strains of NDV has revealed two distinct NDV genotype groups, class I and II, consisting of at least nine genotypes, designed 1-9 and I-IX, respectively [32, 46]. Class I viruses are distributed worldwide and have been isolated mainly from aquatic birds and live-bird markets, and most of them are avirulent [23]. Only class II viruses are responsible for fatal diseases in poultry [33]. The genotypes VI and VII are further divided into seven (VIa-g) and five (VIIa-e) subgenotypes, respectively [9, 27]. Genotypes V, VI, and VII of virulent viruses are the predominant genotypes circulating worldwide [32, 33]. Of these, genotype VII is particularly important, given that it has been associated with many of the most recent outbreaks in Asia, Africa, and the Middle East [22, 23, 27]. Thus, phylogenetic analysis of NDV is a powerful tool for investigating epidemiological relationships among NDV isolates present in various parts of the world.

Despite the extensive and strict vaccination policy for prevention and control of NDV infections, ND is regarded as an endemic disease in some parts of the world, posing a constant threat to the poultry industry. In 2010, infection by vNDV was confirmed in domestic poultry in countries of Asia, Africa, Europe and North and South America [35]. However, very little data is available on virulent NDVs responsible for recent outbreaks in Iran. As the northwestern part of the country shares borders with two post-Soviet states, Armenia and Azerbaijan, besides Turkey and Iraq, it has been speculated that the diversity of vNDVs is high in that region. In order to evaluate the degree of genetic diversity of NDV strains circulating in commercial poultry in this part of Iran and to estimate their relationship to other NDV isolates, a part of the F gene from NDV isolates was characterized phylogenetically.

Materials and methods

Sampling and virus isolation

Tissue samples from four clinically diseased chickens suspected to have ND were collected from each of 19 different farms in various parts of northwestern Iran in 2010. Clinical signs observed on farms affected by ND in 2010 outbreaks included torticollis, paralysis of the legs and wings, tremors, diarrhea, lack of muscular coordination, and lethargy. Specimens including brain, spleen, and intestines of chickens with suspected infection were transferred to Razi Vaccine and Serum Research Institute, Iran. Filtrates of pooled homogenized tissues were used to inoculate fertilized specific-pathogen-free (SPF) chicken eggs (Lohmann, Australia) using standard procedures [27, 36]. The second egg passage of virus stocks was used for serological analysis. Also, allantoic fluid samples were harvested, divided into aliquots, stored at -70 °C and used as a working stock for molecular analysis. The identity of the virus was confirmed by haemagglutination inhibition assay with anti-NDV La Sota hyperimmune serum [27, 35] and F-gene-targeted reverse transcription polymerase chain reaction (RT-PCR) [21].

Virus pathotyping

The virulence of each NDV isolate was evaluated by mean death time (MDT) index [5, 18] and F-gene-based pathotype-specific primers as described previously [21]. All manipulations of live viruses were performed in a biosafety level 3 laboratory containment facility.

RNA extraction and F gene amplification

Genomic RNA was extracted directly from infected allantoic fluid using a commercial RNA extraction kit (Roche, Germany) according to the manufacturer's instructions. Afterwards, a Titan One Tube RT-PCR System (Roche, Germany) was used to prime synthesis of first-strand cDNA and to perform PCR in one step. The reaction mixture (7 μL of purified template RNA, 4 μL of dNTPs mix [2.5 mM each], 1 μL of each forward and reverse primer [10 pM], 2.5 μL of DTT [100 mM], 0.5 μL of protector RNase inhibitor, 10 μL of 5X RT-PCR buffer, 1.5 mM $MgCl_2$, and 1 μL of titan enzyme mix) were mixed in a final volume of 50 μL Using the previously published primers 5′-TTGATGGCAGGCCTCTTGC-3′ [21] and 5′-TTTGTAGTGGCTCTCATCTG-3′ [48], a 1561-bp product of the F gene was amplified. The thermal cycle profile was 45 °C for 45 min and 94 °C for 2 min, followed by 35 cycles of amplification (consisting of denaturation at 94 °C for 1 min, annealing at 60 °C for 75 s, and extension at 68 °C for 135 s) and a final extension step at 68 °C for 10 min. The 1561-bp products were subjected to electrophoresis on a 1 % agarose gel, and the DNA bands were excised from the gel and purified using a gel extraction kit (Roche, Germany).

Nucleotide sequencing and phylogenetic analysis

The purified products were submitted to the MWG-Biotech Company (Germany) for sequencing with the same

Table 1 Characteristics of vNDVs isolated during outbreaks in northwestern Iran in 2010

Isolates	Date of isolation	Host	Age (days)	F_0 cleavage site (aa[a] 112-117)	MDT[b]	Pathotype	Genotype	Lineage	GenBank accession no.
NR_2	17/2/2010	Chicken (broiler)	49	R-R-Q-K-R/F	48.3	V[c]	VIIb	5b	KC161979.1
NR_3	9/3/2010	Chicken (broiler)	35	R-R-Q-K-R/F	44.3	V	VIIb	5b	KC161980.1
NR_6	26/5/1010	Chicken (broiler)	39	R-R-Q-K-R/F	52.7	V	VIIb	5b	KC161981.1
NR_7	20/6/2010	Chicken (broiler)	45	R-R-Q-K-R/F	43.6	V	VIIb	5b	KC161982.1
NR_9	24/7/2010	Chicken (broiler)	21	R-R-Q-K-R/F	54.2	V	VIIb	5b	KC161983.1
NR_10	15/8/2014	Chicken (broiler)	38	R-R-Q-K-R/F	45.9	V	VIIb	5b	KC161984.1
NR_11	17/9/2010	Chicken (broiler)	41	R-R-Q-K-R/F	51.0	V	VIIb	5b	KC161985.1
NR_13	27/10/2010	Chicken (broiler)	35	R-R-Q-K-R/F	44.6	V	VIIb	5b	KC161986.1
NR_14	2/11/2010	Chicken (broiler)	32	R-R-Q-K-R/F	57.9	V	VIIb	5b	KC161987.1
NR_15	23/12/2010	Chicken (broiler)	45	R-R-Q-K-R/F	47.1	V	VIIb	5b	KC161988.1

[a] Amino acid

[b] Mean death time in eggs in hours

[c] Velogenic

primers from both ends. A 1478-bp sequence of the F gene was deposited in the GenBank database under accession numbers listed in Table 1. Nucleotide sequence editing and alignment and prediction of deduced amino acid sequences were conducted using BioEdit software version 7.0.9 [17]. Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis version 6.0 (MEGA 6.0) [49]. Evolutionary distances were inferred using the neighbor-joining algorithm [43] with 1000 bootstrap replicates (Fig. 1). For comparison, the corresponding sequences of representative NDV strains retrieved from the GenBank database were also included (Table 2).

Results

Identification and pathogenicity assessment of the NDV strains

Ten vNDVs were isolated from 19 ND outbreaks occurring on different farms. The isolates were confirmed using an HI test and by amplification of the F gene by PCR (data not shown) [2]. The MDT varied from 43.6 to 57.9 (Table 1). Additionally, a 254-bp amplicon produced from all of the isolates using primers specific for virulent strains, indicated that these viruses were probably highly pathogenic for chickens (data not shown) [2]. A 1561-bp amplicon corresponding to nucleotide positions 141-1701 of the F gene of NDV encompassing the FPCS was generated from all 10 virulent NDV isolates using oligonucleotide primers. Following nucleotide sequencing, the predicted amino acid sequence of the F protein was determined for each isolate.

Comparison of F gene nucleotide sequences of NDVs and their corresponding amino acid sequences

Comparison of nucleotide sequences of the 10 NDV isolates showed high sequence similarity (98.3 to 99.6 % identity at the nucleotide level and 97.3 to 98.9 % at the amino acid level) among them. Sequence analysis of the 10 NDVs based on partial F gene sequences showed 98.5 to 99 % nucleotide sequence identity and 86.4 to 87.3 % amino acid sequence identity to previously characterized MK13/75 NDV isolate from Iran. Comparison of the F gene sequences of NDVs isolated in the present study to those of reference strains from GenBank revealed a high level of similarity of the isolated NDVs to members of genotype VII of NDV. A high level of nucleotide and amino acid sequence identity was found among the isolates of the present study and some NDV strains (98.3 to 98.9 % nucleotide identity to strains chicken/Sweden/97, chicken/Itali/3286/00 and VOL95/Russia and 97.8 to 98.1 % nucleotide sequence identity to strain Sterna/Astr/2755/2001/Russia]. The isolated NDVs had low nucleotide and amino acid sequence similarity to the NDV vaccines commonly used on Iranian poultry farms (84 to 85.1 % identity to the La Sota vaccine and 84.4 to 85.5 % identity to the B1 vaccine at the nucleotide sequence level).

Proteolytic cleavage site of the F_0 protein and virulence

The sequences of nucleotides 330–347 of the F0 gene corresponding to amino acid residues 112–117 at the F_0 protein cleavage site of 10 NDV isolates are shown in Table 1. The MDT values were in accordance with those predicted based on the sequence of the FPCS. The FPCS

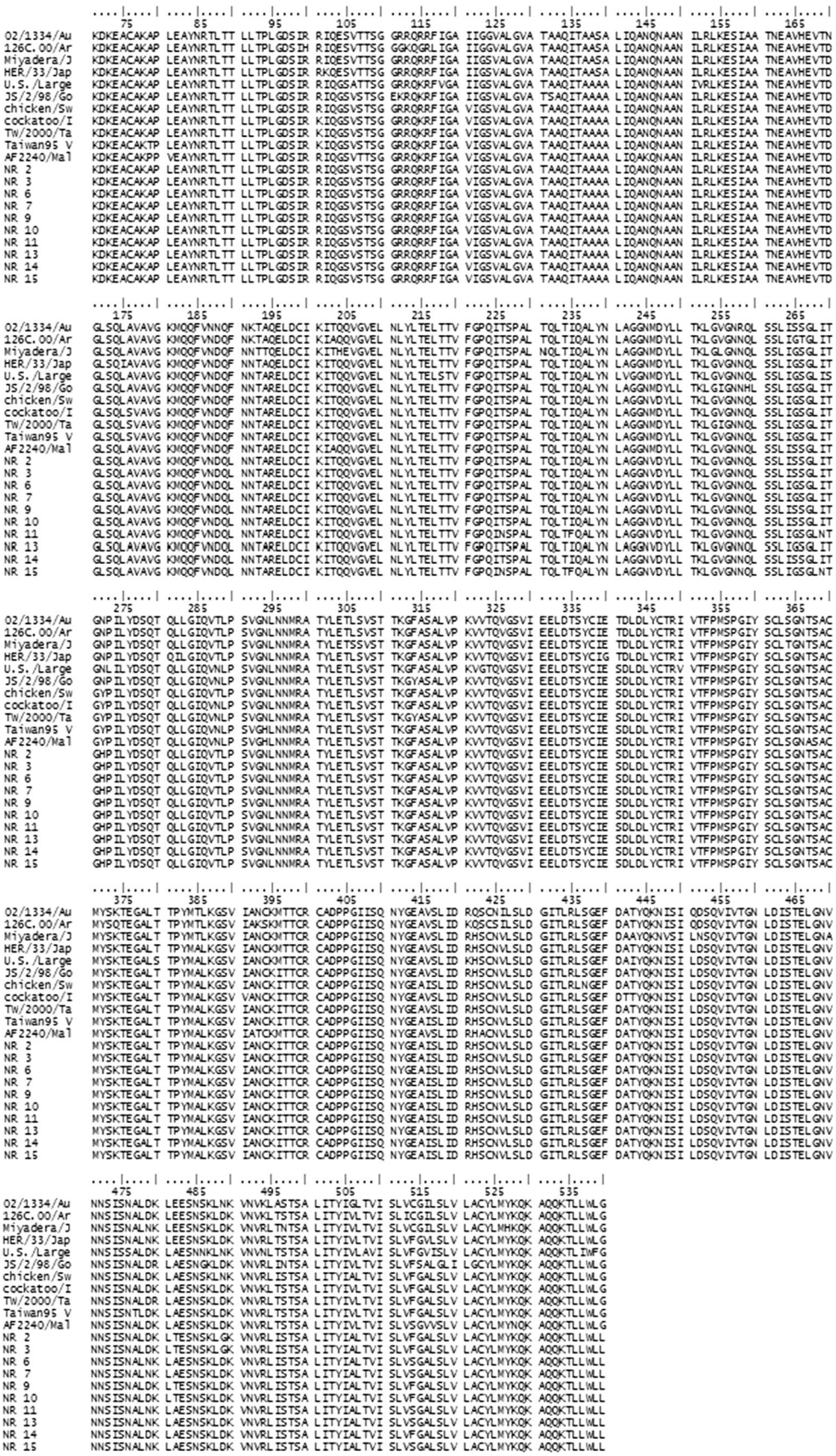

Fig. 1 Alignment of the predicted amino acid sequences of the fusion proteins of NDV isolates. Partial sequences for 10 Iranian NDV isolates and 11 representative NDV strains from GenBank are shown. The sequences were derived by translating primary nucleotide sequences obtained by direct sequencing of RT-PCR products

Table 2 Previously published class II NDVs used for phylogenetic analysis

Strain name	Accession no.	Genotype	Host	Country
Japan/Ishii/62	AB465607.1	I	Chicken	Japan
NDV016540	EU258651.1	I	Chicken	China
QUE/66	M24693.1	I	Chicken	Japan
chicken/N.Ireland/Ulster/67	AY562991.1	I	Chicken	UK/Ireland
02-1334	AY935490.1	I	Chicken	Australia
PHY-LMV42	DQ097394.1	I	Chicken	Hungary
Lasota	DQ195265.1	II	Vaccinal	USA
D58	EU330230.1	II	Chicken	India
VG/GA	EU289028.1	II	Turkey	USA
APMV-1/chicken/U.S.(TX)/GB/1948	GU978777.1	II	Chicken	USA
126C.00	AY727882.1	II	Chicken	Argentina
Beaudette C/45	X04719.1	II	Chicken	
Clone 30	AF099661.1	II	Vaccinal	
ZJ/2000	AF534997.1	II	Chicken	China
AUS/32	M24700.1	III	Chicken	Australia
Miyadera	M18456.1	III	Chicken	Japan
Mukteswar	EF201805.1	III	Chicken	China
chicken/SPVC/Karachi/NDV/1/1974	GU182327.1	III	Chicken	Pakistan
Australia-Victoria		III	-	Australia
Herts/33	AY741404.1	IV	Chicken	Netherland
Italien	EU293914.1	IV	Chicken	China
NDV-2/chicken/Namakkal/Tamil Nadu	GU187941.1	IV	Chicken	India
HER/33	M24702.1	IV	Chicken	Japan
NDV/2K36/peacock/Chennai	HQ011508.1	IV	Peacock	India
2K3/Chennai/Tamil Nadu	FJ986192.2	IV	-	India
mixed species/U.S./Largo/71	AY562990.1	V	Pigeon	USA
NDV-P05	HM117720.1	V	Chicken	Mexico
anhinga/U.S.(Fl)/44083/93	AY562986.1	V	Chicken	USA
cormorant/Canada/95DC02150/1995	FJ705460.1	V	Chicken	Canada
chicken/U.S.(CA)/1083(Fontana)/72	AY562988.1	VI	Chicken	USA
Warwick	Z12111.1	VI	Chicken	Germany
dove/Italy/2736/00	AY562989.1	VI	Dove	Italy
pigeon/Belgium/248VB/1998	EF026583.2	VI	Pigeon	Belgium
STP96	DQ417113.1	VI	Pigeon	China
JS/2/98/Go	AF456439.1	VI	Goose	China
s-1	FJ865434.1	VI	Pigeon	China
W4	HM063423.1	VI	White-breasted water hen	China
chicken/Sweden/97	GU585905.1	VIIb	Chicken	Sweden
Sterna/Astr/2755/2001	AY865652.1	VIIb	*Sterna albifrons* Pallas	Russia
Bareilly	HQ589257.1	VIIb	Chicken	India
MK13/75	AY928933.1	VIIb	Chicken	Iran
chicken/BYP/Pakistan/2010	JN682210.1	VIIb	Chicken	Pakistan
chicken/SPVC/Karachi/NDV/26/2005	GU182329.1	VIIb	Chicken	Pakistan
cockatoo/Indonesia/14698/90	AY562985.1	VIIc	Chicken	Indonesia
SWS03	DQ227254.1	VIIc	Chicken	China
ZJ1	AF431744.3	VIId	Chicken	China
TW/2000	AF358786.1	VIId	Chicken	Taiwan
NR_87	JX129801.1	VIId	Chicken	Iran

Table 2 continued

Strain name	Accession no.	Genotype	Host	Country
NR_92	JX129805.1	VIId	Chicken	Iran
NR_98	JX129809.1	VIId	Chicken	Iran
Taiwan95	U62620.1	VIIe	Chicken	Taiwan
QH4	FJ751919.1	VIII	Chicken	China
Trenque Lauquen	AY734534.1	VIII	Chicken	Argentina
AF2240	AF048763.1	VIII	Chicken	Malaysia

sequence of the 10 NDV isolates had four basic amino acids [^{112}R-R-Q-R-R^{116}] at the C-terminus of the protein and a phenylalanine (F) at residue 117, representing a virulence motif according to the definition of the OIE (Table 1) [35].

Analysis of the nucleotide and deduced amino acid sequences of the F protein gene

The partial predicted amino acid sequences of the F proteins of 10 NDV isolates were 470 residues in length (aa 70 to 540) (Fig. 1). Cysteine residues in the partial F protein were conserved in all NDV isolates. There were 11 cysteine residues located at positions 76, 199, 338, 347, 362, 370, 394, 399, 401, 424, and 523 in the F protein. The predicted N-glycosylation sites of 10 NDV isolates were conserved. All of the isolates had five potential N-glycosylation sites, Asn-X-Ser/Thr (N-X-S/T), located at positions 85NRT87, 191NNT193, 366NTS368, 447NIS449, and 471NNS473. At least 11 neutralizing epitopes, positioned at residues 75, 79, 158, 159, 160, 164, 165, 168, 171, 343, and 378 in the partial F protein, which are believed to be critical for the structure and function of the F protein, have been identified in all examined isolates [34, 51, 52]. The GC content of the partial sequenced F gene was estimated to be 43.57 to 44.05 %.

Although there were similarities among NDV isolates in the present study and their worldwide counterparts, as shown in the phylogenetic tree, the newly characterized NDVs still contained several amino acid substitutions. For instance, substitutions of ^{190}F to L in all 10 newly characterized NDV isolates, ^{265}G to S in NR_2, NR_3, NR_9, NR_10, and NR_14, ^{479}D to N in NR_6, NR_7, NR_11, NR_13, and NR_15, ^{514}F to S in all isolates except NR_2 and NR_3, ^{489}N to G in NR_2 and NR_3, ^{226}T to N, ^{235}I to F, and ^{269}I to N in NR_11 and NR_15 were unique to these isolates. Moreover, analysis of unique residue substitution revealed that the 190th amino acid position of NDVs analyzed from northwestern Iran have leucine (L) replaced by phenylalanine (F), which has never seen in other strains of genotype VIIb.

Phylogenetic analysis

The evolutionary distances among the 2010 NDV isolates from northwestern Iran and other NDV isolates were assessed by phylogenetic analysis. A 1478-bp region corresponding to nucleotides 189-1666 of the F gene was compared to those of other strains belonging to genotypes I to VIII of class II, together with sequences of isolates representing subtypes VIIa, VIIb, VIIc, VIId, and VIIe. The resulting phylogenetic tree is shown in Fig. 2. From the topology of the tree, it was apparent that all NDV isolates under study belonged to genotype VIIb, or lineage 5b, the prevalent pathogen involved in ND outbreaks in northwest of Iran in 2010 to 2011. These viruses were related to chicken/Sweden/97 and Sterna/Astr/2755/2001/Russia as possible progenitor-type viruses. Analysis of the deduced amino acid sequences of the collected samples and data obtained from GenBank did not show any difference in the phylogenetic grouping of samples (results not shown).

Discussion

From 2009 to the middle of 2012, outbreaks of ND on poultry farms throughout Iran were frequently reported to World Organization for Animal Health [35]. Because it shares long borders with several countries, northwestern Iran is considered one of the main entry portals of NDV.

In the present study, the molecular characteristics of 10 vNDV isolates recovered from northwestern Iran during 2010 outbreaks were investigated. Sequence analysis of a 1487-bp fragment of the F gene from each of the NDV isolates, showed that they clustered with virulent NDVs belong to genotype VIIb and were closely related to some Iranian (AY928933), Swedish (GU585905) and Russian isolates (AY865652). Moreover, the predominant subgenotype VIIb existed in Iran and Indian subcontinent countries during 2008-2011 [14]. The close nucleotide and amino acid sequence similarity among the newly isolates and the representative virulent isolates from Iran supports the idea of a local evolutionary trend with regional viral dissemination [14, 44]. Therefore, it could be hypothesized

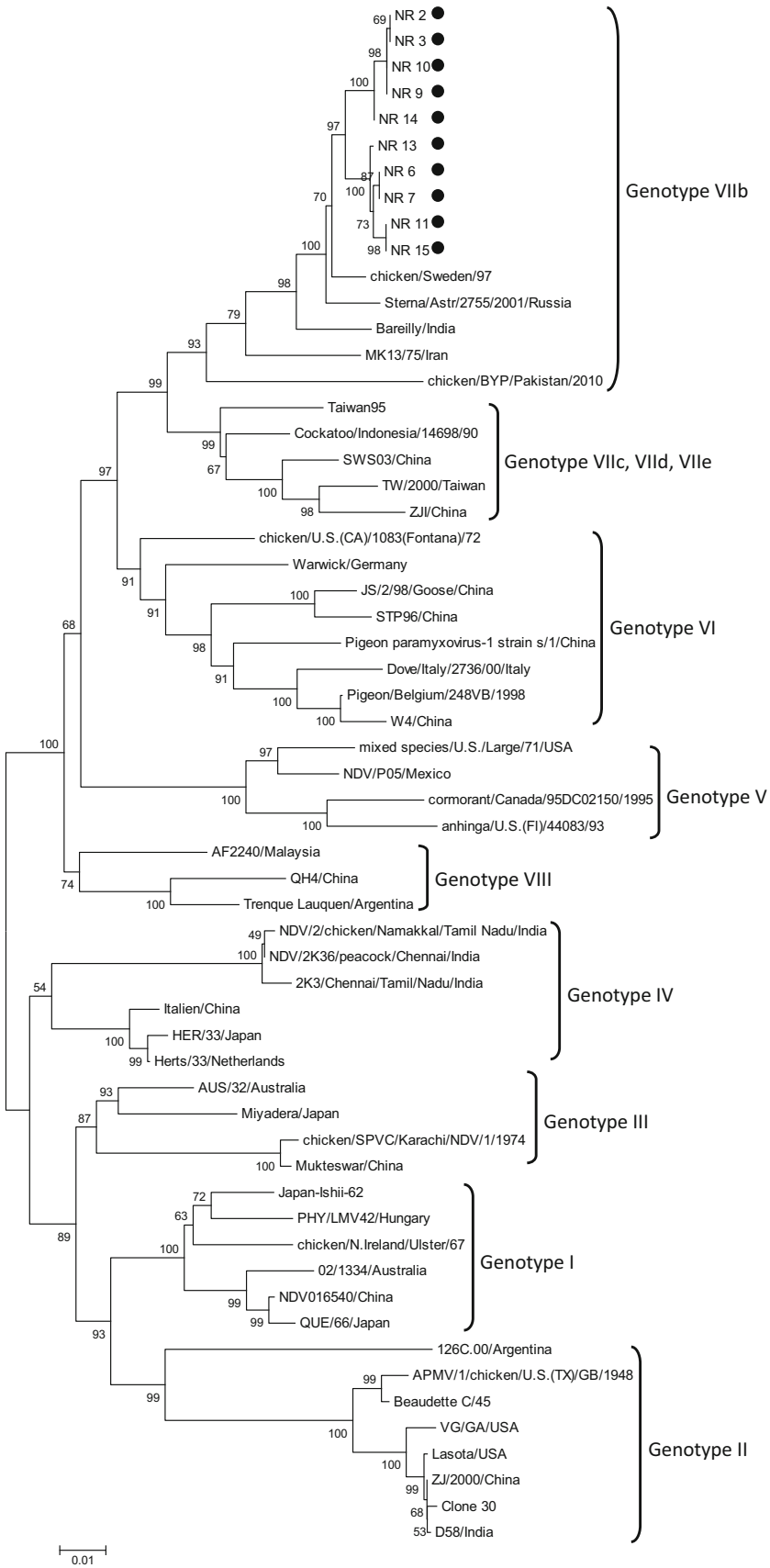

Fig. 2 Phylogenetic comparison of NDV isolates based on their partial F gene sequences (nt189 to 1666) constructed using the neighbor-joining method with 1000 bootstrap replicates (numbers at branching points are bootstrap values given as a percentage). Northwestern Iran isolates from this study are indicated by a solid circle. Provisional designations of the genotypes are shown at the right

that genotype VIIb NDV was present later and is still circulating in the region. A possibility for the maintenance of the virus in the northwestern region of Iran is the existence of a large bird population as the virus reservoir. In contrast, vNDVs obtained from the 2010 to 2011 outbreaks in Shiraz, a city in the south of the country in Iran's Region 2, were phylogenetically different from prior isolates, and consequently, from the isolates from northwestern Iran. NDV isolates in the present study possessed the velogenic motif ^{112}R-R-Q-K-R/F^{117} at the FPCS and clustered in genotype VIId with some Chinese strains, possibly as a result of the import of agricultural products from China to Iran [10]. Conversely, the vNDVs isolated from Industrial poultry throughout the country in the 2011 and 2012 outbreaks, including Ardabil (in the northwest of the country in Iran's Region 3) and Isfahan (in the center of the country in Iran's Region 2) as well as five other provinces, were related to some Chinese and Palestinian isolates based on the motif ^{112}R-R-Q-K-R/F^{117} at the FPCS [42]. In addition, phylogenetic analysis revealed a close association among the prevailing vNDVs isolated from industrial poultry of Isfahan province almost at the end of 2012 with isolates from India (AY339401.1) and Kazakhstan (HQ445947.1). They carried a ^{112}R-R-Q-R-R/F^{117} motif at their cleavage sites [41].

Based on available data, different vNDVs strains have circulated in Iran [11, 31, 44]. Emergence of new strains of vNDVs might be attributed either to the increased trade of agricultural products including poultry or to the movement of migratory birds from the north to the east of the world, involving Europe, Asia, and the Middle East. The presence of multiple velogenic strains of NDV at different times and in various regions of the country emphasizes the need for continued isolation and epidemiological investigation of NDVs circulating across Iran to find the dominant patterns of vNDVs. In a study in Pakistan, a neighboring country to the southeast of Iran, NDVs isolated from commercial poultry were clustered within sub-lineage 5b and were closely related to the viruses isolated in the present study [47]. Therefore, it could be estimated that if all suspicious cases of ND throughout Iran had been evaluated, more diversity of vNDV strains, including the isolates prevailing before 2010, might have been identified.

Although the vNDVs involved outbreaks of the northwest of Iran in 2010 originated from identical or quite similar velogenic strains of the virus, they contained several point mutations, among which the unique residue substitution ^{190}L→F was recognized as a specified marker in these isolates and was reported for the first time in VIIb NDVs. Based on the published phylogenetic data, the only other L-to-F substitution in the vNDV fusion protein was at amino acid 23 in genotype VIId reported by Lien et al. [26], which differs in position and genotype from our findings. Therefore, the importance of the ^{190}L→F substitution in virus pathogenicity and vaccine escape could not be determined in the present study due to the lack of information. Furthermore, amino acid sequence analysis revealed that the fusion-inducing hydrophobic stretch at the N-terminus of the F1 protein (amino acid residues 117 to 142) and cysteine at position 76 are important for disulphide bond formation between F1 and F2. The results also showed that amino acids at potential glycosylation sites were conserved in all 10 ND viruses, as previously reported by other researchers [45, 50]. All of these conserved regions have a considerable role in the precise conformation of the fusion protein vis-à-vis survival of the virus in nature. The FPCS sequence is considered the molecular determinant of NDV pathogenicity in chickens [3, 40]. A typical cleavage site sequence (^{112}R-R-Q-R-R/F^{117}) of virulent NDV present in all 10 isolates in the present study remained conserved. Furthermore, the presence of a phenylalanine (F) residue at position 117 is also potentially associated with neurological signs [25].

In northwestern Iran and the rest of the country as well, a rigorous vaccination program using live (during the rearing and laying period) and killed vaccines (only in the rearing period) are employed in order to induce high levels of maternal antibodies to protect breeders against egg production losses and clinical disease [11, 20, 29]. Nonetheless, ND outbreaks in vaccinated broilers reared under field conditions has increased, and these outbreaks are responsible for high mortality rate and production losses in the poultry industry [15, 20]. As all NDV isolates belong to the same serotype, ND vaccines prepared with any NDV should protect poultry from clinical disease and mortality in the event of a virulent challenge with heterologous strains of different genotypes [35, 39]. La Sota and Clone 30, the most extensively used live vaccines in Iran, belong to genotype II and are different from the prevalent genotype VII. The inability of live vaccines to elicit a protective immune response might be due to several reasons, such as an improper cold chain supply, an inappropriate route of vaccination, or uneven vaccination schedules [47].

In summary, the presence of velogenic NDVs belonging to genotype VIIb has been confirmed in northwestern Iran. Genotype VII of NDV is now regarded as the major pathogen responsible for the fourth panzootic of ND [1, 7, 9, 12, 13, 19, 24, 28, 36, 37]. Therefore, the development and administration of new NDV vaccines that are closely related to predominant VII viruses may confer better protection than conventional vaccines. Further investigation of the molecular epidemiology of NDV and ND vaccinal strains is recommend to explore circulating

NDVs in the region in order to develop the most effective and protective methods.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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