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RESEARCH ARTICLE

Genotypic and Pathogenic Characterization of Newcastle Disease Viruses Isolated from Domestic Ducks in China [1]

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Abstract

In order to determine the prevalence of Newcaste Disease Virus (NDV) in ducks in Guangdong province of China, 10 NDVs were isolated in domestic ducks. Eight isolates were pathogenic as determined by MDT, ICPI and cleavage site of F protein. The F genes of 10 isolates were sequenced, all the genes and their deduced amino acids were compared with 32 reference strains. 5 isolates were clustered in genotype VII (VIId), 3 isolates were classified as genotype IX and 2 isolates were classified as genotype. The subgenotype VIId isolates possessed the motif 112R-R-Q-K-R-F117, and the genotype IX isolates possessed the motif 112R-R-Q-R-R-F117. The 8 NDV isolates exhibited a high ICPI, they were classified as velogenic type of NDVs and were collected between the years of 2006 and 2010. The genotype I isolates possessing the motif 112G-K-Q-G-R-L117 were collected in 2005, they were exhibited a low ICPI and were classified as lentogenic type of NDVs. The NDVs of subgenotype VII are now dominant and have been implicated in most of the recent ND outbreaks in duck farms in Guangdong province. These findings provide data on genetics and molecular evolution of NDV in ducks in China and emphasize importance of NDV surveillance for improving of strategies for the control of the disease.

Keywords: *Newcastle Disease Virus, Duck, Genotype, Pathogenicity*

Çin'de Evcil Ördeklerden İzole Edilen Newcastle Hastalığı Viruslarının Genotipik ve Patojenik Karakterizasyonu

Özet

Çin'in Guangdong Bölgesindeki ördeklerde Newcastle Hastalığı Virusu (NDV) prevalansını belirlemek amacıyla evcil ördeklerden 10 NDV izole edildi. MDT, ICPI ve F proteininin ayrılma yeri dikkate alınarak sekiz izolatın patojenik olduğu belirlendi. 10 izolatın F genlerinin sekansları yapıldı, tüm genler ve onlara ait amino asitler 32 referans türleri ile karşılaştırıldı. 5 izolat genotip VII'de toplanırken (VIId), 3 izolat genotip IX ve 2 izolat genotip olarak sınıflandırıldı. Subgenotip VIID izolatları 112R-R-Q-K-R-F117 motifine sahipken genotip IX izolatları 112R-R-Q-R-R-F117 motifi gösterdi. 8 NDV izolatı yüksek ICPI sergilerken bunlar NDV'nin velojenik tipi olarak sınıflandırıldı ve bu örnekler 2006 ile 2010 yılları arasında toplandı. 112G-K-Q-G-R-L117 motifine sahip olan genotip I izolatları 2005 yılında toplandı. Bunlar düşük ICPI gösterdi ve NDV'nin lentojenik tipi olarak sınıflandırıldı. Subgenotip VII'nin NDV'leri şimdi dominant olup Guangdong Bölgesindeki ördek çiftliklerinde son zamanlardaki çoğu ND salgınlarında belirlenmiştir. Bu bulgular Çin'de ördeklerdeki NDV'ün genetik ve moleküler gelişimi hakkında bilgi vermekte ve hastalığın kontrol stratejileri hakkında NDV takibinin önemini ortaya koymaktadır.

Anahtar sözcükler: *Newcastle Hastalığı Virusu, Ördek, Genotip, Patojenite*

INTRODUCTION

Newcastle disease (ND), which is caused by ND virus (NDV), is one of the most serious diseases affecting the commercial poultry industry around the world and has caused significant economic loss. NDV belongs to the

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Avulavirus genus within the family Paramyxoviridae and is designated avian paramyxovirus type 1(APMV-1), one of 12 identified APMVs serotypes [1,2]. The virus is enveloped with negative-stranded RNA genome of approximately 15 kb that encoding six structural proteins, including the nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), an attachment protein, the hemagglutinin-neuraminidase, and a large polymerase protein (L). Additionally, two non-structural proteins, V and W are derived from P gene by a process called RNA editing respectively [3-6].

NDV strains can be classified into two major classes (class I and II) on base of genetic and antigenic analyses. The class I (1-9 genotypes) viruses are distributed world-wide in wild birds and chickens, and the class II (IX genotypes) viruses include most virulent viruses, some avirulent viruses and vaccine viruses [7,8]. The NDVs currently circulating worldwide exhibit multiple lineages and highly diversive geneticity [6,9].

ND has been classified as an Office International des Epizootics (OIE) "List A" disease [1]. Although NDV isolates are of a single serotype, and numerous live and inactivated vaccines have been developed to control the disease, naturally occurring pathogenicities from avirulent (lentogenic) to mildly virulent (mesogenic) and highly virulent (velogenic) are still in a wide range [9]. Highly virulent strains of NDV are not only found in poultry in China, but also circulate in a large number of countries worldwide. In addition, more avian species are naturally or experimentally susceptible to NDV, and some NDV strains are now becoming particularly virulent in avian species, e.g. duck, which had generally been resistant to clinical disease with virulent NDV infections [10].

The incidences of ND outbreaks in domestic ducks have gradually increased in different regions since the 1990s. It becomes more difficult to prevent and control the disease when NDV is circulating in different avian species [11-13]. In China, many isolates have been isolated from domestic ducks in recent years. Most of isolates are lentogenic and have not lead to great losses to duck farms. Occasionally when a velogenic strain is isolated, it is particularly urgent to evaluate potential risk to domestic ducks [14,15]. While there have insufficient knowledge of pathogenic characterizations and molecular analyses of NDV isolated from domestic ducks in China, and we also have limited knowledge of the relationship between the Chinese representative NDV isolated from chickens and the strains isolated recently from domestic ducks in China. Therefore, continuous surveillance of domestic ducks may help better understand the NDVs circulating in China.

In this study, to clarify the genotypic and pathogenic characterization, 10 NDVs were isolated from outbreaks in domestic ducks in China from 2005-2010, the full-length F gene of the isolates was amplified by RT-PCR and sequenced. F protein cleavage site sequences were analyzed. Molecular genetic analysis was performed to compare the evolution epidemiology and pathotype of these genes with those of reference strains published in GenBank.

MATERIAL and METHODS

Virus Isolation and Biological Characterization

Between the years of 2005 and 2010, ND-suspected field samples from different duck farms were collected. The viruses were isolated and purified by the limiting dilution method in 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs (ECE) using standard procedures. The virus identity was confirmed by haemagglutination-inhibition (HI) assay with polyclonal chicken antiserum to NDV.

Pathogenicity Test

Intra-cerebral pathogenicity index (ICPI) tests in 1 day-old chicks were done as previously described. Initial pathotyping of the isolates involved virus inoculation of 9-day-old embryonated SPF chicken eggs to determined the mean death time (MDT) for the embryos. All tests were performed using the standard procedures devised to distinguish these viruses [1].

RNA Isolation and RT-PCR

Viral RNA was extracted directly from the allantoic fluid of the NDV-inoculated embryos with using HP total RNA purification kit (Omiga, US). The sense primer was F-A (5'-GCCATTGCYAAATACAATCC-3'), and the sequence S1-R (5'- GGCTCCTCTKACCG -TTCTAC-3') was used as antisense primer. The expected size of a PCR-amplified fragment is 1993 bp in length. Briefly, the PCR was performed in 50 uL reaction mixture. The PCR reactions were subjected to 30 cycles consisting of denaturation for 1 min at 94°C, annealing for 30 s at 53°C, and extension for 90 s at 72°C followed by a final extension cycle at 72°C for 8 min.

Cloning and Sequencing of PCR Products

RT-PCR products were analyzed on 1% agarose gels and sequenced after cloning into the pMD18-T (Takara, Dalian, China). F nucleotide in each NDV isolate was sequenced in the forward and reverse directions at least five times and the consensus sequence was determined.

Analysis of Nucleotide and Deduced Amino Acid Sequences

32 NDVs were chosed as reference strains which including current vaccine strains such as LaSota and Clone 30, classical viruses and typical prevailing strains isolated in China and other counties. The reference strains were classified as genotyes I-IX, and at least 2 strains were chosed every genotype. The detailed information and Genbank

accession numbers of NDV reference strains were shown in *Table 1*. Nucleotide and deduced amino acid sequences of full-length F genes of 10 NDV isolates and reference strains were aligned using MEGALIGN program in DNAStar software in this study. In all NDV stains (including 10 isolates and reference strains), the deduced amino acid residues (1-125) of F genes were aligned and compared, and the variation of amino acid residues (including F cleavage sites) were analyzed.

Phylogenetic Analysis

Comparative analysis of part of F aa residues (1-125) in all NDVs obtained in this study (including 10 isolates and 32 references strains). A phylogenetic tree was constructed using MEGA4.1 software (Molecular Evolutionary Genetics Analysis, version 4.0) by Neighbor-Joining method. The evolutionary distances were computed by Pair wise Distance method using the Maximum Composite Likelihood Model.

RESULTS

Virus Isolation and Identification

All 10 NDV strains were isolated from ND-suspected field samples from ducks of different breed and age in

Table 1.*The NDV preference strains and their accession numbers used for phylogenetic analysis*
Tablo 1. *NDV referans tipleri ve patojenite analizi için erişim numaraları*

NDV Isolates	Genotype	Host	Accession Number	Cleavage Site	Country
PHY-MLV42	I	Chicken	DQ097394	GKQGRL	Hungary
Ulster-67	I	Chicken	AY562991	GKQGRL	Ireland
V4	I	Chicken	AF217084	GKQGRL	Australia
B1	II	Chicken	AF375823	GRQGRL	American
Clone30	II	Chicken	Y18898	GRQGRL	American
LaSota	II	Chicken	AY845400	GRQGRL	China
AUS-Victoria-32	III	\	M21881	RRQKRF	Australia
Mukteswar	III	Chicken	EF201805	RRQRRF	N
Herts-33	IV	Chicken	AY741404	RRQRRF	England
Italien	IV	Chicken	EU293914	RRQRRF	Italien
Largo/71	V	Pet birds	AY562990	RRQKRF	American
44083/93	V	Anhinga	AY562986	RRQKRF	American
211472/02	V	Gamefowl	AY562987	RRQKRF	American
Argentian-97	VI	Pigeon	AY734536	RRQKRF	Argentina
Belgium248VB	VI	Pigeon	EF026584	RRQKRF	Belgium
Pigeon-1	VI	Pigeon	AJ880277	RRQKRF	Hungary
Dove Italy	VI	Dove	AY562989	RRQKRF	Italy
FP1-02	VIId	Duck	FJ872531	RRQKRF	China
JSD0812	VIId	Duck	GQ849007	RRQKRF	China
GM	VIId	Chicken	DQ486859	RRQKRF	China
SD09	VIId	Duck	HQ317395	RRQKRF	China
SDWF02	VIId	Duck	HM188399	RRQKRF	China
SF02	VIId	Goose	NC_005036	RRQKRF	China
ZJ	VIId	Goose	AF431744	RRQKRF	China
TW2000	VIIe	Chicken	AF358786	RRQKRF	China/Taiwan
UAE-AE232-96	VIIb	Chicken	AF109884	KRQRRF	Britain
D-83-95	VIIa	\	AF001118	RRQKRF	Hungary
TW-84C	VIIc	\	AF083965	RRQKRF	China/Taiwan
AF2240	VIII	\	AF048763	RRQKRF	Malaysia
F48E9	IX	Chicken	AY508514(F)	RRQRRF	China
JS-1-97	IX	Chicken	FJ436305	RRQRRF	China
FJ-1-85	IX	Chicken	FJ436304	RRQRRF	China

Note: *GKQGRL, Gly-Lys-Gln-Gly-Arg-Leu; GRQGRL, Gly- Arg -Gln-Gly-Arg-Leu, RRQKRF; Arg - Arg -Gln- Lys -Arg-Leu; RRQRRF, Arg - Arg -Gln- Arg -Arg-Leu; KRQRRF, Lys - Arg -Gln- Arg -Arg-Leu*

2005-2010 in Guangdong province *(Table 2)*. The isolates were characterized by HI assays, and conformed by genomic sequencing and the nucleotides BLASTn analysis.

Pathogenicity Analysis

Table 2 presents the initial biological characterizations of 10 NDV isolates, including ICPI and MDT. 8 Guangdong field isolates of NDV with MDT of 45.8-58.8 h and with ICPI of 1.7-1.96 were classified as velogenic NDVs, 2 isolates were classified as lentogenic NDVs with MDT beyond 168 h and with ICPI of 0.15 and 0.21. The results were consistent with cleavage site motifs. *Table 2* also lists the initial biological characterizations of the 10 NDV isolates.

Phylogenetic Analysis

A phylogenetic tree was constructed based on the nt sequences of the full-length F gene in all the 10 field isolates of NDV and the corresponding region of the other 32 NDV strains retrieved from GenBank. The field isolates and reference strains were classified as genotyes I-IX. 5 field isolates were classified into genotype VII and all these NDVs were further subclassified into subgenotype VIId (Duck/CH/GD/SD-06, Duck/CH/GD/SD-06 II, Duck/CH/GD/SD-09, Duck/CH/GD/JY-08, Duck/CH/GD/SS-10). 3 isolates were classified into genotype IX (Duck/CH/GD/FS-06, Duck/CH/GD/YF-09, Duck/CH/GD/NH-10) and 2 isolates (Duck/CH/GD/SH, Duck/CH/GD/SZ-05) were classified into genotype I *(Fig. 1)*.

The Divergence of nt and aa Sequences

The nt and deduced aa sequences of the 10 isolates were compared. The nt and aa sequences data of 32 NDV reference strains obtained from the GenBank database *(Table 1)* were used for comparison. The results of sequence analysis showed that the total length of F gene is 1617 bp, the homology of nt and aa sequences between 10 isolates and 32 reference strains were 83.5%-99.8% and 86.5%-99.5%, respectively. Further analysis was also carried out. 5 isolates (Duck/CH/GD/SD-06, Duck/CH/GD/SD-06, Duck/CH/GD/JY-08, Duck/CH/GD/SD-09, Duck/CH/GD/SS-10) showed great nt and aa identities (96.9-98.8% and 96.8-98.9% respectively) with the velogenic NDV GM strain (Accession number DQ486859, genotype VII). 3 isolates (Duck/CH/GD/FS-06, Duck/CH/GD/YF-09, Duck/CH/GD/NH-10) were highly nt and aa sequences similar (99.5-99.7% and 99.1-99.3%, respectively) to NDV F48E9 strain (Accession number AY508514, genotype IX). 10 isolates had homology of 83.5-89.2% and 86.6-92.6% respectively at nt and aa level with strain LaSota (Accession number AY845400, genotype II), the common vaccine strain used in China. Of all the 10 isolates, 2 strains (Duck/CH/GD/SH-05, Duck/CH/GD/SZ-05) shared greater nt and aa identities (88.9-89.2% and 92.4-92.6%, respectively) with LaSota than other 8 NDV strains *(Fig. 2)*.

Amino acid Variation of the F Proteins of Recent NDV Isolates

Proteolytic cleavage site motifs (residues 112-117) of F protein in the 10 isolates were analyzed. The F cleavage sites in 5 NDV strains isolated in 2008, 2009 and 2010 (Duck/CH/GD/SD-06, Duck/CH/GD/SD-06 II, Duck/CH/GD/SD-09, Duck/CH/GD/JY-08, Duck/CH/GD/SS-10) possessed aa sequence ^{112}R-R-Q-K-R-F^{117}. The motif is commonly found in strains that are highly virulent in chickens, especially in genotype VII viruses. The 3 NDVs (Duck/CH/GD/FS-06, Duck/CH/GD/YF-09, Duck/CH/GD/NH-10) isolated in 2006, 2009 and 2010 exhibited the sequence motif ^{112} R-R-Q-R-R-F ^{117}, which is another common motif in the other virulent NDVs including strain F48E9 (a genotype IX virus). Additionally, 2 NDV strains (Duck/CH/GD/SH-05, Duck/CH/GD/SZ-05) isolated in 2005 were shown to have a lentogenic motif (^{112}G-K-Q-G-R-L^{117}) composed of 2 basic amino acids at the F cleavage site *(Table 2)*.

Table 3 shows the aa residues 1-125 of F protein of different genotypes. An important aa residue substitution at the highly conserved region of Q114K was noted in the five isolates (Duck/CH/GD/SD-06 I, Duck/CH/GD/SD-06 II, Duck/CH/GD/SD-09, Duck/CH/GD/JY-08, Duck/CH/

Table 2. *Details of the 10 NDV isolates investigated in this study*
Tablo 2. *Bu çalışmada araştırılan 10 NDV izolatın detayları*

Strain	City	Year of Isolation	MDT/h	ICPI	Cleavage Site	Genotype
Duck/CH/GD/SH-05	Sihui	2005	>168h	0.15	GRQGRL	I
Duck/CH/GD/SZ-05	Shenzhen	2005	>168h	0.21	GKQGRL	I
Duck/CH/GD/SD-06 I	Shunde	2006	51.75h	1.94	RRQKRF	VII
Duck/CH/GD/SD-06 II	Shunde	2006	54.86h	1.78	RRQKRF	VII
Duck/CH/GD/FS-06	Foshan	2006	48h	1.95	RRQRRF	IX
Duck/CH/GD/JY-08	Jieyang	2008	45.8	1.96	RRQKRF	VII
Duck/CH/GD/YF-09	Yunfu	2009	57.6	1.93	RRQRRF	IX
Duck/CH/GD/SD-09	Shunde	2009	56.4	1.95	RRQKRF	VII
Duck/CH/GD/SS-10	Sanshui	2010	51.6	1.89	RRQKRF	IX
Duck/CH/GD/NH-10	Nanhai	2010	58.8	1.7	RRQRRF	VII

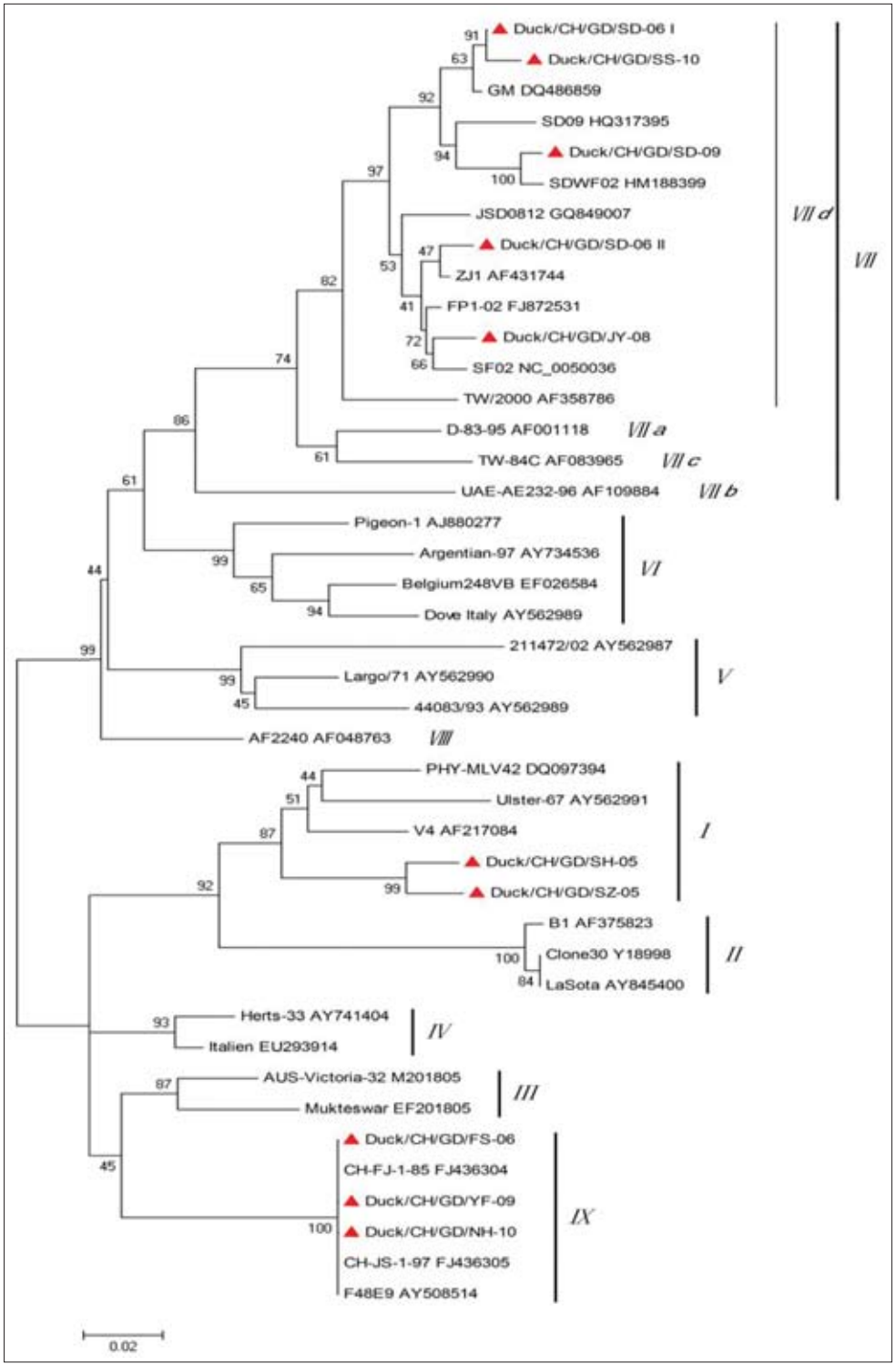

Fig 1. Phylogenetic tree of the nt sequences of NDV strains on a variable portion (nt1-374) of the F gene. (▲) The recent Chinese NDV strained were isolated in domestic ducks in this study in 2005-2010. Accession number and other background information of the reference strains are in *Table 1* and *Table 2*

Şekil 1. F geninin değişken kısmındaki (nt1-374) NDV'nin nt sekansının filogenetik ağacı. (▲) Bu çalışma kapsamında güncel Çin NDV türleri 2005-2010 yılları arasında evcil ördeklerden izole edildi. Referans türlerinin erişim numarası ve diğer bilgileri *Tablo 1* ve *Tablo 2*'de yer almaktadır

GD/SS-10) of subgenotype VIId of NDVs. The aa residue changed at position 3 (S to F) in 3 isolates and at position 24 (S to G) in 2 isolates in the 5 isolates mentioned above. It is interesting to note that one aa residue is unique to 3 isolates of genotype IX at aa position 3 (S to P). One aa residue is changed at amino acid position 24 (S to C) to 2 isolates of genotype. These mutations may alter the pathogenicity and antigenicity of recent Guangdong NDVs isolated in domestic ducks *(Table 3)*.

DISCUSSION

The first ND outbreak was recorded in poultry in 1926. Since then, four major epizootics occurred in the world causing considerable economic losses to poultry industry [16,17]. In China, outbreak of Newcastle disease (ND) was first reported in poultry in 1928, the standard strain F48E9 was isolated in 1948 for the first time. There often, ND became endemic in chicken flocks and large-

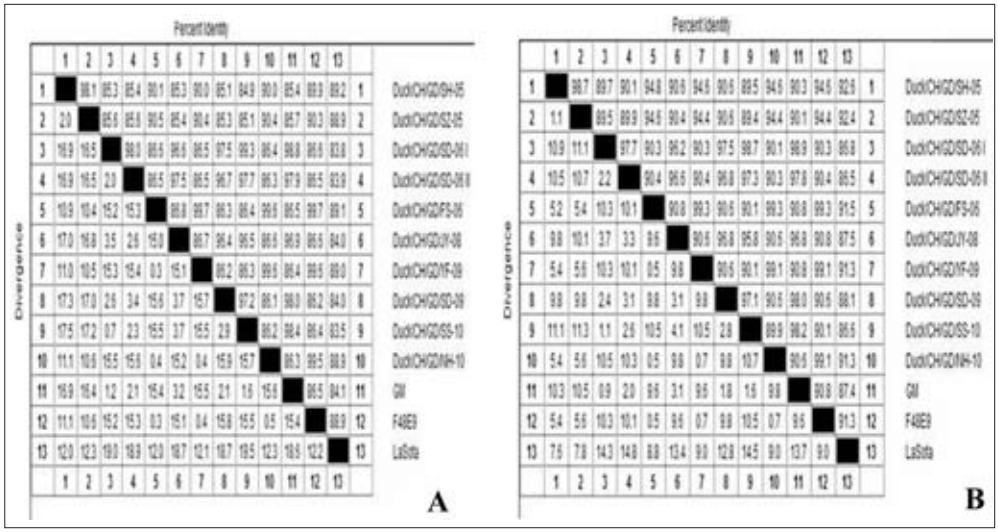

Fig 2. A- Analysis of nucleotide sequences (nt 1-375) between the 10 isolates and 3 reference strain (GM, LaSota and F48E9), **B-** Analysis of amino acid sequences (aa 1-125) between the 10 isolates and 3 reference strains (GM, LaSota and F48E9)

Şekil 2. A- 10 izolat ile 3 referans türü (GM, LaSota and F48E9), arasında nükleotid sekanslarının analizleri (nt 1-375), **B-** 10 izolat ile 3 referans türü (GM, LaSota and F48E9), arasında amino asit sekanslarının (aa 1-125) analizi

scale ND outbreaks were controlled with the wide spread use of ND vaccines. However, ND is still recognized as a major disease of poultry, for the disease is enzootic in some areas and non-typical ND infections occur frequently [18-20].

In the past twenty years, more and more hosts were found susceptible to NDVs. At present, over 200 avian species are naturally or experimentally susceptible to NDVs, e.g. duck, geese, green-winged teal and wood duck. Wild aquatic bird species are considered the natural reservoir of NDVs, some reports have revealed that wild aquatic birds may play an important role in the evolution of NDV [8,21]. The birds mostly harbor lentogenic NDVs, the strains apparently have the potential to become velogenic after transmission and circulation, and the virulence of these viruses has increasing trends. In ducks, many NDVs have been isolated in different country in recent years, studies on genetic diversity among the isolates revealed that most of the NDVs belong to class I with low virulence, occasionally a velogenic strain is isolated [15,22-24]. Due to the lack of surveillance, little is known about distribution, pathogenic and molecular characterizations of NDV in domestic ducks.

This study presents the characterization of NDVs isolated from domestic ducks in Guangdong province of China. It highlights the importance of domestic ducks in the epizootiology of ND. It has been reported that the virulence of NDV is associated with ICPI and the cleavage site in the F protein. ICPI of 0.7 or greater in 1 day-old chicken or presence of three basic amino acids (R or K) at the F protein cleavage site between aa residues 113 and 116 indicate the virulent form of NDV. In this study, we determined the virulence of 10 isolates on base of the criteria. Our results showed that the F cleavage sites in 5 NDV isolates possessed a virulent amino acid sequence 112R-R-Q-K-R-F117. 3 isolates exhibited another virulent sequence motif 112 R-R-Q-R-R-F117, the 8 field isolates in current study exhibited MDT of 45.8-58.8 h in embryonated chicken eggs and ICPI of 1.7-1.96 were classified as velogenic NDVs. The rest of 2 NDV isolates were shown to have a lentogenic motif (112G-K-Q-G-R-L117) composed of 2 basic amino acids at the F cleavage site, they were classified as lentogenic NDVs also with MDT beyond 168 h and with ICPI of 0.15 and 0.21. The finding indicate that the domestic ducks can harbour virulent strains of NDV and that it consequently may constitute a serious threat to the commercial duck farms. Further analysis showed that some aa residues in F protein of different genotypes changed. These mutations may alter the pathogenicity and antigenicity of recent Guangdong NDVs isolated in domestic ducks [25,26].

Sequence comparison and phylogenetic analysis of the 8 NDV isolates were used to predict the genotypes and to determine the origin of NDV outbreaks. Our results showed that 5 isolates belong to genotype VIId, and indicated great nt and aa identities (96.9-98.8% and 96.8-98.9%, respectively) with the velogenic strain GM. 3 isolates are genotype IX, shared high nt and aa similarities (99.1-99.7% and 99.1-99.3%, respectively) to F48E9. Two strains belonged to genotype I had a higher homology than 8 isolates mentioned above at nt and aa level with strain LaSota (genotype II). These results are not in agreement with some studies reporting the detection of lentogenic NDVs in wild birds and domestic ducks [7,8,22,24,27,28]. But the results are consistent with some other reports with the detection of velogenic NDVs in wild birds and domestic ducks, for example, Zhang et al.[15], reported subgenotype VIId of NDV as a predominant genotype spread in waterfowl in China and Yang et al.[29] discovered the presence of VII genotype NDVs in wild in Serbia.

In China, the most commonly used live vaccine LaSota and Clone-30 belong to genotype II, while in the past decade, the predominant NDV strains were genotype VII viruses in China. The prevailing field NDV strains have significant differences from the current vaccine strains in their biology, serology and genetics, which might

Table 3. *Amino acid variation of F protein (aa 1-125) of different genotypes*
Tablo 3. *Değişik genotiplerin F proteininin (aa 1-125) amino asit varyasyonu*

Majority Sequences	**Positions**																								
	3	**4**	**5**	**8**	**9**	**11**	**16**	**17**	**19**	**20**	**22**	**24**	**26**	**30**	**52**	**71**	**101**	**104**	**106**	**107**	**112**	**115**	**117**	**121**	**124**
	S	**K**	**P**	**R**	**I**	**A**	**I**	**T**	**I**	**M**	**I**	**S**	**I**	**S**	**I**	**K**	**R**	**G**	**V**	**T**	**R**	**K**	**F**	**I**	**S**
Ulster-67	-	R	S	-	-	V	T	V	V	A	E	-	V	-	-	-	-	E	-	-	G	G	L	-	G
PHY-MLV42	P	R	S	-	T	-	T	V	L	V	A	-	V	-	-	-	-	E	-	-	G	G	L	-	G
V4	-	R	S	-	-	V	T	V	V		A	-	V	-	-	-	-	E	-	-	G	G	L	-	G
Duck/CH/GD/SH-05	-	R	S	-	-	V	T	V	V	A	A	C	V	-	-	-	-	E	-	-	G	G	L	-	G
Duck/CH/GD/SZ-05	-	R	S	-	-	V	T	V	V	A	A	C	V	-	-	-	-	E	-	-	G	G	L	-	G
Clone30	-	-	-	K	N	-	T	I	V	A	V	-	-	N	-	-	-	E	-	-	G	G	L	-	G
B1	-	R	-	K	N	-	T	I	V	A	V	-	-	N	-	-	-	E	-	-	G	G	L	-	G
LaSota	-	R	-	K	N	-	T	I	V	A	V	-	-	N	-	-	-	E	-	-	G	G	L	-	G
AUS-Victoria-32	P	R	S	-	-	I	T	I	-	A	A	-	V	-	-	-	-	E	-	-	-	-	-	-	-
Mukteswar	P	R	S	-	-	V	T	I	-	T	A	-	V	-	-	-	-	E	-	-	-	R	-	-	-
Italien	-	R	S	-	-	V	-	I	-	A	T	-	-	-	-	-	-	E	-	-	-	R	-	-	-
Herts-33	-	R	S	-	-	V	-	I	-	V	T	-	-	-	-	-	-	E	-	-	-	R	-	-	-
211472/02	-	-	-	W	-	V	-	-	T	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-
44083/93	-	R	-	-	L	V	T	-	-	T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-
Largo/71	-	-	-	-	L	V	-	-	-	T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-
Dove Italy	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-
Belgium248VB	-	-	-	-	-	-	-	-	-	T	V	-	-	-	-	-	-	-	-	S	-	-	-	-	-
Argentian-97	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-
Pigeon-1	-	-	-	-	-	V	-	-	T	T	-	-	-	-	-	-	-	-	-	S	G	-	-	-	-
D-83-95	-	-	-	-	T	V	-	-	-	-	-	-	-	-	-	-	K	-	-	S	-	-	-	V	-
UAE-AE232-96	-	-	-	-	-	-	S	N	V	-	-	-	-	-	-	-	-	E	-	S	K	R	-	V	-
TW-84C	-	-	S	G	S	V	-	-	-	-	-	-	-	-	-	-	K	-	-	S	-	-	-	V	-
JSD0812	-	-	-	-	-	-	-	-	T	-	-	G	-	-	V	R	K	-	-	S	-	-	-	V	-
ZJ	-	-	L	-	-	-	-	-	-	-	-	G	-	-	V	R	K	-	-	S	-	-	-	V	-
TW2000	-	R	S	-	-	-	-	-	-	-	-	-	-	-	V	-	K	-	-	S	-	-	-	V	-
SF02	-	R	-	-	-	-	-	-	-	-	-	G	-	-	V	R	K	-	-	S	-	-	-	V	-
SDWF02	-	-	-	W	-	-	-	-	-	-	-	-	-	-	V	R	K	-	-	S	-	-	-	V	G
SD09	-	-	-	-	-	-	-	-	A	-	-	-	-	-	V	R	K	-	-	S	-	-	-	V	-
GM	F	-	-	-	-	-	-	-	-	-	-	-	-	-	V	R	K	-	-	S	-	-	-	V	-
FP1-02	-	-	-	-	-	-	-	-	-	-	-	G	-	-	V	R	K	-	-	S	-	-	-	V	-
Duck/CH/GD/SD-06 I	F	-	-	-	-	-	-	-	-	-	-	-	-	-	V	R	K	-	-	S	-	-	-	V	-
Duck/CH/GD/SD-06 II	F	-	L	-	-	-	-	-	-	-	-	G	-	-	V	R	K	-	-	S	-	-	-	V	-
Duck/CH/GD/JY-08	-	-	-	-	-	-	V	-	-	-	-	G	-	-	V	R	K	-	-	S	-	-	-	V	-
Duck/CH/GD/SD-09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	R	K	-	-	S	-	-	-	V	G
Duck/CH/GD/SS-10	F	-	-	-	-	-	-	-	-	-	-	-	-	-	V	R	K	-	-	S	-	-	-	V	-
AF2240	-	-	S	-	-	T	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-
F48E9	-	-	S	N	V	-	T	V	-	A	A	-	V	N	-	-	-	E	A	-	-	R	-	-	-
JS-1-97	-	-	S	N	V	-	T	V	-	A	A	-	V	N	-	-	-	E	A	-	-	R	-	-	-
FJ-1-85	-	-	S	N	V	-	T	V	-	A	A	-	V	N	-	-	-	E	A	-	-	R	-	-	-
Duck/CH/GD/FS-06	P	-	S	N	V	-	T	V	-	A	A	-	V	N	-	-	-	E	A	-	-	R	-	-	-
Duck/CH/GD/YF-09	P	-	S	N	V	-	T	V	-	A	A	-	V	N	-	-	-	E	A	-	-	R	-	-	-
Duck/CH/GD/NH-10	P	-	S	N	V	-	T	V	-	A	A	-	V	N	-	-	-	E	A	-	-	R	-	-	-

Only sequences that differ from the majority sequences are shown. Strains in bold are isolated and characterized in the present study. The representative strains are from published data and their source reference is presented in Table 1

be considered as major reasons for the ND outbreaks, and brought enormous pressure for NDV control and prevention.

In summary, we have demonstrated that more than one genotype of NDVs are circulating in the domestic ducks in Guangdong province of China, and NDV isolates of subgenotype VIId are predominant strains. It is of particular importance to characterize epidemic strains in domestic ducks and identify new candidates for vaccine upgrades which are efficacious and provide adequate cross protection.

REFERENCES

1. Alexander DJ: Newcastle disease and other avian paramyxoviruses. *Rev Sci Tech - Office International des Epizooties,* 19, 443-462, 2000.

2. Werner O, Oberdorfer A, Kollner B: Characterization of avian paramyxovirus type I strains isolated in Germany during 1992 to 1996. *Avian Pathol,* 28, 79-88, 1999.

3. Czegledi A, Ujvari D, Somogyi E, Wehmann E, Werner O, Lomniczi B: Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Res,* 120, 36-48, 2006.

4. de Leeuw O, Peeters B: Complete nucleotide sequence of Newcastle disease virus: Evidence for the existence of a new genus within the subfamily paramyxovirinae. *J Gen Virol,* 80, 131-136, 1999.

5. Kolakofsky D, Roux L, Garcin D, Ruigrok RW: Paramyxovirus mRNA editing, the "rule of six" and error catastrophe: A hypothesis. *J Gen Virol,* 86, 1869-1877, 2005.

6. Steward M, Vipond IB, Millar NS, Emmerson PT: RNA editing in Newcastle disease virus. *J Gen Virol,* 74, 2539-2547, 1993.

7. Kim LM, King DJ, Curry PE, Suarez DL, Swayne DE: 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, 12641-12653, 2007.

8. Liu XF, Wan HQ, Ni XX, Wu YT, Liu WB: Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China during 1985-2001. *Arch Virol,* 148, 1387-1403, 2003.

9. Patti JM, Eduardo LD, Claudio LA: Newcastle disease: Evolution of genotypes and the related diagnostic challenges. *Infect Genet Evol,* 10, 26-35, 2010.

10. Marin MC, Villegas P, Bennett JD, Seal BS: Virus characterization and sequence of the fusion protein gene cleavage site of recent Newcastle disease virus field isolates from the southeastern United States and Puerto Rico. *Avian Dis,* 40, 382-390, 1996.

11. Naresh J, Yogesh C, Ashok K C, Marthade A, Patrick TR, Sagar MG: Phylogenetic analysis of Newcastle disease viruses isolated from waterfowl in the Upper Midwest Region of the United States. *J Virol,* 81, 12641-12653, 2007.

12. Liu XW, Wang XQ, Wu S, Hu SL, Peng Y, Xue F, Liu XF: Surveillance for avirulent Newcastle disease viruses in domestic ducks (*Anas platyrhynchos* and *Cairina moschata*) at live bird markets in Eastern China and characterization of the viruses isolated. *Avian Pathol*, 38, 377-391, 2009.

13. Lee EK, Jeon WJ, Kwon JH, Yang CB, Choi KS: Molecular epidemiological investigation of Newcastle disease virus from domestic ducks in Korea. *Vet Microbiol,* 134, 241-248, 2009.

14. Liu H, Wang Z, Wang Y, Sun C, Zheng D, Wu Y: Characterization of Newcastle disease virus isolated from waterfowl in China. *Avian Dis,* 52, 150-155, 2008.

15. Zhang SP, Wang XT, Zhao CG, Liu DH, Hu YX, Zhao JX, Zhang GZ: Phylogenetic and pathotypical analysis of two virulent Newcastle Disease Viruses isolated from domestic ducks in China. *PLos One*, 6, e25000, 2011.

16. Abolnik C, Horner RF, Bisschop SP, Parker ME, Romito M, Viljoen GJ: Aphylogenetic study of South African Newcastle disease virus strains isolated between 1990 and 2002 suggests epidemiological origins in the Far East. *Arch Virol,* 149, 603-619, 2004.

17. Czegledi A, Herczeg J, Hadjiev G, Doumanova L, Wehmann E, Lomniczi B: The occurrence of five major Newcastle disease virus genotypes (II, IV, V, VI an d VIIb) in Bulgaria between 1959 and 1996. *Epidemiol Infect,* 129, 679-688, 2002.

18. Alexander DJ: Gordon Memorial Lecture: Newcastle disease. *Br Poult Sci,* 42, 5-22, 2001.

19. Liang R, Cao DJ, Li JQ, Chen J, Guo X, Zhuang FF, Duan MX: Newcastle disease outbreaks in western China were caused by the genotypes VIIa and VIII. *Vet Microbiol,* 87 (3): 193-203, 2002.

20. Zanetti F, Berinstein A, Carrillo E: Effect of host selective pressure on Newcastle disease virus virulence. *Microbial Pathog,* 44, 135-140, 2008.

21. King DJ, Seal BS: Biological and molecular characterization of Newcastle disease virus isolates from surveillance of live bird markets in the northeastern United States. *Avian Dis,* 41, 683-689, 1997.

22. Hua BX, Huang YY, He YF, Xu CT, Lu XS, Zhang W, Meng B, YanSG, Zhang XM: Avian influenza virus and Newcastle disease virus (NDV) surveillance in commercial breeding farm in China and the characterization of Class I NDV isolates. *Vet Microbiol,* 144, 82-86, 2010.

23. Kouji S, Genki S, Orie T, Yuko W, Alam J, Masayuki N, Kazuaki T: Characterization of Newcastle disease virus isolated from Northern Pintail (Anasacuta) in Japan. *J Vet Med Sci,* 69, 1307-1311, 2007.

24. Jorgensen PH, Handberg KJ, Ahrens P, Therkildsen OR, Manvell RJ, Alexander DJ: Strains of avian paramyxovirus type 1 of low pathogenicity for chickens isolated from poultry and wild birds in Denmark. *Vet Rec,* 154, 497-500, 2004.

25. Collins MS, Bashiruddin JB, Alexander DJ: Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Arch Virol,* 128, 363-370, 1993.

26. Huang Y, Wan HQ, Liu HQ, Wu YT, Liu XF: Genomic sequence of an isolate of Newcastle disease virus isolated from an outbreak in geese: A novel six nucleotide insertion in the non-coding region of the nucleoprotein gene. *Arch Virol,* 149, 1445-1457, 2004.

27. Lomniczi B, Wehmann E, Herczeg J, Ballagi-Pordány A, Kaleta EF, Werner O, Meulemans G, Jorgensen PH, Manté AP, Gielkens AL, Capua I, Damoser J: Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). *Arch Virol,* 143, 49-64, 1998.

28. Muhammad M, Muhammad A, Muhammad TK, Siamak Z, Mikael B: Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. *J Virol,* 9, 46, 2012.

29. Yang CY, Shieh HK, Lin YL, Chang PC: Newcastle disease virus isolated from recent outbreak s in Taiwan phylogenetically related to viruses (genotype VII) from recent outbreaks in western Europe. *Avian Dis,* 43, 125-130, 1999.