Veterinary Microbiology 180 (2015) 237–244

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Genetic, pathogenic and antigenic diversity of Newcastle disease viruses in Shandong Province, China

Yanyan Huang[a,b], Shaohua Yang[a,b], Beixia Hu[a,b], Chuantian Xu[a,b], Dandan Gao[c], Manling Zhu[a,b], Qinghua Huang[a,b], Lin Zhang[a,b], Jiaqiang Wu[a,b,*], Xiumei Zhang[a,b], Marzhar I. Khan[d]

[a] Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China
[b] Shandong Key Lab of Animal Disease Control and Breeding, Jinan, Shandong, China
[c] College of Animal Science, Shandong Agricultural University, Tai'an, Shandong, China
[d] Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, USA

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 29 July 2015
Accepted 2 September 2015

Keywords:
Virus diversity
Phylogenetic analysis
Virus pathogenicity
Virus antigenicity

ABSTRACT

Thirty-one Newcastle disease viruses (NDVs) isolated from domestic and wild birds in Shandong Province, China (2006–2014) were characterized genetically, pathogenically and antigenically. Phylogenetic analysis classified the viruses into a single genotype under Class I, and four genotypes under Class II. The nineteen viruses classified in genotype VII of Class II were velogenic, while the other viruses were either mesogenic or lentogenic to chickens. Some NDV isolates (17/23) showed no neutralizing reactivity with a monoclonal antibody developed against the HN protein of the LaSota strain, reflecting the mutation at the related antigenic epitope. When challenged with two genotype VII NDV isolates, LaSota-vaccinated SPF chickens were prevented from disease development, but virus shedding was detected for at least 5 days post challenge. Circulation of the same NDV isolate for up to 13 years suggested the role of an environmental reservoir in NDV perpetuation, and reinforced the importance of strict biosecurity measures in addition to vaccination for disease control.

1. Introduction

Newcastle disease virus (NDV) is a group of negative-sense RNA viruses belonging to the family *Paramyxoviridae*, and was designated as type-1 avian Paramyxovirus (Mayo, 2002a,b). NDV is the causative agent of Newcastle disease (ND), which can infect domestic and wild birds, and is one of the most hazardous diseases infecting poultry, especially chickens. The genome of NDV is about 15.2 kb, encoding genes for the six major structural proteins (3′-NP-P-M-F-HN-L-5′) and two nonstructural proteins (V and W) generated by P-gene mRNA editing (Samson et al., 1991; Steward et al., 1993). Hemagglutinin–neuraminidase protein (HN) and fusion protein (F) are two NDV surface proteins functioning in NDV infection, pathogenicity and antigenicity (De Leeuw et al., 2005; Kim et al., 2011). F protein is also widely used for NDV phylogenetic classification (Aldous et al., 2003; Cattoli et al., 2010; Kim et al., 2007; Liu et al., 2003). The unified genotyping system separates NDVs into 2 Classes, which includes 2 genotypes in Class I and 18 genotypes in Class II (Briand et al., 2014; Courtney et al., 2012; Diel et al., 2012; Snoeck et al., 2013b).

Based on their pathogenicity in chickens, NDV may be classified as highly virulent (velogenic), moderately virulent (mesogenic) and low virulent (lentogenic), which, respectively, could cause fatal, clinical or subclinical infections in chickens (Kang et al., 2014; Liu et al., 2003; Snoeck et al., 2013a; Wu et al., 2011). The amino acid motif at the F protein cleavage site is the major molecular determinant of NDV virulence. Polybasic amino acids at the F cleavage site allow protein cleavage by ubiquitous fusion-like proteases, therefore facilitating systemic NDV infection (Samal et al., 2012, 2011). Biological assays such as the mean death time (MDT) of chicken embryos after NDV infection, and the intracerebral pathogenicity index (ICPI) in day-old chickens are indeces of NDV pathogenicity in chickens (Liu et al., 2003; Wu et al., 2011).

Many countries, including China, employ vaccination measures to control ND outbreaks in poultry, with the LaSota strain being the most extensively used for attenuated vaccines (Afonso and Miller, 2013), providing efficient protection against NDV infections (Dai

* Corresponding author.
E-mail addresses: 506785625@qq.com (Y. Huang), ysh7865@163.com (S. Yang), hubeixia917@sina.com (B. Hu), 37116976@qq.com (C. Xu), 774798020@qq.com (D. Gao), 411041991@qq.com (M. Zhu), huangqih@163.com (Q. Huang), lzhang3406@163.com (L. Zhang), wujiaqiang2000@sina.com (J. Wu), zxm820410@163.com (X. Zhang), mazhar.khan@uconn.edu (M.I. Khan).

http://dx.doi.org/10.1016/j.vetmic.2015.09.002
0378-1135/

et al., 2008; Liu et al., 2003). However, with the accumulation of viral mutations (Cho et al., 2008; Umali et al., 2014; Wu et al., 2011), more reports are being seen in recent years showing compromised protection efficacy against NDV infection in immunized poultry (Jeon et al., 2008; Rehmani et al., 2015; Yi et al., 2011).

Located in eastern China, Shandong Province is the largest center for poultry production and processing in the country. In this study, we explored the genetics, pathogenicity and antigenicity of 31 NDV isolates from Shandong to provide information on ND epidemiology, virus evolution and vaccine efficacy in China.

2. Materials and methods

2.1. Virus stocks

Thirty-one Newcastle disease viruses (NDVs) were isolated from Shandong Province, China between 2007 and 2014 (Table 1) through the inoculation of 10-day-old embryonated specific-pathogen-free (SPF) chicken eggs. Among the viruses, 22 were derived from clinical specimens sampled from farmed chickens or ducks, 2 viruses were from diseased farmed pigeons, and the other 7 were isolated from fecal swabs of wild ducks (Table 1). The virus-containing allantoic fluids were harvested and analyzed for the existence of NDV by haemagglutination (HA) and haemagglutination-inhibition (HI) assays using NDV-specific antiserum. The isolates were then plaque-purified three times on primary chicken embryo fibroblasts, and amplified by two passages in 10-day-old SPF chicken embryos. The purified viruses were tested negative by RT-PCR to exclude the existence of avian influenza virus (AIV), infectious bronchitis virus (IBV) and infectious bursal disease virus (IBDV). The virus-containing allantoic fluids were harvested and stored at −80 °C until use.

2.2. F gene sequencing and phylogenetic analysis

Viral genomic RNA was extracted from the allantoic fluid with Trizol LS Reagent (Invitrogen, Carlsbad, CA, USA), and used as the template for RT-PCR. Degenerate primers (sequence information available upon request) and the PrimeScript™ One Step RT-PCR Kit (TaKaRa, Dalian, China) were used to amplify the complete coding sequences (cds) of the F genes. To achieve a more accurate consensus sequence of the genes, 3 independent PCRs were performed for each RNA sample. The RT-PCR product from each reaction was purified using the Gel Extraction kit (Omega Biotek Inc., USA), and then ligated with pEASY-T3 vector (Transgene Co., Beijing). The ligation mixtures were used to transform DH5α competent cells. One recombinant clone from each of the three PCRs was selected and sequenced in both directions. Sequencing was performed by Beijing Genomics Inc. (Beijing, China). The sequence data was compiled, analyzed and predicted for the encoded amino acid sequences using Lasergene v7.1 (DNASTAR Inc., Madison, WI).

Pair-wise sequence alignments of the 31 F genes were performed with Lasergene v7.1 (DNAStar, Inc., Madison, WI) using the Jotun–Hein method. Also, Nucleotide BLAST was performed to analyze highly related genes (≥99% nucleotide identity). The 31 F genes in this study and 78 reference genes, representing all complete F genes detected from the Shandong Province available in the GenBank database until December 2014, were analyzed for their phylogeny using the 1662 base pair (bp)-whole coding region of the F gene. Also included in the phylogenetic analysis were 40 reference F genes of established genotypes according to the unified classification system (Diel et al., 2012). The GenBank accession numbers of the genes are displayed in the tree. Phylogenetic trees were constructed with the distance-based

Table 1
Thirty-one Newcastle disease viruses characterized in this study.

NDV isolate	Year	Host	F cleavage site	MDT[a] (h)	ICPI[b]	Class	Genotype	GenBank accession no. of F gene
SH Y06	2006	Chicken	RRQKRF	52	2.0	II	VIId	EU597811
Chicken/China/SD2/2007	2007	Chicken	RRQKRF	57.1	1.80	II	VIId	HM748944
Chicken/China/SD2/2008	2008	Chicken	RRQKRF	48.5	1.89	II	VIId	HM748945
Chicken/China/SD3/2008	2008	Chicken	RRQKRF	56	1.71	II	VIId	HM748946
Chicken/China/SD4/2008	2008	Chicken	RRQKRF	58.2	1.68	II	VIId	HM748947
Pigeon/China/SD5/2008	2008	Pigeon	RRQKRF	82	1.35	II	VI	HM748948
Ch/D10	2009	Duck	ERQERL	105	0.36	I	–	KF055275
Ch/D58	2009	Duck	ERQERL	116	0.40	I	–	KF055276
Duck/China/SD03/2009	2009	Duck	RRQKRF	52	1.87	II	VIId	JN400895
Chicken/China/SDLY01/2010	2010	Chicken	RRQKRF	46	1.85	II	VIId	JN400897
Duck/China/SDFC07/2011	2011	Duck	GKQGRL			II	I	JQ029740
Chicken/China/SDSG01/2011	2011	Chicken	RRQKRF	52.6	1.74	II	VIId	JN400896
Chicken/China/SDWF07/2011	2011	Chicken	RRQKRF	56.7	1.74	II	VIId	JQ015295
Chicken/China/SD04/2011	2011	Chicken	RRQKRF	58.6	1.56	II	VIId	JQ015296
Chicken/China/SDYT03/2011	2011	Chicken	RRQKRF	60.4	1.72	II	VIId	JQ015297
Pigeon China SDLC 2011	2011	Pigeon	RRQKRF	86	1.3	II	VI	JQ979176
Ch/SD704/12	2012	Chicken	RRQKRF	57.2	1.84	II	VIId	JX840454
Ch/SD754/12	2012	Chicken	RRQKRF	60	1.67	II	VIId	JX840452
Ch/SD755/12	2012	Chicken	RRQKRF	57.6	1.76	II	VIId	JX840453
Ch/SD758/12	2012	Chicken	RRQKRF	58.8	1.82	II	VIId	JX840455
Ch/SD834/12	2012	Chicken	RRQKRF	58.2	1.82	II	VIId	KC489471
SD01/13	2013	Wild duck	GKQGRL	–	–	II	II	KM670002
SD21/13	2013	Wild duck	ERQERL	–	–	I	–	KM669995
Ch/SD883/13	2013	Chicken	RRQKRF	48	1.91	II	VIId	KF208469
Ch/SD889/13	2013	Chicken	RRQKRF	47.5	1.80	II	VIId	KF208470
Ch/SD01/13	2013	Chicken	RRQKRF	50	2.0	II	VIId	KF055273
SD22/13	2013	Wild duck	ERQERL	–	–	I	–	KM669996
SD07/14	2014	Wild duck	GKQGRL	–	–	II	I	KM670004
SD08/14	2014	Wild duck	GKQGRL	–	–	II	I	KM670005
SD09/14	2014	Wild duck	GKQGRL	–	–	II	II	KM670001
SD04/14	2014	Wild duck	GKQGRL	–	–	II	II	KM670003

[a] The mean death time of chicken embryos.
[b] ICPI represents intracerebral pathogenicity index in 1-day-old chickens.