Genetic characterization and pathotypical analysis of two strains of pigeon Newcastle disease virus of Jiangsu in China

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Abstract

Pigeon Newcastle disease (ND) is a severe infectious disease caused by pigeon Newcastle disease virus (NDV) or Paramyxovirus type 1 (PPMV-1). Genotype VI NDV is a major cause of ND in *Columbiformes* (i.e. pigeons and doves). In this study, two strains of NDV (Pigeon/China/JS/VI-NJ/2006 and Pigeon/China/JS/VI-HZ/2017) were isolated from diseased pigeons. The virus particles were about 120 nm observed under the transmission electron microscope. The MDT and ICPI values of the two viruses were typically mesogenic. The amino acid sequence at cleavage site of F protein of Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains were ^{112}RRQKRF^{117} and ^{112}KRQKRF^{117}, with the molecular characteristics of virulent strains. The whole genome homology analysis showed that the two strains had high homology with strains of genotype VI (92.52%~99.13%). Furthermore, based on the phylogenetic tree of the F gene, Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 were closely related to the sub-genotype VI 2.1.1.2.1 and VI.2.2.2, respectively. The comparison of the amino acid positions of the F and HN proteins from Pigeon/China/JS/VI-HZ/2017 strain showed that there were four mutant sites (20, 22, 235, 514) on the F protein and four mutant sites (253, 280, 288, 326) on the HN protein, demonstrating that genetic variation had occurred. The survival rate of pigeons infected with the two strains were 40% and 20%, with lesions in multiple tissues, indicating that these strains had strong ability to spread and high pathogenicity. In summary, the results showed that the prevalence of genotype VI NDV was contributed by strains from diverse sub-genotypes, suggesting the importance of pigeon NDV surveillance in China.

1. Introduction

Newcastle disease (ND) is highly contagious and can be devastating, particularly in immunologically naïve poultry(1) (Alexander et al., 2012). ND is caused by virulent strains of Newcastle disease viruses (NDV), commonly known as Avian Paramyxoviruses 1 (APMV-1) of the genus *Avian orthoavulavirus 1* (AOAV-1) belonging to the family *Paramyxoviridae* (2). NDV has a negative-sense, single-strand, non-segmented RNA genome composed of 3'-NP-P/V/W-M-F-HN-L-5', encoding six structural proteins, namely, nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and large protein (L), as well as two non-structural proteins (V and W) through RNA-editing from P gene.

Genetic analysis of the complete F gene nucleotide sequences is the basis for a unified nomenclature and classification system for assigning NDV isolates (3). NDV strains are divided into two classes (I and II), of which class I contains only one genotype (genotype 1) and class II includes at least twenty distinct genotypes (I-XXI, except XV) (3). Class I viruses are lentogenic or non-virulent in wild birds and poultry. Several class II strains (genotype II, III, VI, VII and IX) are virulent and could cause devastating economic losses to the poultry industry worldwide (4, 5).

Genotype VI NDV, traditionally called pigeon paramyxovirus type-1 (PPMV-1), is a major cause of pigeon ND in *Columbiformes* (i.e. pigeons and doves) (5, 6). This genotype NDV was first detected in the Middle East in 1978 in captive pigeons raised for meat (7) During the early 1980s, the virus was subsequently detected in outbreaks in pet and racing pigeons from private lofts, as well as free-ranging feral pigeons in Europe and North America. It caused periodic transmission to domestic poultry through the contamination of feed with pigeon feces (8). The virus is currently thought to be maintained in pigeons raised in squab facilities and in private lofts with regular transmission among pigeons during shows or racing events (5, 9). Recent studies have shown that the pigeon genotype VI NDV strains circulating in China are generally isolated in several provinces (6, 10–15).

After infection with genotype VI NDV, pigeons would show clinical signs similar to neurotropic ND-infected chickens, with a morbidity ranged from 30–70%, and a mortality no more than 10% (16), which emphasized the importance of continuous genotype VI NDV surveillance in pigeons. In this study, we analyzed genomic sequences of two NDV isolates from diseased pigeon flocks in Jiangsu province of China, along with the genetic characterization, virulence and pathogenicity of these viruses.

2. Materials And Methods

2.1. Virus isolation and pathogenicity tests

The NDV strain Pigeon/China/JS/VI-NJ/2006 and Pigeon/China/JS/VI-HZ/2017 (designated VI-NJ06 strain and VI-HZ17 strain in this study) were obtained from diseased pigeons in Jiangsu province of China in 2006 and 2017, respectively. The strains were isolated through the inoculation of 9-day-old specific pathogen-free (SPF) chicken eggs (Nanjing Tech-bank Bio-industry Co.,Ltd, Nanjing, China. Voucher number: SCXK(zhe)2021-0005) (17). The allantoic fluids were harvested and analyzed for the existence of NDV by haemagglutination (HA) and haemagglutination-inhibition (HI) assays using NDV-specific antiserum and avian influenza virus (AIV) H5, H7 and H9 subtype sera (Harbin Weike Biotechnology Development Company, Harbin, China). The isolates were then plaque-purified for three times on primary chicken embryo fibroblasts (CEF) and amplified in 9-day-old SPF chicken embryos. Virus titer were expressed as 50% embryo infectious dose (EID_{50}/ml) by the end-point method of Reed and Muench. The virus-containing allantoic fluids were harvested and stored at − 80°C until use.

For negative staining transmission electron microscopy (TEM), the samples were negatively stained with 1% phosphotungstic acid and dried by aspiration on a copper gird, and then visualized with a Hitachi H-7650 transmission electron microscope (Hitachi Ltd, Japan).

Intra-cerebral pathogenicity index (ICPI) tests in 1-day-old SPF chickens (Nanjing Tech-bank Bio-industry Co.,Ltd, Nanjing, China) and the mean death time (MDT) in 9-day-old SPF chicken eggs were performed according to the Office International des Epizooties manual of standards (17).

2.2. Viral RNA extraction, Reverse transcription PCR and full length genome sequencing

The viral genomic RNA was extracted from the harvested allantoic fluids using an SteadyPure Virus DNA/RNA Extraction Kit (Accurate Biotechnology (Hunan) Co.,Ltd., Changsha, China) according to the manufacturer's instructions. RNA pellets were resuspended in 50 µL RNase-free water and reverse transcribed with

Evo M-MLV Plus 1st Strand cDNA Synthesis Kit (Accurate Biotechnology (Hunan) Co.,Ltd., Changsha, China). The cDNAs were used as template to generate eleven successive and overlapping DNA fragments by PCR using primer pairs specific for genotype VI NDV strains (Table S1). To determine the 3'- and 5'-ends of the viral genomes, rapid amplification of cDNA end (RACE) was performed by SMARTer RACE 5'/3' Kit (Takara Biomedical Technology Co., Ltd, Beijing, China) according to the manufacturer's instructions.

The recovered PCR products were connected to pMD™19-T vector (Takara Biomedical Technology Co., Ltd, Beijing, China) and transformed into *Escherichia coli* DH5α competent cells (Takara Biomedical Technology Co., Ltd, Beijing, China). At least three clones for each segment were sent to General Biosystems Co., Ltd (Chuzhou, China) for sequencing.

2.3. Sequence and phylogeny analysis

The nucleotide sequences of the whole genome were aligned by the ClustalW software (version 2.0.10) and compared with the corresponding whole genome sequences of other 21 NDV strains from different genotypes in GenBank (Table S2). These alignments were subjected for nucleotide and deduced amino acid sequence analyses as implemented in the BioEdit software (version 7.2). The nucleotide homology analysis and the key amino acid site mutation of F and HN proteins analysis were performed by MEGA software (version 7).

Phylogenetic analysis was performed based on the complete F gene (1662 nt) sequences with 43 other strains from different genotypes or sub-genotypes (Table S2) using MEGA software (version 7) (18). The evolutionary history was inferred by RaxML (version 8) (19), which utilized the Maximum Likelihood (ML) method based on the General Time Reversible (GTR) model with a discrete gamma distribution (+ G) allowing for invariant sites and 1000 bootstrap replicates. All data and accession numbers of NDV strains used in this study were listed in Table S2.

2.4. Clinicopathologic assessment in pigeons

A total of 30 clinically healthy 30-day-old pigeons (Voucher number: SCXK(su)2021-0012) were randomly divided into three groups. All the pigeons were lack of NDV-specific HI antibodies and were negative for NDV genome in cloacal swab samples. Pigeons were inoculated with Pigeon/China/JS/VI-NJ/2006 and Pigeon/China/JS/VI-HZ/2017 strains via the intranasal route with 10^6 EID_{50} of each virus in 0.2 mL saline. The negative control group was inoculated with 0.2 mL normal saline. Pigeons in the experimental groups were housed in negative-pressure condition and were provided with food and water ad libitum. All the pigeons were clinically monitored every day for signs of disease and mortality. Two pigeons in each group were sacrificed on day5 post inoculation (dpi) and tissue samples (brain, trachea, lung, stomach, liver, spleen, small intestine, and bursa of fabricius) were collected and fixed in 10% neutral buffered formalin for histological observation. All sections were counterstained with hematoxylin and visualized by light microscopy.

The samples were also homogenized, and the supernatants were inoculated into 9-day-old SPF chicken eggs to monitor virus shedding. 72 h after inoculation the allantoic fluid was collected, and the virus was tittered using a standard HA assay (Oie, 2008). The samples with HA values higher than or equal to 4 $\log_2$ were recorded as positive.

3. Results

3.1. Virus isolation and identification

The samples were inoculated to embryonated chicken eggs and the embryo allantoic liquid was collected to detect the HA activity of the isolated strains. The HA values of each virus ranged from 7 $\log_2$ to 8 $\log_2$ and two viruses can react with the serum that is positive to NDV HI test, rather than AIV H5, H7 and H9 subtype sera. After several rounds of plaque purification, two NDV strains were successfully isolated and identified, namely, Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017. Through TEM observation, both strains showed encapsulated viral particle of around 120 nm diameter, which was the characteristic structure of NDV (Fig. 1). Detailed information about the two isolates is provided in Table 1. The MDT and ICPI values of Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains were typically in accordance with the feature of mesogenic isolates.

Table 1
Two pigeon NDV isolates characterized in this study.

Strain	F cleavage site	MDT [a] (h)	ICPI[b]	HA titer	$TCID_{50}$/mL	EID_{50}/mL	GenBank accession no.
Pigeon/China/VI-NJ/2006	^{112}RRQKR↓F^{117}	68	1.71	8 $\log_2$	$10^{8.67}$	$10^{8.7}$	MZ409510
Pigeon/China/VI-HZ/2017	^{112}KRQKR↓F^{117}	84	1.58	7 $\log_2$	$10^{7.33}$	$10^{7.6}$	MW412840

[a] Mean death time (MDT) was determined by inoculating in 9-day-old SPF chicken eggs (hours) (< 60, velogenic / highly virulent strain; 60–90, mesogenic / moderately virulent strain; >90, lentogenic / low virulent strain).

[b] Values equal to 0.7 or greater are identified as virulent NDV strain.

3.2. Genomic, homology and phylogenetic analysis

In total, 11 overlapping DNA segments of 1200 bp ~ 1700 bp were obtained through RT-PCR. The lengths of 3' leader and 5' trailer were 55 nt and 114 nt, respectively. Sequence analysis of Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains consisted of 15,192 nt were assembled through software (GenBank number: MZ409510 and MW412840, respectively). The sequences of the two strains possessed the gene order 3'-NP-P/V/W-M-F-HN-L-5'. The open reading frame (ORF) sequence of the six structural proteins (NP, P, M, F, HN, L) were 1470 bp, 1188 bp, 1095 bp, 1662 bp, 1716 bp and 6615 bp, respectively. P

gene encoded two non-structural protein V and W proteins through RNA editing. The ORF of V and W proteins were 720 bp and 684 bp. The amino acid sequence at cleavage site of F protein of Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains were ^{112}RRQKRF^{117} and ^{112}KRQKRF^{117} (Table 1), which were characteristic for virulent strains.

The whole genome nucleotide homology analysis of the two isolates with 21 reference strains of each genotype showed that the homology with the reference strains remain distinct (58.48%~99.13%). Among the 21 strains, the current two isolates were particularly related to three strains of genotype VI, XX and XXI (92.52%~99.13% for genotype VI, 90.91%~91.65% for genotype XX and 90.63% for genotype XXI). Their homogeneity with the predominant genotype VII strains circulating in China were 87.20%~87.86% (Fig. 2), while the homogeneity with Class I strains were only 58.48%~59.29%. In addition, the homology within the two isolates was 91.98% (Fig. 2).

A phylogenetic tree based on F gene sequences was presented in Fig. 3. The phylogenetic analysis showed that the two isolates were both located in the branches of Class II genotype VI strains. Pigeon/China/VI-NJ/2006 strain was more closely related to the sub-genotype VI 2.1.1.2.1 strain and Pigeon/China/VI-HZ/2017 strain had the closest genetic relationship with the sub-genotype VI.2.2.2 strain (Fig. 3).

Further analysis displayed amino acid sequence differences. F and HN mutations of the two isolates were similar to VI 2.1.1.2.1 and VI.2.2.2 strains (Table 2 and Table 3). Besides, the Pigeon/China/JS/VI-HZ/2017 isolate also displayed novel mutations (I20, M22, V235, S515 mutation in F protein and I253, H280, I326, S365 mutation in HN protein). The amino acid substitutions of the F and HN proteins were shown in Table 2 and Table 3in detail.

Table 2
Summary table of amino acid substitutions in the F protein of two pigeon NDV detected in this study comp

Accession no.	Strain	Genotype	6	11	13	20	22	28	30	111	112	208	235	294	341	349
FJ410145	Pigeon/USA/NY/1984	VI.1	S	V	L	M	I	P	S	G	R	I	I	N	S	R
MG018211	Collared dove/USA/1185/2015	VI.2.1.1.1	S	T	L	T	I	L	S	V	R	V	I	N	S	R
FJ766527	Pigeon/China/JS16/2007	VI.2.1.1.2.1	H	A	L	T	V	L	S	G	R	I	I	S	S	R
MG840654	Pigeon/China/Ningxia/2068/2016	VI.2.1.1.2.2	H	A	P	T	V	S	S	G	R	I	I	N	S	R
JX518532	Dove/Kenya/B2/Isiolo/2012	VI.2.1.2	S	A	L	T	I	P	S	G	R	I	I	N	S	R
FJ410148	Pigeon/USA/Texas/1998	VI.2.2.1	S	A	P	M	I	Q	S	E	R	I	I	N	S	K
KJ808820	Pigeon/China/SD/2012	VI.2.2.2	S	V	P	M	I	P	G	E	K	V	I	N	T	K
MZ409510	Pigeon/China/VI-NJ/2006	VI.2.1.1.2.1	H	A	L	T	V	L	S	G	R	I	I	S	S	R
MW412840	Pigeon/China/VI-HZ/2017	VI.2.2.2	S	V	P	I	M	P	G	E	K	V	V	N	T	K

Note: novel mutations compared to genotype VI NDV strains.

Table 3
Summary table of amino acid substitutions in the HN protein of two pigeon NDV detected in this stu

Accession no.	Strain	Genotype	7	14	19	34	38	41	45	57	62	63	66	84	120	145	147
FJ410145	Pigeon/USA/NY/1984	VI.1	R	E	N	V	A	A	V	V	V	I	T	I	N	T	N
MG018211	Collared dove/USA/1185/2015	VI.2.1.1.1	K	E	N	V	A	A	V	V	V	I	V	I	N	T	G
FJ766527	Pigeon/China/JS16/2007	VI.2.1.1.2.1	K	E	S	V	A	V	V	I	M	V	A	V	N	V	G
MG840654	Pigeon/China/Ningxia/2068/2016	VI.2.1.1.2.2	K	E	S	V	A	V	A	V	M	V	V	I	N	I	G
JX518532	Dove/Kenya/B2/Isiolo/2012	VI.2.1.2	K	E	N	M	A	A	V	V	V	V	M	I	N	I	D
FJ410148	Pigeon/USA/Texas/1998	VI.2.2.1	R	E	N	M	V	V	V	V	V	I	T	I	N	T	D
KJ808820	Pigeon/China/SD/2012	VI.2.2.2	R	K	N	I	V	A	A	I	V	I	T	I	S	T	D
MZ409510	Pigeon/China/VI-NJ/2006	VI.2.1.1.2.1	K	E	S	V	A	V	V	I	M	V	A	V	N	V	G
MW412840	Pigeon/China/VI-HZ/2017	VI.2.2.2	R	K	N	I	V	A	A	I	V	I	T	I	S	T	D

Note: novel mutations compared to genotype VI NDV strains.

Table 4
Detection of virus from pigeons' cloaca route[a]

Group	3 d	5 d	7 d	10 d	14 d
Pigeon/China/VI-NJ/2006	5/10	10/10	8/8	3/3	2/2
Pigeon/China/VI-HZ/2017	3/10	8/10	9/9	7/7	4/4
Control	0/10	0/10	0/10	0/10	0/10

[a] Samples with HA values higher than or equal to 4 $\log_2$ were recorded as positive.

3.3. Clinical symptoms and gross pathology

Clinical signs in Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains infected pigeons were observed since 2 dpi. The birds showed the decreased appetite at 5 dpi, and became more severe with progression of the disease, consisting of depression with somnolence, green-white soft faeces and diarrhoea. Two infected pigeons of each groups were therefore euthanatized and sampled at 5 dpi for histological observation. From 6 dpi, the pigeons infected with Pigeon/China/VI-NJ/2006 strain exhibited 80% (8/10) ended up with death (Fig. 4). In the meanwhile, the pigeons infected Pigeon/China/VI-HZ/2017 strain also showed the mortality of 60% (6/10) (Fig. 4). Pigeons in the negative control did not induce gross lesions.

Observation of histopathological sections of euthanatized pigeons infected with Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains showed brain edema (Fig. 5a and 5b). Necrosis and desquamation of mucous epithelial cells were seen in tracheas (Fig. 5d and 5e). A large number of inflammatory cells gathered in some areas of the lung (Fig. 5g and 5h), and congestion and focal inflammatory reaction of the liver (Fig. 5m and 5n) were observed. The bleeding spots could be detected in stomachs (Fig. 5j). Broken villi, dropout of epithelium, and hemorrhage were presented in the small intestine, and the lamina propria had an inflammatory reaction (Fig. 5p and 5q). The lymphocytes in the bursa of fabricius had vacuolar necrosis (Fig. 5s and 5t). In comparison, all tissues samples from the control group had no apparent histological changes.

The cloacal swabs of Pigeon/China/VI-NJ/2006 strain infected pigeons were positive for NDV at 3 dpi and the viral load was highest at 5 dpi. As to Pigeon/China/VI-HZ/2017 group, all the swabs were positive at 7 dpi. The samples of control group were all negative for NDV throughout the experiment (Table. 4).

In general, the Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 strains were both neurotropic and invasive to gastrointestinal tract, with strong ability to spread and high pathogenicity.

4. Discussion

Pigeon NDV or PPMV-1 is a highly pathogenic and severe infectious disease. Both young and adult pigeons can be infected, which caused significant economic losses in the pigeon industry (5, 15). Although some strains of PPMV-1 are non-pathogenic in chickens, they cause morbidity in pigeons. The disease signs in pigeons are consistent with that in chickens, and generally include a series of nervous disorders: bilateral or unilateral locomotor disturbances of wings or legs, torticollis, and watery green diarrhea. If pigeons were infected during breeding or molting, the mortality would tend to increase.

Most pigeon-derived virulent isolates belong to class II of NDV. NDV strains of several genotypes were isolated and detected in pigeons, including genotype VI, VII and XXI (13, 20, 21). In China, the strains that infect pigeons are mainly genotype VI (11, 13–15).. The two strains also belong to genotype VI, suggesting that genotype VI is the dominant strain in pigeon in China. The genotype VI strains can infect many kinds of birds, including pigeons, chickens, turkeys, quails, and geese (5). However, it would not cause obvious symptoms in other species (22). There are still potential hazards to the aquaculture industry. Therefore, it is very important to implement epidemiological testing for PPMV-1.

The classification of NDV genotypes were renewed by Dimitrov et al (3). Namely, former VIc, VII, VIi, VIg and VIm sub-genotypes were confirmed and renamed as genotype XX and XXI (3). Thus, the homogeneity of the two strains were with a close genetic distance from these three genotypes (VI, XX and XXI). Moreover, genotype VI strains can be further divided into at least seven sub-genotypes (VI.1, VI.2.1.1, VI.2.1.1.2.1, VI.2.1.1.2.2, VI.2.1.2, VI.2.2.1, VI.2.2.2) (3). At present, the genotypes of PPMV-1 that are prevalent in other countries are VI.1, VI.2.1.1, VI.2.1.2, VI.2.2.1. The genotypes prevalent in China are mainly VI.2.2.2, VI.2.1.1.2.1, VI.2.1.1.2.2(3). Tian et al. found that ten PPMV-1 viruses isolated in China during 1996–2019 belonged to sub-genotypes VI.2.2.2, VI.2.1.1.2.1, VI.2.1.1.2.2 and genotype VII (13). Additionally, Zhan et al. reported that 21 PPMV-1 isolates belonged to sub-genotypes VI.2.1.1.2.1 and VI.2.1.1.2.2 in China from 2007 to 2019 (14). In this study, we identified two strains of PPMV-1 from Jiangsu province in China in 2006 and 2017, they were classified into sub-genotype VI.2.1.1.2.1 and VI.2.2.2. The above results indicated that the prevalence of PPMV-1 was the result of different genotype strains circulating in China. Besides, several novel mutations were also detected (I20, M22, V235, S515 mutation in F protein and I253, H280, I326, S365 mutation in HN protein). It indicated that the overall variation rate of the genotype VI strain amino acids was rather fast. It should be noticed that all the novel mutations were in the sub-genotype VI.2.2.2 strain, suggesting it might become a new sub-genotype.

In summary, the full genomes of two genotype VI viruses isolated from pigeons in Jiangsu Province were identified and analyzed based on a recently unified phylogenetic classification system. The sub-genotype VI.2.1.1.2.1 and VI.2.2.2 strains showed high virulence and shedding efficacy in pigeons. The results showed that the prevalence of genotype VI NDV was contributed by strains from diverse sub-genotypes. Although these isolates were only found in Jiangsu Province, it had undergone a certain degree of variation, suggesting the importance of NDV prevalence and evolution surveillance in China. It would further lay a foundation for the subsequent development of pigeon ND vaccine.

Declarations

Authorship contribution statement

Jing Qian, Pengju Chen and Hongbo Zhou designed study. Jing Qian, Xue Liu and Zhengxu Tang performed the experiments. Jing Qian wrote the main manuscript text. Jiankun Wang, Yongshan Wang and Wei Wang analyzed data. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Research involving in animal participants

This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China. The protocols for animal studies were approved by the Committee on the Ethics of Animal Experiments of Jiangsu Academy of Agricultural Sciences (Voucher number: SCXK(zhe)2021-0005 and SCXK(su)2021-0012).

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Figures

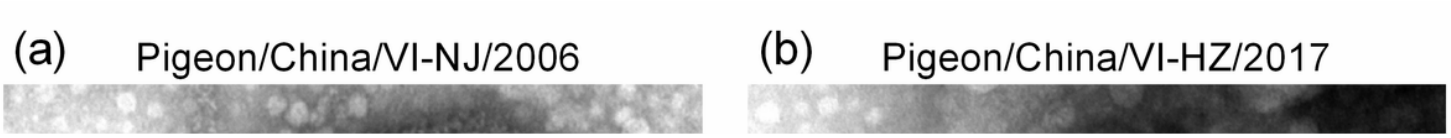

Figure 1

Negative staining electron microscopies of two pigeon NDV particles. (a) Pigeon/China/VI-NJ/2006 strain. (b) Pigeon/China/VI-HZ/2017 strain.

Note: The scale bar represents 100 nm.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	Pigeon/China/VI-NJ/2006		0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2	Pigeon/China/VI-HZ/2017	91.98		0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
3	Class I FJ794269.1/Duck/China/NDV08-004/2008	59.29	58.48		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
4	Class II-I JX524203.1/Chicken/Australia/V4/1966	83.16	83.77	62.37		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
5	Class II-II AF077761/Chicken/USA/Lasota/1946	80.49	80.28	58.16	87.36		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6	Class II-III EF201805/Avian/Mukteswar/1940	83.12	82.29	60.46	90.25	85.91		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
7	Class II-IV AY741404/Fowl/UK/Herts/1933	86.58	87.00	59.20	91.40	87.17	92.13		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
8	Class II-V JX974435/Chicken/Mexico/2010	85.96	85.79	58.58	83.91	82.23	84.96	87.90		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
9	Class II-VI KJ808820/Pigeon/China/SD/2012	92.52	99.13	58.94	83.77	80.11	82.29	87.00	85.94		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
10	Class II-VII DQ659677/Goose/China/NA1/1999	87.86	87.20	59.15	84.68	80.10	84.19	87.65	86.68	87.42		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
11	Class II-VIII FJ751918/Chicken/China/QH1/1979	87.58	87.35	61.04	86.06	82.41	85.73	89.12	87.88	87.42	87.34		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
12	Class II-IX FJ436302/Chicken/China/F48E8/1948	84.02	83.84	58.90	90.80	86.89	91.07	92.47	85.61	83.76	84.84	86.14		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
13	Class II-X JN872171/Turkey/USA/17531-3/2010	79.58	79.60	60.43	88.00	86.43	86.28	86.86	81.10	79.52	80.78	82.09	87.10		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
14	Class II-XI HQ266603/Ckicken/Madagascar/MG1992/2008	78.99	78.46	56.92	82.60	79.63	83.00	88.60	80.89	78.38	79.49	80.97	84.49	79.30		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
15	Class II-XII KC551967/Goose/China/Guangdong/2010	86.54	86.59	60.84	82.77	79.19	82.49	85.60	84.50	86.22	88.62	86.34	83.15	79.47	77.44		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
16	Class II-XIII MF422129.1/Chicken/India/41016A/2016	84.41	84.91	59.92	82.08	79.33	81.21	84.85	84.46	84.60	87.28	85.07	82.46	79.01	77.61	87.50		0.01	0.01	0.01	0.01	0.01	0.01	0.01
17	Class II-XIV KT948996/Duck/Nigeria/NG695/2009	83.02	84.49	58.70	79.12	75.80	79.08	82.95	83.79	84.33	87.00	83.86	79.71	77.45	75.17	86.42	85.26		0.01	0.01	0.01	0.01	0.01	0.01
18	Class II-XVI JX119193/Chicken/Dominican Republic/49931/2008	80.42	80.88	56.85	80.97	78.20	81.29	84.25	82.90	80.72	80.69	82.75	81.17	79.32	76.26	80.89	79.90	78.01		0.01	0.01	0.01	0.01	0.01
19	Class II-XVII KY171991/Quail/Nigeria/861/2004	85.75	85.57	58.57	82.45	79.46	82.33	85.61	85.96	85.57	88.63	84.59	83.56	79.47	78.69	88.34	87.01	87.44	79.66		0.01	0.01	0.01	0.01
20	Class II-XVIII MH392227/Chicken/Nigeria/914/2009	86.07	86.79	59.35	82.39	79.30	82.98	85.70	85.60	86.86	88.50	85.29	83.65	80.33	78.83	88.50	87.23	87.44	80.09	90.38		0.01	0.01	0.01
21	Class II-XIX JN941993/Pelican/USA/W241508/2008	83.23	81.92	58.54	81.13	78.90	81.28	83.92	89.93	82.08	83.42	84.40	81.45	78.84	77.24	81.58	81.71	79.76	79.76	82.66	82.26		0.01	0.01
22	Class II-XX AB853928/Chicken/Japan/SM87/1987	90.91	91.65	59.10	85.71	81.41	84.55	88.93	87.25	92.05	89.25	88.91	85.73	80.83	80.78	86.95	86.62	84.14	82.47	87.56	88.29	83.39		0.01
23	Class II-XXI KT962979/Pigeon/Russia/777/2010	90.63	90.63	58.59	83.46	79.55	82.93	86.42	85.32	90.70	87.77	87.43	83.93	79.38	77.97	85.78	84.29	83.44	80.40	85.33	85.96	82.94	90.62	

Figure 2

Nucleotide homological analysis of the whole genome sequences of two pigeon NDV detected in this study and other reference NDV strains (n=21).

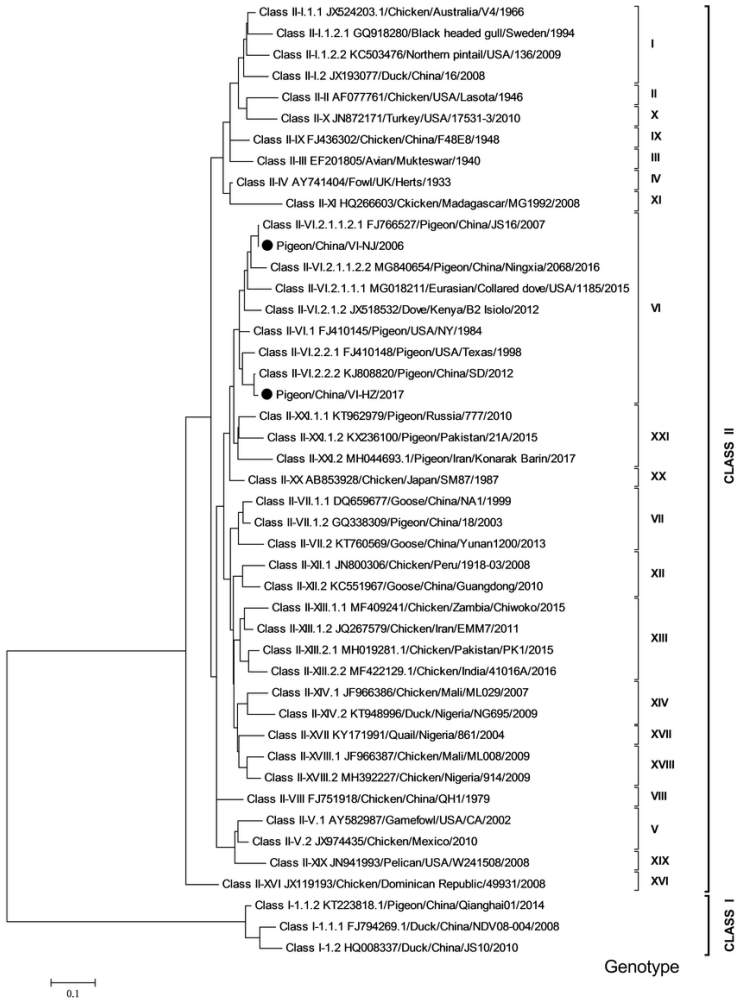

Figure 3

Phylogenetic analysis of the F region of two pigeon NDV detected in this study and other reference NDV strains (n=43). A phylogenetic tree was constructed based on the complete F gene sequences using the maximum likelihood (ML) method with 1000 bootstrap replicates and Poisson model in the MEGA 7.0 software.

Note: The Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017 in this study are labeled with a black solid circle (●).

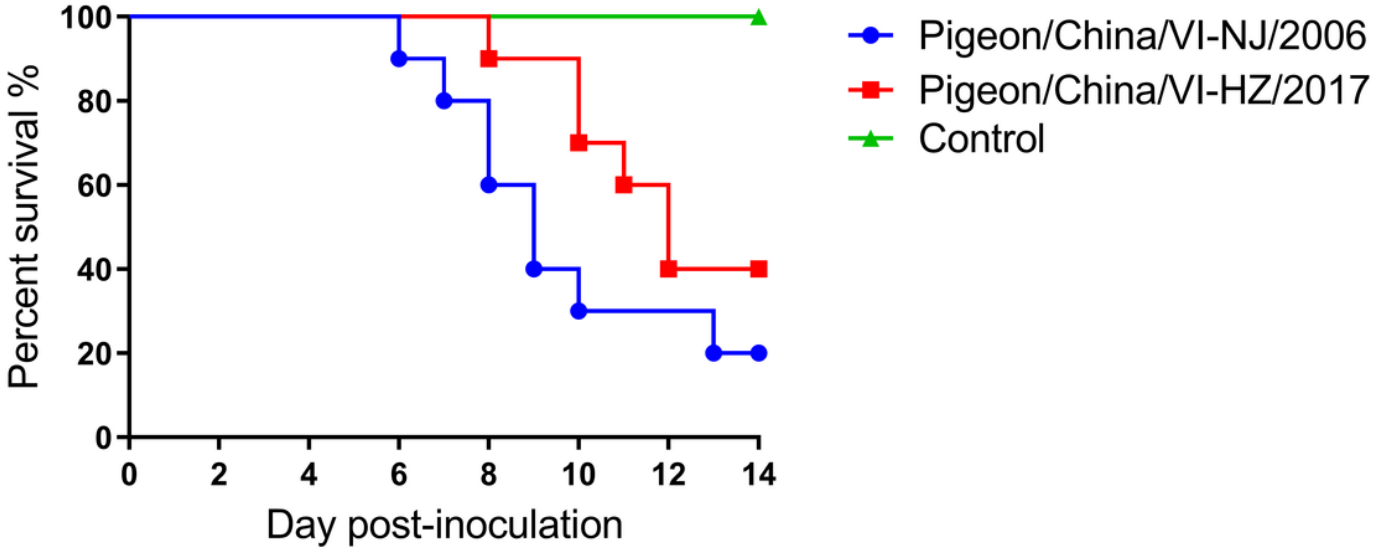

Figure 4

Survival rate of pigeons inoculated with Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017.

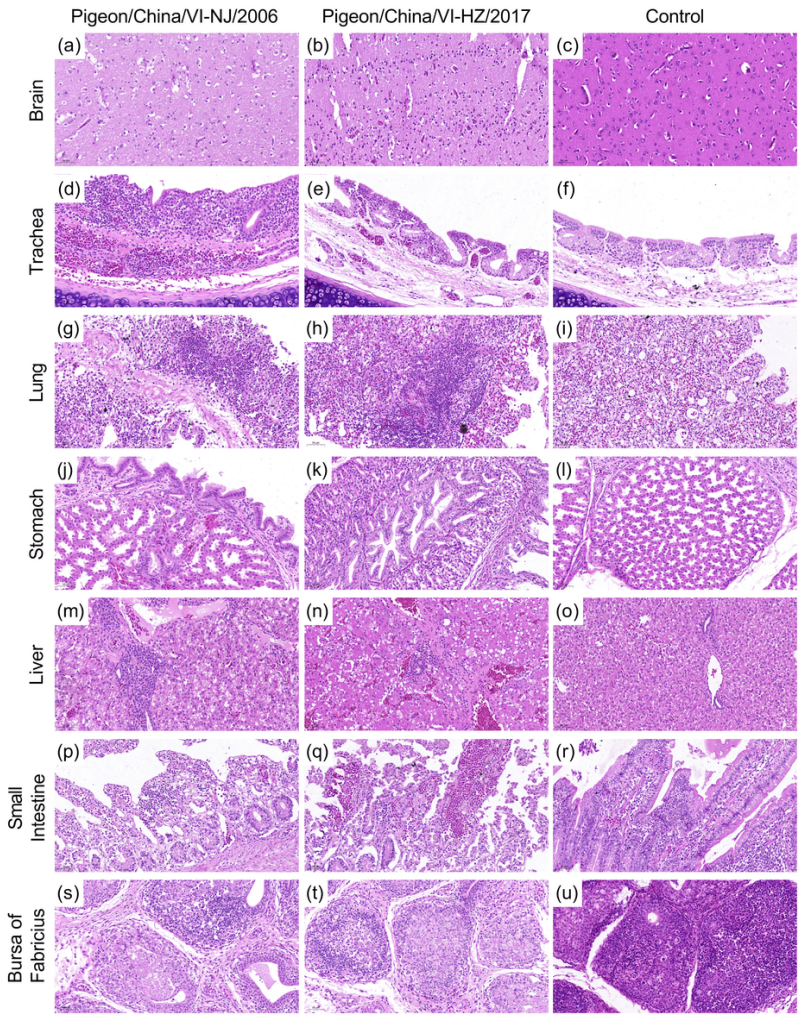

Figure 5

Histopathology observation of tissues from pigeons inoculated with Pigeon/China/VI-NJ/2006 and Pigeon/China/VI-HZ/2017. (a) and (b) Edema in the brain. (c) No abnormality in the brain. (d) and (e) Necrosis and desquamation of mucous epithelial cells in the trachea. (f) No abnormality in the trachea. (g) and (h) A large number of inflammatory cells gathered in some areas of the lung. (i) No abnormality in the lung. (j) Bleeding spots in the stomach. (k) and (l) No abnormality in the stomach. (m) and (n) Congestion and focal inflammatory reaction in the liver. (o) No abnormality in the liver. (p) and (q) Broken villi, dropout of epithelium, hemorrhage and lamina propria inflammatory reaction in the small intestine. (r) No abnormality in the small intestine. (s) and (t) Vacuolar necrosis of lymphocytes in the bursa of fabricius. (u) No abnormality in the bursa of fabricius.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.doc
- TableS2.doc