AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

High Genetic Diversity of Newcastle Disease Virus in Wild and Domestic Birds in Northeastern China from 2013 to 2015 Reveals Potential Epidemic Trends

Pingze Zhang,[a] Guangyao Xie,[a] Xinxin Liu,[b] Lili Ai,[a] Yanyu Chen,[a] Xin Meng,[a] Yuhai Bi,[c] Jianjun Chen,[d] Yuzhang Sun,[e] Tobias Stoeger,[f] Zhuang Ding,[a] Renfu Yin[a]

Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Jilin University, Changchun, Jilin, China[a]; College of Food Science and Engineering, Jilin University, Changchun, Jilin, China[b]; CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China[c]; CAS Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Hubei, China[d]; China Animal Health and Epidemiology Center, Qingdao, Shandong, China[e]; Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum Muenchen, Neuherberg/Munich, Germany[f]

Newcastle disease (ND), caused by the virulent Newcastle disease virus (NDV), is one of the most important viral diseases of birds globally, but little is currently known regarding enzootic trends of NDV in northeastern China, especially for class I viruses. Thus, we performed a surveillance study for NDV in northeastern China from 2013 to 2015. A total 755 samples from wild and domestic birds in wetlands and live bird markets (LBMs) were collected, and 10 isolates of NDV were identified. Genetic and phylogenetic analyses showed that five isolates from LBMs belong to class I subgenotype 1b, two (one from wild birds and one from LBMs) belong to the vaccine-like class II genotype II, and three (all from wild birds) belong to class II subgenotype Ib. Interestingly, the five class I isolates had epidemiological connections with viruses from southern, eastern, and southeastern China. Our findings, together with recent prevalence trends of class I and virulent class II NDV in China, suggest possible virus transmission between wild and domestic birds and the potential for an NDV epidemic in the future.

Newcastle disease virus (NDV) is synonymous with avian paramyxovirus type 1 and is a member of the genus *Avulavirus* within the family *Paramyxoviridae* (1). The virulent NDVs are the causative agents of Newcastle disease (ND), which is one of the most devastating diseases to the poultry industry worldwide (1). NDV is an enveloped virus containing a nonsegmented, single-stranded, negative-sense RNA genome 15.2 kb in size. The genome contains six genes, which encode the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), RNA-dependent RNA polymerase (L), and two additional nonstructural proteins (V and W, produced by a frameshift within the coding region of P) (1).

NDV is considered to be enzootic in both domestic and wild bird species (1). Based on the genomic length and phylogenetic analysis of the F gene, NDVs have been historically classified into two major groups, class I and class II, within a single serotype (2). Class I NDV is distributed globally, usually thought to be avirulent, and frequently isolated from wild birds and live bird markets (LBMs) (3). Class II NDV is widely distributed in multiple bird species. Some class II strains are avirulent or are vaccine viruses, but virulent strains of NDV also mainly group into class II and infections can cause significant economic losses to the poultry industry (2). Class I viruses were previously classified into nine genotypes (1 to 9), based on phylogenetic analysis using parts of the F gene (3, 4), but are now classified into a single phylogenetic group (genotype 1) based on the new classification criteria using the complete F gene sequence (5). Due to their high genetic diversity, class II viruses are classified into 18 genotypes (I to XVIII) (5, 6).

The scale and magnitude of ND outbreaks in China have been decreasing yearly since 2005 (Fig. 1). Interestingly, an increasing number of class I NDVs have been isolated from domestic poultry in southern, eastern, and southeastern China, as well as worldwide, in recent years (4, 7–10). However, no class I NDV was isolated in China before 2002, since they may escape detection by many conventional assays (2, 4). Furthermore, surveillance studies have demonstrated that the ecology of avirulent NDV in wild birds appears to be similar to that of low-pathogenicity avian influenza virus in that both viruses seem to enhance their pathogenicity in birds by accumulated mutations at the fusion protein cleavage site (3, 11–14).

In this study, NDVs from wild birds and domestic poultries in LBMs were sequenced and analyzed in order to understand the current epidemiology and gene evolution of NDV in northeastern China. We then analyzed the epidemic trends of class I NDV in China and elucidated the potential ecological relationships between class I and class II NDVs, and the results are presented here.

MATERIALS AND METHODS

Sample collection, virus isolations, and identification. During the surveillance of NDV from April 2013 to June 2015, a total of 755 tracheal or cloacal swab samples were collected from clinically healthy birds in 16 LBMs and three wetlands in Jilin Province of northeastern China. Of

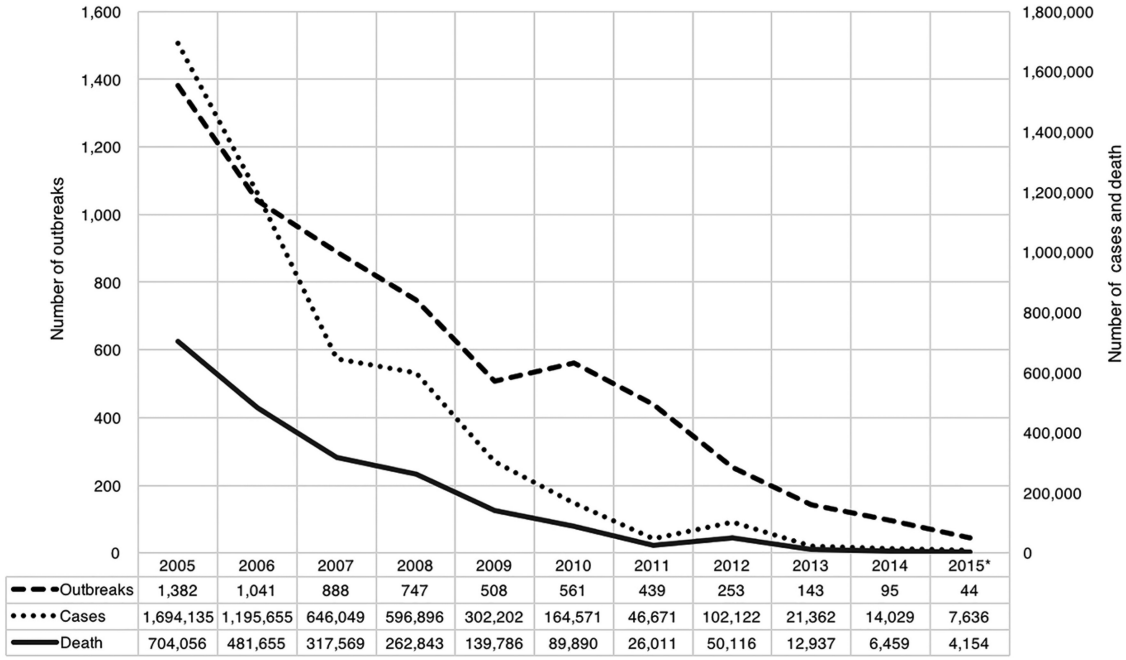
Received 19 October 2015 **Accepted** 16 December 2015

Accepted manuscript posted online 28 December 2015

Citation Zhang P, Xie G, Liu X, Ai L, Chen Y, Meng X, Bi Y, Chen J, Sun Y, Stoeger T, Ding Z, Yin R. 2016. High genetic diversity of Newcastle disease virus in wild and domestic birds in northeastern China from 2013 to 2015 reveals potential epidemic trends. Appl Environ Microbiol 82:1530–1536. doi:10.1128/AEM.03402-15.

Editor: D. W. Schaffner, Rutgers, The State University of New Jersey

Address correspondence to Renfu Yin, yin@jlu.edu.cn.



	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015*
Outbreaks	1,382	1,041	888	747	508	561	439	253	143	95	44
Cases	1,694,135	1,195,655	646,049	596,896	302,202	164,571	46,671	102,122	21,362	14,029	7,636
Death	704,056	481,655	317,569	262,843	139,786	89,890	26,011	50,116	12,937	6,459	4,154

FIG 1 ND outbreak events in China between 2005 and 2015. The asterisk indicates data cutoff on June 2015. All data were collected from the OIE official website (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail).

these, 340 were from wild birds, and 415 were from domestic poultries. Details of collection for the NDV isolates are given in Fig. 2 and Table 1.

All samples were inoculated into the allantoic cavities of 9- to 11-day-old specific-pathogen-free (SPF) chicken embryos (Beijing Merial Vital Laboratory Animal Technology Co., Ltd., Beijing, China) and incubated 96 h at 37°C. Allantoic fluids from inoculated eggs were harvested either when the embryos were killed or after the two passages; the presence of NDV was confirmed by hemagglutination, as well as hemagglutination inhibition (HI) assay, using La Sota-specific polyclonal sera (Harbin Weike Biotechnology Development Company, Harbin, China) according to the standardized OIE protocols for NDV (15).

Nucleic acid extraction, PCR, and sequencing. For genetic characterization of NDV, total RNA was extracted from infectious allantoic fluid using TriPure RNA isolation reagent (Hoffmann-La Roche, Ltd., Basel, Switzerland) according to the manufacturer's instructions. The extracted RNA was used for reverse transcription with random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Promega Corporation, Madison, WI) according to the manufacturer's instructions.

The amplification for partial F genes of class I and II strains are performed as described in previous studies (16, 17). Conditions for PCR of complete F genes was as follows: 95°C for 3 min, followed by 35 cycles at 95°C for 1 min, 51°C for 45 s, and 72°C for 2 min 30 s, with a final

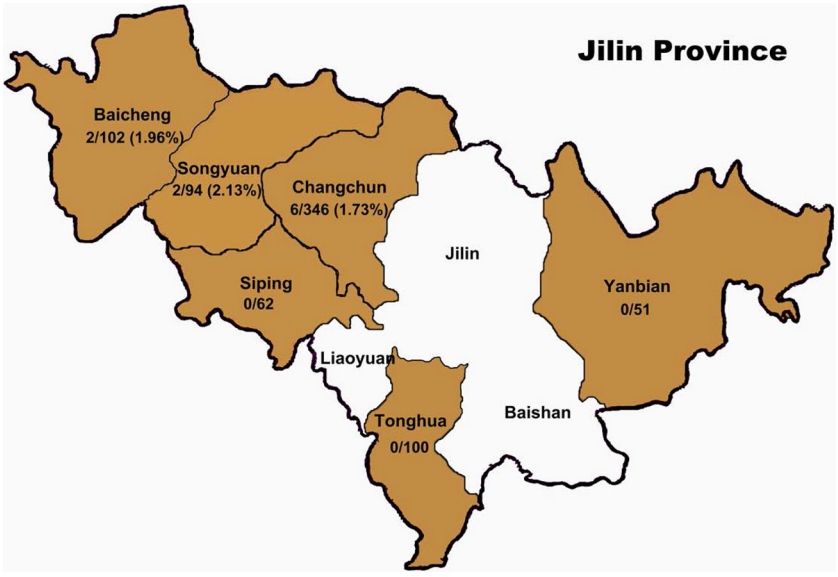


FIG 2 Sample collection sites in Jilin Province. Sampled cities or areas are indicated by color. The number of samples and positive rates of NDV are annotated.

TABLE 1 Samples and isolates obtained from birds in different ecological groups in Jilin Province from 2013 to 2015[a]

Bird	No. (%) of samples obtained during various seasons			No. (%) of samples obtained in LBMs	Total no. (%) of samples
	Autumn migration	Wintering	Spring migration, nesting, and postnesting movements		
Anseriformes					
Swan goose (*Anser cygnoides*)	1/12	0/9	1/217		2/238 (0.84)
Hooded crane (*Grus monacha*)			2/102		2/102 (1.96)
Gaoyou duck (*Anas platyrhynchos domestica*)				2/132	2/132 (1.52)
Galliformes					
Big bone chicken (*Gallus gallus domestica*)				4/283	4/283 (1.41)
Total	1/12 (8.33)	0/9 (0)	3/319 (0.94)	6/415 (1.45)	10/755 (1.32)

[a] The results are presented as the number of samples alone or the total number/number of isolated viruses. The percentages given in parentheses represent the percentages of positive samples.

extension step at 72°C for 10 min. The primer pair sequences used are listed in Table 2. Sequencing of PCR amplicons was conducted by Majorbio (Shanghai, China).

Phylogenetic analysis. In this study, the sequences of class I NDV reference strains of 1a, 1b, and 1c subgenotypes were obtained from GenBank (http://www.ncbi.nlm.nih.gov/GenBank). The accession numbers of the reference NDVs are shown in the phylogenetic trees. Alignment and comparison of the nucleotide and amino acid sequences were performed using the MegAlign program in the Lasergene package (DNASTAR, Inc., Madison, WI). A maximum-likelihood tree was generated using MEGA 6.06 (18).

Pathogenicity tests. The intracerebral pathogenicity index (ICPI) in 1-day-old SPF chickens and mean time to death (MDT) in 9- to 11-day-old SPF embryonated chicken eggs (Beijing Merial Vital Laboratory Animal Technology Co., Ltd., Beijing, China) were determined according to standard OIE procedures (15).

RESULTS AND DISCUSSION

A total of 755 samples were obtained from 16 LBMs and three wetlands from April 2013 to June 2015. Ten NDVs (six from LBMs and four from wild birds) were isolated; five isolates from LBMs were found to cluster with class I subgenotype 1b, two isolates (one from wild bird and one from LBMs) grouped with the vaccine-like class II genotype II, and three (all from wild birds) belonged to class II subgenotype Ib, based on complete F gene sequences (Fig. 3). No virulent NDV strain was isolated. Isolation rates for class I and II NDV were 0.66% (5/755), indicating that both viruses were equally prevalent in the sampled bird population.

All five class II isolates showed the typical lentogenic sequence motifs $_{112}$GRQGR*L$_{117}$ and $_{112}$GKQGR*L$_{117}$ (the asterisk indicates the cleavage site of the F0 precursor protein into its F1 and F2 subunits) (Table 3). The genotype II strain Swan goose/China/Jilin/CC01/2015, which was isolated from a swan goose in the wetlands of Changchun, Jilin Province, in 2015, has a high similarity of complete F gene sequence compared to the duck-origin NDV strain Duck/China/Jilin/SY01/2014 (nucleotide sequence homologies of 100%), which was isolated from LBMs in Songyuan, Jilin Province, in 2014. Both strains share a 99.9% nucleotide similarity in complete F gene sequence to two commonly used live vaccine strains in China, La Sota and clone 30, suggesting that the swan goose and duck strains may be reisolation of a vaccine strain. The remaining three class II NDVs (Swan goose/China/Jilin/CC02/2013, Hooded crane/China/Jilin/BC01/2015, and Hooded crane/China/Jilin/BC02/2015), isolated from wild birds in Baicheng and Changchun of Jilin Province, were clustered into subgenotype Ib, which phylogenetically closed to both wild waterfowl isolates in the Far East (Russia, South Korea, and Japan) and recent isolates from domestic ducks in Asia (Eastern China, Japan, and South Korea) (8, 9, 19–21). Highly similar genotype II NDVs isolated from distinct species in different cities of Jilin Province suggested that the virus may be transmitted between wild birds and domestic poultries in the region and that the NDV isolates from wild birds detected in this study will likely be detected from domestic birds in the future.

The complete F gene of four class I NDVs (Chicken/China/Jilin/CC02/2015, Chicken/China/Jilin/CC03/2014, Duck/China/Jilin/CC04/2015, and Chicken/China/Jilin/CC05/2015) isolated from LBMs shared high genetic identities (nucleotide sequence homologies of 99.7 to 100%) and formed a cluster within subgenotype 1b, suggesting that these isolates had a similar ancestor (Fig. 3). The remaining F gene sequence characterized in this study (Chicken/China/Jilin/SY02/2015) was also nested within subgenotype 1b. Chicken/China/Jilin/SY02/2015 had a typical cleavage site of avirulent class I subgenotype 1b strains, $_{112}$ERQER*L$_{117}$, while Chicken/China/Jilin/CC02/2015, Chicken/China/Jilin/CC03/2014,

TABLE 2 Primers used in this study

Fragment	Primer sequence (5′–3′)	Position	Source or reference
Class I F	CACCAAGCTGGAGAAAGGGCATAC	4285	17
Class I R	CAGTATGTTTGCAGCATTCTGGTTGG	5011	17
Class I 2F	GGAGAAAGGGCATACTATTG	4351	8
Class I 2R	TCTACCCGTATATCTTCACC	6322	8
Class II F	CCATTGCTAAATACAATCCTTTCA	4353	This study
Class II R	CTGCCACTGCTAGTTGTGATAATCC	5060	16
Class II 2R	GCTTCTCTCTCCTCATTCTC	6448	This study

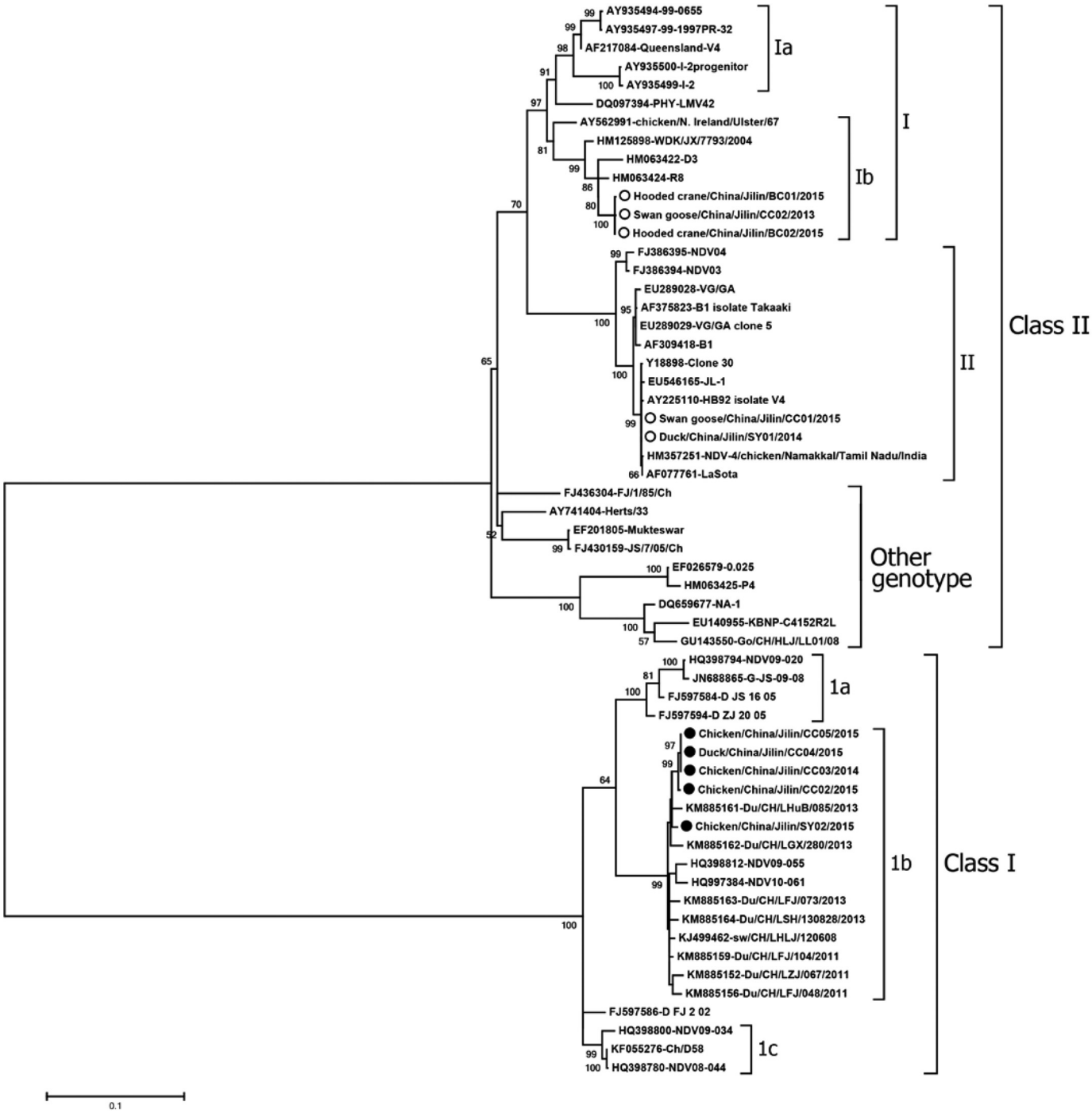


FIG 3 Phylogenetic analysis of complete F gene sequences (1,662 nucleotides). Class I and II sequences are indicated as gray and white circles, respectively. Only bootstrap values of ≥50% are shown. The evolutionary history was inferred by using the maximum-likelihood method based on the general-time-reversible model. The tree with the highest log likelihood (−11262.9423) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum-composite-likelihood approach. A discrete gamma distribution was used to model evolutionary rate differences among sites (four categories [+G, parameter = 0.6973]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.0000% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 58 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1,662 positions in the final data set. Evolutionary analyses were conducted using MEGA 6.06.

Duck/China/Jilin/CC04/2015, and Chicken/China/Jilin/CC05/2015 displayed a motif of $_{112}$ARQER*L$_{117}$ due to a nonsynonymous adenine-to-cytosine substitution at nucleotide position 335. All class I strains belonged to the subgenotype 1b cluster found mainly in domestic ducks and chickens in eastern, southern, and southeastern China (7, 8). Interestingly, similar subgenotype 1b strains were not detected in poultries in northeastern China, even during more intensive sampling periods during 2013 to 2014, suggesting these strains

TABLE 3 Detailed information of NDVs isolated from northeastern China from 2013 to 2015

NDV strain	Host[a]	Yr	Class	Genotype or subgenotype	F cleavage site	MDT (h)	ICPI	Accession no.
Chicken/China/Jilin/CC02/2015	Big bone chicken*	2015	I	1b	ARQERL	>120	0.11	KT892746
Chicken/China/Jilin/CC03/2014	Big bone chicken*	2014	I	1b	ARQERL	>120	0.09	KT892748
Chicken/China/Jilin/SY02/2015	Big bone chicken*	2015	I	1b	ERQERL	>120	0.18	KT892752
Duck/China/Jilin/CC04/2015	Gaoyou duck*	2015	I	1b	ARQERL	>120	0.11	KT892750
Chicken/China/Jilin/CC05/2015	Big bone chicken*	2015	I	1b	ARQERL	>120	0.16	KT892749
Swan goose/China/Jilin/CC01/2015	Swan goose†	2015	II	II	GRQGRL	>120	0.14	KT892747
Duck/China/Jilin/SY01/2014	Gaoyou duck*	2014	II	II	GRQGRL	>120	0.25	KT892751
Swan goose/China/Jilin/CC02/2013	Swan goose†	2013	II	I	GKQGRL	>120	0.34	KT892753
Hooded crane/China/Jilin/BC01/2015	Hooded crane†	2015	II	I	GKQGRL	>120	0.30	KT892754
Hooded crane/China/Jilin/BC02/2015	Hooded crane†	2015	II	I	GKQGRL	>120	0.23	KT892755

[a] *, birds obtained in a live bird market; †, birds obtained in the wild.

may have been introduced into northeast China from eastern, southern, or southeastern China via the poultry trade. To our knowledge, wild birds are the natural reservoirs of NDV (1). Hence, NDV transmitted between migratory birds and domestic poultries also cannot be excluded (22), even though class I NDV was not isolated in Japan and South Korea after 2006 (20). These countries, including China, are all in the East Asian-Australasian flyway, which provides the opportunity for NDV transmission from wild birds to domestic waterfowl via the stopover wetland sites and vice versa (20, 23).

Class I NDV was first isolated in Fujian and Zhejiang Province in 2002 (9) and since then has been spread into most regions of China, including the south, east, and southeast regions, as well as Hong Kong (Fig. 4) (4, 7–9). Class I NDVs are always considered avirulent for chickens; however, a waterfowl-source avirulent virus, goose/Alaska415/91, became virulent after serial passaging in SPF chicken eggs (24, 25). Therefore, class I NDV has the potential risk to virulent by the viral amino acid substitutions and may need to be monitored in the future (24, 25). In addition, previous study indicated that vaccination with avirulent class II NDV vaccine strains, such as La Sota and V4, may fail to prevent virus shedding of ducks infected with class I virus due to considerable differences in their antigenicity (8). Thus, a killed vaccine based on class I NDV may be necessary to protect the domestic poultry industry from potentially virulent strains of class I NDV.

Based on hemagglutination inhibition (HI) and virus neutralization (VN) tests, class I NDV showed a broader cross-antigenicity to other genotype NDV strains (8, 26) and displayed higher HI and VN titers against both class I and class II virulent NDV strains than the current class II vaccine strain La Sota (26). Therefore, to some extent, the wide distribution of class I NDV may protect infected poultry against disease caused by virulent class II NDVs and may be one of the reasons for the decreased incidence of ND outbreaks in China from 2005 to 2015.

NDV recombination has also been reported in some reports during recent years (27–32), though even these events are quite controversial (30, 33). A high rate of NDV coinfection will increase the chance for recombination; therefore, the growing prevalence of class I NDV in wild waterfowl and domestic poultries may recombine with other class I and II circulating viruses to generate new strains with unpredictable phenotypes. More research is needed to elucidate the frequency and impact of NDV recombination among wild and domestic birds.

In conclusion, both class I and II NDVs were prevalent in northeastern China from 2013 to 2015. Some class II viruses of

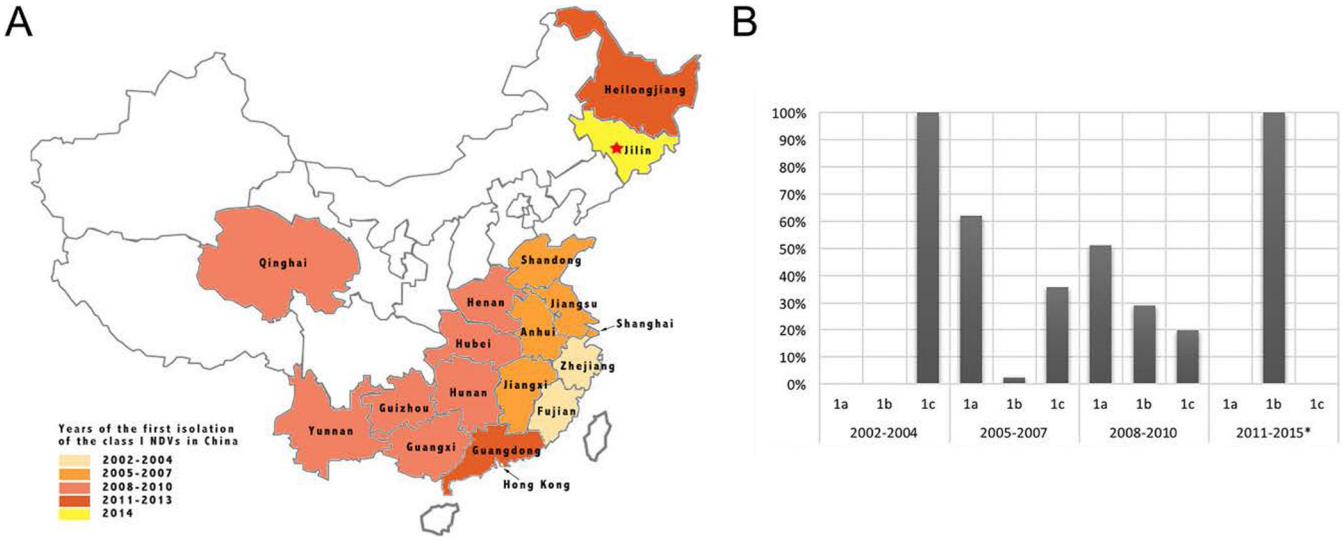


FIG 4 Isolation sites and years (A) and percentages (B) of class I NDV clusters in China. Sampled provinces in this study are indicated by a star in panel A. The asterisk in panel B indicates the data cutoff on 20 November 2015. All data were obtained from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/).

wild bird origin were closely related to the NDV from domestic poultry, suggesting interspecies transmission and the potential for virus recombination into more virulent strains, necessitating the demand for constant surveillance.

ACKNOWLEDGMENTS

This study was partly supported by two grants from National Science Foundation of China (31402195 to R.Y. and 31472195 to Z.D.), two grants from Jilin Provincial Science Technology Department (20140520171JH and 20160414029GH to R.Y.) and one grant from Chinese Special Fund for Agri-scientific Research in the public interest (201303033 to Z.D.), three grants from Jilin University (4305050102TS and 450060501486 to R.Y. and 4305050102S4 to X.L.). None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

FUNDING INFORMATION

Jilin Provincial Science Technology Department provided funding to Renfu Yin under grant numbers 20140520171JH and 20160414029GH. Chinese Special Fund for Agri-scientific Research provided funding to Zhuang Ding under grant number 201303033. National Natural Science Foundation of China (NSFC) provided funding to Renfu Yin under grant number 31402195. National Natural Science Foundation of China (NSFC) provided funding to Zhuang Ding under grant number 31472195. Jilin University (JLU) provided funding to Renfu Yin under grant numbers 4305050102TS and 450060501486. Jilin University (JLU) provided funding to Xinxin Liu under grant number 4305050102S4.

REFERENCES

1. **Swayne DE.** 2013. Diseases of poultry, 13th ed. John Wiley & Sons, Ames, IA.
2. **Czeglédi A, Ujvári D, Somogyi E, Wehmann E, Werner O, Lomniczi B.** 2006. Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. Virus Res **120:** 36–48. http://dx.doi.org/10.1016/j.virusres.2005.11.009.
3. **Miller PJ, Decanini EL, Afonso CL.** 2010. Newcastle disease: evolution of genotypes and the related diagnostic challenges. Infect Genet Evol **10:**26–35. http://dx.doi.org/10.1016/j.meegid.2009.09.012.
4. **Kim LM, King DJ, Suarez DL, Wong CW, Afonso CL.** 2007. Characterization of class I Newcastle disease virus isolates from Hong Kong live bird markets and detection using real-time reverse transcription-PCR. J Clin Microbiol **45:**1310–1314. http://dx.doi.org/10.1128/JCM.02594-06.
5. **Diel DG, da Silva LH, Liu H, Wang Z, Miller PJ, Afonso CL.** 2012. Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Infect Genet Evol **12:**1770–1779. http://dx.doi.org/10.1016/j.meegid.2012.07.012.
6. **Snoeck CJ, Owoade AA, Couacy-Hymann E, Alkali BR, Okwen MP, Adeyanju AT, Komoyo GF, Nakoune E, Le Faou A, Muller CP.** 2013. High genetic diversity of Newcastle disease virus in poultry in West and Central Africa: cocirculation of genotype XIV and newly defined genotypes XVII and XVIII. J Clin Microbiol **51:**2250–2260. http://dx.doi.org/10.1128/JCM.00684-13.
7. **Zhu J, Xu H, Liu J, Zhao Z, Hu S, Wang X, Liu X.** 2014. Surveillance of avirulent Newcastle disease viruses at live bird markets in Eastern China during 2008-2012 reveals a new sub-genotype of class I virus. Virol J **11:** 211. http://dx.doi.org/10.1186/s12985-014-0211-2.
8. **Wu W, Liu H, Zhang T, Han Z, Jiang Y, Xu Q, Shao Y, Li H, Kong X, Chen H, Liu S.** 2015. Molecular and antigenic characteristics of Newcastle disease virus isolates from domestic ducks in China. Infect Genet Evol **32:**34–43. http://dx.doi.org/10.1016/j.meegid.2015.02.016.
9. **Liu X, Wang X, Wu S, Hu S, Peng Y, Xue F, Liu X.** 2009. 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. http://dx.doi.org/10.1080/03079450903183637.
10. **Kim LM, King DJ, Curry PE, Suarez DL, Swayne DE, Stallknecht DE, Slemons RD, Pedersen JC, Senne DA, Winker K, Afonso CL.** 2007. 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. http://dx.doi.org/10.1128/JVI.00843-07.
11. **Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y.** 1992. Evolution and ecology of influenza A viruses. Microbiol Rev **56:**152–179.
12. **Senne DA, Panigrahy B, Kawaoka Y, Pearson JE, Suss J, Lipkind M, Kida H, Webster RG.** 1996. Survey of the hemagglutinin (HA) cleavage site sequence of H5 and H7 avian influenza viruses: amino acid sequence at the HA cleavage site as a marker of pathogenicity potential. Avian Dis **40:**425–437. http://dx.doi.org/10.2307/1592241.
13. **Gould AR, Kattenbelt JA, Selleck P, Hansson E, Della-Porta A, Westbury HA.** 2001. Virulent Newcastle disease in Australia: Molecular epidemiological analysis of viruses isolated prior to and during the outbreaks of 1998-2000. Virus Res **77:**51–60. http://dx.doi.org/10.1016/S0168-1702(01)00265-9.
14. **Alexander DJ, Campbell G, Manvell RJ, Collins MS, Parsons G, McNulty MS.** 1992. Characterisation of an antigenically unusual virus responsible for two outbreaks of Newcastle disease in the Republic of Ireland in 1990. Vet Rec **130:**65–68. http://dx.doi.org/10.1136/vr.130.4.65.
15. **OIE.** 2008. Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds, and bees, 6th ed, p 1092–1106. International Office of Epizootics, World Organization for Animal Health, Paris, France.
16. **Yang CY, Shieh HK, Lin YL, Chang PC.** 1999. Newcastle disease virus isolated from recent outbreaks in Taiwan phylogenetically related to viruses (genotype VII) from recent outbreaks in western Europe. Avian Dis **43:**125–130. http://dx.doi.org/10.2307/1592771.
17. **Wu S, Wang W, Yao C, Wang X, Hu S, Cao J, Wu Y, Liu W, Liu X.** 2011. Genetic diversity of Newcastle disease viruses isolated from domestic poultry species in Eastern China during 2005-2008. Arch Virol **156:** 253–261. http://dx.doi.org/10.1007/s00705-010-0851-5.
18. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: molecular evolutionary genetics analysis, version 6.0. Mol Biol Evol **30:** 2725–2729. http://dx.doi.org/10.1093/molbev/mst197.
19. **Sakai K, Sakabe G, Tani O, Watanabe Y, Jahangir A, Nakamura M, Takehara K.** 2007. Characterization of Newcastle disease virus isolated from northern pintail (*Anas acuta*) in Japan. J Vet Med Sci **69:**1307–1311. http://dx.doi.org/10.1292/jvms.69.1307.
20. **Lee EK, Jeon WJ, Kwon JH, Yang CB, Choi KS.** 2009. Molecular epidemiological investigation of Newcastle disease virus from domestic ducks in Korea. Vet Microbiol **134:**241–248. http://dx.doi.org/10.1016/j.vetmic.2008.08.020.
21. **Kim BY, Lee DH, Kim MS, Jang JH, Lee YN, Park JK, Yuk SS, Lee JB, Park SY, Choi IS, Song CS.** 2012. Exchange of Newcastle disease viruses in Korea: the relatedness of isolates between wild birds, live bird markets, poultry farms and neighboring countries. Infect Genet Evol **12:**478–482. http://dx.doi.org/10.1016/j.meegid.2011.12.004.
22. **Muzyka D, Pantin-Jackwood M, Stegniy B, Rula O, Bolotin V, Stegniy A, Gerilovych A, Shutchenko P, Stegniy M, Koshelev V, Maiorova K, Tkachenko S, Muzyka N, Usova L, Afonso CL.** 2014. Wild bird surveillance for avian paramyxoviruses in the Azov-black sea region of Ukraine (2006 to 2011) reveals epidemiological connections with Europe and Africa. Appl Environ Microbiol **80:**5427–5438. http://dx.doi.org/10.1128/AEM.00733-14.
23. **Takakuwa H, Ito T, Takada A, Okazaki K, Kida H.** 1998. Potentially virulent Newcastle disease viruses are maintained in migratory waterfowl populations. Jpn J Vet Res **45:**207–215.
24. **Shengqing Y, Kishida N, Ito H, Kida H, Otsuki K, Kawaoka Y, Ito T.** 2002. Generation of velogenic Newcastle disease viruses from a nonpathogenic waterfowl isolate by passaging in chickens. Virology **301:**206–211. http://dx.doi.org/10.1006/viro.2002.1539.
25. **Tsunekuni R, Ito H, Otsuki K, Kida H, Ito T.** 2010. Genetic comparisons between lentogenic Newcastle disease virus isolated from waterfowl and velogenic variants. Virus Genes **40:**252–255. http://dx.doi.org/10.1007/s11262-009-0427-1.
26. **Meng C, Qiu X, Jin S, Yu S, Chen H, Ding C.** 2012. Whole genome sequencing and biological characterization of Duck/JS/10, a new lentogenic class I Newcastle disease virus. Arch Virol **157:**869–880. http://dx.doi.org/10.1007/s00705-012-1248-4.
27. **Han GZ, He CQ, Ding NZ, Ma LY.** 2008. Identification of a natural multi-recombinant of Newcastle disease virus. Virology **371:**54–60. http://dx.doi.org/10.1016/j.virol.2007.09.038.

28. **Qin Z, Sun L, Ma B, Cui Z, Zhu Y, Kitamura Y, Liu W.** 2008. F gene recombination between genotype II and VII Newcastle disease virus. Virus Res **131:**299–303. http://dx.doi.org/10.1016/j.virusres.2007.10.001.
29. **Miller PJ, Kim LM, Ip HS, Afonso CL.** 2009. Evolutionary dynamics of Newcastle disease virus. Virology **391:**64–72. http://dx.doi.org/10.1016/j.virol.2009.05.033.
30. **Chong YL, Padhi A, Hudson PJ, Poss M.** 2010. The effect of vaccination on the evolution and population dynamics of avian paramyxovirus-1. PLoS Pathog **6:**e1000872. http://dx.doi.org/10.1371/journal.ppat.1000872.
31. **Zhang R, Wang X, Su J, Zhao J, Zhang G.** 2010. Isolation and analysis of two naturally occurring multi-recombination Newcastle disease viruses in China. Virus Res **151:**45–53. http://dx.doi.org/10.1016/j.virusres.2010.03.015.
32. **Han GZ, Worobey M.** 2011. Homologous recombination in negative sense RNA viruses. Viruses **3:**1358–1373. http://dx.doi.org/10.3390/v3081358.
33. **Afonso CL.** 2008. Not so fast on recombination analysis of Newcastle disease virus. J Virol **82:**9303. http://dx.doi.org/10.1128/JVI.01231-08.