

Complete Genome Sequence of a Subgenotype VIId Newcastle Disease Virus Circulating Predominantly in Chickens in China

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At least 11 genotypes of class II viruses have been identified since the discovery of Newcastle disease virus (NDV) in 1926. Here, we reported the complete genome sequence of a prevalent NDV variant from China, belonging to subgenotype VIId in class II. The similar viruses have been the predominant strains circulating in China for the past decade, which occupied over 80% of Chinese prevalent strains and were phylogenetically different from currently available vaccines.

N ewcastle disease (ND), caused by Newcastle disease virus (NDV), is one of the most important infectious diseases of poultry because of its worldwide distribution and the potential for devastating losses (1, 8). NDV belongs to the genus *Avulavirus* in the family *Paramyxoviridae*, and its genome is a negative-sense, single-stranded, nonsegmented, enveloped RNA virus of approximately 15.2 kb that codes for six major structural proteins (1, 2, 8, 9, 12).

Based on genetic and antigenic analyses of NDV strains, the viruses could be classified into two distinct clades: class I and class II (2, 3). To date, at least 11 genotypes (I to XI) have been identified in class II, and among them, strains of genotype VII were responsible for most of the outbreaks of ND reported in recent years in many countries throughout the world (1, 3-9, 11). Constant epidemiological surveillance and proactive characterization of circulating strains are needed to detect future variability worldwide.

The YZCQ/Liaoning/08 (YZCQ) virus was isolated from a vaccinated broiler flock with up to 60% mortality in the Liaoning province of China in 2008 (8). The whole genome of YZCQ was amplified by reverse transcription (RT)-PCR as reported previously (12). The 18 segments were cloned into pMD18-T vector (TaKaRa, Japan) and sequenced at BGI (Beijing, China). Sequence alignment was performed using the MegAlign program of DNAstar version 3.1 (Madison, WI). A phylogenetic tree was constructed with the program MEGA4.0 using the neighbor-joining and maximum-likelihood methods (10). The genome of the YZCQ isolate was found to be 15,192 nucleotides (nt) in length [excluding the poly(A) tail], which contains a previously described 6-nt insert in the downstream untranslated region of the NP gene, compared to the common vaccine strains LaSota, Clone-30, and B1 (12).

The sequence of the F protein cleavage site is a major determinant of NDV pathogenicity, and virulent NDV strains usually contain multiple basic residues at the site. Strain YZCQ had a cleavage site sequence of $^{112}\underline{R}-\underline{R}-Q-\underline{K}-\underline{R}\downarrow F^{117}$, which was the most common motif of virulent NDVs, especially in genotype VII viruses (8).

The complete genome of YZCQ had 95.40% to 98.16% nucleotide sequence identity with the genomes of the velogenic reference strains reported in GenBank (4, 8, 12). By BLAST analysis, the obtained sequence was closest to that of chicken/China/ SDSG01/2011 (accession number JN400896), a subgenotype VIId isolate from the Shandong province of China. The isolate showed lower nucleotide sequence homologies of 82.71% and 82.64%, respectively, with strain LaSota and Clone-30. The similar variants have been predominantly circulating in China (8, 12). Phylogenetic analysis further indicated that strain YZCQ belonged to subgenotype VIId in genotype VII of class II and was genetically distinct and phylogenetically distant from the vaccine strains (Lasota, AY845400; Clone-30, Y18898), and clustered in different groups. Large phylogenetic and antigenic distances between vaccines and current circulating virulent strains may facilitate the evolution of virulent NDV, which should be a major reason for the continuous outbreaks of ND in China.

Nucleotide sequence accession number. The complete genome sequence of YZCQ/Liaoning/08 has been deposited in GenBank under the accession number JX867334.

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Received 26 September 2012 Accepted 27 September 2012 Address correspondence to Guozhong Zhang, zhanggz@cau.edu.cn. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JVI.02663-12 fication of an original genotype possibly deriving from a died out ancestor of genotype IV. PLoS One 5:e13987. doi:10.1371/journal.pone.0013987.

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