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GENOME ANNOUNCEMENT

Complete Genome Sequence of a Newcastle Disease Virus Strain Isolated from Broiler Breeder Flocks in China

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In 2010 and 2011, several devastating Newcastle disease (ND) outbreaks occurred in China, affecting broilers, layers, and breeders. The CK-JSX1-201005 virus was isolated from broiler breeder flocks vaccinated with the classical ND virus (NDV) vaccine program, but laying rate decreased from 80% to 30 to 40% in the clinic. Here, we report the complete genome sequence and molecular characteristic of the CK-JSX1-201005 NDV. These findings provide additional insights into the genetic variation of NDV circulating in China and are useful for vaccine development for NDV.

Newcastle disease (ND), caused by Newcastle disease virus (NDV), is included in list A of the Office of International Epizootes (OIE) (2). NDV strains can be classified as highly virulent, intermediate, or nonvirulent based on their pathogenicity in embryonating eggs or chickens (1, 3). Ten genotypes (I to X) have been identified to date for NDV strains by molecular approaches, and genotype VII can be further divided into five (VIIa to VIIe) subgenotypes (5, 6, 9). The complication of the NDV genome and the extensive use of live NDV vaccines resulted in diversity of the NDV epizootiology.

We had isolated 42 NDV isolates from different farms of nine provinces of China from 2010 to 2011. The CK-JSX1-201005 virus was isolated from broiler breeder flocks immunized with live vaccines and boosted with inactive vaccines, with the laying rate decreased from 80% to 30 to 40% in the clinic. The whole sequence of the CK-JSX1-201005 NDV was amplified and cloned into the pMD19-T vector (TaKaRa Bio Inc., Japan), followed by sequencing three times using an ABI 3730 Sanger-based genetic analyzer (Carlsbad, CA). The sequences were assembled using DNAStar (version 7). Multiple-sequence alignment was performed with Clustal X (BioEdit version 7). A phylogenetic tree was constructed for genome sequences using the MEGA 5.1 program (12).

The entire genome sequence of the CK-JSX1-201005 NDV was 15192 nucleotides in length and encoded six genes with an order of 3′-NP-P-M-F-HN-L-5′. There was a 6-nt insert (TCCCAC) in the 5′-noncoding sequence region (NCR) of the NP gene, which was considered to be a genetic marker of class II genotype V to VIII NDV strains (4). The F protein cleavage site, indicative of pathogenicity, was ^{112}RRQRR ↓ F^{117}, which met the characteristic of highly virulent NDV strains (7, 10, 13). The HN protein of the CK-JSX1-201005 isolates was 571 amino acids long, which was the characteristic of the III to VIII virulent strains (11). The F protein of NDVs had six potential *N*-glycosylation sites, five in the ectodomain (at amino acids 85, 191, 366, 447, and 471) and one in the cytoplasmic domain at amino acid 542, that were highly conserved among NDV isolates (8). But the 542 *N*-glycosylation site of the CK-JSX1-201005 NDV was deleted because of a novel single amino acid substitution (T to A) at residue 543. Phylogenetic analysis indicated that the CK-JSX1-201005 NDV belonged to genotype VII and more specifically to subgenotype VIId. Genotype VIId NDV had been the predominant pathogen responsible for most Newcastle disease outbreaks in China. In addition, CK-JSX1-201005 was approximately 95.0% to 97.5% homologous with the virulent NDV sequences in GenBank but only about 82.5% to 88.4% homologous with the LaSota, B1, Mukteswar, and F48E8 vaccine strains used to date in China. These results suggested that CK-JSX1-201005 was significantly different from the vaccine strains and could potentially lead to poor protection by vaccination. Therefore, genotype-homologous vaccines should be developed in countries that experience significant economical burdens due to ND outbreaks.

Nucleotide sequence accession number. The complete genome sequence of the CK-JSX1-201005 is available from GenBank under accession number JX519467.

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