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GENOME SEQUENCES

Complete Genome Sequences of 11 Newcastle Disease Virus Isolates of Subgenotype VII.2 from Indonesia

Iryna V. Goraichuk,[a] Dawn Williams-Coplin,[a] Michael H. Wibowo,[b] Peter A. Durr,[c] Widya Asmara,[b] Sidna Artanto,[b] Kiril M. Dimitrov,[a] Claudio L. Afonso,[a] David L. Suarez[a]

[a]Exotic and Emerging Avian Viral Disease Research Unit, Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, ARS, USDA, Athens, Georgia, USA
[b]Department of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
[c]CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia

ABSTRACT We report the complete genome sequences of 11 virulent Newcastle disease viruses. The isolates were obtained from vaccinated broiler and layer chickens in three different provinces of Indonesia in 2013 and 2014. Phylogenetic analysis revealed that all isolates belong to subgenotype VII.2 in the class II cluster.

Virulent Newcastle disease virus (vNDV; genus *Orthoavulavirus*, subfamily *Avulavirinae*, family *Paramyxoviridae*) causes Newcastle disease, and the first reported outbreaks of this severe disease of poultry occurred in 1926 in Java, Indonesia (1). Since the 1950s, vaccination has been an important control strategy for reducing the clinical disease associated with infection, but vaccination has not been an effective eradication tool, and Newcastle disease is endemic in Indonesia and many other countries (2, 3).

Eleven vNDVs were isolated from vaccinated broiler and layer flocks in three Indonesian provinces (Table 1). The presence of NDV in swabs was confirmed by the detection of viral RNA using the real-time reverse-transcription PCR described previously (4). The isolates were propagated in 9-day-old specific-pathogen-free embryonating chicken eggs, following standard procedures (5). Viral RNA was isolated from allantoic fluid using the QIAamp viral RNA minikit (Qiagen, USA). The Illumina libraries were prepared using the KAPA stranded RNA sequencing (RNA-Seq) library preparation kit (Kapa Biosystems, USA) as per the manufacturer's instructions. The distribution size and concentration of the prepared libraries were checked on a Bioanalyzer 2100, using a high-sensitivity (HS) DNA kit (Agilent Technologies, Germany), and Qubit fluorometer, using a double-stranded DNA (dsDNA) HS assay kit (Life Technologies, USA), respectively. Next-generation paired-end sequencing (2 × 150 bp) was performed on a MiSeq instrument using the 300-cycle MiSeq reagent kit v. 2 (Illumina, USA). Sequence data were assembled using MIRA3 v. 0.0.1 (6) within a customized workflow on the Galaxy platform (7), as described previously (8, 9). The MiSeq run generated from 34,698 to 6,631,803 total paired-end reads per sample (Table 1). All final consensuses were called from the raw reads that were aligned to the *de novo*-generated contig using BWA-MEM (10), were 15,192 nucleotides (nt) long (100% genome coverage as estimated based on the size of NDV isolates in NCBI RefSeq accession number NC039223), and had 47% GC content. The complete genome sequences comply with the paramyxovirus "rule of six" (11) and contain six open reading frames (3′-NP-P-M-F-HN-L-5′) that were identified using Geneious 11.1 and confirmed by alignment with published NDV genomes. Phylogenetic analysis in MEGA v. 7.0.26 revealed that the full genomes of the 11 presented isolates had 0.00 to 1.6% pairwise nucleotide distance compared to each other, which indicates a high level of identity. Initial BLAST comparison to the currently available full-length NDV genome sequences showed the highest (99.00 to

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Address correspondence to David L. Suarez, david.suarez@usda.gov.

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TABLE 1 Isolates, sampling locations, dates, sequencing metrics, and accession numbers of genomes of the virulent Newcastle disease viruses in this report

Isolate name	Collection date (mo/day/yr)	Location (province)	Total no. of raw read pairs	No. of mapped reads	Median coverage depth (reads)	Mean read length (nt)	GenBank accession no.	SRA accession no.
broiler/Indonesia/Muntilan-1P-I1/972/2014	2/7/2014	Central Java	934,601	814,170	8,134	149	MN557401	SRR10259372
broiler/Indonesia/Muntilan-2P-I2/973/2014	2/19/2014	Central Java	250,076	213,676	8,134	145	MN557402	SRR10259371
broiler/Indonesia/Muntilan-2L-I3/974/2014	2/19/2014	Central Java	1,159,772	1,050,511	10,780	154	MN557403	SRR10259369
layer/Indonesia/KP-145-I4/975/2013	12/3/2013	Yogyakarta	2,566,465	80,771	821	164	MN557404	SRR10259368
layer/Indonesia/GK-SR1-I5/976/2013	11/15/2013	Yogyakarta	6,631,803	1,351,918	14,976	175	MN557405	SRR10259367
layer/Indonesia/Jatim3-I6/977/2014	5/3/2014	East Java	34,698	31,540	331	158	MN557406	SRR10259366
layer/Indonesia/GK-SR2-I7/978/2013	11/15/2013	Yogyakarta	1,740,267	1,343,434	14,424	166	MN557407	SRR10259365
layer/Indonesia/Jatim-SDD-I8/979/2014	6/26/2014	East Java	533,641	342,590	4,123	203	MN557408	SRR10259364
layer/Indonesia/BYL1-I9/980/2014	1/12/2014	Central Java	893,981	640,443	8,134	202	MN557409	SRR10259363
layer/Indonesia/BYL2-I10/981/2014	1/12/2014	Central Java	775,809	640,419	6,715	162	MN557410	SRR10259362
layer/Indonesia/BYL3-I11/982/2014	1/12/2014	Central Java	420,674	365,239	8,134	146	MN557411	SRR10259370

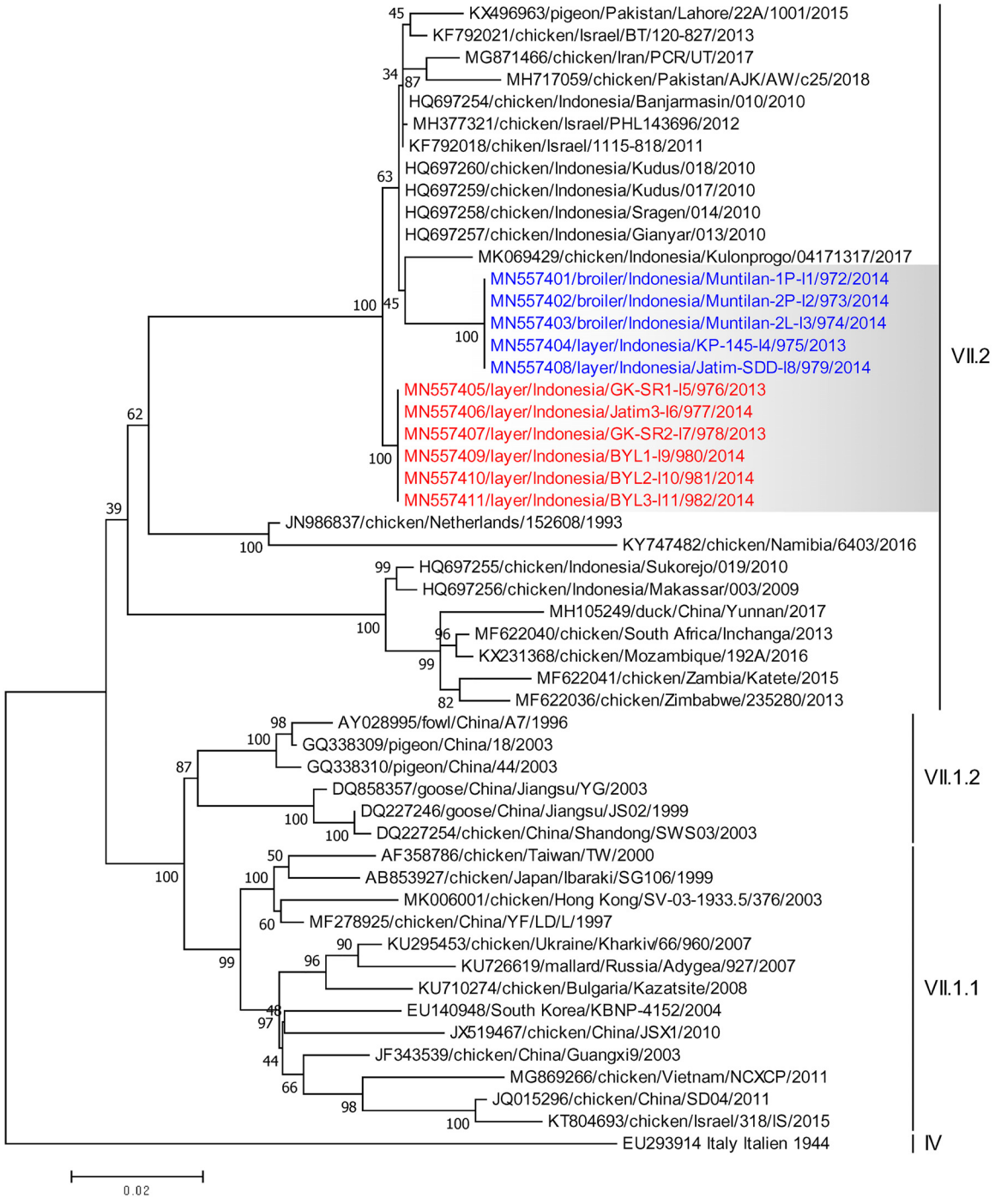

FIG 1 Phylogenetic analysis of NDV isolates of genotype VII based on the complete fusion gene sequences constructed with the maximum likelihood method, based on the general time-reversible model in MEGA v. 7.0.26. The tree with the highest log likelihood (−7,821.76) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The initial tree(s) for the heuristic search was obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with the superior log likelihood value. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.4168]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 33.07% of sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 52 nucleotide sequences (the sequence from genotype IV is included as an outgroup). All positions containing gaps and missing data were eliminated. There were a total of 1,662 positions in the final data set. The isolates used in this study, which cluster into two distinct branches, are shown in blue and red.

99.29%) nucleotide identity to the highly vNDV strain chicken/Indonesia/Banjarmasin/010/2010 (GenBank accession number HQ697254) (12). Detailed phylogenetic analysis based on the complete fusion gene classified all 11 isolates as members of subgenotype VII.2 together with other NDV isolates from Indonesia, Pakistan, and the Middle East (13) (Fig. 1). The phylogenetic tree revealed that the 11 Indonesian isolates characterized in this study cluster into two distinct branches.

Amino acid analysis showed that the fusion protein cleavage sites of all 11 isolates (major molecular determinant of virulence for NDV) (5, 14) contained a polybasic amino acid motif and a phenylalanine at position 117 (^{113}RQKR ↓ F^{117}), which is typical for vNDV. The sequence data described here provide evidence that vNDV strains were circulating among vaccinated flocks in three different provinces of Indonesia during 2013 to 2014. Vaccination can prevent or reduce clinical disease, but NDV can still circulate in vaccinated flocks (15–17), as also demonstrated here. These facts highlight the need for continuous vaccine evaluation and development of improved vaccines for disease control (18, 19).

Data availability. The complete genome sequences of all 11 isolates have been deposited in GenBank under the accession numbers MN557401 through MN557411. The raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA576938.

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