Here, we report two genomes of newly emerged strains of Newcastle disease virus (NDV), Chicken/Indonesia/Tangerang/004WJ/14 and Chicken/Indonesia/VD/003WJ/11, from disease outbreaks in chickens in Indonesia. Phylogenetic study results of the fusion (F) protein’s gene-coding sequences of different genotypes of NDV revealed that these two strains belong to genotype VII.2 in the class II cluster of avian paramyxoviruses.

Newcastle disease (ND) still causes high mortality and reduces profitability in the chicken industry in Southeast Asia and is an endemic disease in Indonesia. Newcastle disease virus (NDV) is a member of the genus Avian orthoavulavirus 1 within the new subfamily Avulavirinae of the family Paramyxoviridae (1). Genotypes VII.1.1 (subgenotypes b, d, e, j, and l) and VII.2 (subgenotypes a, h, i, and k) caused an ND panzootic in Africa, Europe, the Middle East, and Asia (2–4). In this study, we compared the full-length genomes of newly emerged strains of genotype VII NDVs to that of the currently used vaccine strain, LaSota. Strains Chicken/Indonesia/Tangerang/004WJ/14 (Tangerang) and Chicken/Indonesia/VD/003WJ/11 (VD) were isolated from the brain samples of two chickens vaccinated against NDV with the live LaSota vaccine. The viruses were isolated from two layer farms with high mortality located in different geographical locations in West Java, Indonesia, in 2011 and 2014. The viruses were isolated by inoculating embryonated specific-pathogen-free (SPF) chicken eggs and harvesting allantoic fluid according to the World Organisation for Animal Health (OIE) standard protocol (5). The pathogenicity of these strains was measured by mean death time (MDT) assay according to the Food and Agriculture Organization (FAO) manual (6). A QIAamp viral RNA minikit (Qiagen, USA) was used for RNA extraction, and the extracted RNA was submitted to the Australian Cancer Research Foundation (ACRF) for RNA sequencing. The cDNA library and sequencing were performed by the ACRF using a random hexamer approach (stranded mRNA-Seq kit; Kapa Biosystems, USA) as per the manufacturer’s recommendations. The Illumina MiSeq platform v3 was used for sequencing cDNA libraries and generated 2 × 300-nucleotide (nt) reads. After removing the adaptors and low-quality reads with Trimmomatic v0.36 software (7), Unicycler v0.4.4 software was used for de novo assembly of a total of 817,686 reads for sample 1 and 764,502 reads for sample 2. The final assembled reads were visualized using Bandage (8). Contigs were compared to the
FIG 1 Molecular phylogenetic analysis by the maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (14). The bootstrap consensus tree (Continued on next page)
nucleotide collection using NCBI BLAST, and two NDV contigs for Tangerang and VD were identified, with 46.52% and 46.49% genome GC contents, 15,096 and 15,179 nucleotide lengths, and 818-fold and 534-fold coverages, respectively. These contigs were compared to the Indonesian genotype VII strain Chicken/Sukorejo/019/10 (Sukorejo; GenBank accession number HQ697255.1) and showed similarities of 97.90% and 98.95%, respectively. In the contig of Tangerang, some contaminating sequences of Pseudomonas spp. were observed after an NCBI BLAST search at the end of the sequence; they were removed with BioEdit, and reverse transcriptase PCR (RT-PCR; OneStep Ahead RT-PCR, Qiagen, USA) and Sanger sequencing were used to close the detected gaps in this sequence after alignment to Sukorejo (GenBank accession number HQ697255.1). The Clustal X (9) and Genericus Primer (10) software programs were used to align and annotate genes. All tools were run with default parameters.

Both Tangerang and VD are associated with severe neurological symptoms in infected chickens and had a mean death time (MDT) of 60 to 67 h. These two strains are similar at the C terminus of the F protein cleavage site, which is a key molecular marker for NDV pathogenicity (11, 12). The 111RRKRF117 amino acid sequence motif is the same as that in Sukorejo, as the reference strain for the Indonesian genotype VII of NDVs. Phylogenetic analysis of F gene sequences carried out using MEGA7 software (13) suggests that these strains circulating in Indonesia belong to genotype VII.2 (Fig. 1), the main genotype causing recent NDV outbreaks in Indonesia. Importantly, the amino acid sequences for the viral N, P, M, F, HN, and L proteins for these two strains were similar and have percent identities of 92%, 81%, 88%, 89%, 85%, and 94% to strain LaSota (GenBank accession number AF077761.1), respectively.

These significant differences in the amino acid identities of circulation viruses and strain LaSota, as the most common vaccine strain used in Indonesia, shed more light on the probable reason for vaccine failure against these NDV strains and highlight the urgent need for updated vaccine development strategies in Southeast Asia.

Data availability. The GenBank accession numbers for Tangerang and VD are MN699677 and MN699676, respectively. The BioSample SRA run numbers are SRR11593162 for Tangerang and SRR11593166 for VD.

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