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GENOME SEQUENCES

First Complete Genome Sequence of a Subgenotype Vd Newcastle Disease Virus Isolate

Iryna V. Goraichuk,[a,b] Peter L. M. Msoffe,[c] Gaspar H. Chiwanga,[d] 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]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine
[c]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, Tanzania
[d]Tanzania Veterinary Laboratory Agency, Mtwara, Tanzania

ABSTRACT A Newcastle disease virus was isolated from a chicken from a live bird market in the Mbeya region of Tanzania. Complete genome characterization of the isolate identified it as a member of subgenotype Vd. This is the first complete genome sequence of this subgenotype.

Newcastle disease virus (NDV), a member of the genus *Avulavirus* within the family *Paramyxoviridae*, has a negative-sense single-stranded nonsegmented RNA genome (1). Although it represents a single serotype, NDV has at least 19 different genotypes divided into two distinct classes (2, 3). To date, only partial genomes of subgenotype Vd viruses are available in public databases (4, 5). In this study, we report the first isolation of a subgenotype Vd virus from Tanzania. This virus was isolated from a chicken in 2012, and the obtained sequence represents the first subgenotype Vd complete genome.

A virulent NDV from a cloacal swab collected from a chicken at a live bird market in the Mbeya region of Tanzania in 2012 was isolated at the Southeast Poultry Research Laboratory of the USDA in Athens, GA. The virus was propagated in 9-day-old specific-pathogen-free embryonating chicken eggs, and the intracerebral pathogenicity index (ICPI) was estimated following standard procedures (6). Viral RNA was isolated from allantoic fluid using the QIAamp viral RNA minikit (Qiagen, USA). The Illumina libraries were prepared using a stranded RNA-Seq library preparation kit (KAPA Biosystems, USA) 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 a Qubit fluorometer, using a double-stranded DNA (dsDNA) HS assay kit (Life Technologies, USA), respectively. Next-generation paired-end sequencing (2 × 250 bp) was performed on a MiSeq instrument using the 500-cycle MiSeq reagent kit v.2 (Illumina, USA). Sequence data were assembled using a *de novo* approach and utilizing MIRA v.3.4.1 (7) within a customized workflow on the Galaxy platform (8), as described previously (9). A total of 805,566 raw paired-end reads were generated. A single NDV contig was assembled. The final consensus was 15,180 nucleotides (nt) long (99.9% genome coverage), was called from 508,118 NDV raw reads using BWA-MEM (10), and had 46.6% GC content. The median read depth coverage of the NDV assembly was 5,508×, and the maximum depth coverage was 9,354×. Six nucleotides missing at both the 5′ and 3′ ends of the obtained consensus sequence were sequenced utilizing a single 3′-nucleotide tailing reaction of both the genomic RNA and the full-length positive-sense antigenomic RNA, followed by a single reverse transcription reaction targeted to the common polynucleotide tails, as described previously (11). The final genome consensus of the isolated

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

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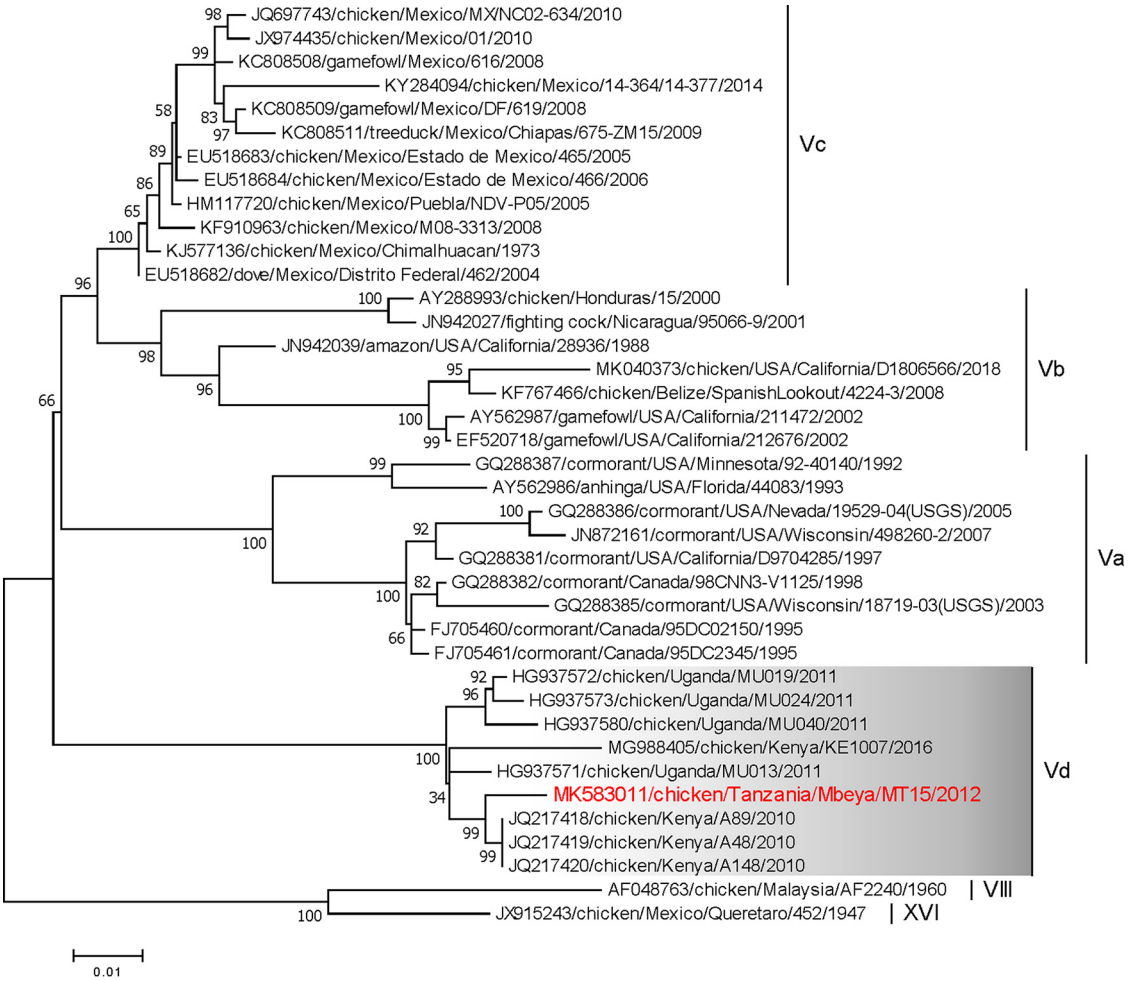

FIG 1 Phylogenetic analysis of NDV isolates of genotype V; this was based on the complete fusion gene sequences constructed with the maximum likelihood method from the general time-reversible model in MEGA version 7.0. The tree with the highest log likelihood (−7,045.63) is shown. The percentage for which the associated taxa clustered together is shown next to each branch. The initial tree(s) for the heuristic search was obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then the topology with the superior log likelihood value was selected. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.4593]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 33.48% of the sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 39 nucleotide sequences (sequences from genotypes VIII and XVI are included as an outgroup). All positions containing gaps and missing data were eliminated. There were a total of 1,646 positions in the final data set. The isolate used in this study is shown in red.

strain, designated chicken/Tanzania/Mbeya/MT15/2012 (here, MT15), comprises 15,192 nucleotides and was annotated using the annotation tool of Geneious 9.1.8. The genome complies with the paramyxovirus "rule of six" (12) and contains six open reading frames (ORFs) (3′-NP-P-M-F-HN-L-5′) of 1,470 nt, 1,188 nt, 1,095 nt, 1,662 nt, 1,716 nt, and 6,615 nt in length, respectively. The ORFs were identified using Geneious and confirmed by alignment with published NDV genomes. A preliminary BLAST search of the complete genome sequence showed 99% nucleotide identity with the genotype V incomplete genomes of chicken/Kenya/A89/2010 (14,888 nt; GenBank accession number JQ217418) and chicken/Kenya/A48/2010 (14,945 nt; GenBank accession number JQ217419). Detailed phylogenetic analysis based on the complete fusion gene (13) classified MT15 as a member of subgenotype Vd, together with isolates from Kenya (obtained in 2010 to 2016) and Uganda (obtained in 2011) (Fig. 1). The MT15 isolate shared 98.3% and 97.6% nucleotide identity with the Kenyan and Ugandan viruses, respectively.

According to the World Organisation for Animal Health, an ICPI value of 0.7 or

greater or the presence of at least three basic amino acids at the fusion protein cleavage site indicates a virulent form of NDV (6), while values above 1.5 are typical for velogenic viruses (14). Analysis of the deduced amino acid sequence of the fusion protein cleavage site (6, 15) of MT15 showed a polybasic amino acid motif and a phenylalanine at position 117 (^{112}RRQKR↓F^{117}), which is typical for virulent NDV strains. This result was consistent with the ICPI value of 1.86 (6). Velogenic Newcastle disease viruses are important pathogens, and the sequence information provides valuable information for characterizing and tracking the viruses.

Data availability. The complete genome sequence of chicken/Tanzania/Mbeya/MT15/2012 has been deposited in GenBank under the accession number MK583011. Raw data were deposited in the SRA under accession number SRR9071773, BioSample number SAMN11660474, and BioProject number PRJNA543308.

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