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Complete genome sequence of seven virulent Newcastle disease virus isolates of sub-genotype XIII.1.1 from Tanzania

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ABSTRACT We report the complete genome sequences of seven virulent Newcastle disease viruses (NDVs) that were isolated from chickens from live bird markets in the Arusha, Iringa, Mbeya, and Tanga regions of Tanzania in 2012. Phylogenetic analysis revealed that all isolates belong to sub-genotype XIII.1.1.

KEYWORDS Newcastle disease virus, NDV, Avian avulavirus 1, Avian orthoavulavirus 1, orthoavulavirus javaense, XIII.1.1, complete genome, next-generation sequencing, live bird market, Tanzania

Newcastle disease virus (NDV) is a single-stranded, non-segmented RNA virus that belongs to the family *Paramyxoviridae* (1). It has a single serotype and at least 22 different genotypes that can be divided into two classes (2–4). Outbreaks of velogenic NDV (vNDV) occur worldwide, and the disease is endemic in many countries in Africa, Asia, the Middle East, and Central and South America (5–7).

In this study, 36 vNDVs were isolated from 796 oropharyngeal swabs collected from chickens at live bird markets in six regions of Tanzania in 2012, as described previously (8). Out of these 36 vNDVs, seven isolates were chosen for whole-genome sequencing (Fig. 1A). The viruses were propagated in 9-day-old specific-pathogen-free embryonating chicken eggs, and the intracerebral pathogenicity index (ICPI) was estimated following standard procedures (9). Viral RNA was isolated from the allantoic fluid using the Trizol LS Reagent (Invitrogen, USA). The Illumina libraries were prepared using the KAPA Stranded 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 the Agilent High Sensitivity DNA Kit (Agilent Technologies, Germany) and a Qubit fluorometer, using the dsDNA HS Assay Kit (Life Technologies, USA), respectively. Next-generation paired-end sequencing was performed on an Illumina 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 version 3.4.1 (10) within a customized workflow on the Galaxy platform (11), as described previously (12, 13).

The MiSeq run generated from 671,067 to 2,263,648 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 contigs using BWA-MEM (14) and were 15,192 nucleotides long with 100% genome coverage. Phylogenetic analysis in MEGA 7.0.26 revealed that all isolates had a 0.003 to 0.065% pairwise distance value (*P*-distance) compared to each other, which indicates a high level of nucleotide identity (93.5 to 99.7%) among them. The initial NCBI BLASTn (15) comparison to the currently available full-length NDV genome sequences showed that four isolates (AC53, N1, IM40, and N34) had the highest nucleotide identity (98.93 to 99.58%) to a previously published vNDV strain chicken/Tanzania/Tanga/N38/2012 (GenBank accession number MK673140) (8), while the other three isolates (MT1, N6, and AK5) showed the highest nucleotide identity (94.46

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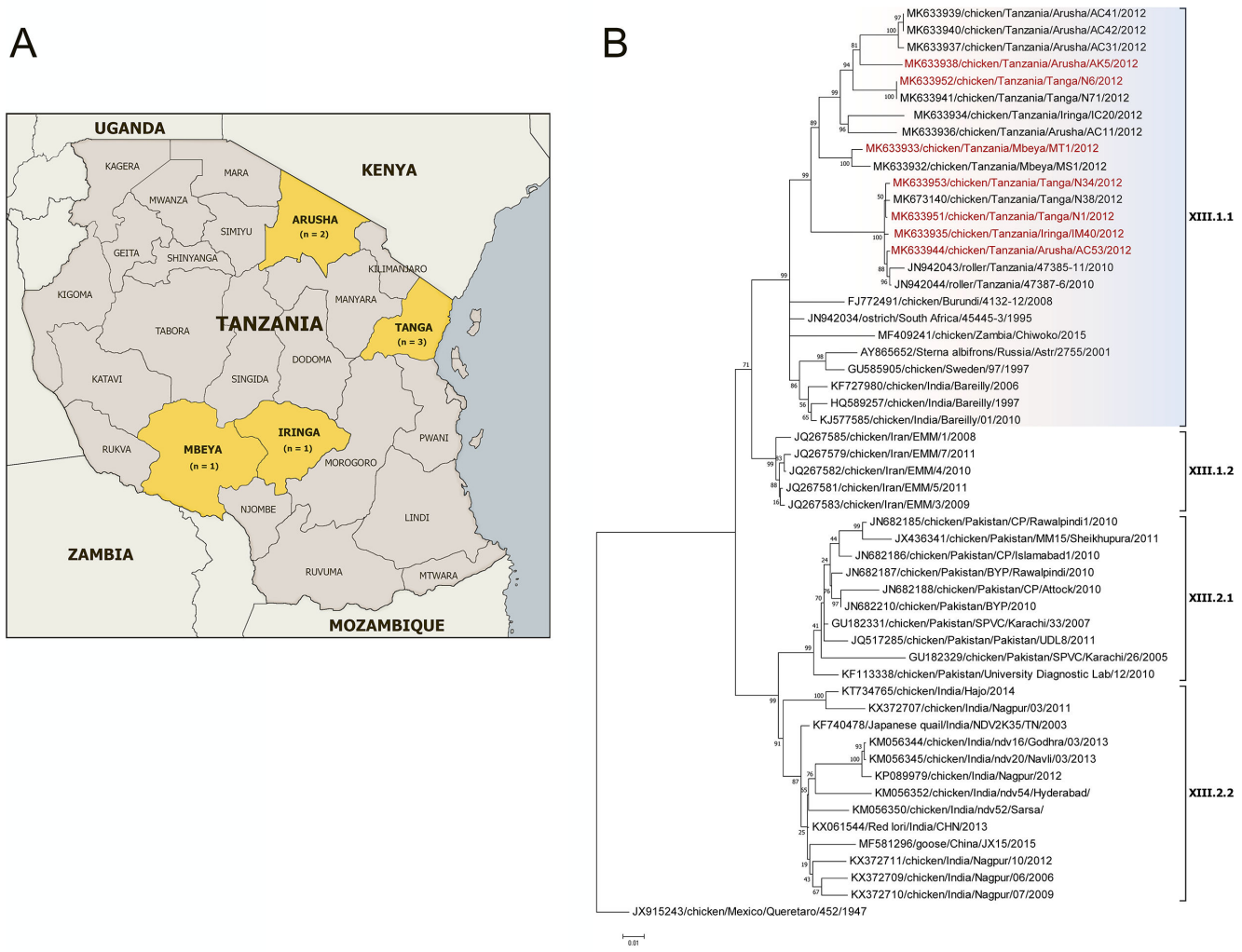

FIG 1 (A) Locations for the live bird market surveillance. (B) Phylogenetic analysis of NDV isolates of genotype XIII based on the complete fusion gene sequences constructed with the Maximum Likelihood method based on the General Time Reversible model in MEGA version 7.0.26. The tree with the highest log likelihood (−9896.03) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying 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 superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0. 4735)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+I), 38.62% sites]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 54 nucleotide sequences (sequence from genotype XVI 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 dataset. The isolates used in this study are shown in red.

to 95.2%) to the vNDV strain chicken/India/Bareilly/01/2010 (KJ577585) (16). Detailed phylogenetic analysis based on the complete fusion gene classified all seven isolates as members of sub-genotype XIII.1.1 (Fig. 1B). The phylogenetic tree revealed that all publicly available fusion gene sequences of sub-genotype XIII.1.1 isolates from Tanzania cluster into a distinct branch from XIII.1.1 isolates detected in other countries (8, 16–21).

Analysis of the deduced amino acid sequence of the fusion protein cleavage sites (9, 22) of isolates from this study showed a polybasic amino acid motif ^{112}RRQKR↓F^{117}, which is typical for vNDV. This result was consistent with the ICPI values ranging between 1.78 and 1.95 (9). Overall, this study provides valuable sequence information on vNDVs from Tanzania and sheds light on their genetic diversity and virulence.

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

Isolate name	Collection date	Location (region)	Total no. of raw read pairs	No. of mapped read pairs	Median coverage depth (reads)	ICPI[a]	GenBank accession no.	SRA accession no.
Chicken/Tanzania/Mbeya/MT1/2012	05/30/2012	Sokomatola (Mbeya)	2,263,648	687,157	7,300	1.88	MK633933	SRR24142067
Chicken/Tanzania/Iringa/IM40/2012	06/04/2012	Mashine tatu (Iringa)	671,067	449,556	5,366	1.88	MK633935	SRR24142063
Chicken/Tanzania/Arusha/AK5/2012	05/27/2012	Kilombero (Arusha)	1,006,401	454,248	5,287	1.78	MK633938	SRR24142066
Chicken/Tanzania/Arusha/AC53/2012	05/27/2012	Arusha Central (Arusha)	1,325,590	917,398	10,854	1.83	MK633944	SRR24142065
Chicken/Tanzania/Tanga/N1/2012	05/21/2012	Ngamiani (Tanga)	813,624	316,250	3,292	1.95	MK633951	SRR24142064
Chicken/Tanzania/Tanga/N34/2012	05/21/2012	Ngamiani (Tanga)	881,511	437,253	5,182	1.88	MK633953	SRR24142062
Chicken/Tanzania/Tanga/N6/2012	05/21/2012	Ngamiani (Tanga)	844,044	484,424	5,873	1.88	MK633952	SRR24142061

[a]ICPI = intracerebral pathogenicity index.

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DATA AVAILABILITY

The complete genome sequence of seven isolates has been deposited in GenBank under the accession numbers MK633933, MK633935, MK633938, MK633944, MK633951, MK633952, and MK633953. Raw data were deposited in the NCBI Sequence Read Archive (SRA) under accession numbers SRR24142061, SRR24142062, SRR24142063, SRR24142064, SRR24142065, SRR24142066, and SRR24142067 under the BioProject number PRJNA543308.

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