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GENOME SEQUENCES

Whole-Genome Sequence of *Avian coronavirus* from a 15-Year-Old Sample Confirms Evidence of GA08-like Strain Circulation 4 Years Prior to Its First Reported Outbreak

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ABSTRACT Here, we report the complete genome sequence of an *Avian coronavirus* strain GA08-like isolate from a fecal sample from a broiler chicken collected in Georgia in 2004. The viral genome in this 15-year-old sample provides evidence for the circulation of the GA08-like strain at least 4 years before its first report in 2008.

Avian infectious bronchitis virus (IBV) is a positive-sense, single-stranded RNA coronavirus (genus *Gammacoronavirus*, family *Coronaviridae*) that causes a highly contagious and economically significant disease in chickens worldwide (1–3). Currently, 7 genotypes exist that together comprise 35 distinct viral lineages and numerous strains of IBV, and new variants emerge due to frequent point mutations and recombination events in the viral genome (4–7). In 2008, a novel IBV strain was detected in Georgia broiler chickens with acute chronic respiratory disease and colibacillosis (8, 9). The GA08 virus became a predominant IBV strain at that time in the southeastern United States (10, 11). Over the years, its incidence and severity have increased dramatically, resulting in significant losses. In this study, we report the complete sequence of an IBV GA08-like strain from a 15-year-old sample.

A fecal sample from a 22-day-old broiler chicken was collected at a commercial farm in Georgia in 2004. Feces were first diluted 3:7 in sterile phosphate-buffered saline and then centrifuged for 10 min at 3,200 rpm. The supernatant was further passed sequentially through 1.2-μm- and 0.45-μm-pore-size filters (Merck Millipore, USA) to remove bacteria and large-cell particles. The filtrate was then stored at −70°C for 15 years. In 2019, preserved filtered lysate was DNase treated using the Turbo DNA-free kit (Ambion, USA) to remove host DNA, followed by total nucleic acid extraction using the DNeasy blood and tissue kit (Qiagen, Germany) according to the manufacturer's recommendations. Sequence-independent single-primer amplification (12) was used to produce random amplicons that were processed using the Nextera XT DNA library preparation kit (Illumina, USA). Paired-end (2 × 150-bp) sequencing was performed on an Illumina MiSeq instrument using the 300-cycle MiSeq reagent kit v2 (Illumina, USA). A total of 2,088,901 raw paired-end reads were generated. A customized workflow on the Galaxy platform (13) was used to perform preprocessing and assembly of the raw sequencing reads, as described previously (14, 15). Briefly, the raw read quality was assessed using FastQC v0.63 (16), and residual adapter sequences were trimmed using Cutadapt v1.6 (17). *De novo* assembly was performed utilizing MIRA3 v0.0.1 (18) with default settings. The contigs of interest were subjected to a BLASTn search and mapped using BWA-MEM (19) to the full-length reference genome GA9977/2019 (GenBank accession number MK878536) to obtain a draft genome scaffold. The consensus genome sequence of the strain, designated GA/1472/2004, was then recalled based on BWA-MEM mapping of 207,352 trimmed IBV reads to the genome scaffold. The median read depth of the IBV assembly was 558 reads. The final genome consensus was 27,639 nucleotides long, excluding the poly(A) tail (100% genome coverage as estimated based on the size of the IBV GA08

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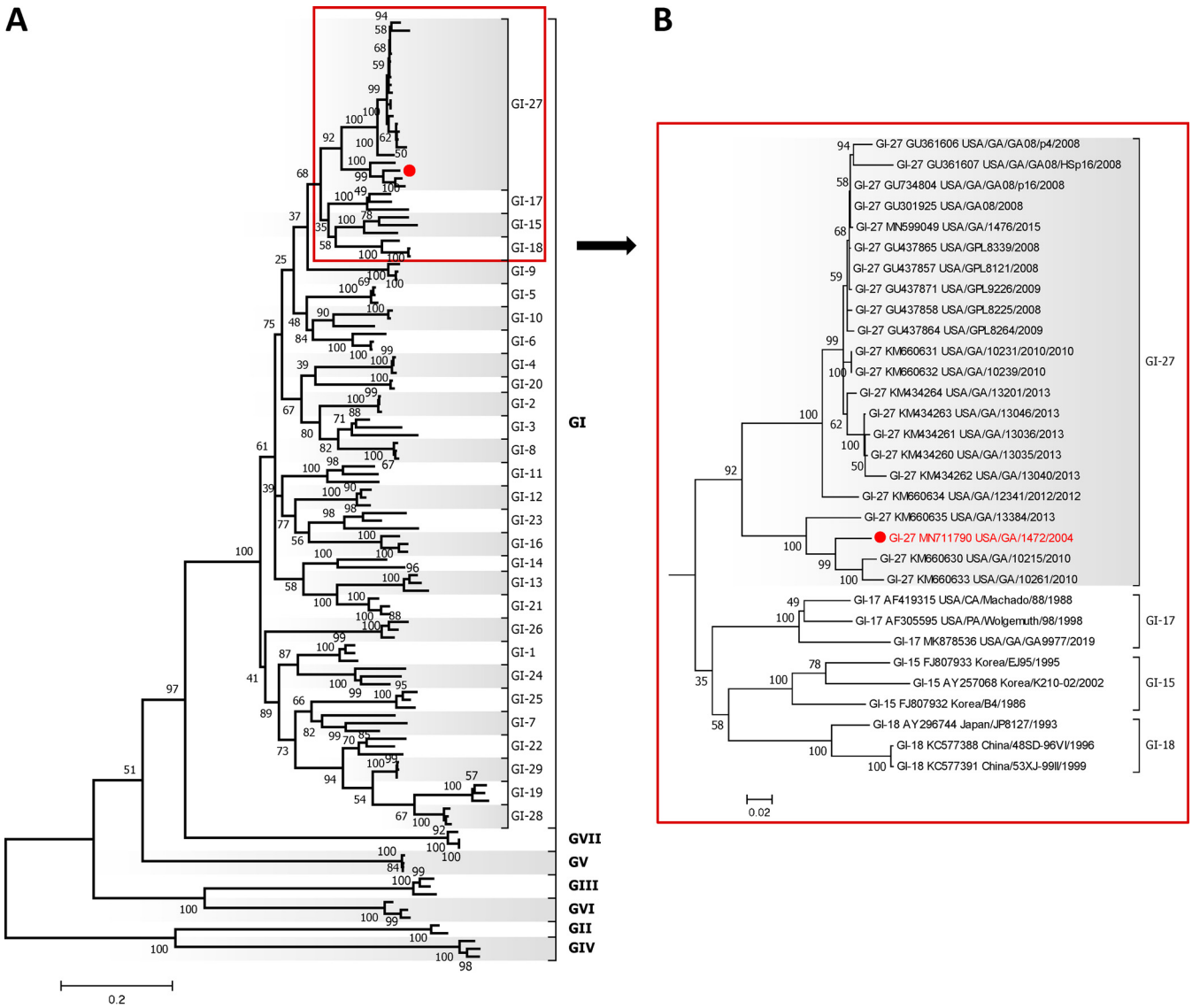

FIG 1 Phylogenetic analysis of IBV isolates of all 35 lineages (121 nucleotide sequences) (A) and GI-27 isolates (B) based on the complete S1 gene sequences constructed with the maximum likelihood method based on the general time-reversible model in MEGA v7.0.26. The tree with the highest log likelihood (−40755.95) is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to each branch. The tree is drawn to scale, with the branch lengths measured in the number of substitutions per site, and the tree is midpoint rooted. All positions containing gaps and missing data were eliminated. There were a total of 1,347 positions in the final data set. The isolate used in this study is shown in red.

reference genome GA/1476/2015; GenBank accession number MN599049.1), and had 38.2% GC content (20). The open reading frames (ORF) were identified using Geneious v11.1.5 and confirmed by alignment with published IBV genome sequences. The genome has the typical genetic structure of all IBV strains and contains 13 ORF (5′-1a/1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3′). A preliminary BLAST comparison of the GA/1472/2004 genome sequence to the currently available full-length IBV genome sequences showed the highest (94.08%) nucleotide identity to the DMV/1639 strain GA9977/2019 belonging to GI-17 (21). However, a new classification of IBV genotypes based on the phylogenetic analysis of the complete spike glycoprotein S1 coding region has been proposed (4). Therefore, classification using complete S1 nucleotide sequences of all IBV lineages revealed that the GA/1472/2004 isolate clustered with GA08 strains belonging to the GI-27 lineage (Fig. 1), which form two distinct clades of vaccine-type isolates and isolates circulating in backyard birds (9, 20, 22). The IBV isolate characterized in this study clustered together with isolates GA/13384/2013, GA/10215/2010, and GA/10261/2010 (GenBank accession numbers KM660635, KM660630, and KM660633,

respectively) (23) from backyard birds with nucleotide identities ranging from 92.31% to 94.75%.

Therefore, the phylogenetic analysis of the GA/1472/2004 isolate revealed that the IBV GA08-like strain was already circulating in Georgia at least 4 years prior to the first report in 2008. This complete genome sequence information of the 15-year-old IBV GA08 strain would be useful for an in-depth understanding of IBV evolution as well as planning vaccination strategies.

Data availability. The complete genome sequence of the *Avian coronavirus* GA/1472/2004 isolate of strain GA08 has been deposited in GenBank under the accession number MN711790. The raw data were deposited in the SRA under accession number SRR10500281, BioSample accession number SAMN13337568, and BioProject accession number PRJNA556282.

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