



# A 25-Year-Old Sample Contributes the Complete Genome Sequence of *Avian Coronavirus* Vaccine Strain ArkDPI, Reisolated from Commercial Broilers in the United States

 Iryna V. Goraichuk,<sup>a</sup> James F. Davis,<sup>b</sup> Arun B. Kulkarni,<sup>b</sup>  Claudio L. Afonso,<sup>a</sup>  David L. Suarez<sup>a</sup>

<sup>a</sup>Exotic and Emerging Avian Viral Disease Research Unit, Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, ARS, USDA, Athens, Georgia, USA

<sup>b</sup>Georgia Poultry Laboratory Network, Gainesville, Georgia, USA

**ABSTRACT** Here, we report the complete genome sequence of *Avian coronavirus* strain ArkDPI of the GI-9 lineage, isolated from broiler chickens in North Georgia in 1994. This is the complete genome sequence of this vaccine strain, reisolated from broilers in the United States.

**A** *avian coronavirus* infectious bronchitis virus (IBV) (family *Coronaviridae*, genus *Gammacoronavirus*) is a respiratory pathogen that causes severe economic losses in the poultry industry worldwide (1–4). Numerous IBV variants have been reported in the United States (5). However, the Arkansas type is one of the most common IBV serotypes isolated from chickens in the field (6, 7). Currently, only Arkansas Delmarva Poultry Industry (ArkDPI) attenuated live vaccine is commercially available against the Ark-IBV serotype (6). It has been shown that the ArkDPI live attenuated vaccine can persist in flocks (8), causing a rolling reaction by continuing transmission of the vaccine virus to the unvaccinated chickens, which results in increased virulence of the vaccine and vaccine reactions in the flock (6, 9). This is unique to the ArkDPI vaccine and is due to a minor subpopulation in the vaccine, exhibiting polymorphisms in the spike 1 (S1) protein. Reisolation of the ArkDPI vaccine virus from chickens has shown that two amino acid changes in the S1 protein, Y43H and Δ344, are the most common mutations observed (7, 10–13). In this study, we report the complete genome of the ArkDPI vaccine virus, reisolated from chickens in the United States.

The ArkDPI-like IBV was isolated from the feces of broiler chickens collected at a commercial farm in North Georgia in 1994 (14). The feces were homogenized and passed sequentially through 1.2- $\mu$ m- and 0.45- $\mu$ m-pore-size filters (Merck Millipore, USA) to remove bacteria. The filtrate was inoculated into specific-pathogen-free embryonating chicken eggs. The embryos died 48 to 96 h postinoculation and were harvested, quick frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$ . The Ark-IBV was previously detected in the infected embryo using monoclonal antibodies (14). Total nucleic acids were isolated from a preserved 25-year-old pancreas sample of a chicken experimentally inoculated with homogenized infected embryo using the DNeasy blood and tissue kit (Qiagen, Germany), followed by DNase treatment with the TURBO DNA-free kit (Ambion, USA) to remove host DNA according to the manufacturer's recommendations. Sequence-independent single-primer amplification (15) was used to produce random amplicons that were processed using the Nextera XT DNA library preparation kit (Illumina, USA). Next-generation paired-end sequencing ( $2 \times 150$  bp) was performed on an Illumina MiSeq instrument using the 300-cycle MiSeq reagent kit v2. A total of 2,146,321 raw paired-end reads were generated. A customized workflow on the Galaxy platform (16) was used to perform preprocessing and assembly of the raw sequencing reads, as described previously (17, 18). Briefly, the raw read quality was assessed using FastQC v0.63 (19), and the residual adapter sequences were trimmed

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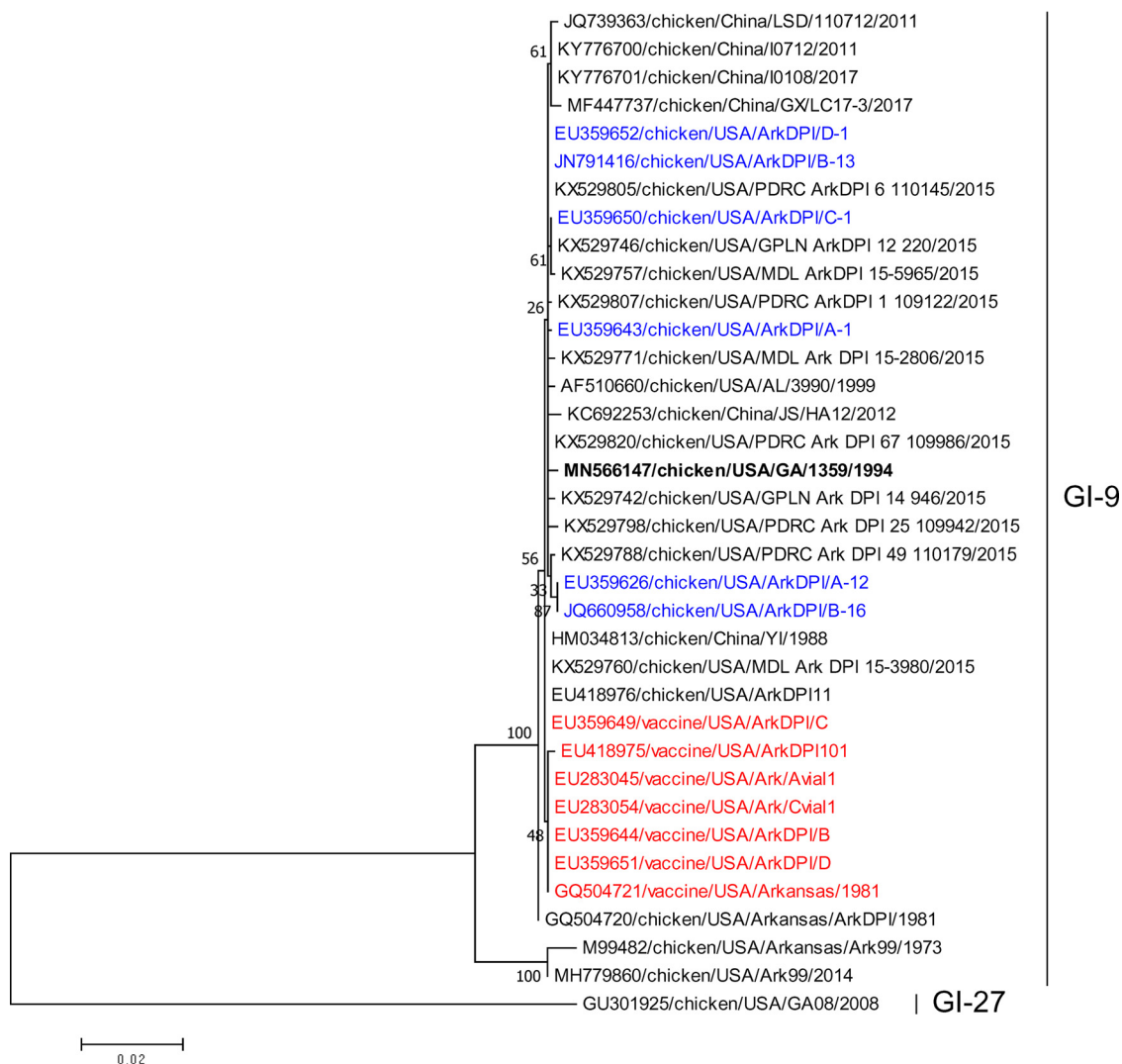
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Address correspondence to David L. Suarez, david.suarez@usda.gov.

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**FIG 1** Phylogenetic analysis of IBV isolates of the Arkansas-type variant based on the complete S1 gene sequences. The S1 gene sequences of 35 IBV isolates were downloaded from the NCBI GenBank database. Together with the sequence obtained in the current study, all sequences were subjected to multiple alignment using the ClustalW algorithm. The phylogenetic tree was constructed by using the maximum likelihood method based on the general time-reversible model in MEGA v7.0.26. The tree with the highest log likelihood ( $-3,705.46$ ) is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 36 nucleotide sequences (the sequence from the GI-9 lineage is included as an outgroup). All positions containing gaps and missing data were eliminated. There were a total of 1,617 positions in the final data set. The ArkDPI-derived vaccine, the reisolated ArkDPI-like strains from experimentally vaccinated chickens, and the strain used in this study are shown in red, blue, and bold, respectively.

using Cutadapt v1.6 (20). After the host and control library reads were removed, the overlapping read pairs were joined with PEAR v0.9.6.1 (21). Digital normalization via median k-mer abundance was performed using the khmer package v1.1-1 (cut-off = 100, kmer size = 20) (22). *De novo* assembly was performed utilizing MIRA3 v0.0.1 (23) with default settings. The contigs of interest were subjected to a BLASTn search and aligned with the full-length reference genome ArkDPI11 (GenBank accession number [EU418976](#)) to obtain a draft genome scaffold. The genome consensus was then recalled from 183,511 raw IBV reads using BWA-MEM (24) mapping of trimmed but unnormalized reads to the genome scaffold. The median read depth of the IBV assembly was 444. The final genome consensus of the isolate, designated GA/1359/1994, was 27,617 nucleotides long, excluding the poly(A) tail (100% genome coverage based on reference genome ArkDPI11), and had a 38% GC content. The open reading

frames (ORFs) were identified using Geneious v11.1.5 and confirmed by alignment with published IBV genomes. The genome has the typical genetic structure of all IBV strains and contains 13 ORFs (5'-1a/1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3'). A preliminary BLAST comparison to the currently available full-length IBV genome sequences showed the highest (99.87%) nucleotide identity to the virulent Arkansas strain ArkDPI11 ([EU418976](https://doi.org/10.1093/mbe/mnq011)), belonging to the GI-9 lineage (25, 26). Detailed phylogenetic analysis based on the complete coding sequence of the S1 gene (27) confirmed that GA/1359/1994 is a member of the GI-9 lineage, clustering in one group along with the lineage prototype strain Ark99/1973 (96.62% nucleotide identity; [M99482](https://doi.org/10.1093/mbe/mnq011)) (Fig. 1). Certain polymorphisms in the S1 gene can often be found in viruses reisolated from chickens vaccinated with the ArkDPI attenuated vaccine. The Y43H and  $\Delta$ 344 mutations are critical for vaccine virus fitness in chicks, as changes at these two positions are most frequently seen in field reisolated viruses compared to the parent vaccine. The S1 gene of GA/1359/1994 had both the Y43H and  $\Delta$ 344 amino acid changes. Despite the ArkDPI vaccine persisting in U.S. flocks (9, 13, 28), there are only sequences of the S1 gene available and no full genomes. This complete genome sequence information would be useful for in-depth understanding of the role that live vaccines play in the recombination of IBVs, which may enhance the virus fitness in chickens.

**Data availability.** The complete genome sequence of the GA/1359/1994 isolate of the ArkDPI-like strain has been deposited in GenBank under the accession number [MN566147](https://doi.org/10.1093/mbe/mnq011). The raw data were deposited under SRA accession number [SRR10742607](https://doi.org/10.1093/mbe/mnq011), BioSample number [SAMN13020879](https://doi.org/10.1093/mbe/mnq011), and BioProject number [PRJNA556282](https://doi.org/10.1093/mbe/mnq011).

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