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GENOME SEQUENCES

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

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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.

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-μm- and 0.45-μm-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°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 × 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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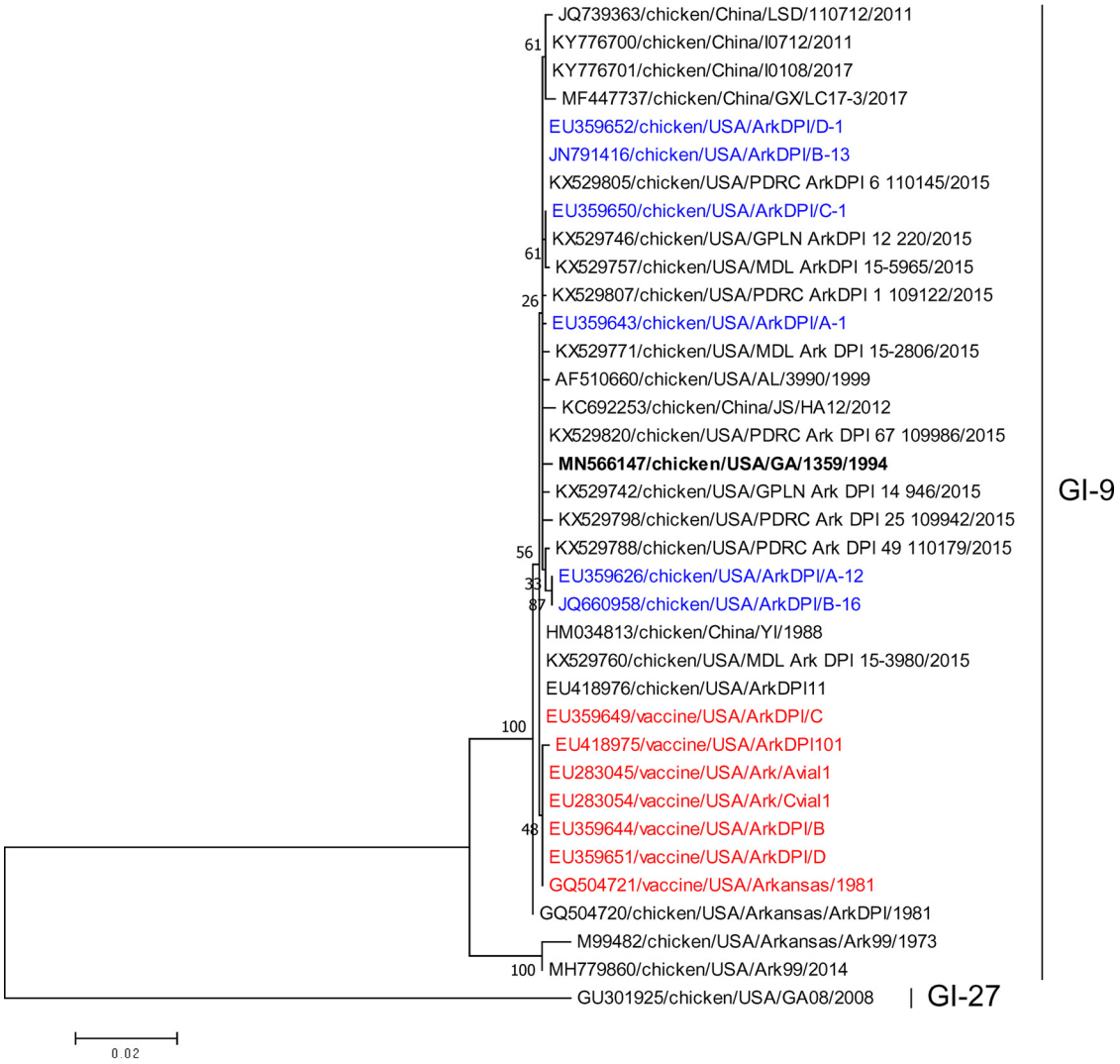

FIG 1 Phylogenetic analysis of IBV isolates of the Arkansas-type variant based on the complete S1 gene sequences. The S1 gene sequences of 35 IBV isolates were downloaded from the NCBI GenBank database. Together with the sequence obtained in the current study, all sequences were subjected to multiple 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-27 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), 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) (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 Δ344 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 Δ344 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. The raw data were deposited under SRA accession number SRR10742607, BioSample number SAMN13020879, and BioProject number PRJNA556282.

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REFERENCES

1. Jackwood MW, de Wit S. 2013. Infectious bronchitis, p 139–159. *In* Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair VL (ed), Diseases of poultry, 13th ed. John Wiley and Sons, Inc., Oxford, United Kingdom.
2. Cavanagh D. 2007. Coronavirus avian infectious bronchitis virus. Vet Res 38:281–297. https://doi.org/10.1051/vetres:2006055.
3. Naqi S, Gay K, Patalla P, Mondal S, Liu R. 2003. Establishment of persistent avian infectious bronchitis virus infection in antibody-free and antibody-positive chickens. Avian Dis 47:594–601. https://doi.org/10.1637/6087.
4. King AMQ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE, Gorbalenya AE, Harrach B, Harrison RL, Junglen S, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Nibert ML, Rubino L, Sabanadzovic S, Sanfacon H, Siddell SG, Simmonds P, Varsani A, Zerbini FM, Davison AJ. 2018. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). Arch Virol 163:2601–2631. https://doi.org/10.1007/s00705-018-3847-1.
5. Jackwood MW, Hilt DA, Lee CW, Kwon HM, Callison SA, Moore KM, Moscoso H, Sellers H, Thayer S. 2005. Data from 11 years of molecular typing infectious bronchitis virus field isolates. Avian Dis 49:614–618. https://doi.org/10.1637/7389-052905R.1.
6. Leyson CM, Hilt DA, Jordan BJ, Jackwood MW. 2017. Minimum infectious dose determination of the Arkansas Delmarva Poultry Industry infectious bronchitis virus vaccine delivered by hatchery spray cabinet. Avian Dis 61:123–127. https://doi.org/10.1637/11474-072216-ResNote.
7. Nix WA, Troeber DS, Kingham BF, Keeler CL, Jr, Gelb J, Jr. 2000. Emergence of subtype strains of the Arkansas serotype of infectious bronchitis virus in Delmarva broiler chickens. Avian Dis 44:568–581. https://doi.org/10.2307/1593096.
8. Jackwood MW, Hilt DA, McCall AW, Polizzi CN, McKinley ET, Williams SM. 2009. Infectious bronchitis virus field vaccination coverage and persistence of Arkansas-type viruses in commercial broilers. Avian Dis 53:175–183. https://doi.org/10.1637/8465-090308-Reg.1.
9. Jackwood MW, Lee DH. 2017. Different evolutionary trajectories of vaccine-controlled and non-controlled avian infectious bronchitis viruses in commercial poultry. PLoS One 12:e0176709. https://doi.org/10.1371/journal.pone.0176709.
10. Leyson C, França M, Jackwood M, Jordan B. 2016. Polymorphisms in the S1 spike glycoprotein of Arkansas-type infectious bronchitis virus (IBV) show differential binding to host tissues and altered antigenicity. Virology 498:218–225. https://doi.org/10.1016/j.virol.2016.08.030.
11. McKinley ET, Hilt DA, Jackwood MW. 2008. Avian coronavirus infectious bronchitis attenuated live vaccines undergo selection of subpopulations and mutations following vaccination. Vaccine 26:1274–1284. https://doi.org/10.1016/j.vaccine.2008.01.006.
12. Toro H, van Santen VL, Jackwood MW. 2012. Genetic diversity and selection regulates evolution of infectious bronchitis virus. Avian Dis 56:449–455. https://doi.org/10.1637/10072-020212-Review.1.
13. van Santen VL, Toro H. 2008. Rapid selection in chickens of subpopulations within ArkDPI-derived infectious bronchitis virus vaccines. Avian Pathol 37:293–306. https://doi.org/10.1080/03079450802043783.
14. Davis JF, Castro AE, de la Torre JC, Barnes HJ, Doman JT, Metz M, Lu H, Yuen S, Dunn PA, Teng MN. 1996. Experimental reproduction of severe hypoglycemia and spiking mortality syndrome using field-derived and embryo-passaged preparations. Avian Dis 40:158–172. https://doi.org/10.2307/1592385.
15. Chrzastek K, Lee DH, Smith D, Sharma P, Suarez DL, Pantin-Jackwood M, Kapczynski DR. 2017. Use of sequence-independent, single-primer-amplification (SISPA) for rapid detection, identification, and characterization of avian RNA viruses. Virology 509:159–166. https://doi.org/10.1016/j.virol.2017.06.019.
16. Afgan E, Baker D, van den Beek M, Blankenberg D, Bouvier D, Čech M, Chilton J, Clements D, Coraor N, Eberhard C, Grüning B, Guerler A,

Hillman-Jackson J, Von Kuster G, Rasche E, Soranzo N, Turaga N, Taylor J, Nekrutenko A, Goecks J. 2016. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res 44:W3–W10. https://doi.org/10.1093/nar/gkw343.

17. Goraichuk IV, Kulkarni AB, Williams-Coplin D, Suarez DL, Afonso CL. 2019. First complete genome sequence of currently circulating infectious bronchitis virus strain DMV/1639 of the GI-17 lineage. Microbiol Resour Announc 8:e00840-19. https://doi.org/10.1128/MRA.00840-19.
18. Dimitrov KM, Sharma P, Volkening JD, Goraichuk IV, Wajid A, Rehmani SF, Basharat A, Shittu I, Joannis TM, Miller PJ, Afonso CL. 2017. A robust and cost-effective approach to sequence and analyze complete genomes of small RNA viruses. Virol J 14:72. https://doi.org/10.1186/s12985-017-0741-5.
19. Andrews S. 2020. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
20. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10–12. https://doi.org/10.14806/ej.17.1.200.
21. Zhang J, Kobert K, Flouri T, Stamatakis A. 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30:614–620. https://doi.org/10.1093/bioinformatics/btt593.
22. Crusoe MR, Alameldin HF, Awad S, Boucher E, Caldwell A, Cartwright R, Charbonneau A, Constantinides B, Edvenson G, Fay S, Fenton J, Fenzl T, Fish J, Garcia-Gutierrez L, Garland P, Gluck J, González I, Guermond S, Guo J, Gupta A, Herr JR, Howe A, Hyer A, Härpfer A, Irber L, Kidd R, Lin D, Lippi J, Mansour T, McA'Nulty P, McDonald E, Mizzi J, Murray KD, Nahum JR, Nanlohy K, Nederbragt AJ, Ortiz-Zuazaga H, Ory J, Pell J, Pepe-Ranney C, Russ ZN, Schwarz E, Scott C, Seaman J, Sievert S, Simpson J, Skennerton CT, Spencer J, Srinivasan R, Standage D, et al. 2015. The khmer software package: enabling efficient nucleotide sequence analysis. F1000Res 4:900. https://doi.org/10.12688/f1000research.6924.1.
23. Chevreux B, Wetter T, Suhai S. 1999. Genome sequence assembly using trace signals and additional sequence information, p 45–56. *In* Wingender E (ed), Computer science and biology: proceedings of the German Conference on Bioinformatics (GCB '99). GBF-Braunschweig, Department of Bioinformatics, Braunschweig, Germany.
24. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
25. Ammayappan A, Upadhyay C, Gelb J, Jr, Vakharia VN. 2008. Complete genomic sequence analysis of infectious bronchitis virus Ark DPI strain and its evolution by recombination. Virol J 5:157. https://doi.org/10.1186/1743-422X-5-157.
26. Ammayappan A, Upadhyay C, Gelb J, Jr, Vakharia VN. 2009. Identification of sequence changes responsible for the attenuation of avian infectious bronchitis virus strain Arkansas DPI. Arch Virol 154:495–499. https://doi.org/10.1007/s00705-009-0325-9.
27. Valastro V, Holmes EC, Britton P, Fusaro A, Jackwood MW, Cattoli G, Monne I. 2016. S1 gene-based phylogeny of infectious bronchitis virus: an attempt to harmonize virus classification. Infect Genet Evol 39:349–364. https://doi.org/10.1016/j.meegid.2016.02.015.
28. Ndegwa EN, Toro H, van Santen VL. 2014. Comparison of vaccine subpopulation selection, viral loads, vaccine virus persistence in trachea and cloaca, and mucosal antibody responses after vaccination with two different Arkansas Delmarva Poultry Industry-derived infectious bronchitis virus vaccines. Avian Dis 58:102–110. https://doi.org/10.1637/10609-070613-Reg.1.