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GENOME SEQUENCES

Complete Coding Sequences of Three Chicken Parvovirus Isolates from the United States

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ABSTRACT Parvoviruses are commonly found in U.S. poultry and are associated with clinical disease. Here, we report the complete coding sequences of three chicken parvoviruses from broiler chickens from commercial farms in the state of Georgia.

Chicken parvovirus (ChPV) and turkey parvovirus (TuPV) are members of the species *Galliform aveparvovirus 1* in the genus *Aveparvovirus* of the *Parvoviridae* family (1, 2). Aveparvoviruses are small, nonenveloped viruses with linear, single-stranded DNA genomes of approximately 5.3 kbp in length (1). Both ChPV and TuPV are widespread in commercial chicken and turkey flocks across the world (3–10). These viruses are highly infectious in young poultry but result in uncertain pathology (2). Infected birds excrete virus in feces at a high titer as early as 4 days of age (11). Aveparvoviruses have been associated with the occurrence of malabsorption syndrome in chickens and enteritis in turkeys (12–16). Despite the proposed association, the contribution of aveparvoviruses to such syndromes is unclear, since ChPV genomes have also been detected in healthy chickens (6, 17). In this study, we report the coding-complete genome sequences of three ChPV isolates from the United States.

Fecal samples from broiler chickens with severe hypoglycemia were collected at a commercial farm in the state of Georgia during 2003 to 2005 (Table 1). Three fecal samples were selected for whole-genome random sequencing. The feces were first diluted 3:7 in sterile phosphate-buffered saline and then centrifuged for 10 min at 3,200 rpm. The supernatants were further passed sequentially through 1.2- and 0.45-μm-pore-size filters (Merck Millipore, USA) to remove bacteria and large particles. Total nucleic acids were isolated from the preserved filtered lysate 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. Briefly, 20 μl of proteinase K and 200 μl of animal tissue lysis (ATL) buffer were added to the sample, which was then incubated at 56°C for 10 min. Subsequently, 200 μl of 96% ethanol was added; the mixture was pipetted to a spin column and centrifuged at 8,000 rpm for 1 min. Then, the column was washed with AW1 and AW2 buffers. Finally, bounded nucleic acids were eluted in 100 μl of AE buffer. Sequence-independent single-primer amplification (18) was used to produce random amplicons that were processed using the Nextera XT DNA library preparation kit (Illumina, USA). The distribution size and concentration of the prepared library were checked on a Bioanalyzer 2100 instrument using the high-sensitivity DNA kit (Agilent Technologies, Germany) and a Qubit fluorometer with the double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Life Technologies, USA), respectively. Next-generation paired-end (2 × 150-bp) sequencing was performed on an Illumina MiSeq instrument using the 300-cycle MiSeq reagent kit version 2. The sequence data were assembled using a *de novo* approach and utilizing MIRA3 version 0.0.1 (19) within a

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TABLE 1 Sequencing data for the chicken parvovirus isolates in this report

Isolate name	Collection date (mo/day/yr)	Host	Total no. of raw read pairs	No. of mapped reads	Median coverage depth (no. of reads)	Mean read length (nt)[a]	GC content (%)	GenBank accession no.	SRA accession no.
GA/1478/2003	4/28/2003	18-day-old broiler	1,303,453	136,601	908	130	43.7	MN782010	SRR10566435
GA/1472/2004	10/5/2004	22-day-old broiler	2,088,901	26,973	279	136	43.5	MN782008	SRR10500281
GA/1477/2005	9/29/2005	16-day-old broiler	2,446,408	629,453	27,140	111	43.8	MN782009	SRR10566436

[a] nt, nucleotides.

customized workflow on the Galaxy platform (20), as described previously (21, 22). The MiSeq run generated from 1,303,453 to 2,446,408 total paired-end reads per sample (Table 1). All final consensuses were called from the raw reads that were mapped to the *de novo*-generated contigs using BWA-MEM (23), and all three were 4,615 nucleotides

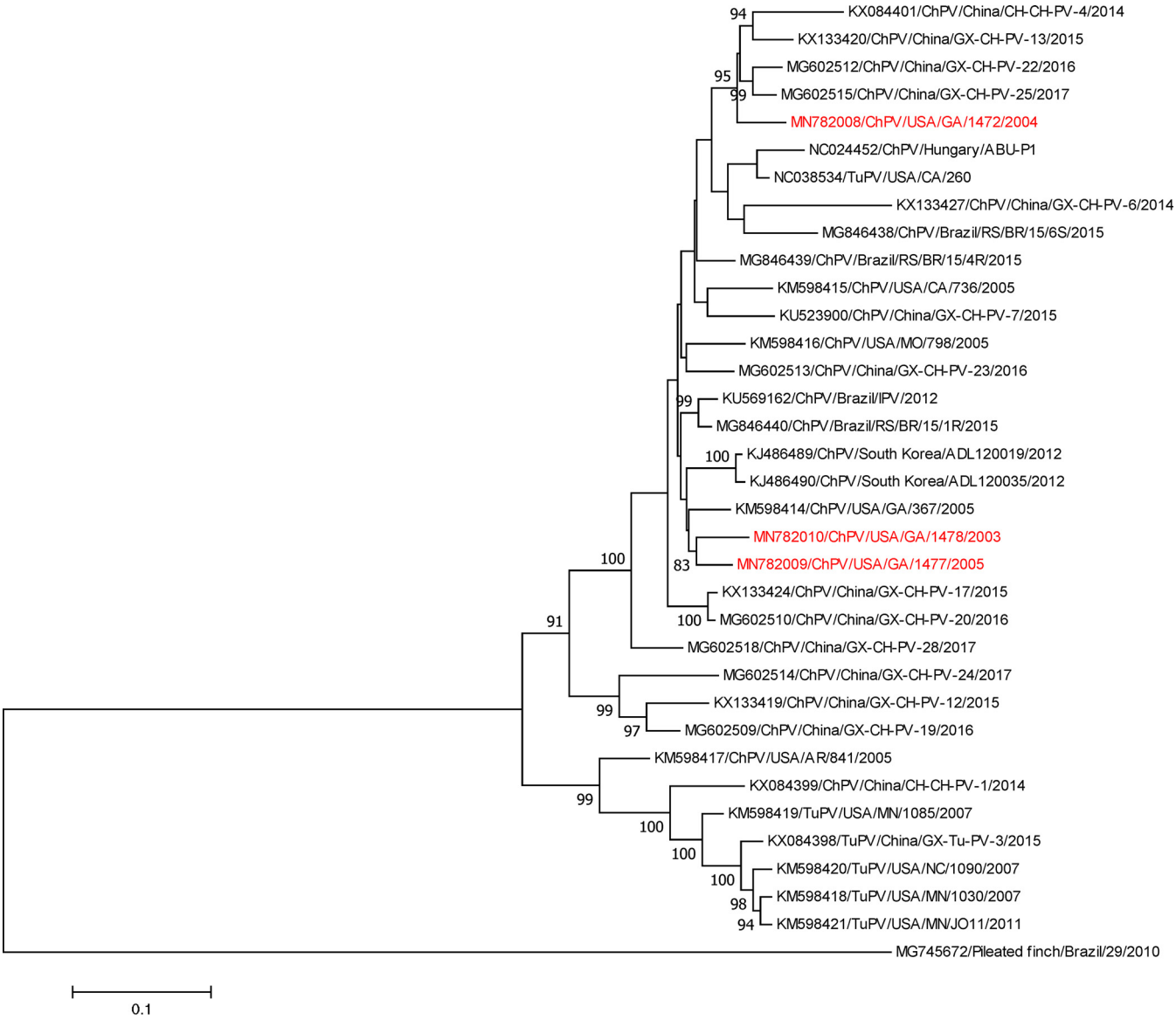

FIG 1 Phylogenetic analysis of chicken parvovirus (ChPV) and turkey parvovirus (TuPV) isolates based on the concatenated complete-coding amino acid sequences of the NS and VP proteins constructed with the neighbor-joining method in MEGA version 7.0.26. The optimal tree with a sum of branch length of 2.59922420 is shown. The tree is drawn to scale; units are the number of amino acid substitutions per site. 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. Bootstrap values of 70% are shown at the branch nodes, and the scale bar represents nucleotide substitutions per site. The analysis involved 35 amino acid sequences (the sequence of pileated finch aveparvovirus is included as an outgroup). All positions containing gaps and missing data were eliminated. There was a total of 1,315 positions in the final data set. The isolates used in this study are shown in red.

(nt) long (100% coding-complete coverage; GC content, ~44%), missing 410 nt at the 5′ end and 232 nt at the 3′ end of the genome compared to the reference genome, ABU-P1 (NCBI RefSeq accession number NC_024452.1). The open reading frames (ORF) were identified using the Geneious version 11.1.5 and confirmed by alignment with published chicken parvovirus genomes. The genomes of all three isolates have the typical genetic structure of all parvoviruses and contain two major ORF that encode nonstructural (NS) and structural capsid (VP) viral proteins (2). Phylogenetic analysis based on the concatenated coding-complete amino acid sequences of the NS and VP proteins revealed that all three isolates sequenced in this study clustered together with other members of the *Aveparvovirus* genus (Fig. 1). BLAST comparison of the designated Georgia/1478/2003, Georgia/1472/2004, and Georgia/1477/2005 nucleotide sequences to the currently available full-length ChPV genome sequences showed the highest (95.93%, 95.95%, and 95.26%, respectively) identity to Brazil/RS/BR/15/1R/2015, China/GX-
CH-PV-25/2017, and USA/GA/367/2005 (GenBank accession numbers MG846440.1, MG602515.1, and KM598414.1), respectively. Isolates GA/1478/2003 and GA/1477/2005 were more similar to one another (96.21% nucleotide identity) than to GA/1472/2004 (93.39% and 93.33% nucleotide identity, respectively). Amino acid analysis showed that the NS protein of all three isolates possessed a well-conserved phosphate-binding loop (P-loop) motif ^{392}GPANTGKT^{399} and downstream residues involved in nucleoside triphosphate (NTP) binding, Walker B motif ^{436}EE^{437} (15, 24, 25).

The chicken and turkey parvoviruses were not cultured, so Koch's postulates cannot be fulfilled to replicate clinical disease. Therefore, genomic sequence data from flocks with sufficient metadata can help in understanding the epidemiology of the virus and its association with clinical disease in the United States.

Data availability. The coding-complete sequences of all 3 isolates have been deposited in GenBank under the accession numbers MN782008 to MN782010. The raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA590745.

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