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GENOME SEQUENCES

Complete Genome Sequence of an *Avian Metapneumovirus* Subtype B Strain from Hungary

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ABSTRACT Avian metapneumoviruses (aMPVs), which have been reported in many countries, cause acute upper respiratory tract disease in chickens and turkeys. Using next-generation sequencing, we report here the complete genome sequence of an aMPV subtype B strain that was isolated from a turkey in Hungary in 1989.

Avian metapneumoviruses (aMPVs) cause respiratory and reproductive disorders in poultry, most notably turkeys, chickens, and ducks, and are a major economic concern for the industry (1, 2). aMPV is a member of the genus *Metapneumovirus* within the subfamily *Pneumovirinae* of the family *Paramyxoviridae* and contains a negative-sense, nonsegmented RNA genome that is approximately 13.5 to 14 kb long (3, 4). Although only one serotype of aMPV has been described, four subtypes (A, B, C, and D) are recognized based on the levels of genetic and antigenic differences (5, 6). Recently, two distinct aMPVs, isolated from a monk parakeet (7) and a gull (8), were identified and proposed as new subtypes. aMPVs of subtypes A and B are present in Europe and many countries in the world, excluding the United States (9–13). aMPV-C is present mainly in the United States and has been observed to a limited degree in France, South Korea, and China (14–18). To date, aMPV-D has been observed only in France (5, 19). In this study, we report the complete genome of an aMPV-B strain from Hungary.

The aMPV strain Hungary/657/4, which was isolated from a turkey in Hungary in 1989, was obtained from the National Veterinary Services Laboratories of the Animal and Plant Health Inspection Service, U.S. Department of Agriculture (20, 21). The virus was propagated in Vero cells and purified as discussed previously (22). Viral RNA was isolated from the supernatant of virus-infected Vero cells using the QIAamp viral RNA minikit (Qiagen, USA), 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 (23) was used to produce random amplicons, which were processed using the Nextera XT DNA library preparation kit (Illumina, USA). The distribution size and concentration of the prepared libraries were checked with a Bioanalyzer 2100, using the high-sensitivity DNA kit (Agilent Technologies, Germany), and a Qubit fluorometer, using the double-stranded DNA (dsDNA) high-sensitivity assay kit (Life Technologies, USA), respectively. Next-generation paired-end sequencing (2×250 bp) was performed on an Illumina MiSeq instrument using the 500-cycle MiSeq reagent kit v2. The MiSeq run generated 1,209,676 total paired-end reads. A customized workflow on the Galaxy platform (24) was used to perform preprocessing and assembly of the raw sequencing reads, as described previously (25, 26). Briefly, raw read quality was assessed using FastQC v0.63 (27), and residual adapter sequences were trimmed using Cutadapt v1.6 (28). After host and control library reads were removed, overlapping read pairs were joined with PEAR v0.9.6. (29). Digital normalization via median k-mer abundance was

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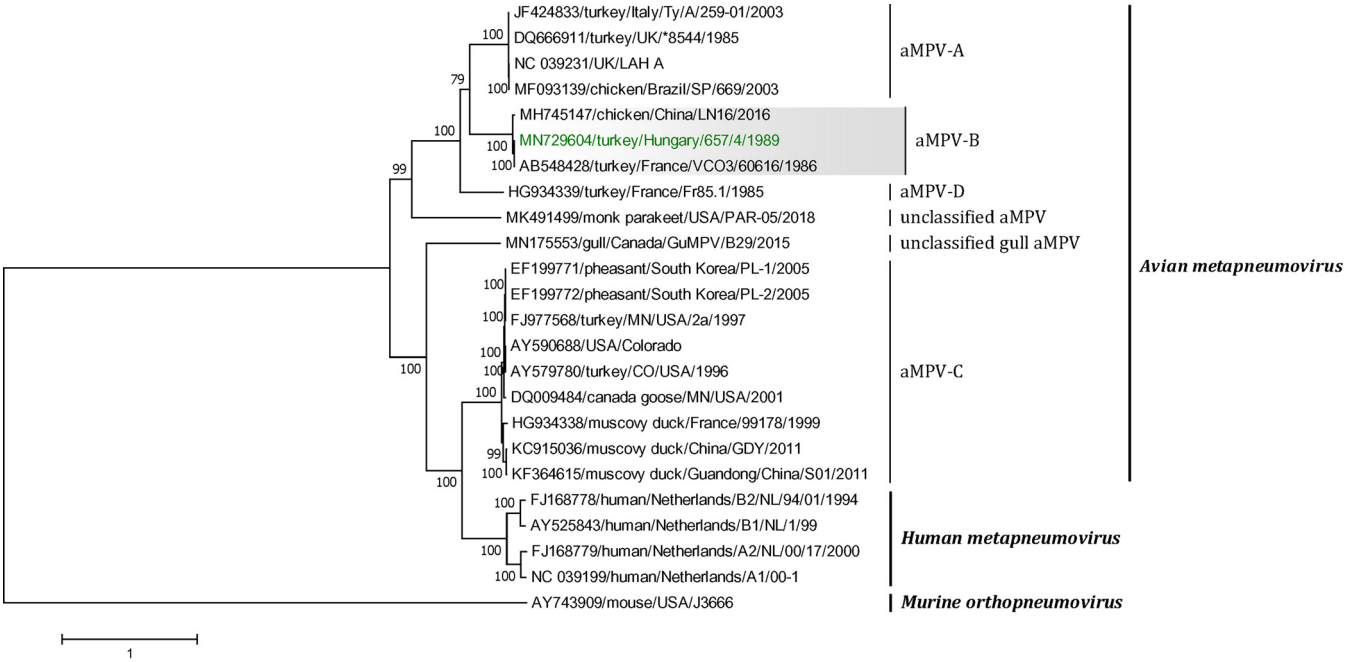

FIG 1 Phylogenetic analysis of the aMPV-B Hungary/657/4 isolate within the genus *Metapneumovirus*, based on the complete genome sequences constructed with the maximum likelihood method using the general time reversible model in MEGA v7.0.26. The tree with the highest log likelihood (−113,969.82) is 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 24 nucleotide sequences (sequences from *Murine orthopneumovirus* are included as an outgroup). All positions containing gaps and missing data were eliminated. There was a total of 12,392 positions in the final data set.

performed using the khmer v1.1-1 package (cutoff, 100; k-mer size, 20) (30). *De novo* assembly was performed utilizing MIRA3 v0.0.1 (31) with default settings. The final genome consensus was called from the raw aMPV reads, which were aligned with the *de novo*-generated contig by BWA-MEM (32) mapping of trimmed but unnormalized reads to the genome scaffold. This consensus had an average depth of coverage of 49-fold, with a maximum of 456-fold. The complete genome sequence of the isolate designated Hungary/657/4 was 13,508 nucleotides (nt) long (100% genome coverage, based on the VCO3/60616 reference genome [GenBank accession number AB548428]) and had a GC content of 43.2%. Genome annotation was performed using Geneious v11.1.5 and was confirmed by alignment with published aMPV genomes, all with default settings. The genome has the typical genetic structure of all metapneumovirus strains and contains eight open reading frames (3′-N-P-M-F-M2-SH-G-L-5′), which encode nine proteins of 1,176 nt, 840 nt, 765 nt, 1,617 nt, 561/222 nt, 543 nt, 1,245 nt and 6,015 nt. BLAST comparison to the currently available full-length aMPV genome sequences showed the highest (99.92%) nucleotide identity with the pathogenic strain VCO3/60616 (33), belonging to subtype B (Fig. 1). The VCO3/60616 strain is from the seventh passage of the field strain 86004, which was isolated from a turkey affected by turkey rhinotracheitis in France in 1986 (34, 35). Detailed analysis showed that Hungary/657/4 had the same 18 nucleotide substitutions as the pathogenic strain VCO3/60616, distinguishing them from the attenuated vaccine strain VCO3/50, which was established by another 50 passages in Vero cells (33).

Amino acid analysis showed that the fusion protein cleavage site contained 4 basic amino acids (^{99}RKKR ↓ F^{102}), which are conserved among all wild-type aMPV-B strains (35, 36). Currently, there are only two aMPV-B full-genome sequences available, from strains that were isolated in France in 1986 (GenBank accession number AB548428) (33) and in China in 2016 (GenBank accession number MH745147) (10). This complete genome sequence information is useful for in-depth understanding of the evolution of aMPV, as well as for planning vaccination strategies.

Data availability. The complete genome sequence of aMPV-B Hungary/657/4 has been deposited in GenBank under accession number MN729604. Raw data were deposited in the SRA under accession number SRR10518066, BioSample number SAMN13354755, and BioProject number PRJNA590745.

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