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GENOME SEQUENCES

Coding-Complete Genome Sequence of *Avian orthoavulavirus 16*, Isolated from Emperor Goose (*Anser canagicus*) Feces, Alaska, USA

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ABSTRACT We sequenced the coding-complete genome of an avian orthoavulavirus serotype 16 (AOAV-16) isolate recovered from emperor goose (*Anser canagicus*) feces collected in Alaska. The detection of AOAV-16 in North America and genomic sequencing of the resultant isolate confirms that the geographic distribution of this virus extends beyond Asia.

A viral isolate of a novel serotype of avian orthoavulavirus, avian orthoavulavirus serotype 16 (AOAV-16; family *Paramyxoviridae*, subfamily *Avulavirinae*), formerly taxonomically classified within *Avian paramyxovirus* (1), was recovered from wild bird feces collected in South Korea during 2014 and genomically characterized (AOAV-16/WB/Korea/UPO216/2014 [Korea/2014]; NCBI RefSeq accession number NC_039016.1 [2]). Subsequent reanalysis of archived samples collected from wild birds in Kazakhstan during 2006 revealed additional detections of AOAV-16, including the recovery of an isolate from white-fronted goose (*Anser albifrons*) feces (AOAV-16/white fronted goose/Central Kazakhstan/1791/2006 [Kazakhstan/2006]; GenBank accession number MH423285.2 [3]). Here, we report the coding-complete genome sequence for another AOAV-16 isolate which was recovered from emperor goose (*Anser canagicus*) feces collected in North America.

On 21 September 2019, fecal samples from emperor geese were collected along the shores of Cold Bay, Alaska (55.2567°N, 162.7056°W), as part of annual influenza A virus (IAV) research and surveillance within and adjacent to Izembek National Wildlife Refuge. Samples were screened via real-time reverse transcriptase PCR (rRT-PCR) for IAV and propagated in eggs using previously described methods (4, 5). RNA from hemagglutination assay-positive isolates was tested by rRT-PCR for IAV subtypes H5 and H7 (4–6). Viral RNA from isolate AK19-296 yielded an H5 cycle threshold value of 35.9, and the isolate was sent to the National Veterinary Services Laboratories for characterization. IAV cDNA was not identified when sequencing was attempted using an IAV-specific amplification protocol (7).

To characterize the hemagglutinating virus, RNA was extracted from isolate AK19-296 for sequencing using the MagMAX-96 viral RNA isolation kit. Sequence-Independent, Single-Primer-Amplification (SISPA [8]) was used to generate and enrich, via random primers, viral cDNA, followed by library preparation using the Ion Xpress Plus fragment library kit and sequencing on an Ion 520 chip using the Ion Chef and Ion S5 systems. The sequencing run generated 397,134 reads with a mean read length of 242 nucleotides (nt). DNAStar SeqMan NGen v14 was used to perform reference-guided mapping with default parameters for Ion Torrent read technology and 80% minimum match percentage for the first iteration. Representative consensus sequences for avian paramyxovirus types 1 through 20 were used for reference input, with reads

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TABLE 1 Comparison of the Alaska/2019 genome sequence to other AOAV-16 strains

Comparison sequence	Data for:													
	Genome[a]		Nucleoprotein		Phosphoprotein		Matrix		Fusion		Hemagglutinin-neuraminidase		Large polymerase	
	Length (nt)	Nucleotide identity (%)	ORF length (nt)[d]	Nucleotide identity (%)	ORF length (nt)[d]	Nucleotide identity (%)	ORF length (nt)[d]	Nucleotide identity (%)	ORF length (nt)[d]	Nucleotide identity (%)	ORF length (nt)[d]	Nucleotide identity (%)	ORF length (nt)[d]	Nucleotide identity (%)
Kazakhstan/2006	15,177[b]	98.1	1,476	98.5	1,200	97.2	1,095	98.4	1,656	98.2	1,857	98.2	6,609	98.4
Korea/2014	15,159[b,c]	97.0	1,476	98.3	1,200	95.9	1,095	97.5	1,656	96.8	1,857	97.5	6,609	97.3

[a] Includes noncoding regions; regions of incomplete coverage or deletion were trimmed from the alignments.
[b] Region of incomplete coverage trimmed from alignment.
[c] Region of identified deletion trimmed from alignment.
[d] All ORFs for AOAV-16/emperor goose/Alaska/AK19-296/2019 are equal in length to those of both AOAV-16/white fronted goose/Central Kazakhstan/1791/2006 and AOAV-16/white fronted goose/Central Kazakhstan/1791/2006.

mapping to AOAV-16, specifically Kazakhstan/2006. The alignment was visualized in DNAStar SeqMan Pro v14. The raw reads were remapped with the obtained consensus to confirm the final sequence. Overall, 326,658 reads were mapped, resulting in a median depth of coverage of 4,755×.

The final length of the isolate designated AOAV-16/emperor goose/Alaska/AK19-296/2019 (Alaska/2019) was 15,177 nt; it had a G+C content of 45% and was confirmed by alignment with currently available full-length AOAV-16 genome sequences using Geneious v11.0.4. At the 5′ terminus of Alaska/2019, 21 nt were not determined (coverage, 0×). This near-complete genome sequence (15,198 nt) complies with the paramyxovirus "rule of six" (9) and contains six open reading frames (ORFs; 3′-NP-P-M-F-HN-L-5′), determined via reference to Korea/2014 and Kazakhstan/2006. The Alaska/2019 sequence did not have an 18-nt deletion within the noncoding regions between the HN and L protein ORFs, which was reported for Korea/2014 but not for Kazakhstan/2006 (3). The W protein (140 amino acids [aa]) was coded for within the P protein ORFs of both Alaska/2019 and Korea/2014, while this protein was not predicted within the Kazakhstan/2006 ORF (3).

Nucleotide identities were calculated between genome sequences and between strains for each of the six protein-coding regions. As previously reported (3), the greatest differences in nucleotide identity were observed between the P protein sequences, while the NP comparisons were the most akin (Table 1). Genomic and protein ORF nucleotide sequences for Alaska/2019 were more similar to those of Kazakhstan/2006 (genome, 98.1%; protein ORFs, 97.2% to 98.5%) than to those of Korea/2014 (genome, 97.0%; protein ORFs, 95.9% to 98.3%) (Table 1) despite the closer temporal and geographic proximity of sample collection efforts in Alaska and South Korea.

The detection of AOAV-16 from waterfowl feces collected in North America confirms that the geographic distribution of this virus extends beyond Asia. Additional screening of wild bird samples for AOAV-16 is required to better understand the distribution and evolution of this viral agent.

Data availability. The consensus data for the Alaska/2019 genome sequence have been deposited in GenBank under the accession number MW161159. The raw sequence data were deposited in the NCBI SRA under BioProject accession number PRJNA670691.

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