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VIRUSES

First Complete Genome Sequences of Anopheles A Virus of the Genus *Orthobunyavirus*

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ABSTRACT Here, we report the first complete genome sequence of Anopheles A virus (ANAV) that was isolated from Colombia in 1940, and we include the first description of the medium and large segments. The ANAV medium and large segments share the highest identity with serogroup member Lukuni virus, which causes human infection.

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The Anopheles A serogroup of the genus *Orthobunyavirus*, in the family *Peribunyaviridae*, includes the prototype Anopheles A virus (ANAV), along with Lukuni and Tacaiuma viruses (1). All members of the family *Peribunyaviridae* have a negative sense, single-stranded, tripartite RNA genome composed of the small (S), medium (M), and large (L) segments. The S segment encodes the nucleocapsid (2), the M segment encodes two structural glycoproteins (3), and the L segment encodes the viral RNA-dependent RNA polymerase (4). The process of segment reassortment, which has been shown to be a factor in the evolution of several *Orthobunyavirus* serogroups (5), and lack of thorough genomic sequencing have confounded the detection and characterization of many bunyaviruses. Here, we describe the complete genome sequences for the original ANAV isolated in Meta Villavicencio, Colombia, in 1940.

The ANAV was originally isolated and propagated in suckling mouse brain. Viral RNA was extracted from the ninth passage of suckling mouse brain from lyophilized stock virus in the Division of Vector-Borne Diseases (DVBD) Arbovirus Reference Collection using the QIAamp viral RNA minikit (Qiagen). cDNA was generated using the Ovation RNA sequencing (RNA-seq) system version 2 (NuGEN). Libraries were made using the Ion Xpress Plus genomic DNA (gDNA) fragment library preparation kit (Life Technologies) by fragmenting cDNA for 2 min, generating fragments of 250 bp, on average. Libraries were barcoded using Ion Xpress Barcodes (Life Technologies) and quantified using the Ion library quantitation kit (Life Technologies). Libraries were prepared for sequencing using the Ion OneTouch 2 system with Ion PGM Hi-Q OT2 kit (Life Technologies). Sequencing was performed on the Ion Torrent Personal Genome Machine using the Ion PGM Hi-Q sequencing kit and Ion 316 Chip version 2 (Life Technologies). The sequences were assembled into contigs using *de novo* assembly in CLC Genomics Workbench (Qiagen) and subjected to NCBI BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Contigs were obtained that had high similarity to published ANAV S segment (95%) and to the Anopheles A virus serogroup member, Lukuni virus M (69%) and L segments (71%). Consensus contigs for each segment were extracted from CLC Genomics Workbench and used in templated assemblies in SeqMan NGen (DNAStar) that were able to generate final assemblies which spanned the 5′ to 3′ ends. The predicted open reading frames (ORFs) were found to be 738 nucleotides (nt) for the S segment (6), 4,218 nt for the M segment, and 6,729 nt for the L segment. The S and L segments were found to have the canonical terminal ends, in coding sense,

5′-AGTAGTGTACT. . .AGCACACTACT-3′, while the M segment terminal ends were 5′-AGTAGTGTACT. . .AGATACATACA-3′. The final assemblies have been annotated and submitted to GenBank.

With the frequent recognition of reassorted bunyaviruses, thorough molecular characterization is imperative in this diverse virus family. With members of the ANAV serogroup known to cause human infection and disease (7, 8), our sequence data will aid in surveillance, as arboviruses continue to be a frequently emerging public health threat.

Accession number(s). Sequence data have been deposited in GenBank with the numbers KY793537, KY793538, and KY793539 for the L, M, and S segments, respectively.

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The mention of trade names or products is solely for the purpose of providing specific information and does not imply endorsement by the CDC. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the CDC.

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