

Complete Genome Sequencing of Four Geographically Diverse Strains of Batai Virus

Allison Groseth,^a Keita Matsuno,^a Eric Dahlstrom,^b Sarah L. Anzick,^b Stephen F. Porcella,^b and Hideki Ebihara^a

Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA,^a and RML Genomics Unit, Research Technology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA^b

Batai virus (BATV) is a widely distributed but poorly studied member of the *Orthobunyavirus* genus in the family *Bunyaviridae* and is of particular interest as a known participant in natural reassortment events. Both research and surveillance efforts on this and other related viruses have been hampered by the lack of available full-length sequence data covering all three genomic segments. Here, we report the complete genome sequence of four BATV strains (MM2222, Chittoor/IG-20217, UgMP-6830, and MS50) isolated from various geographical locations. Based on these data, we have determined that strain MS50 is in fact unrelated to BATV and likely represents as a novel genotype in the genus *Orthobunyavirus*.

Batai virus (BATV) is a member of the *Orthobunyavirus* genus within the family *Bunyaviridae*. It is a single-stranded negative-sense RNA virus whose genome is divided into 3 segments: the S segment, M segment, and L segment. BATV is widely distributed throughout large parts of Africa, Asia, and Europe; however, infections in humans are rare, with patients developing a febrile illness (4). Importantly, based on partial sequencing data, BATV was identified as the M-segment donor for a reassortment event that produced the recently identified and highly virulent Ngari virus (NRIV) (1, 6). Given its broad geographical distribution, and also the broad range of mosquito species it can infect, it is possible that BATV could be a participant in other reassortments that have yet to be identified. However, despite the potential health concern posed by bunyavirus reassortment, as emphasized by the recent emergence of Schmallenberg virus (3), a lack of complete sequencing data for many of these viruses complicates surveillance and virus identification efforts. Indeed, full-length genome sequence data are available for very few orthobunyaviruses, and while full-length sequencing of a single BATV strain was reported in 2009, the data have not been made publically available (5).

To determine the complete genome sequence of BATV, the sequences of two strains (MM2222 and MS50) were first obtained using the 454 FLX pyrosequencing technology platform (454 Life Sciences). Library construction was performed using previously described methods (2), and genome assembly/analysis was conducted using various publically available algorithms. Based on partial sequencing, MM2222 and MS50 appeared highly divergent, despite both being isolated in Malaysia (from *Culex gelidus* and *Aedes curtipes*, respectively). Overlapping primer sets were designed based on the 454-determined sequences to allow confirmation by Sanger sequencing. These primer sets were then also used to determine the complete genome sequence of two additional BATV strains (Chittoor/IG-20217 and UgMP-6830), isolated in India (from *Anopheles barbirostris*) and Uganda (from *Aedes abnormalis*), respectively.

Analysis of homology among the segments indicates that MS50 is not closely related to any of the other BATV strains analyzed, with nucleotide identity values of 77 to 78% (S segment), 64% (M segment), and 73% (L segment). By comparison, other BATV strains all showed >90% identity to one another across all segments. MS50 also showed comparably low levels of identity (56 to

82%, 54 to 73%, and 61 to 74%, respectively) to the other Bunyamwera serogroup members for which full-length sequence data are available. These data indicate that BATV strain MS50 is not related to other BATVs and that its classification needs to be revisited. Indeed, the data suggest that this virus may represent a novel genotype within the Bunyamwera virus species of the *Orthobunyavirus* genus. These findings emphasize the need for fulllength characterization of all three genomic segments of bunyaviruses in order to better identify newly emerging viruses with potential significance for human and/or animal health.

Nucleotide sequence accession numbers. The genome sequences of all BATV strains were deposited in GenBank under the following accession numbers (S segment, M segment, and L segment): JX846595, JX846596, and JX846607 (MM2222), JX846598, JX846599, and JX846600 (Chittoor/IG-20217), JX846601, JX846602, and JX846603 (UgMP-6830), and JX846604, JX846605, and JX846606 (MS50).

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