

Complete Genome Sequencing of Mosquito and Human Isolates of Ngari Virus

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Ngari virus (NRIV) is a recently described, naturally occurring reassortant between two other orthobunyaviruses, Bunyamwera virus (BUNV) and Batai virus (BATV). Intriguingly, this reassortment was associated with the acquisition of heightened virulence, although the molecular basis for this is not understood. Here we report the first complete genome sequences of Ngari virus. We include five isolates from various geographical locations, as well as samples isolated from both mosquitos and human cases. Based on an analysis of these sequence data, NRIVs are clearly genetically distinct from all known BUNV and BATV strains but are very closely related to one another regardless of their source.

Ngari virus (NRIV) is a member of the *Orthobunyavirus* genus in the family *Bunyaviridae*. As such, it is a single-stranded negative-sense RNA virus whose genome is divided into 3 segments: the S segment, M segment, and L segment. NRIV has been identified as a naturally occurring reassortant between the Batai virus (BATV) M segment and the Bunyamwera virus (BUNV) S and L segments, and intriguingly, this reassortment event is associated with increased virulence (1–3, 5). However, the molecular basis for the increased virulence of NRIV remains unclear and research on this topic is hampered by the lack of complete genome sequence data for any NRIV strain. NRIV infection in humans has resulted in at least two very large outbreaks in Central Africa, with infection resulting in hemorrhagic fever in some cases (2, 3). The first recognized outbreak occurred in Kenya, Somalia, and Tanzania between 1998 and 1999 and produced the 9800535 and 9800521 strains, both of which were isolated from human cases (2, 3). Retrospectively, a previous outbreak from 1988 in Sudan, from which the SUD-HKV66 and SUD-HKV141 isolates derive, was also identified as NRIV (2, 7). The DakArD28542 strain represents an early mosquito (*Aedes simpsoni*) isolate from Senegal, isolated in 1979 (6), prior to NRIV's recognition as a significant human pathogen.

Based on available partial genome data, there appeared to be a high degree of sequence conservation between NRIV genome segments and strains of the parental viruses involved in the reassortment process (i.e., BUNV and BATV). Therefore, we generated multiple overlapping reverse transcriptase PCR (RT-PCR) primer sets based on alignments of available BUNV S- and L-segment sequences and BATV M-segment sequences. Using these primer sets, overlapping PCR fragments sufficient to cover each genome segment were amplified for Sanger sequencing. The 5' and 3' termini of the genome were amplified and sequenced using RNA ligation as described previously (4).

Based on the full-length genome analysis, it is clear that all analyzed NRIV strains have the genetic composition $S_{\text{BUNV}}-M_{\text{BATV}}-L_{\text{BUNV}}$ and that these sequences are divergent from available BUNV and BATV sequences. In particular, the S segment of NRIV is notably distinct from the prototype BUNV (strain 6547-8) sequence, forming an entirely separate phylogenetic branch. In addition, while our data confirm BATV (strain UgMP-6830) as the closest known relative of the NRIV M segment, these sequences still form their own discrete clade. It is also of interest to note that, regardless of which segment is analyzed, all five NRIVs

form a closely related group with similar genetic distances between isolates from different human outbreaks or between human and mosquito isolates. The availability of these complete genome sequences will be crucial for future studies aimed at investigating the molecular determinants associated with virulence acquisition during bunyavirus reassortment, as well as for surveillance efforts and epidemiological studies targeting these viruses.

Nucleotide sequence accession numbers. The genome sequences of all NRIV strains were deposited in GenBank under the following accession numbers (S segment, M segment, and L segment): [JX857316](#), [JX857317](#), and [JX857318](#) (DakArD28542), [JX857319](#), [JX857320](#), and [JX857321](#) (SUD-HKV66), [JX857322](#), [JX857323](#), and [JX857324](#) (SUD-HKV141), [JX857325](#), [JX857326](#), and [JX857327](#) (9800521), and [JX857328](#), [JX857329](#), and [JX857330](#) (9800535).

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