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Complete Genome Sequencing of Trivittatus virus

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Abstract

Trivittatus virus (family *Bunyaviridae*, genus *Orthobunyavirus*) represents an important genetic intermediate between the California encephalitis group, and Bwamba/Pongola and Nyando groups. Here, we report the first complete genome sequence of the prototype (Eklund) strain, isolated in 1948, which interestingly shows only few differences compared to partial sequences of modern strains.

Keywords

bunyavirus; orthobunyavirus; genome sequencing; Trivittatus virus; California encephalitis virus group

Trivittatus virus (TVTV; genus *Orthobunyavirus*, family *Bunyaviridae*) is a tri-segmented single-stranded negative-sense RNA virus whose genome is divided into the small (S), medium (M) and large (L) segments. TVTV was first isolated from *Aedes trivittatus* in Bismarck, North Dakota in 1948 [6]. It has since been shown to be widely distributed throughout North America [6], although most isolations continue to be from the American Midwest [7]. Despite serological evidence of human infection [10], little is known about the consequences of infection, although symptomatic illness is suspected [8, 11].

Based on S- and M-segment sequencing data, TVTV appears to represent a genetic intermediate that bridges the California encephalitis virus (CEV) group, which includes several important causes of neurological disease in North America [5], and the Bwamba/Pongola (BWAV/PGAV) group, which includes highly prevalent agents of febrile illness in Africa [9]. Given its relationship to these important agents of human clinical disease, as well as its unique position with respect to the evolution of these groups, we were interested to establish the complete genome sequence for this virus, including the L-segment for which no pre-existing data was available.

Preliminary genome sequences of the prototype Eklund strain of TVTV were obtained using the 454 FLX pyrosequencing technology platform (454 Life Sciences). Library construction was performed using previously described methods [3] and genome assembly/analysis was

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conducted using various publically available algorithms. Based on the preliminary 454-determined sequences, primer sets were designed to allow confirmation by Sanger sequencing, including terminal 3' and 5' RACE using previously described approaches [9].

The sequences obtained in this study were compared to complete S- (973nt) and M-segment (4503nt) data available for this strain in GenBank (Accession # U12803 and AF123491) as well as partial (812nt) S-segment data for two more recent virus isolates from 2003 (Accession # KM215565 and KM215564) [1, 2, 4]. No differences were identified between our S-segment sequence and that previously reported for the Eklund strain and we could confirm the previously identified polymorphism in the 3' terminal genome sequence (3'-AGTAGTGTACYCCAC). Only a few isolated differences were observed in the M-segment sequence (99.2% nucleotide identity), compared to the previously published sequence. Further, the S-segment sequences of the more recent strains (strains ND3702 and ND3374) were also found to be highly similar (99.3% and 99.0% nucleotide identity, respectively). Phylogenetic analysis showed a clear clustering of the TVTV sequences into a single clade and showed consistent placement of these sequences between the CEV and BWAV/PGAV groups, for all three genome segments (Figure 1). Further, genetic divergence on the amino acid level clearly supported the classification of TVTV as a distinct virus species with only modest levels of amino acid identity [66.4 – 75.8% (nucleoprotein; N), 51.1 – 68.7% (glycoprotein; GPC) and 63.9 – 81.9% (polymerase; L)] being identified to existing virus species of the closely related BWAV/PGAV and CEV clades (Online Resource 1).

The availability of this type of full-length genome data is critical for the accurate genetic classification of bunyaviruses, and the availability of broad datasets, including genetically intermediate viruses like TVTV, are an important resource for researchers seeking to understand the evolution of these virus groups.

The genome sequences of TVTV (strain Eklund) determined in this study were deposited in GenBank under the accession numbers KR149247 (S-segment), KR149248 (M-segment) and KR149249 (L-segment).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Phylogenetic relationship of Trivittatus virus to other Orthobunyaviruses
 Maximum likelihood trees were constructed based on the amino acid sequences of the nucleoprotein, glycoprotein and polymerase, as indicated. Bootstrap values based on 1,000 replicates are also indicated. Sequences generated in this study are shown in bold.