JVI Journals.ASM.org

GENOME ANNOUNCEMENT

Full Genomic Sequence of the Prototype Strain (M64) of Rio Bravo Virus

Evgeniya Volkova,[a,b,c] Robert B. Tesh,[a,b,c] Thomas P. Monath,[d,e] and Nikos Vasilakis[a,b,c]

Center for Biodefense and Emerging Infectious Diseases and Department of Pathology[a] and Center for Tropical Diseases,[b] University of Texas Medical Branch, Galveston, Texas, USA; Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas, USA[c]; PaxVax, Inc., Menlo Park, California, USA[d]; and Kleiner Perkins Caufield & Byers, Menlo Park, California, USA[e]

***Rio Bravo virus* (RBV) is a member of the family *Flaviviridae*, genus *Flavivirus*. It belongs to a group of viruses in the genus with no known vector. In this report, we analyze the complete genome of the prototype RBV, strain M64.**

Rio Bravo virus (RBV) is a member of the family *Flaviviridae*, genus *Flavivirus* (4, 9); it was first isolated in 1954 from the submaxillary salivary glands of an adult male Mexican free-tailed bat, *Tadarida brasiliensis mexicana*, captured in Rio Bravo School, Kern County, CA (6–8). RBV belongs to a group of flaviviruses with no known vector (NKV) (2, 9), because it has never been isolated from either wild-caught or laboratory-infected arthropods and it does not replicate in a number of mosquito cell lines *in vitro* (reviewed in reference 5). Bats are the only known amplification and reservoir host and are believed to become persistently infected (1, 3) with RBV. Transmission probably occurs by direct contact (saliva) (10) and/or indirectly via fomites or aerosols (3). Evidence for the aerosol route is suggested by reports of a number of accidental human infections acquired in the laboratory (11). The pathogenesis of RBV in humans is not well understood, but the limited number of documented human infections suggest a febrile illness with central nervous system involvement and orchitis or oophoritis as commonly observed complications (11).

The complete genome of the prototype M64 virus, obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch (passage history: 9 intracerebral passages in suckling mice and once in Vero cells), was sequenced with an ABI 3500 genetic analyzer using the Sanger method and based on 4 overlapping amplicons (100–3514, 2555–4995, 4551–7609, and 7008–10020). The complete 5′- and 3′-terminal sequences were determined by their ligation and sequencing with the RLM random amplification of cDNA ends kit (Ambion). The complete M64 genome is 10,742 nucleotides (nt) long; the 5′- and 3′-noncoding regions (NCR) are 116 nt and 486 nt long, respectively. The open reading frame (ORF) is 10,140 nt long and encodes three structural proteins, capsid (C; 102 amino acids [aa]), premembrane/membrane (prM/M; 165 aa), and envelope (E; 484 aa), and seven nonstructural (NS) proteins, NS1 (353 aa), NS2A (229 aa), NS2B (130 aa), NS3 (617 aa), NS4A (122 aa), NS4B (281 aa), and NS5 (898 aa). The ORF of M64 has 21 nt that are different from the RiMAR strain (GenBank accession number AF144692) (2), 11 of which are concentrated within a short 25-nt region in the NS5 gene (nt 7685 to 7710), resulting in 99% sequence homology with the RiMAR strain at both the nucleotide and amino acid levels. There is only 1 nt difference between M64 and RiMAR in the 3′-NCR. The complete genome sequence of M64 will be helpful for the development of a reverse genetics system to study the unique aspects of the RBV life cycle *in vivo* and *in vitro*, particularly of the ability to establish noncytopathic persistent infections.

Nucleotide sequence accession number. The GenBank accession number of RBV prototype M64 strain is JQ582840.

ACKNOWLEDGMENTS

This work is supported in part by the Department of Pathology start-up funds (N.V.), NIH contract HHSN272201000040I/HHSN27200004/D04 (R.B.T.), and a PaxVax, Inc., contract (N.V., E.V., and T.P.M.).

REFERENCES

1. **Baer GM, Woodall DF.** 1966. Bat salivary gland virus carrier state in a naturally infected Mexican freetail bat. Am. J. Trop. Med. Hyg. **15**:769–771.
2. **Billoir F, et al.** 2000. Phylogeny of the genus Flavivirus using complete coding sequences of arthropod-borne viruses and viruses with no known vector. J. Gen. Virol. **81**:2339.
3. **Constantine DG, Woodall DF.** 1964. Latent infection of Rio Bravo virus in salivary glands of bats. Public Health Rep. **79**:1033–1039.
4. **De Madrid AT, Porterfield JS.** 1974. The flaviviruses (group B arboviruses): a cross-neutralization study. J. Gen. Virol. **23**:91–96.
5. **Hendricks DA, Patick AK, Petti LM, Hall AJ.** 1988. Biochemical and biophysical characteristics of Rio Bravo virus (Flaviviridae). J. Gen. Virol. **69**:337–347.
6. **Johnson HN.** 1967. Ecological implications of antigenically related mammalian viruses for which arthropod vectors are unknown and avian associated soft tick viruses. Jpn. J. Med. Sci. Biol. **20**(Suppl.):160–166.
7. **Johnson HN.** 1960. Ecology of viral diseases of man transmitted by arthropods. Bol. Ofic. Sanit. Panam. **48**:134–140.
8. **Karabatsos N (ed).** 1985. International catalogue of arboviruses including certain other viruses of vertebrates, 3rd ed, p 859–860. American Society of Tropical Medicine and Hygiene, San Antonio, TX.
9. **Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB.** 1998. Phylogeny of the genus *Flavivirus*. J. Virol. **72**:73–83.
10. **Price JL.** 1978. Isolation of Rio Bravo and a hitherto undescribed agent, Tamana bat virus, from insectivorous bats in Trinidad, with serological evidence of infection in bats and man. Am. J. Trop. Med. Hyg. **27**:153–161.
11. **Sulkin SE, Burns KF, Shelton DF, Wallis C.** 1962. Bat salivary gland virus: infections of man and monkey. Tex. Rep. Biol. Med. **20**:113–127.

Received 7 February 2012 Accepted 8 February 2012

Address correspondence to Nikos Vasilakis, nivasila@utmb.edu.

doi:10.1128/JVI.00331-12