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Address for correspondence: Glenn Morris, Emerging Pathogens Institute, 2055 Mowry Rd, PO Box 100009, Gainesville, FL 32610, USA; e-mail: jgmorris@epi.ufl.edu

# Metatranscriptomic Identification of Trubanaman Virus Sequences in Patient with Encephalitis, Australia

Krispin Hajkowicz, John Woodford, Elango Subramonia Pillai, Andrea Henden, Kym Lowry, Mary E. Petrone, Patrick N.A. Harris, Edward C. Holmes

Author affiliations: Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia (K. Hajkowicz, E. Subramonia Pillai, A. Henden, P.N.A. Harris); University of Queensland Centre for Clinical Research, Brisbane (K. Hajkowicz, K. Lowry, P.N.A. Harris); The School of Medical Sciences, University of Sydney, Sydney, New South Wales, Australia (K. Hajkowicz, M.E. Petrone, E.C. Holmes); Ipswich Hospital, Ipswich, Queensland, Australia (J. Woodford); Queensland Institute of Medical Research-Berghofer, Brisbane (J. Woodford, A. Henden); Queensland Paediatric Infectious Diseases Sakzewski Laboratory, Brisbane (K. Lowry); Pathology Queensland, Brisbane (P.N.A. Harris)

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Using metatranscriptomics, we identified Trubanaman virus in cerebrospinal fluid from a severely immunocompromised man who died of encephalitis in Queensland, Australia. Virus sequences were related to orthobunyaviruses previously detected in mosquitoes in Australia. Testing for other causes yielded negative results, suggesting that Trubanaman virus was the cause of this fatal encephalitis case.

Approximately 50% of global encephalitis cases remain undiagnosed by conventional testing (*1*). Metagenomic next-generation sequencing (mNGS), particularly metatranscriptomics (i.e., total RNA sequencing), is an emerging approach to infection diagnosis that reveals all nucleic acid in a sample, making it ideal for detecting novel and emerging pathogens (*2*).

*Orthobunyavirus* (order Bunyavirales) is a diverse genus of negative-sense single-stranded RNA viruses recognized to cause febrile illness and encephalitis in humans globally (*3*). The best described orthobunyaviruses are La Crosse virus and Jamestown Canyon virus, both of which rarely cause encephalitis, permanent neurologic sequalae, or death (*4*,*5*). Jamestown Canyon virus is associated with meningoencephalitis in immunocompromised persons (*5*), whereas the emerging Oropuche virus is associated with fever, headache, myalgias, and rare cases of meningoencephalitis and has recently expanded its range in Central and South America (*6*). We used metatranscriptomics to investigate a case of encephalitis in an immunocompromised person in Australia.

The study was approved by the Metro-North Health Human Research Ethics Committee and written informed consent was obtained from the patient and his next of kin. Metatranscriptomic sequencing and analysis methods are detailed (Appendix, https://wwwnc.cdc.gov/EID/article/31/12/25-1190-App1.pdf).

A man in his 50s who lived in West Moreton, Queensland, Australia, was admitted for a volunteer unrelated donor allogeneic hemopoietic stem cell transplantation with posttransplant cyclophosphamide and tacrolimus for B-cell acute lymphoblastic leukemia in complete remission one. There was no central nervous system involvement. He received 8 cycles of rituximab-hyper cyclophosphamide, vincristine, doxorubicin, and dexamethasone before transplantation. The transplant was complicated by a polymicrobial bloodstream infection that was successfully treated with intravenous daptomycin, as well as mucositis and diarrhea.

On day 18 after the hemopoietic stem cell transplantation, the patient experienced fever to 38.8°C, tachycardia to $10^9$ beats/min, muscular pain, intermittent headache, and confusion manifesting as slow and tangential answers to questions, difficulty word-finding, reduced oral intake, disorientation to time and place, and delusions such as thinking that he had been in a car accident. The onset coincided with recovery of his neutrophil and lymphocyte

count. His confusion fluctuated but generally deteriorated. Twenty-two days later, a cerebrospinal fluid (CSF) examination was performed (Table). Magnetic resonance imaging (MRI) of the brain was also performed, and results were unremarkable. However, results of an electroencephalograph were abnormal, showing mild, diffuse cortical dysfunction but no epileptiform activity. Results of a nasopharyngeal nucleic acid amplification test (NAAT) were positive for rhinovirus. Stool, blood, and urine culture and NAAT results were negative for viruses, bacteria, and fungi (Table). He was unresponsive to corticosteroids, and during the next few months, his level of consciousness, function, and speech declined; serial MRIs showed progressive cerebral atrophy. He died 6 months after the onset of confusion.

Metatranscriptomic sequencing of the patient's CSF using NovaSeq (Illumina, https://www.illumina.com) generated a total of 57,452,775 paired reads, of which 74 matched the M glycoprotein precursor of Trubanaman, Murrumbidgee, and Buffalo Creek viruses (i.e., the Mapputta group, which likely represents a single species within the genus *Orthobunyavir*us). E-values were $<10^{-116}$. From some of those reads, we assembled a single contig of 270 bp (GenBank accession no. PV702715), denoted Trubanaman virus West Moreton (Figure). We did not recover reads from the RNA-dependent RNA polymerase (RdRp) or other virus genes. The water control was negative for bunyaviruses. Similarly, metatranscriptomic analysis was negative for other known or putative human pathogenic viruses, bacteria, fungi, and parasites, and no other candidate pathogens were identified.

Using metatranscriptomics, we identified Trubanaman virus sequences in a CSF sample from a person with encephalitis. Extensive testing for other infectious, autoimmune, and malignant causes yielded negative results. In the context of a high-risk immunocompromised person with typical clinical manifestations of encephalitis, our findings support, but do not confirm, that Trubanaman virus was the cause of the patient's encephalitis. PCR could not be performed on the original sample because it was fully depleted for conventional testing and sequencing, although no viable routes to sample contamination existed. Follow-up testing of CSF collected 6 weeks later was negative by both metatranscriptomics and orthobunyavirus-specific PCRs targeting the N protein and RdRp.

Trubanaman and related viruses have been detected in mosquito populations throughout Australia (*7*). Patients with a suspected arthropodborne virus infection in New South Wales exhibited neutralizing antibody prevalences of 4.7% to Gan Gan virus (GGV) and 1.4% to Trubanaman virus (*8*). GGV was associated with an acute febrile illness and polyarthritis in 3 persons in Australia who had significant titer rises in paired serum samples, as well as GGV-specific IgM (*9*). In addition, serologic evidence suggests that kangaroos, feral animals, and domestic horses are reservoirs for orthobunyaviruses in Australia (*9*). Of note, 2 bunyavirus-associated cases of fatal meningoencephalitis in immunocompromised persons were recently described in the United States using CSF mNGS (*10*). Further research is required to establish the pathogenic role of Trubanaman virus as a cause of encephalitis in Australia and to determine the arthropod vectors, zoonotic reservoirs, and seroprevalence. However, our findings suggest that Trubanaman virus was the cause of this fatal encephalitis case, and clinicians should be aware of the possibility of infection with this virus in similar cases.

**Table.** Results of cerebrospinal fluid testing in an immunocompromised patient with encephalitis in study of metatranscriptomic identification of Trubanaman virus, Australia*

| Test | Result |
|---|---|
| Leukocytes, $\times 10^6$ cells/L (reference range <5) | 2 |
| Erythrocytes, $\times 10^6$ cells/L (reference range <5) | 61 |
| Protein, mg/L (reference range 150–500) | 570 |
| Glucose, mmol/L (reference range 2.2–3.9) | 5.3 |
| Microscopy and culture | No bacteria or fungi detected |
| NAAT for herpes simplex 1 and 2, cytomegalovirus, varicella zoster virus, human herpesvirus 6, John Cunningham virus, enteroviruses, and parechoviruses | Negative |
| NAAT for *Escherichia coli*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Cryptococcus* spp. | Negative |
| Cryptococcal antigen in cerebrospinal fluid | Negative |
| Neuronal antibodies Hu, Ri, Yo, and Purkinje cell cytoplasmic types 2 and Tr | Negative |
| N-methyl-D-aspartate, GABA-B, and AMPA receptors | Negative |
| CV2/collapsin response mediator protein 5 | Negative |
| Ma/Ta, glutamic acid decarboxylase, and amphyphysin antibodies | Negative |

*NAAT, nucleic acid amplification test.

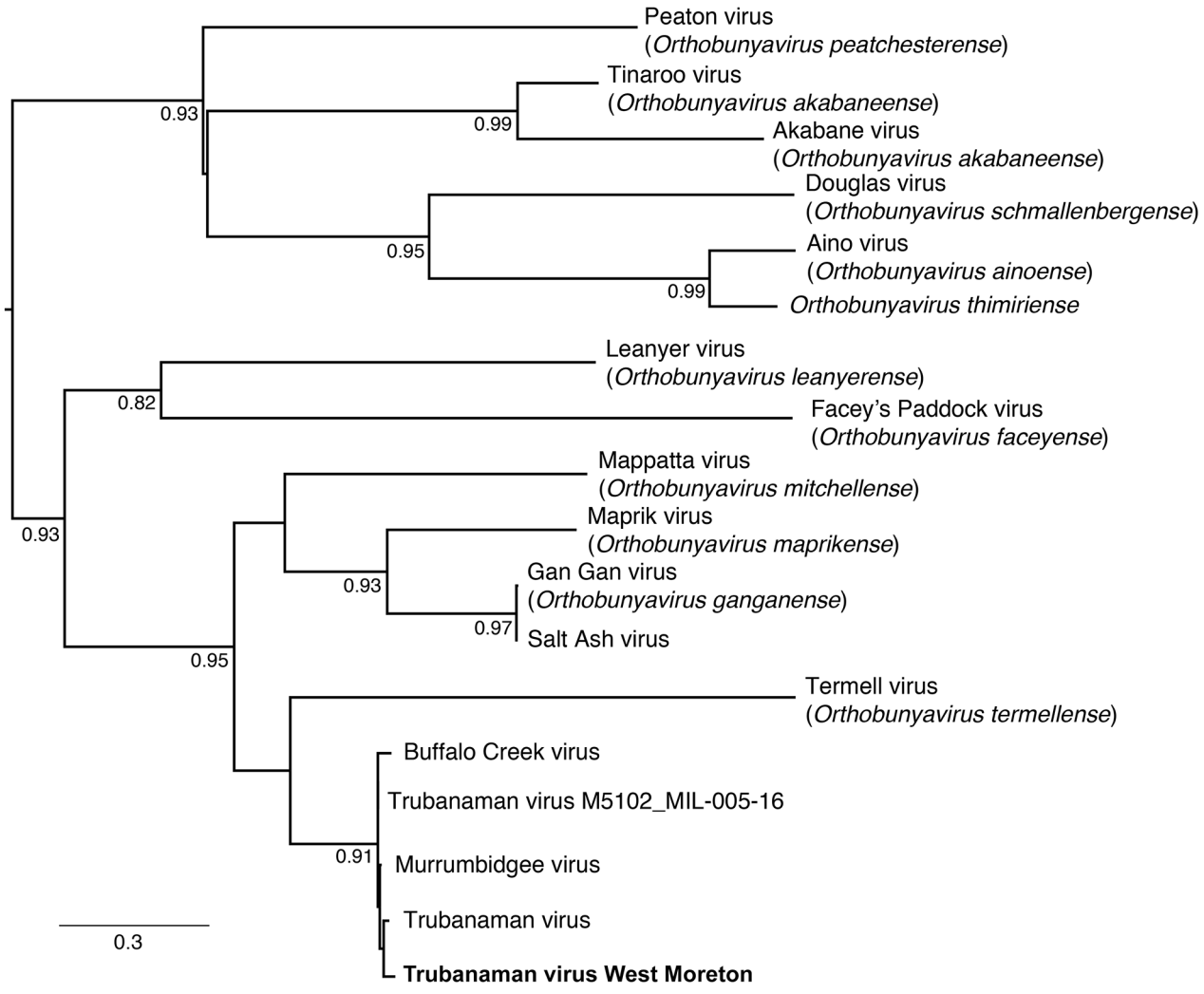


**Figure.** Phylogenetic tree of orthobunyavirus M segment sequences from this study and previously collected mosquito samples in study of metatranscriptomic identification of Trubanaman virus in patient with encephalitis, Australia. Bold font indicates the human sequence from this study; other sequences are from mosquito orthobunyaviruses previously identified in Australia. Sequences were aligned using MAFFT (https://mafft.cbrc.jp/alignment/server/index.html). The phylogeny was estimated using the maximum-likelihood approach in PhyML (http://atgc.lirmm.fr/phyml), by using the general time reversible model of nucleotide substitution and gamma-distributed rate variation among sites. Bootstrap support values are displayed at nodes. Scale bar indicates nucleotide substitutions per site.

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## About the Author

Mr. Hajkowicz is a senior staff specialist and former director of the Infectious Diseases Unit at Royal Brisbane and Women's Hospital and Clinician Researcher at the University of Queensland Centre for Clinical Research and a PhD student at the University of Sydney School of Medical Sciences. His research interests include detection of emerging viral infections in Australia, including mpox and Zika virus, and pandemic response.

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Address for correspondence: Krispin Hajkowicz, School of Medical Sciences, University of Sydney, Biomedical Bldg C81, Central Avenue, Everleigh, NSW 2015, Australia; email: khaj2604@uni.sydney.edu.au

# Carbapenem-Resistant *Salmonella Typhi* Infection in Traveler Returning to Germany from India, 2024

Sandra Simon, Eva Trost, Jan Lennings, Julia Enkelmann, Julia Kuhn, Michael Pietsch, Antje Flieger

Author affiliations: Robert Koch Institute, Wernigerode, Germany (S. Simon, E. Trost, M. Pietsch, A. Flieger); Public Health Department of Stuttgart, Stuttgart, Germany (J. Lennings); Robert Koch Institute, Berlin, Germany (J. Enkelmann); Ministry of Social Affairs, Health and Integration Baden-Wuerttemberg, Stuttgart (J. Kuhn)

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We report on a carbapenem-, extended spectrum β-lactam-, fluoroquinolone-, and tetracycline-resistant *Salmonella enterica* serovar Typhi strain in a patient returning to Germany from India. Considering the recent emergence of extensively drug-resistant *Salmonella* Typhi strains, further expansion of antibiotic resistance to carbapenems poses a serious threat for typhoid fever treatment.

Extensively drug-resistant (XDR) *Salmonella enterica* serovar Typhi, belonging to the H58 haplotype, was first identified in Sindh, Pakistan, in 2016 (*1*). Since then, those strains have been reported worldwide, mainly in association with travel to Pakistan. XDR *Salmonella* Typhi exhibit a multidrug-resistant (MDR) phenotype, including resistance to chloramphenicol, ampicillin, and sulfamethoxazole/trimethoprim, along with additional resistance to fluoroquinolones and third-generation cephalosporins. Consequently, therapeutic options for treating infections caused by XDR strains are primarily limited to the macrolide azithromycin and carbapenems. *Salmonella* Typhi strains resistant to carbapenems, azithromycin, or both have been reported occasionally. Carbapenem-resistant strains isolated in Pakistan harbored genes encoding VIM, GES, or NDM-5 carbapenemases (*2*,*3*). The respective NDM-5–positive strain showed the XDR phenotype and was phylogenetically assigned to the H58 haplotype. Recently, another case study described a non–XDR NDM-5–producing *Salmonella* Typhi isolate from India, which revealed additional resistance to fluoroquinolones and third-generation cephalosporins but remained susceptible to chloramphenicol, sulfamethoxazole/trimethoprim, and azithromycin (*4*).

We report an NDM-5–producing *Salmonella* Typhi strain isolated from a patient from Germany after returning from India. The patient, an experienced traveler to India who was last vaccinated with the typhoid polysaccharide vaccine in June 2021, undertook a 4-week round trip through several states in southwest India in September and October 2024. Upon return to Germany, the patient had onset of mild gastrointestinal symptoms, including diarrhea and abdominal pain. Symptoms gradually worsened over 2 weeks, prompting the patient to seek outpatient medical attention at a medical practice and a clinic, where stool and blood samples were collected. Blood tests were suggestive of a bacterial infection, and an empiric 3-day course of ciprofloxacin was commenced. However, after completion of the antibiotic therapy, the patient's condition further deteriorated. Specifically, the patient had onset of fever (38.5°C) and severe headaches. Molecular stool diagnostics provided positive PCR signals for *Salmonella* and *Shigella* spp., but stool culture only resulted in growth of *Salmonella* spp. We subtyped the retrieved isolate (no. 24-09143) as *Salmonella* Typhi; phenotypic antimicrobial susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing guidelines determined resistance to