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Case Report

Undiagnosed West Nile virus lineage 2d infection in a febrile patient from South-west Uganda, 2018

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ABSTRACT

We report the retrospective identification and subsequent recovery of a near-complete West Nile Virus lineage 2 genomes from a hospitalized patient with acute febrile illness in Uganda, using a combination of degenerate primer polymerase chain reaction screening and a novel 1200bp nanopore-based whole-genome amplicon sequencing scheme. This represents the first West Nile virus genome to be recovered from a human in Uganda since its discovery in 1937. Basic molecular rather than serological surveillance methods could be more widely deployed in the region to better diagnose febrile infections.

West Nile virus (WNV) is an arbovirus belonging to the family *Flaviviridae* and genus *Orthoflavivirus* [1]. Disease associated with the virus is frequently mild, asymptomatic, and undiagnosed; however, severe or fatal cases may present with neurological pathologies, including encephalitis, meningitis, and acute flaccid paralysis [2]. WNV is phylogenetically divided into two major lineages, 1 and 2, both with broad geographic distribution principally described in Africa, Europe, and North America, and seven further infrequently detected and characterized lineages 3-9 [3].

Except for the original 1937 WNV isolate, subsequently serially passaged before sequencing in the 1980s [4], to our knowledge there is no other recorded genomic sequence of WNV isolated from humans in Uganda. However, a WNV lineage 2 genome has been more recently recovered from the *Culex neavei* species collected at the Mweya Queen Elizabeth National Park, located in Western Uganda in 2009 [5]. In addition, serological studies indicating acute viral infection have since been recorded [6]. Continuous immunoglobulin (Ig)M-based serological surveillance indicates that WNV remains endemic in the region [7], however, molecular detection of WNV by polymerase chain reaction (PCR) is rarely undertaken [8].

We thus retrospectively applied molecular investigation to a cohort of 1090 serum and whole blood samples from patients with acute febrile illness yet negative for Arbovirus IgM enzyme-linked immunosorbent assay results from a broader cohort described previously [7]. We reasoned that some patients may be in the IgM-negative pre-seroconversion 'window' phase of an arboviral infection with detectable genomic RNA. RNA was extracted and complementary DNA (cDNA) was generated from the samples initially in pools of five, then individually to identify the positive source in a positive pool (see Supplementary File). We applied pan-flavivirus genus degenerate primers [9] to carry out conventional screening PCR for all known flaviviruses, followed by Sanger sequencing, identifying a single positive pool and subsequent source sample 1633 (see Supplementary Figure 1). Nucleotide basic local alignment search tool (BLASTn) analysis of the resulting sequence indicated a putative lineage 2 WNV positive sample most closely related to the aforementioned spatiotemporally-related WNV sequence detected in the recent Ugandan study of arbovirus vector species [5].

Using the contemporary complete WNV genome as a template (GenBank accession ID KY523178 [5]), we generated a 200-800 bp am-

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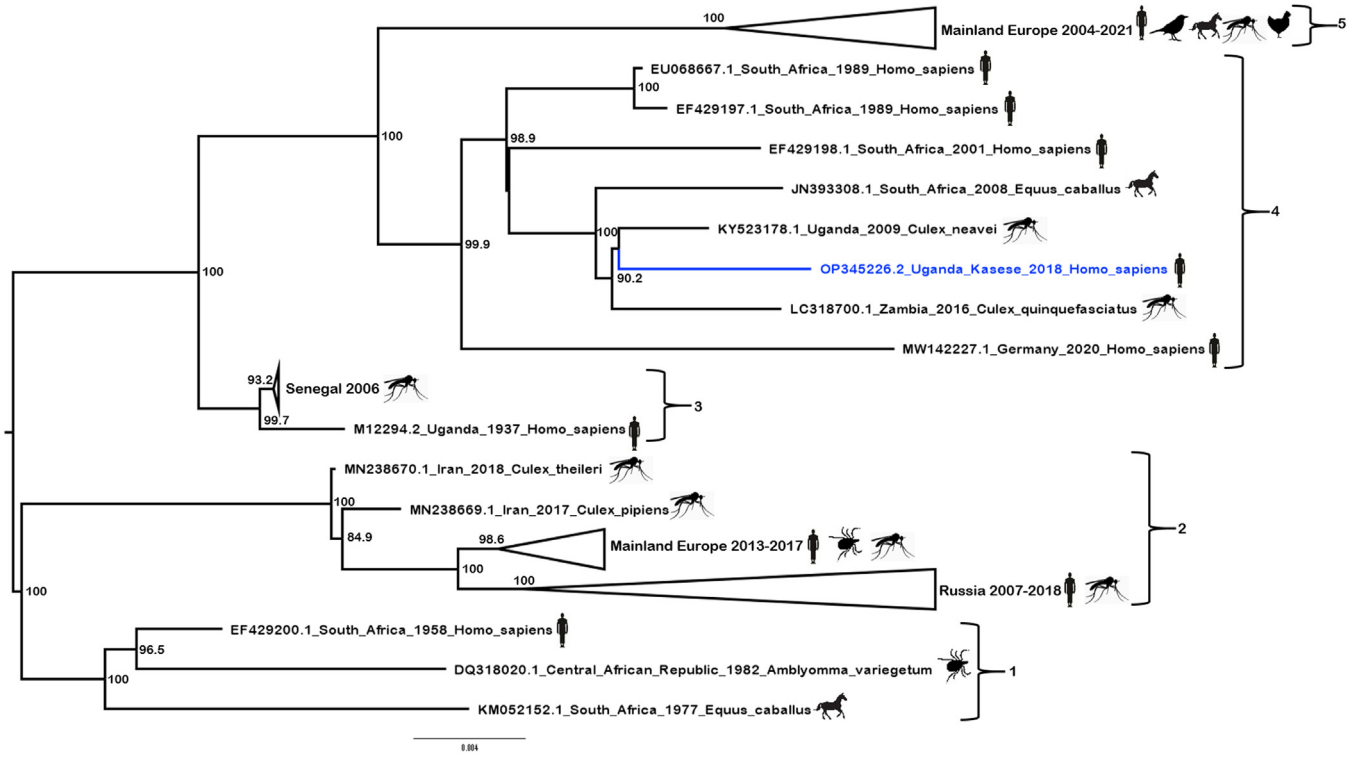

Figure 1. Molecular phylogenetic analysis of full-length West Nile Virus (WNV) using the maximum-likelihood method with GTR+F+I+R2 model in IQ-TREE2. Analysis was completed for 211 representative WNV nucleotide sequences principally selected by Mencattelli et al. [11] to represent lineage 2 clade d, with individual clusters highlighted (1-5). Bootstrapping based on 1000 replications is shown next to each branch at a scale of 0.004, with bootstrap values below 70 omitted. Tree is midpoint rooted and silhouettes indicate viral sequence hosts.

plicon sequencing scheme and continued to use a conventional PCR and Sanger amplification and sequencing strategy (see Supplementary File). This initially recovered 9.8kb (89%, GenBank accession number OP345226.1) of the genome with four short internal gaps. A second 1200bp scheme was then attempted (see Supplementary Table 1) and applied using a standard nanopore-based amplicon sequencing approach [10] generating a near-complete genome (OP345226.2), spanning positions 30-10368 (93.57%) of the reference KY523178.

The recovered WNV sequence phylogenetically grouped with other exemplary strains of lineage 2d as expected (Supplementary Figure 2). A second phylogenetic tree constructed with a focused WNV lineage 2, clade d cohort (Figure 1) indicated the greatest degree of similarity with other sequences predominantly from the African continent in the well-supported cluster 4 (Figure 1, using grouping terminology from Mencattelli et al. [11]). Most closely related were the spatiotemporally-related vector-derived Ugandan sequence from 2009 (KY523178), a 2016 Zambian mosquito isolate (LC318700), and also a brain-derived isolate from a fatal infection of a horse sampled in South Africa in 2008 (JN393308 [12]). The ancestral 1937 ancestor M12294, formed part of the distinct 2d Cluster 3, alongside more recent West African vector-derived sequences (Figure 1).

At the amino acid level, 26/3434 sites (0.76%) displayed heterogeneity among the three Ugandan isolates sampled over 80 years apart (Supplementary Table 2). Of these, 23 were conserved between the 2009 mosquito and the 2018 study sequences, two between 2009 and 1937, and one between 2018 and 1937. Most striking was a four amino acid insertion and adjacent substitution (NYSTQ) at positions 154-158 of the envelope gene in the recent sequences relative to the 1937 sequence (also present in the designated West Nile Virus Lineage 2 reference sequence NC_001563.2, data not shown). This position has previously been demonstrated to influence avian host and vector competence phenotypes, independent of E-protein glycosylation status, with NYS glycosylated viruses showing higher *in vitro* viral replication, and lower pH and temperature sensitivity than viruses with the site deleted, as well as other variants [13].

Demographic data showed that the sample was collected in 2018 from the Kasese district in Western Uganda, several 100 kms from the West Nile region of Uganda, but circa. 50 km from the Mweya site where the 2009 mosquito sample was identified [5]. The patient was a 20-year-old farm worker, hospitalized in June 2018 with fever, an axillary temperature of 38.4°C, headache, skin rash, vomiting, nausea, diarrhea, intense fatigue, and abdominal, muscle, joint, and chest pain. Epidemiological data indicated that they had a history of direct contact with their farm animals (pigs, chickens, goats, and rabbits). Serological testing of the patient's serum was negative for WNV IgM antibodies [7]. The negative IgM test can probably be attributed to the time lag between virus infection, dissemination, and the production of IgM antibodies by the adaptive immune system as shown experimentally in mice [14]. The clinical presentation of the study patient further underscores the lineage 2 strain's capacity to cause severe disease [2,3]. In 2010, for example, Greece's WNV lineage 2 outbreak was associated with 197 neuroinvasive cases and 33 fatalities (16.75% fatality rate) [15]. Possible reservoir sources of WNV infection in the patient may have been from their farm animals, as WNV has been described elsewhere in chickens likely vectored by either *Culex spp.* mosquitoes or potentially *Ornithonyssus sylviarum* chicken mites [3,5].

This study indicates that WNV is likely implicated in the undiagnosed burden of acute febrile illnesses in Uganda and may be missed by solely IgM-based serological diagnostics [3,7]. This highlights the need to improve regional availability of molecular assays and pathogen genome sequencing for not only timely diagnosis for the patient but also enhanced epidemiology for a global One Health understanding [8].

Declarations of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Timothy Byaruhanga: Formal analysis, Investigation, Data curation, Writing – original draft. **Stuart Astbury:** Methodology, Formal analysis, Data curation, Writing – review & editing, Visualization. **Jack D. Hill:** Validation, Formal analysis, Writing – review & editing, Visualization. **Theocharis Tsoleridis:** Validation, Formal analysis, Writing – review & editing. **Joseph G. Chappell:** Validation, Data curation, Writing – review & editing. **John T. Kayiwa:** Resources, Data curation. **Irene J. Ataliba:** Resources, Data curation. **Annet M. Nankya:** Resources, Data curation. **Jonathan K. Ball:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Julius J. Lutwama:** Resources, Supervision, Funding acquisition. **C. Patrick McClure:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

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Ethical approval statement

Ethical approval was waived due to the existing approval granted by the Uganda Virus Research Institute research ethics committee and the Uganda National Council of Science and Technology to the Department of Arbovirology.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100462.

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