



Short Communication

Detection of West Nile virus lineage 1 sequences in blood donors, Punjab Province, Pakistan



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ABSTRACT

Objectives: This study was performed to determine the presence of West Nile virus (WNV) in mosquito specimens and human blood donors in Pakistan.

Methods: A total of 4150 mosquito specimens were collected using CO₂-baited traps from five selected districts of Punjab Province, Pakistan. The mosquitoes were taxonomically identified using standard morphological keys, resulting in 166 pools. In addition, 1070 serum samples were collected from human blood donors. RNA was extracted from mosquito and human samples and screened for WNV using a reverse transcriptase PCR (RT-PCR).

Results: None of the mosquito pools tested positive for WNV, whereas three samples from asymptomatic humans tested positive. To determine the WNV strains, partial sequences were compared against a global representation of 23 WNV sequences. The study strains were determined to come from WNV lineage 1. **Conclusions:** This study is novel in reporting the circulation of lineage 1 WNV in Pakistan. Given its ability to transmit from human to human via blood transfusion, this highlights the urgent need for nationwide surveillance to assess the distribution and impact of WNV in Pakistan. Determining the source of human infection will require more extensive mosquito sampling.

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Introduction

West Nile virus (WNV) is a mosquito-borne positive-sense, single-stranded RNA virus of the genus *Flavivirus* (Family: *Flaviviridae*) with a worldwide distribution. Phylogenetic analyses

of WNV isolates from different geographic regions currently divide strains upto nine lineages. Of these, lineages 1, 2, and 5 have been associated with significant viral encephalitis outbreaks in humans (Shah-Hosseini et al., 2014; Chancey et al., 2015). The presence of WNV has been reported both within Pakistan (Zohaib et al., 2014; Khan et al., 2018) and in adjacent countries such as Iran, China, and India (e.g., Lu et al., 2014; Shah-Hosseini et al., 2014; Aghaie et al., 2016; Balakrishnan et al., 2017). An outbreak of WNV in humans was recently documented in Karachi; however, the investigators could not detect the viral genome and relied on the presence of anti-WNV IgM antibodies (Khan et al., 2018). Although mosquito

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bite is the major route of WNV infection in humans, other routes of transmission such as blood transfusion and organ transplantation have also been reported (Aghaie et al., 2016; Iwamoto et al., 2003). This study was performed to investigate the presence of WNV in mosquitoes and humans in Punjab Province of Pakistan.

Methods

A total of 4150 mosquitoes were captured in Punjab Province of Pakistan during 2016–2017 using CO₂ traps. The mosquitoes were taxonomically identified using standard morphological keys, trapping site, and time, resulting in 166 pools. In addition, 1070 serum samples were collected from humans during the years 2016–2018 (1045 male, 25 female; mean age 27 years). Viral RNA was extracted from the 166 mosquito pools and human serum using a viral RNA extraction kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. A reverse transcriptase PCR (RT-PCR) assay was conducted to amplify a 408-bp region to sequence the junction between capsid (C) and pre-membrane (prM) (Lanciotti et al., 2000) (see **Supplementary Material**).

Results

The specimens collected in this study included *Culex* (*Cx.*) *pipiens* complex (*Cx. p. pipiens*+*Cx. p. quinquefasciatus*), *Cx. restuans*, *Cx. salinarius*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, and *Anopheles maculatus*. None of the mosquito pools was positive for WNV, whereas three human serum samples tested positive by RT-PCR and were subsequently sequenced (GenBank accession numbers **MH545363–MH545365**). The WNV-positive donors were asked about their history of febrile illness and neurological symptoms in the 2 weeks prior to and 1 month after blood donation. None of the positive donors reported any history of fever or neurological symptoms.

A phylogenetic tree was constructed using 23 representative sequences of WNV and the three nucleotide sequences obtained in this study (Figure 1). The Pakistani sequences grouped into lineage 1, along with other highly pathogenic strains from other regions.

Lineage 1 is responsible for the majority of outbreaks in Australia, India, Europe, and the Middle East (Chancey et al., 2015). Nucleotide sequences from Pakistani strains were 98–99% similar to isolates of mosquito origin from the province of Xinjiang in China (**JX442279**) and a human encephalitis patient from the district of Kerala in India (**KC601756**) (Lu et al., 2014; Balakrishnan et al., 2017). Within Pakistan, isolates Pak-1 and Pak-4 were identical, whereas Pak-1 and Pak-3 differed by three nucleotides. The Pak-3 sequence differed by one nucleotide and Pak-1 differed by four nucleotides and eight nucleotides compared to the Indian and Chinese isolates (**KC601756** and **JX442279**), respectively. However, these were silent mutations. The Pakistani sequences were identical on the basis of translated amino acid residues with several sequences from lineage 1 detected in India, China, the USA, Russia, Morocco, Spain, and Italy.

Discussion

West Nile fever is characterized by a febrile illness and roughly one in 150 infections leads to encephalitis (Chancey et al., 2015). WNV was not detected in mosquito pools from Punjab Province, Pakistan; however, three blood donor specimens tested positive for WNV. None of the WNV-positive donors indicated any recent history of fever or neurological symptoms. This suggests that acute WNV infections may remain undetected and have the potential to be transmitted through routes such as blood transfusion. The transmission of WNV via this route poses a serious health risk, especially for elderly patients (Aghaie et al., 2016; Iwamoto et al., 2003). Recipients and blood banks need to be aware of this possibility.

To the best of our knowledge this is the first report on the circulation of lineage 1 WNV in Pakistan. The study results highlight the need for urgent, nationwide, coordinated surveillance to assess the distribution and impact of WNV in Pakistan. In the meantime, public health measures to prevent viral transmission should be promoted, e.g., vector monitoring and control, information campaigns to improve personal protection, and district-level deployment of screening tests for blood and tissues.

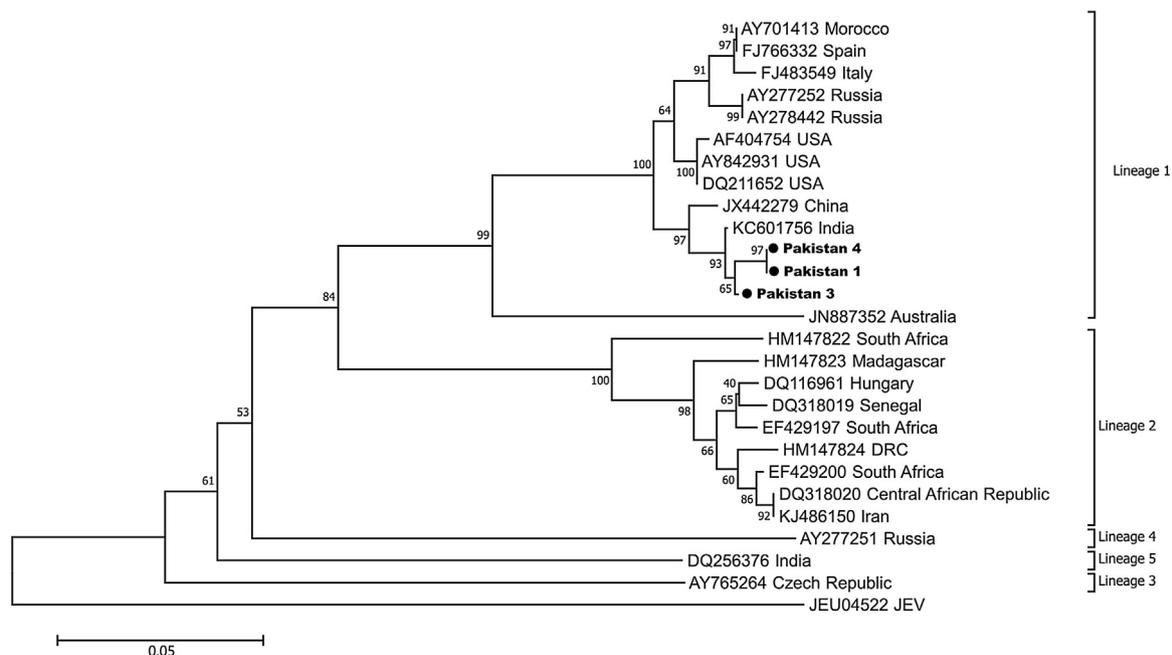


Figure 1. Phylogenetic tree of 23 representative strains of West Nile virus (WNV) and the three sequences from Pakistan detected in this study. This tree was generated using the neighbor-joining method in MEGA7, with bootstrap values along branches for 1000 replicates. A Japanese encephalitis virus sequence was included as an outgroup. Detected strains of WNV are depicted in boldface and identified with circle node markers (●). The scale bar indicates nucleotide substitutions per site.

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Ethical approval

This study was approved by the Institutional Review Board of Wuhan Institute of Virology (China) and by the Ethics Review Committee of the Government College University Faisalabad (Pakistan) (No. GCUF/MICRO/18/1600). Informed consent was obtained from humans for whom identifying information is included in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijid.2019.01.020>.

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