



REVIEW ARTICLE

# Genotype 5 Japanese Encephalitis Virus—Old Genotype, New Threat

Weijia Zhang<sup>1</sup>, Chongxiao Xu<sup>1</sup>, Kai Nie<sup>1</sup> and Huanyu Wang<sup>1,\*</sup>

## Abstract

Japanese encephalitis (JE) is an important viral encephalitis with epidemic status in Asia, which is caused by Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*. JEV is divided into five genotypes. Genotype 5 (G5) is relatively neglected because of the limited number of cases and strains isolated. The first strain of G5 JEV (Muar strain) was isolated in Singapore in 1952 in a patient from Muar, Malaysia. The second strain (XZ0934) was isolated 57 years later in China, thus indicating the re-emergence of G5 JEV. A female patient who had been vaccinated against JE was infected with G5 JEV in Korea in 2015. JE is a vaccine-preventable disease, and its incidence has decreased with vaccination in many Asian countries. G3 JEV is the main candidate for current JE vaccines, which include attenuated, inactivated and chimeric type vaccines. However, the available vaccines do not provide adequate protection against the older G5 JEV lineage. Therefore, more research on this genotype is crucial for developing better detection methods, expanding surveillance to determine the possible chains of viral transmission for this new threat and developing a polyvalent JEV vaccine.

**Keywords:** Japanese encephalitis virus (JEV), Japanese encephalitis (JE), genotype 5, vaccine

## Edited by:

Han Xia, Wuhan Institute of Virology, Chinese Academy of Science

## Reviewed by:

Reviewer 1, Xiaodan Li, School of Medicine, Hunan Normal University  
Reviewer 2 chose to be anonymous

## \*Corresponding author:

E-mail: wanghy@ivdc.chinacdc.cn

<sup>1</sup>Department of Arboviruses, NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, State Key Laboratory for Infectious Disease Prevention and Control, Chinese Center for Disease Control and Prevention, Beijing, 102206, People's Republic of China

Received: April 25 2022

Revised: June 26 2022

Accepted: July 19 2022

Published Online: August 05 2022

## INTRODUCTION

Throughout our history, humans have fought against infectious diseases. The ongoing pandemic of coronavirus disease 2019 has posed a major threat to human society [1]. However, the threats due to previously reported viruses should not be ignored. For instance, Zika virus (ZIKV) was first isolated in Uganda in 1947, and only 14 sporadic cases have been reported to date. However, during the first ZIKV outbreak in Yap Island, in 2007, 49 laboratory-confirmed cases were identified. Since 2015, the ZIKV epidemic has expanded to other geographical areas, including South America, Central America and Southeast Asia. The World Health Organization has declared this epidemic the fourth Public Health Emergency of International Concern [2-6]. Another

example, West Nile virus (WNV), which was first isolated in 1937, was extensively distributed throughout Africa. In 1999, the virus was detected in New York City. Since then, WNV continued to spread, reaching all 48 contiguous states of the US by the year 2004. Currently, WNV disease is the most important arboviral infectious disease in the United States [7]. Since then, the virus has spread across South America and Europe and become a global public health problem [8,9]. Japanese encephalitis virus (JEV), belonging to the genus *Flavivirus* along with ZIKV and WNV, also poses a great threat to human health.

## EPIDEMIOLOGY OF JE

Japanese encephalitis (JE) was first described in Japan in the 1870s as the “summer encephalitis,” and arose from bites from

mosquitos infected with JEV. The disease has been circulating among humans for more than 100 years. JE is endemic in several regions of Asia and the Pacific [10]. However, the detection of JEV sequences in mosquitoes in Europe and in patients in Africa suggests that JEV has the potential to spread globally [11,12]. The global incidence of JE is nearly 50,000 cases annually, and the mortality rates are 30–50%; approximately 50% of infection survivors have neurological sequelae [13]. China is a major region where JE is epidemic. Data have indicated that JE outbreaks occurred in all provinces except for the Xinjiang Uygur autonomous region, Tibet and Qinghai [14]. JE cases have been reported since the 1940s, and between the 1960s and 1970s two major outbreaks have been recorded; the cumulative number of cases has exceeded 1,000,000. During 1976 to 2007, the annual incidence and mortality rates due to JE decreased. The average annual incidence fell to 30.14/100,000 between 2008 and 2018 [15,16]. During the same period, the incidence of the disease showed patterns according to age: the incidence was high in adults in northern China and in children in southern China [17].

## GENOTYPES OF JEV

JEV belongs to the genus *Flavivirus* in the family *Flaviviridae*. It was first isolated from the brain tissues of patients with fatal cases of encephalitis in Japan in 1935 [18]. The JEV genome comprises a single-stranded, positive-sense RNA that encodes three structural proteins (C, prM and E) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) in one open reading frame. JEV is divided into five genotypes (G1, G2, G3, G4 and G5) according to the nucleotide sequences of the E gene [19,20] and the whole genome [21,22]. Early isolates of JEV from the 1930s to the 1950s were mainly G3. During the 1990s and 2000s, G1 and G3 co-circulated in epidemic areas [23]. However, G1 JEV gradually replaced G3 and became the dominant genotype in JE epidemics [24–27]. G2 was distributed mainly in Australia and New Guinea, whereas G4 was distributed mainly in eastern Indonesia [13].

## G5 JEV

### Background

G5 JEV was first isolated from the brain tissues of a patient with viral encephalitis in Malaysia in 1952, and was named the Muar strain [28,29]. The second G5 JEV strain was isolated from the *Cx. tritaeniorhynchus* vector in China in 2009 and was named the XZ0934 strain [21]. In addition, the G5 JEV sequence was also detected in a *Cx. bitaeniorhynchus* vector in Korea in 2010 [30]. Moreover, sequences of six G5 JEV positive pools were detected in *Cx. orientalis* and *Cx. pipiens* vectors, and G5 JEV was confirmed to have been introduced to Korea in 2012 [31]. G5 JEV sequences were identified in mosquito surveillance during 2012 to 2018. Next-generation sequencing in mosquitoes indicated that G5 JEV is circulating in Pyeongtaek and Seoul and may become the dominant JEV genotype in Korea [32,33]. The

third G5 JEV strain (K15P38) was isolated from the convalescent cerebrospinal fluid of a 27-year-old woman who had been vaccinated against JE in Korea in 2015 [34]. The locations of G5 JEV isolation and detection are geographically separated; thus, G5 JEV may spread via migrating birds. To determine whether G5 JEV has caused JE cases in other Asian countries, serum samples were collected from patients with JE in northern Vietnam and Japan in 2014 and 2016, respectively. The G1, G3 and G5 JEV serum cross-neutralization investigation was investigated. No G5 JEV infected patients were found [35,36].

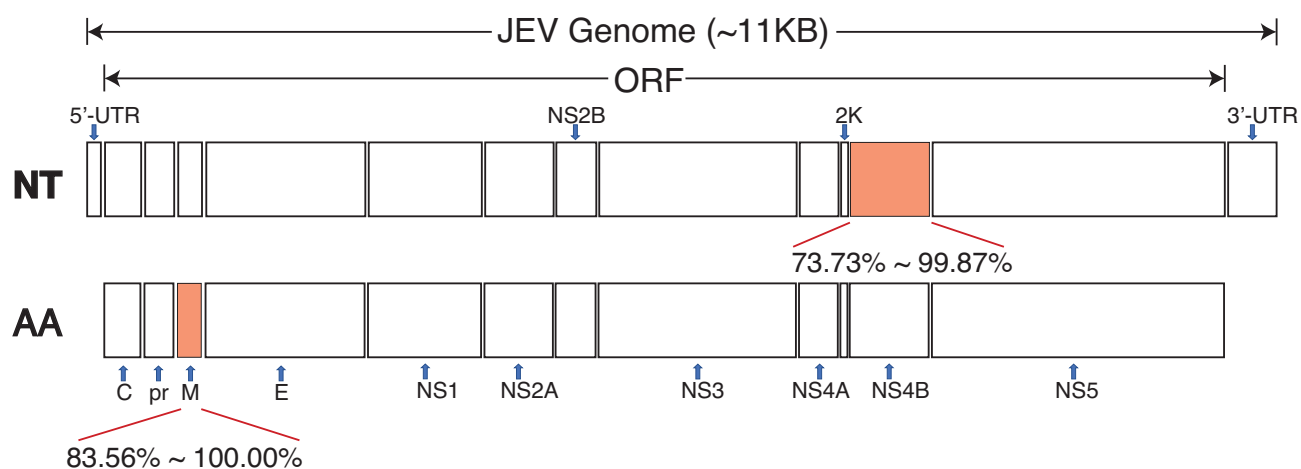
### Identification and Genome Characterization

Regarding G5 JEV genotype characterization, previous serological studies have indicated that the Muar strain is an individual group, on the basis of complement-fixation tests, hemagglutination-inhibition tests and neutralization tests [29,37]. In 1994, the E gene of the Muar strain was sequenced. Nucleotide and deduced amino acid sequence analyses indicated that the Muar strain differed from the other four genotype strains of JEV [38]. In 2001 and 2003, phylogenetic analyses based on the E gene sequence revealed that the strain was the fifth genotype of JEV [19,20]. In 2011, phylogenetic analysis based on the complete nucleotide sequence of the Muar strain confirmed that it belonged to the fifth JEV genotype [22]. The assumption that G5 was the earliest recognized JEV lineage was also based on phylogenetic analysis of the whole genome sequences of Muar and XZ0934 strains in 2015 [39]. Molecular clock analysis of the complete coding sequence of JEV confirmed that the strain is the oldest lineage of JEV, and all five JEV genotypes shared a common ancestor 449.6 years ago [22]. The open reading frame sequences of the four G5 JEV strains from GenBank share a sequence identity of 90.3–100% for nucleotides and 98.1–100% for deduced amino acids. The individual gene fragment NS4b has the lowest nucleotide sequence identity (73.73%), and M has the lowest deduced amino acid sequence identity (83.56%; Fig 1). In contrast to the other genotypes, the insertion of three nucleotides (encoding a serine residue) in the NS4a gene is present in all G5 JEVs [40].

### Virulence and Immunological Characteristics

Studies on the virulence of G5 JEV have investigated several perspectives. Pathogenicity studies in mice have shown that the neuroinvasiveness of the Muar strain is equivalent to that of Beijing-1 (G3) and greater than that of Mie/41/2002 (G1) [41]. Through cDNA-based technology, a live G5 JEV infectious clone has been obtained, and the structural protein region has been found to be associated with greater G5 JEV pathogenicity in mice [42]. Subsequently, fragment constructed infectious clones have confirmed that G5 JEV is highly pathogenic in mice, and the E and prM proteins of G5 JEV are responsible for this increased virulence [43].

JE is a vaccine-preventable disease. In China, for example, the incidence of JE has decreased sharply since 2008, when the Chinese government included the JE vaccine in the



**FIGURE 1** | For the individual gene fragment of four G5 Japanese encephalitis virus (JEV) strains, NS4b has the lowest nucleotide (nt) homology (73.73%) and M has the lowest deduced amino acid (aa) sequence identity (83.56%).

Expanded Program on Immunization [17]. The vaccine is an effective strategy for JE prevention and control at the national level [44]. Four types of JE vaccines are available: 1) inactivated mouse brain-derived JE vaccine; 2) inactivated Vero cell-derived JE vaccine; 3) primary hamster kidney cell-derived, live attenuated vaccine based on the SA14-14-2 strain; and 4) live attenuated recombinant (chimeric) JE vaccine. The mouse brain-derived vaccine has currently been replaced by inactivated Vero cell-derived vaccine and live attenuated vaccine using the SA14-14-2 strain and live chimeric JE vaccines. All strains used for JE vaccines are derived from G3 [45–47]. However, plaque reduction neutralization tests have indicated that the neutralization ability of the JE vaccines against the Muar strain is less than that against the G1 and G3 strains [41]. Results of G3 and G5 cross-neutralizing immune responses in vaccinated humans, as well as cross-protective immune responses in mice, have shown that the current JE vaccine derived from G3 JEV does not provide adequate protection against the XZ0934 strain [48].

## CONCLUSIONS AND PERSPECTIVES

The results in the present study indicate that the spread of the older G5 JEV lineage may bring new threats to humans. Both the currently used JE vaccines are derived from G3 JEV; however, their protective efficacy is insufficient. In South Korea, for instance, G5 JEV has been detected in diverse *Culex* mosquito species since 2010. In 2015, a G5 JEV vaccine breakthrough case was reported. In the future, G5 should be monitored closely throughout JEV epidemic regions to 1) establish detection methods with high sensitivity and specificity for G5 JEV; 2) expand surveillance regions to identify possible chains of virus transmission; 3) increase epidemiological surveillance, molecular detection and serological investigations of G5 JEV in not only mosquitoes but also dead pigs and migrant birds; and 4) develop a polyvalent vaccine to prevent epidemics caused by JEV genotypes G1, G3 and G5.

Thus, the potential new threat of the older G5 JEV lineage warrants global attention.

## ACKNOWLEDGEMENTS

This study was supported by funding from the Development Grant of the State Key Laboratory of Infectious Disease Prevention and Control under award number 2015SKLID505 (H.W.).

## CONFLICTS OF INTEREST

The authors declare they have no actual or potential competing interests.

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Huanyu Wang (PhD) is a professor at the Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention. His research interests are the distribution of arboviruses and their relationship with diseases in China, including pathogen etiology, epidemiology and detection method establishment. He undertakes technical training and guidance for national CDC personnel. He undertakes the National Natural Science Foundation of China “National Science and Technology Major Project” and other projects. He has published more than 200 papers (SCI-88).