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Genotype 5 Japanese Encephalitis Virus - Old Genotype, New Threat

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

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Throughout history, humans have never been separated from the fight against infectious diseases. The ongoing pandemic of coronavirus disease 2019 (COVID-19) posed great threat to human society [1]. However, the threat caused by other “old” viruses should not be neglected. For instance, Zika virus (ZIKV) was first isolated in Uganda in 1947 and only 14 sporadic cases have been reported, so far. However, during the first ZIKV outbreak in Yap Island, in 2007, 49 laboratory-confirmed cases were identified. Later, the ZIKV epidemic expanded to other geographical areas, including South America, Central America and Southeast Asia since 2015. The World Health Organization has declared this epidemic as the fourth Public Health Emergency of International Concern [2-6]. Another example, West Nile virus (WNV) which was first isolated in 1937, had an extensive distribution throughout Africa. In 1999, the virus was detected in New York City. Since then, WNV has continued to spread, by the year 2004 to all 48 contiguous states of the US. Currently, WNV disease is the most important arboviral infectious disease in the United States [7]. Since then, the virus spread across South America and Europe to become a global public health problem [8, 9]. Japanese encephalitis virus (JEV), as an "old" virus, which belongs to the genus *Flavivirus* 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”, due to a mosquito bite contaminated with JEV. The disease has been circulating among humans for over 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, suggest that JEV have the potential to spread globally [11, 12]. The global incidence of JE is close to 50,000 cases annually, with 30 to 50% mortality rate, and around 50% of the infection survivors live with neurological sequelae [13]. China is one of the main regions where JE is epidemic. Data showed that JE outbreaks occurred in all provinces except for Xinjiang Uygur Autonomous, Tibet, and Qinghai [14]. JE cases have been reported since 1940s, and

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between 1960s and 1970s two major outbreaks have been recorded; the accumulated number of cases exceeded 1,000,000. During the period of 1976 to 2007, the incidence and mortality rates due to JE decreased annually. The average annual incidence fell to 30.14/100,000 from 2008 to 2018 [15, 16]. During the same period, the incidence of the disease was altered with age; the incidence in adults increased in northern China, while the incidence was high 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 tissue of fatal cases of encephalitis in Japan in 1935 [18]. The JEV genome is comprised of 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 was divided into five genotypes (G1, G2, G3, G4, and G5) based on 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 genotype 3. During 1990s and 2000s, G1 and G3 were co-circulating in epidemic areas [23]. However, G1 JEV gradually replaced G3 and became the dominant genotype in JE epidemics [24-27]. Genotypes 2 and 4 were distributed mainly in the Australia and New Guinea region, and Eastern Indonesia, respectively [13].

Old genotype-G5 JEV

Background

G5 JEV, an “old” genotype, was first isolated from the brain tissues of a patient with viral encephalitis in Malaysia in 1952, and was named Muar strain [28, 29]. The second G5 JEV strain was isolated from *Cx. tritaeniorhynchus* vector in China in 2009 and was named XZ0934 strain [21]. In addition, G5 JEV sequence was also detected from *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 confirmed that G5 JEV was introduced in 2012 in Korea [31]. G5

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JEV sequences were always identified through mosquito surveillance during 2012 to 2018. Next-generation sequencing (NGS) in mosquitoes ascertained 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 (CSF) of a 27-year-old woman who had been vaccinated against JE in Korea in 2015 [34]. The isolation and detection locations of G5 JEV are geographical separation, so the "old" genotype JEV may spread farther via migrating birds. To determine whether G5 JEV caused JE cases in other Asian countries, serum samples were collected from JE patients in northern Vietnam and Japan in 2014 and 2016, respectively. The G1, G3, G5 JEV sera cross-neutralizing investigation were conducted. No G5 JEV infected patients were found [35, 36].

Identification and genome characterization

There is a process of recognition of G5 JEV, "old genotype". Previous serological studies indicated that the Muar strain was an individual group using the complement-fixation test, hemagglutination-inhibition test, and the neutralization test [29, 37]. In 1994, the E gene of the Muar strain was sequenced. Nucleotide and deduced amino acid sequence analyses showed that the Muar strain was different from the other four genotype strains of JEV [38]. In 2001 and 2003, phylogenetic analyses based on E gene sequence revealed that the strain was the fifth genotype of JEV [19, 20]. In 2011, phylogenetic analysis based on 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 inferenced by phylogenetic analysis based on the whole genomic sequences of Muar and XZ0934 strains in 2015 [39]. Molecular clock analysis of complete coding sequences of JEV confirmed that the strain represents the oldest lineage of JEV and all five JEV genotypes shared a common ancestor 449.6 years ago [22]. The open reading frame (ORF) sequences of the four G5 JEV strains from GenBank share identity of 90.3-100% in nucleotide and 98.1-100% in deduced amino acid. For the individual gene fragment, NS4b has the lowest nt homology (73.73%), M has the lowest

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deduced amino acid (aa) sequence identity (83.56%) (Fig 1). Compared with other genotypes, three nucleotides (encoded with a Serine residue) insertion in the NS4a gene existed in all G5 JEVs [40].

Virulence and immunological characteristics

Different studies on the virulence of G5 JEV were tackled from different angles. Pathogenicity in mice studies showed that the neuroinvasiveness of the Muar strain was equivalent to that of Beijing-1(G3) and higher than that of Mie/41/2002(G1) [41]. Using a cDNA-based technology, a live G5 JEV infectious clone was obtained, and found the structural protein region was associated with an increase in G5 JEV pathogenicity in mice [42]. Subsequently, fragment constructed infectious clones confirmed that the G5 JEV was highly pathogenic in mice, and the E and prM proteins of G5 JEV were responsible for the 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 Expanded Programme on Immunization (EPI) [17]. The vaccine is an effective strategy of JE prevention and control at the national level [44]. There are four types of JE vaccines, including 1) inactivated mouse brain-derived JE vaccine; 2) inactivated Vero cell-derived JE vaccine; 3) primary hamster kidney (PHK) cell-derived, live attenuated vaccine based on the SA14-14-2 strain, and 4) live attenuated recombinant (chimeric) JE vaccine. Mouse brain-derived vaccine is currently replaced by the inactivated Vero cell-derived vaccine and live attenuated vaccine using 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 test (PRNT) indicated that the neutralization ability of the JE vaccines against the Muar strain is less compared with G1 and G3 strains [41]. Results of G3 and G5 cross-neutralizing immune responses in vaccinated humans and, separately, cross-protective immune responses in mice showed that the current JE vaccine derived from G3 JEV did not provide adequate levels of protection against XZ0934 strain [48].

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Conclusions and Perspectives

The results in the present study indicate that the spread of G5 JEV, "old" genotype, may bring new threat to humans. Currently used JE vaccines were both derived from G3 JEV; however, the protective efficacy was not sufficient. In South Korea, for instance, the G5 JEV has been detected in diverse Culex mosquito species since 2010. In 2015, a G5 JEV vaccine breakthrough case was founded. In the future, G5 should be monitored closely throughout JEV epidemic regions: 1) establish detection methods with high sensitivity and specificity focus on G5 JEV; 2) expand surveillance region to seek possible chains of virus transmission; 3) increase epidemiological surveillance objects, molecular detection and serological investigations of G5 JEV should not be limited to mosquitoes but also dead porker and migrant birds; and 4) develop polyvalent vaccine to cover epidemics caused by JEV genotypes G1, G3 and G5. Thus, the potential new threat promotes the world's attention on the "old" genotype, G5 JEV.

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COMPETING INTERESTS

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

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