Vector-Borne Diseases & Treatment

Chapter 6

Japanese Encephalitis Virus: Displacing of Virus Genotype and Efficacy of Vaccination

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Abbreviations: AES: Acute Encephalitis Syndrome; Arbo: Athropod Borne; C6/36: *Aedesalbopictus*C6/36 clone; E: Envelope; G: Genotype; Genotype I: GI; Genotype II: GII; Genotype III: GIII; Genotype IV: GIV; Genotype V: GV. JEV: Japanese Encephalitis Virus; PS: PS: Pig Kidney Epithelial; RD: Human Rhabdomyosarcoma

1. Introduction

Japanese Encephalitis Virus (JEV) is a mosquito-borne virus which causes Acute Encephalitis Syndrome (AES). After more than 60 years since JEV was discovered in Japan in 1935, the virus spread to many Asian countries, Western Pacific countries and North Australia, with approximately over 3 billion individuals lived in epidemic areas in 2011 [2,3,34,36]. JEV is a leading cause of AES for children in Asian countries with a high morbidity and mortality. It was claimed that if there had been no vaccination to prevent human from the disease, its consequence would have been more severe than any other disease. JEV has only one serotype but five genotypes (GI-V). The emergence and distribution of genotypes were varied between different geographical regions and periods. At the time of vaccine development, most of JEV strains isolated in humans belonged to JEV GIII, thus vaccine for humans was developed from GIII strains [1,14,24] **(Table 1).** At the beginning of 21st century, it was predicted that JEV GIII and GII would emerge in Australia and some northern Asian countries, respectively [30]. However, the emergence of genotypes from mosquitoes, pigs, and humans from 1935 to 2016 in Asia, Western Pacific region, and Australia showed that this prediction did not come true. Moreover, the JEV genotype isolated from humans shifted from GIII to GI in the recent decade in most the Asian countries [17,22,25]. Besides this, the appearance of the JEV GV in several Asian countries was also reported in 2000s after long time of first detection in 1952 in

Malaysia [16,28,31] **(Table 1)**. It raises a question about the efficacy of Japanese encephalitis vaccine with the emerging JEV genotypes.

2. Japanese Encephalitis Virus

2.1. Origin, vector, and amplifying hosts

Japanese encephalitis virus was firstly isolated from a human in Tokyo, Japan in 1935. Comparison of *JEV* with other Flaviviruses suggested that jev might evolved from an African ancestral virus, but recent phylogenetic analysis showed results of the origin of JEV might be from its ancestral in the Indonesia-Malaysia region [7,29,30].

JEV belonged to Arbovirus which shared the ability of transmission by Arthropod vectors. JEV is maintained in nature in a cycle between vertebrate host and mosquitoes which primarily from the *Culex* genre. *Culex spp* acts not only as a vector but also as sub-amplifying hosts since mosquitoes can transmit JEV to the next generation through eggs. The vertical transmission JEV in mosquitoes makes virus control in nature become very difficult. Moreover, this species feeds on birds, creates the natural cycle between mosquitoes and avian species. Particularly, *Culex spp* feeds on mammals and transmits JEV to humans and animals [15,34,36]. The role of animals is amplifying factors and subsequent source infection to mosquitoes. Additionally, pigs are considered the most efficient amplifying host of JEV, but other livestock species also act as amplifying hosts such as horses and cattle [21,32,39].

2.2. Morphology

The mature virus particles are 45-50nm in diameter and possess a spherical symmetry consists of the inner core or an icosahedral nucleocapsid protein surrounding the genomic RNA, a lipid bi-layered membrane, and an envelope. The envelope proteins are reported to carry Hemagglutinating (HA) activity and immunogenicity relating to neutralization. The virion is about 69-70 kilodaltons (kDa) in size and 200S of deposition value [8,39,42].

2.3. Molecular characteristics

JEV genome is a single positive-strand RNA genome, approximately 11 kb in length, encodes 10 proteins consisting of three structural proteins and seven non-structural proteins. Structural proteins are C protein (core protein), M protein (membrane), and E protein (envelope) [14,18,42] **(Figure 1)**.

JEV has only a single serotype but is divided into five genotypes (GI-GV) based on the E gene or the complete genome. The genotype distribution is different spartio-temporally [28]. In Vietnam, a long-term phylogenetic study showed that from 1964 to 1988, Vietnamese isolates were classified into one genotype and evolved slowly with evolution rate of ≤3.2%

[11] **(Table 1).**

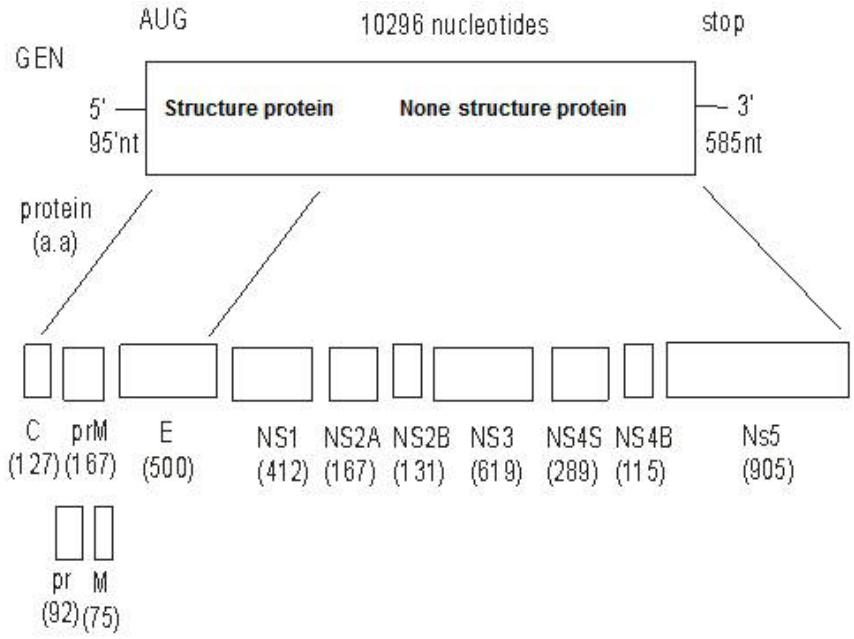

Figure 1: JEV genome schematic and protein translation.

2.4. Virulence

The virulence of the five genotypes differ between them. In 1960s-1970s, JEV detected in humans in Asian countries mainly belonged to GIII. Recently, JEV detected in humans in Malaysia, Indonesia, and Northern Australia belonged to GII. The situation in Thailand, Vietnam, and China was different, since all isolated JEV belonged to GI. The etiological role of GIV and GV in humans had been still unknown, although virus belonging to GIV was detected in mosquitoes in Indonesia [8,9,27]. Moreover, experimental studies showed that GIV virus was less virulence than GIII virus but the GIV has been not detected from humans up to now. Besides that, several GIII strains became less virulence after number of passages, such as SA 14-17-2 strain [16,29,31].

3. Appearance and Replacement of JEV Genotypes

The first JEV strain detected from brain tissue of Japanese patient in 1935 belonged to GIII. After this first detection, GIII was reported as epidemic strains in many Asian countries, such as China, Korea, Vietnam, Thailand, Malaysia, Indonesia, and India **(Table 1).** In 1994, the GI was firstly reported in Japan from mosquitoes. However, some molecular epidemiology studies after that when analyzing strains collected before 1994 showed the much earlier appearance of GI in Northern Asian countries and Vietnam: China in 1979 in mosquitoes, South Korea in 1983 in mosquitoes, Vietnam in 1990 in humans, Australia in 2000, and India 2009 **(Table 1).** After that, GI strains became predominant in these countries where GIII used to be the predominance. It is noted that the first detections of GI were mainly in mosquitoes and swine. This phenomenon suggested that GI might adapt better in mosquitoes and swine than in human. However, recent studies in Vietnam, Japan, China and India showed that GI

was also the agent causing encephalitis in humans [6,12,18]. In Vietnam, although GI was detected in 1990s in humans it suggested that GI might circulate in mosquito's population much earlier **(Table 1).**

Table 1: Emerging of JEV Genotypes in the Asian Pacific Region and Northern Australia in Recent Decades

Country	Year of JEV genotypes emerged or notified				
	GI	GII	GIII	GIV	GV
Japan	1994	Undetected	1935	Undetected	Undetected
China	1979	Undetected	1948	Undetected	2009
Korea	1983	Undetected	1987	Undetected	2010
Vietnam	1990	Undetected	1964	Undetected	2018
Thailand	1963	1983	1964	Undetected	Undetected
Australia	2000	1995	Undetected	Undetected	Undetected
Malaysia	Undetected	1970	1965	1965	1952
Indonesia	Undetected	1981	1979	1981	Undetected
India	2009	Undetected	1956	Undetected	Undetected

JEV GV was detected in the 1950s in Malaysia and Singapore in mosquitoes and birds, respectively. Over 60 years, it was not detected until recently, the new emerging of GV was noticed from mosquitoes in several Asian countries, such as China in 2009, Korea in 2010 [16,31,33], and Vietnam in 2018 (Unpublished data) **(Table 1).**

The prediction of emerging new type or new genotype is also interesting topic of recent decades. For examples, Schuh *et al*. have performed bioinformatics analysis and calculated that time of the most recent common ancestor of GI strains in Vietnam was 1953 [29]. This was the source of widely spread to neighboring countries such as China, Japan, South Korea, Thailand and became the most predominant genotype in these countries [29]. These results showed strong evidence completely different with the previous prediction of the emerging and predominance of GII in Northern Asia and the emerging of GIII in Australia [30].

In the recent decades, the trend of JEV genotype replacement occurred in many countries. Before the 1990s, JEV from humans mainly belonged to GIII. GI was detected only in Thailand in five patients. However, after the 1990s, the emerging of GI was reported as increasing worldwide [11,22,23].

Table 2: The Last Time GII and GIII Strains in Asian Pacific Countries and Australia

Countries	The last time of GII and GIII strains	
	GII	GIII
Japan	Undetected	1994
China	Undetected	2007
South Korea	Undetected	1994
Vietnam	Undetected	2004
Thailand	1992	1992
Australia	2000	Undetected

The surveillance data showed that when GI appeared and spread throughout Asian countries, GI and GIII co-circulated just for a short period of time before GIII disappeared. GI became the single genotype found in Japan, South Korea, Vietnam, and Thailand **(Table 2)**. Similarity, GII appeared in Thailand and Australia in 1983 and 1995 and but disappeared in 1992 and 2000, respectively, while GI appeared and spread **(Table 2)** [4,26,35]. The *in-vitro* studies using different cell-lines originated from humans, mosquitoes and swine could provide the experimental illustration of this phenomenon. JEVs were amplified and maintained by C6/36 cells (originated from mosquitoes) after 10 passages whereas that by RD (originated from humans) and PS (originated from swine) only limited within 8 and 6 passages, respectively. This result showed that GI strain amplified and maintained more efficiently on C6/36 and PS but not RD, whereas GIII strain amplified and maintained more efficiently on RD [5].

One example of the genotype replacement was in Vietnam. A phylogenetic analysis of JEVs in Vietnam from 1964 to 2011 based on 1,500 nucleotide sequences of E gene showed that, the first GIII strain was detected in humans in Vietnam in 1964, and in mosquitoes in 1979, whereas GI strains were first detected in humans and mosquitoes in 1990 and 1994, respectively **(Figure 2)**. After 2004, GI was the only genotype detected in Vietnam, demonstrating that the GIII strains had been displaced by GI strains [4,19,22]. All the Vietnamese GI strains belonged to the GI-b clade defined by Schuh *et al*. including the major GI strains circulating widely in temperate climates, such as China, Japan, Korea, and Thailand. Whereas sub-genotype GI-acontains strains circulating only in the tropical climates, such as Thailand and Australia [28].

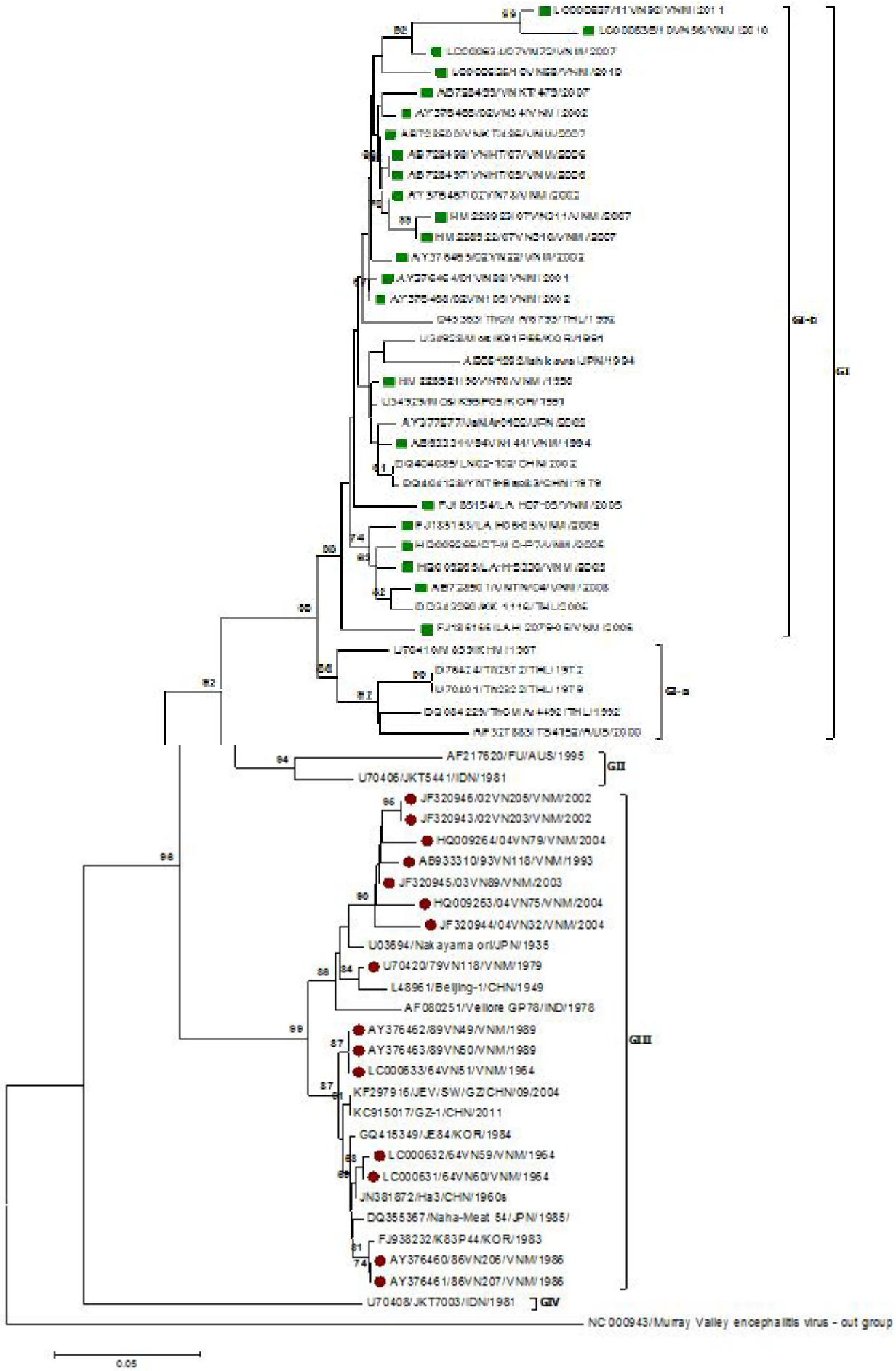

Figure 2: The phylogenetic tree of E gene of GI and GIII strains in Vietnam, 1964–2011. Marked red circle: GI strains; Marked green square: GIII strains [4].

4. Japanese Encephalitis Vaccines

Japanese Encephalitis vaccine was researched and developed by Mitamura *et al*. since 1936. This first licensed vaccine was a mouse brain-derived using Nakayama strain developed in Japan in 1954. In 1965, this vaccine used a combined physics-chemistry method to produce a purified, safe and high effectiveness vaccine [13]. Another mouse brain-derived vaccine using Beijing-1 strains was also used worldwide, especially in Asia, Europe and the USA [40]. Owing the vaccine strains protective efficacy, they have been used worldwide in the last 30 years with the efficacy of 80-91% after the second dose of JE vaccine. Not only produced in Japan, the mouse brain-derived vaccine technology has been transferred to several Asian countries, such as China, India, Taiwan, Vietnam, and Thailand to develop domestic products. However, the antibody declined over time leading to booster doses every 3-4 years till 15 years old. In addition, the cost of a purified process is quite expensive. That is the reason why only some developed countries, such as Japan, Korea had introduced this vaccine to control the disease nationwide [10,20,41]. Another first generation JEV vaccines is cell culture inactivated JEV vaccine. The first cell culture inactivated vaccine cultivated on Primary Hamster Kidney cells (PHK) using Beijing-3, P-3 strain with the efficacy of about 76-95% was used in Chinese nationwide campaigns until the mid-2000s [40]. Lived attenuated SA14-14-2 virus vaccine was replaced the PHK inactivated JE vaccine after that and has been used in China, Nepal, India, Sri Lanka and South Korea [3,40].

After the first generation of JEV vaccine, the development of second generation JEV vaccine has continued. Chimeric live-attenuated JEV vaccine is a recombinant vaccine based on a chimeric of JEV (SA14-14-2 strains) and Yellow Fever Virus (YFV17D) and was approved in Australia and Thailand [40]. It is a two doses therapy, whole-life protection and already commercial but its high cost is a consideration issue for the developing countries in Asia [20]. Vero cell lived-attenuated JEV vaccine using SA14-14-2 strain is licensed in the USA, Europe, Canada, Switzerland and Australia [40]. Inactivated Vero cell- derived vaccine was licensed in China and two other vaccines were licensed in 2009 and 2011 in Japan, respectively [40]. Another one developed by Novartis company, which has been approved and licensed in 2009 by the US Food and Drug Administration (FDA). These new generation vaccines are safe and produce a good sero-conversion rate of more than 83% [13,24,38,40]. In Vietnam, cell-derived JE vaccine has been developed in order to replace the mouse brain-derived vaccine [41].

Nowadays, JE vaccines for human are abundant and available. However, to achieve the target of control Japanese Encephalitis in Asia, it is necessary to have a safe vaccine, ready to produce large number of doses, high immunogenicity, two doses in the life therapy, acceptable price for the impoverish rural in Asia. It is also raised a question about new vaccines for specific populations such as infants or children infected with HIV.

5. Japanese Encephalitis under the Impact of the Vaccine

If the vaccine had been not available for prevent human from the disease, JE would have been one of the most serious health problems in Asia. The surveillance of JE pre- and post-vaccine era in Japan, Korea provided the evidences of this judgment. In Japan, before the introduction of JE vaccine (1949–1958), the incidences of JE were very high with 2,882 cases annually. After JE vaccine was introduced to susceptible population (1986–2000), the incidences of JE were dropped down to only 12.6 cases annually with a 99.6% reduction comparing to previous period. In Korea, in the period when JE vaccine was not used (1949–1958), the incidences of JE were 1,669 cases annually. After JE vaccine was introduced national-wide (1986–2000), the annual incidence reduced 99.9% and there were only 1.1 cases annually. In China, the first big outbreak of JE was recorded in 1966 with 150,000 cases, the second one occurred 5 years later with 180,000 cases were reported. After that, due to the enhancement of introducing JE vaccine, number of JE case was declined significantly with annual incidence of 20,000-40,000. However, in China, another issue needed to be considered is the available of stock vaccine which would be required huge number of doses in the control JE period in the future [10,37,39]. Similarity, in Vietnam, JE vaccine has been introduced in Expanded Program of Immunization (EPI) since 1997 for children at 1 to 5 years of age in high risk areas. The program started with 11 districts of 11 high risk provinces in the North meaning only 1 district in each province used JE vaccine. The coverage rate of JE vaccine by district in Vietnam was 1.63% (11/676 districts) in 1997. Every year, the enhancement of JE vaccination was consolidated. Till 2011, the vaccine coverage rate in district was 75.44% (510/676 districts). As the results of this enhancement, the incidence of suspected viral acute encephalitis syndrome in Vietnam had been declined to 1,000 cases/year, meaning the rate of suspected viral AES reduced from 4.2-4.8/100,000 population before 1997 to 1.2-1.8/100,000 population in the recent years [41].

Hitherto, the Japanese encephalitis vaccine has been produced using genotype III of JEV strain protecting human from the other genotypes of JEV.

6. Concluding Remarks

JEV is a mosquito born virus, the leading etiology cause AES for children with high morbidity, mortality and sequela. JEV has five genotypes, the emerging and circulating of each genotype change by periods and geographical regions. Before 1990, most of human isolates were JEV GIII. Thus, selected JEV GIII strain was used to develop vaccine for human till now. But in recent decades, JEV GI emerged in a lot of Asian countries causing AES in human. The shift genotype was took place when JEV GI appeared and spread in Asia, yielding the quiescent of JEV GIII or GII in areas where those genotypes were circulating before.

To prevent human from JE disease, three generations of JE vaccine were licensed in the world in order to supply purify, safer and high effectiveness vaccines. Several North Asian countries had controlled JE disease thanks to national wide vaccination such as Japan, Korea. And other countries have also controlled JE disease when increasing JE vaccination for children such as Vietnam, China yielding JE incidence rate dramatically reduce. It showed the efficacy of JE vaccine producing from JEV genotype III to protect human from the other genotypes of JEV.

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