Communication

Recent Population Dynamics of Japanese Encephalitis Virus

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Recent Population dynamics of Japanese Encephalitis Virus

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Abstract: Japanese encephalitis virus (JEV) causes acute viral encephalitis in humans and reproductive disorders in pigs. JEV emerged during the 1870s in Japan and since that time, JEV has been transmitted exclusively throughout Asia, according to known reporting and sequencing records. A recent JEV outbreak occurred in Australia which affected commercial piggeries across different temperate southern Australian states and caused confirmed infections in humans. A total of 47 human cases and seven deaths were reported. The recent evolving situation of JEV needs to be reported due to its continuous circulation in endemic regions and spread to non-endemics areas. Here, we reconstructed the phylogeny and population dynamics of JEV using recent JEV isolates for the future perception of disease spread. Phylogenetic analysis shows the most recent common ancestor occurred about 3120 years ago (YA) (95% Highest posterior density [HPD], 2680 to 3715). Our results of the Bayesian skyline plot (BSP) demonstrates that JEV demography lacks fluctuations for the last two decades, but it shows that JEV genetic diversity has increased during the last ten years. This indicates the potential JEV replication in the reservoir host, which is helping it to maintain its genetic diversity, and to continue its dispersal into non-endemic areas. The continuous spread in Asia and recent detection from Australia further support these findings. Therefore, an enhanced surveillance system is needed along with precautionary measures such as regular vaccination and mosquito control to avoid future JEV outbreaks.

Keywords: Japanese encephalitis virus; population dynamic; genetic diversity

1. Introduction

Japanese encephalitis (JE) is a vaccine-preventable disease, caused by the Japanese encephalitis virus (JEV) which is prevalent in Asian countries [1]. JEV has a positive-sense RNA genome belonging to the *flavivirus* genus within the *flaviviridae* family of five geographically and epidemiologically distinct genotypes (genotype I-V) [2]. Its genome contained 10,965 nucleotides and encoded polyprotein is further processed into three structural (capsid, membrane, envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). JEV genotype III (GIII) had been the most abundant genotype which led to several outbreaks in JEV endemic areas until 1990. However, recent data shows the emergence of genotype I (GI) as a dominant JEV genotype and it is gradually displacing GIII. The exact mechanism of this genotype displacement needs to be explored. In nature, the virus can circulate in invertebrate and vertebrate hosts. Invertebrates (mosquitoes) act as vectors and vertebrate hosts such as pigs and aquatic wading birds act as an amplifying/reservoir, and humans and equines are the dead-end hosts [3,4]. Approximately 300 million people live in Asia where JEV is circulating endemically, and they are at risk of JEV infection. Annually, it caused 68,000

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clinical cases, and 10,000-15,000 associated deaths [1,5,6]. Recently, JEV cases have been controlled to a significant extent by the use of JEV vaccines all over Asian countries [7], and the demographic history of JEV has been reported in previous studies [2,8,9]. However, due to the continued spread of JEV to non-endemic areas such as Tibet, Xinjiang, Philippines, and Australia [10–14] and continuous detection from mosquitoes or vertebrate hosts of endemic regions [15–21], we felt that there is a need to reconstruct the molecular phylogeny and population dynamics of JEV using recent isolates (till December 2022) of JEV for the future perception of disease spread.

2. Methodology

All published and publicly available (n=160) complete JEV genomes (till December 2022) were retrieved from GenBank public database (Supplementary table 1). A MUSCLE based multiple sequence alignment (MSA) of the data set was generated using an online tool at EBI server (https://www.ebi.ac.uk/), which was then visualized in BioEdit software [22]. The model of evolution was tested by ModelFinder tool [23], which revealed GTR+F+I+G4 as the best-fit evolutionary model for the dataset judged by Akaike and Bayesian information criterions (AIC and BIC). Timeline phylogeny reconstructions were performed in a Bayesian framework with BEAST 2 [24] using Markov chain Monte Carlo (MCMC) algorithms [25]. The GTR site model with a strict molecular clock and a fixed rate of 1.002×10^{-4} mutations/site/year [18,26] was applied. The MCMC chain was run for 1 billion steps, with sampling of parameters every 2,000 steps. Tracer v1.7.1 was used to assess the MCMC generated results, and an ESS value of >200 was considered as acceptable for all parameters of interest. The maximum clade credibility tree was extracted by TreeAnnotator and it was then visualized and finished in FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

We reconstructed a Bayesian skyline plot (BSP) [27] using BEAST 2 [24]. The BSP was generated with a strict molecular clock and a fixed rate of 1.002×10^{-4} mutations/site/year [18]. The MCMC chain was run for 1 billion steps, with sampling of parameters every 2,000 steps. The ESS for all parameters of interest remained >200 as analyzed in Tracer. Finally, the BSP was visualized and extracted using Tracer v1.7.1 [28].

3. Results

Recently, the epidemiology of JEV is changing and it has been expanded from Asia to other regions of the world such as Papua New Guinea, Australia. The rest of the world such as Europe, South and North America, and Pacific Islands are also receptive due to the presence of JEV competent mosquito vectors. To determine the continuously evolving situation of JEV, we downloaded the available complete genome sequences (n=160) and constructed phylogeny and population dynamics to report the possible threat of JE disease. We constructed a Bayesian molecular timeline phylogenetic tree to investigate the most recent common ancestor (tMRCA) for all genotypes using. Figure 1 explained that the tMRCA occurred about 3120 years ago (YA) (95% Highest posterior density [HPD], 2680 to 3715). The branching of the lineages occurred in the following order: genotype V, at the root of the tree; genotype IV, about 1589 YA (95% HPD, 1375 to 1754); genotype I, II, & III shared their MRCA about 824 YA (95% HPD, 730 to 1093); MRCA of genotype I & II was about 732 YA (95% HPD, 644 to 867). The mean rate of nucleotide substitution for all available (till December 2022) JEV strains isolated from a variety of hosts worldwide, estimated using a Bayesian MCMC approach, was 1.0628E-4 nucleotide substitutions per site per year (95% HPD values 8.7795E-5, 1.2564E-4).

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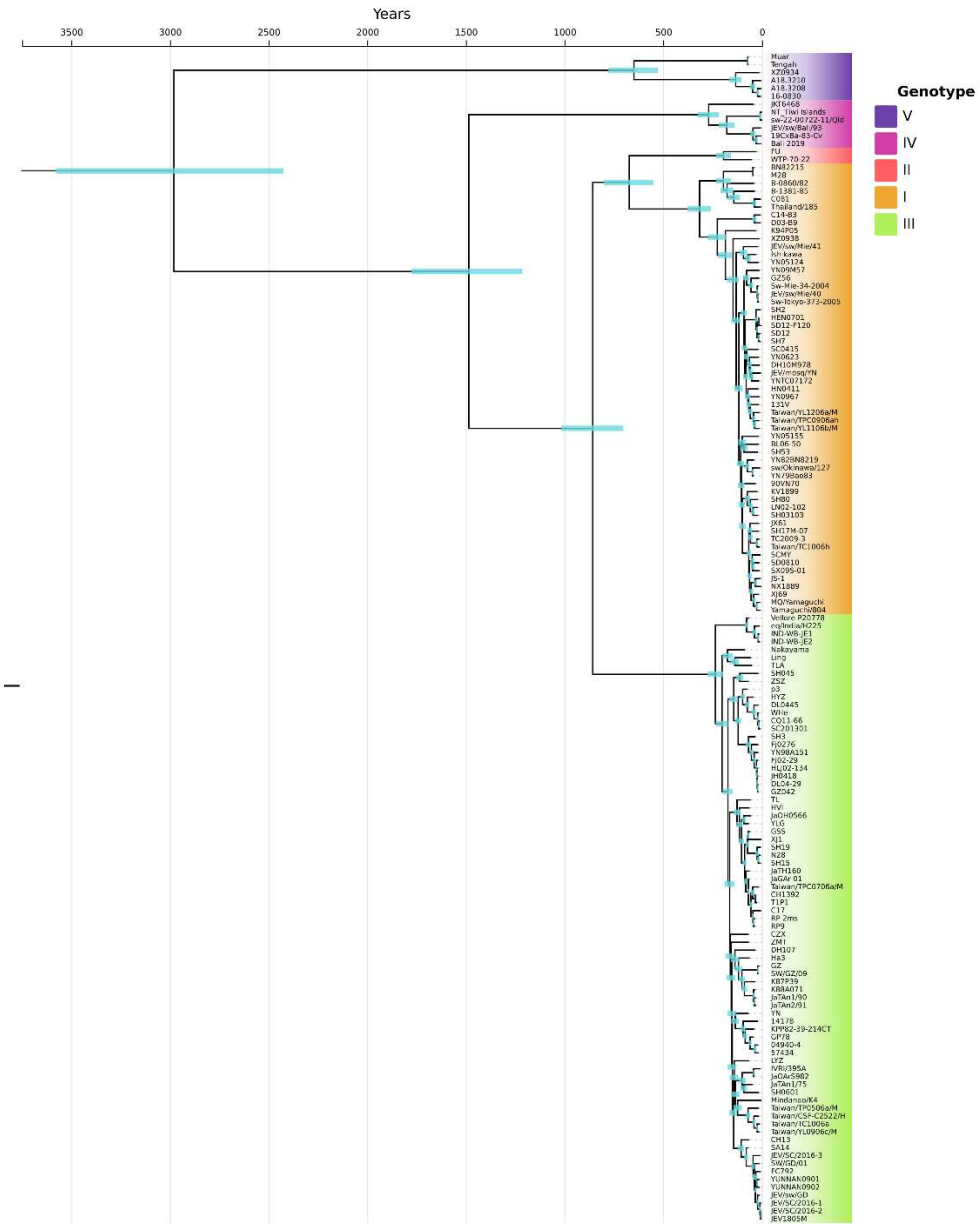

Figure 1. Bayesian inference based phylogenetic tree for all available JEV complete genomes till 2022 was reconstructed in the present study. BEAST Bayesian inference-based chronograms present the divergence time estimates, and phylogenetic relationship among previously reported JEV genomes. Tree was constructed based on C, E, NS2B and NS5 protein coding sequences of all publicly available JEV sequences.

Figure 2a–c, and d illustrate the population dynamic of JEV. The skyline plot showed that the JEV population experienced complicated changes during the process of evolution after the 18th century. Initially, JEV remained relatively stable after its emergence (Figure 2 a). However, a fluctuation was observed in the late 18th century as shown in Figure 2 b. There was a gradual rise in the JEV population observed from 1790 to 1870. It was a time when the first recurrent epidemics of JEV occurred in Japan from 1871 onwards [29]. After 1870 it continued to rise and peaked during 1920 to 1930 and a little fluctuation was seen between 1975 to 1985 as presented in Figure 2 b. After that JEV population remained high (Figure 2 b). Figure 2 c explains that the JEV population is increasing slightly after 2010 to 2022, and genetic diversity has also increased slightly. The overall-JEV-lineage-through-time analysis (Figure 2 d) depicted a similar pattern of JEV population dynamics to that of BSP. It showed that the JEV population remained constant in the beginning, then increased stepwise,

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and finally attained a sharp increase. Whereas, after the sharp increase it makes a plateau-like pattern and maintains it to date (Figure 2 b&c).

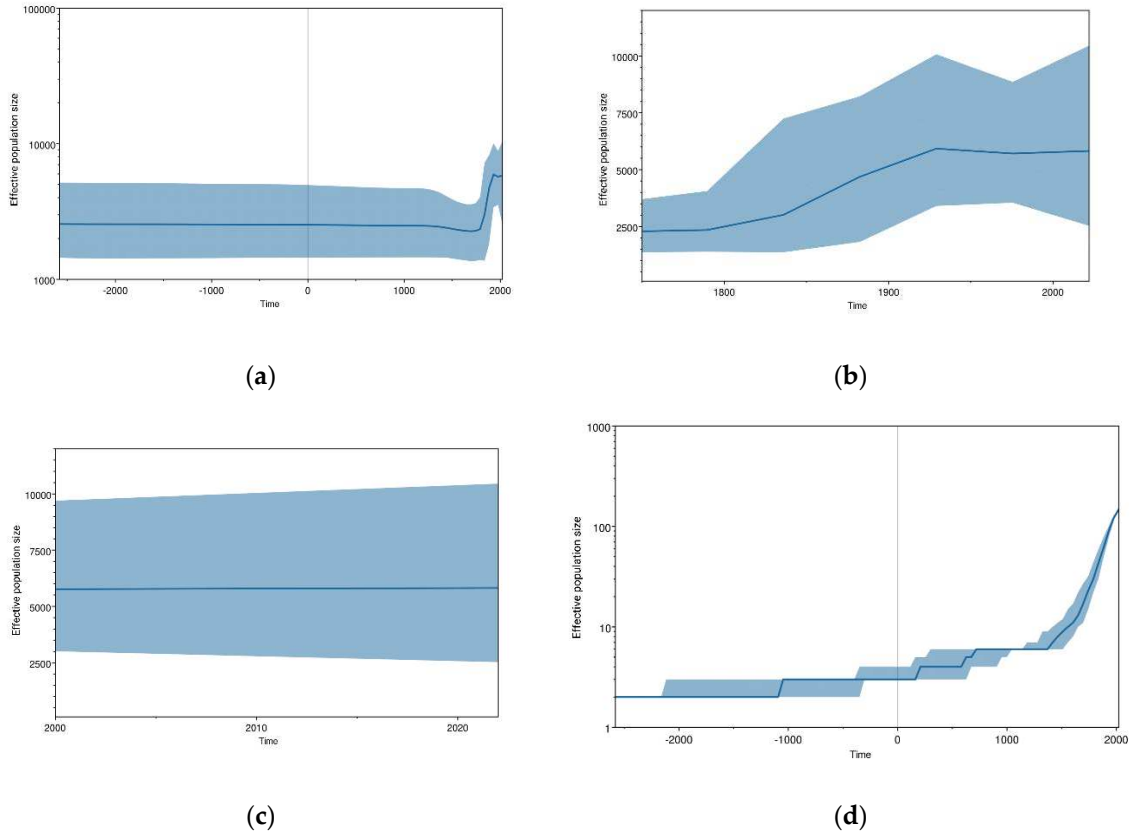

Figure 2. Bayesian skyline plots representing the demographic history of JEV. The central solid blue line represents the median posterior value, and the shaded area represents the 95% HPD intervals. The x-axis corresponds to time (years), while the y-axis represents the effective population size. (**a**) Population modeling during the whole evolutionary history; (**b**) Population trends during 1750-2022, (**c**) Population dynamics from 2000 to 2022, (**d**) JEV lineage through time.

4. Discussion

In the present study, we performed bioinformatic analysis of the available JEV genome sequences (n=160) to construct a timeline phylogenetic tree to determine the tMRCA and also determined the population dynamics of JEV. Our analysis showed that tMRCA occurred about 3120 years ago (YA) (95% Highest posterior density [HPD], 2680 to 3715). Furthermore, population dynamics analysis showed that JEV genetic diversity has been increasing since 2010. Therefore, we need to monitor JEV spread and update vaccines accordingly.

Recently, JEV cases have decreased due to the development and wide-scale application of JEV vaccines [30,31]. Although the JEV outbreak has been controlled, from this molecular data, we can conclude that disease threat still exists. Because a plateau-like pattern at high population diversity demonstrates the high genetic diversity of JEV, which infers that JEV is potentially replicating in the reservoir hosts, and it is a potential threat for future outbreaks (Figure 2 c). Our skyline data show that a fluctuation was observed after 1790 and gradual rise in the JEV population observed from 1790 to 1870 (Figure 2 b). It was a time when the first recurrent epidemics of JEV occurred in Japan from 1871 onwards [29], which is a good interpretation of our data. However, this data is not in line with previous reports where they observed the first rise in JEV population after 1930 [8]. This difference might come due selection of different models for analysis. However, Figure 2 c illustrates that after 2000, a similar trend was observed in previous and present JEV evolutionary studies [8,9,32]. In addition, despite the continuous application of different JEV vaccines, a high level of genetic diversity

infers that the virus is replicating potentially and there are chances of gaining mutations that can lead to the emergence of new and highly pathogenic strains.

Flaviviruses such as West Nile virus (WNV), yellow fever virus (YFV), dengue virus (DENV), tick-borne encephalitis virus (TBEV), Zika virus (ZIKV), and JEV are the most abundant mosquito borne viruses which are causing outbreaks in different regions [33,34]. The evolutionary potential of viruses is determined by the nucleotide substitution rate [35]. In the present study, the mean rate of nucleotide substitution for JEV strains was 1.0628E-4 nucleotide substitutions per site per year (95% HPD values 8.7795E-5, 1.2564E-4) which is comparable with WNV 5.06E-4, TBEV 2.104E-4, and YFV 4.2E-4 substitutions per site per year [36–38] and shows the JEV evolutionary potential among flaviviruses. Recently, JEV GIV caused an outbreak in Australia which highlights its potential to spread to different regions of the world and cause epidemics. JEV GIV was also detected from Indonesia [39,40]. There are multiple factors which might play role in JEV spread to non-endemic areas such as infected mosquitoes transfer through wind-blown, harboring on planes, local mosquitoes can get JEV from infected migratory birds, adaptive changes in virus to produce long time viremia in vertebrate hosts, etc. Previous studies show the role of birds in the JEV zoonotic transmission as a natural reservoir and amplifying host [4,41]. Migratory birds can move hundreds to thousands of kilometers long and can extend across political boundaries [42]. Bird population is increasing because of development in agriculture. A recent study from Korea, reported that the distribution and density of migratory birds are correlated with JE cases in cities and they might be highly potential hosts contributing to transmit JEV in metropolitan areas [43]. JEV GIV infected birds' migration from endemic areas to non-endemic regions might lead to JEV spread to Australia which requires intensive investigation.

Overall, our analysis shows that JEV genetic diversity has increased during the last five to ten years which indicates the potential JEV replication in the reservoir host, which is helping it to maintain its genetic diversity, and to continue its dispersal into non-endemic areas. Therefore, an enhanced surveillance system is needed along with precautionary measures such as regular vaccination and mosquito control to avoid future JEV outbreaks.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: JEV isolates analyzed in this study.

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Ethical Statement: This communication does not contain any studies with human or animal subjects performed by any of the authors.

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