Japanese Encephalitis Virus: An Emerging Threat in the Indo-Pacific Region

Kumar Saurabh Srivastava[1], Vandana Jeswani[1], Nabanita Pal[1], Babita Bohra[1], Vaishali Vishwakarma[1], Atharva Ashish Bapat[1], Yamini Prashanti Patnaik[1], Navin Khanna[2]* and Rahul Shukla[1]*

[1]Division of Virus Research and Therapeutics, CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India

[2]Translational Health, Molecular Medicine Division, International Centre for Genetic Engineering & Biotechnology, New Delhi, India

Running title: *JEV: an emerging threat*

Keywords: *Japanese encephalitis (JE), Japanese Encephalitis Virus (JEV), Therapeutics, Vaccine, Antiviral, Drug*

*** Correspondence:**
NK (navinkhanna5@gmail.com; navin@icgeb.res.in
RS (rahul.shukla1@cdri.res.in)

 doi:10.20944/preprints202212.0581.v1

Abstract

Japanese Encephalitis (JE) is a disease caused by the Japanese Encephalitis Virus (JEV). JEV is an arbovirus that spreads primarily through the bite of a female *Culex spp.* mosquito. JE shows predominance over the Asia-pacific region and has the potential to spread globally with a higher rate of morbidity and mortality. JE, a neuro-invasive disease, initiates with mild fever which may lead to encephalitis with severe neurological sequelae in some cases. Efforts have been made to identify and select various target molecules essential in JEV progression, but until now, no licensed anti-JE drugs have been available. From a prophylactic point of view, a few licensed JE vaccines are available but various factors *viz.* high cost and different side effects imposed by them has narrowed their global use. With an average occurrence of >67,000 cases of JE annually, there is an urgent need to find a suitable antiviral drug to treat patients at the acute phase, as only supportive care is available to mitigate infection. This systematic review highlights the current status of efforts put in to develop antivirals against JE and the available vaccines along with their effectiveness. It also summarizes epidemiology, structure, pathogenesis, and potential drug targets that can be explored to develop a new range of anti-JEV drugs to combat JEV infection globally.

1 Introduction

Japanese Encephalitis (JE) is one of the most common epidemic encephalitic disease in the world which is majorly distributed in South and Southeast Asia [1]. It is caused by a mosquito-borne virus, the Japanese Encephalitis Virus (JEV) which belongs to the family Flaviviridae and genus *Flavivirus* [2]. Other clinically relevant viruses belonging to the same genus include Yellow Fever Virus (YFV]) Murray Valley Encephalitis (MVE), Dengue Virus (DENV), West Nile Virus (WNV), Zika Virus (ZIKV), St. Louis Encephalitis Virus (SLEV) and Tick-Borne Encephalitis Virus (TBEV) [3]. According to the WHO report (2019), almost 68,000 cases of JE with a 20-30% mortality rate were recorded annually. A study based on mathematical modeling with age-stratified case data estimated that approximately 100,308 clinical cases and 20,000–30,000 deaths occurred due to JEV in 2015 across the globe [4]. Children aged 0 to 15 years are comparatively more vulnerable with increased threat of neurological complications than adults [5]. Almost 2 billion people living in endemic countries face constant threat of JE and upsurge in the mosquito population poses risk of JEV expansion to newer geographical areas.

JEV is a single-stranded positive-sense RNA virus, which is primarily transmitted through the bite of infected female mosquito *Culex tritaeniorhynchus*. Other *Culex* species such as *Cx. annulirostris, Cx. vishnui, Cx. pseudovishnui, Cx. gelidus, Cx. sitiens* and *Cx. fuscocephela* are also reported to be involved in the transmission of JEV along with some species of *Anopheles* mosquito such as *Anopheles subpictus, An. Peditaeniatus* and *An. hyrcanus [6]*. The primary reservoirs of JEV are the birds of the family *Ardeidae*, like herons and egrets. Pigs are highly susceptible to the virus where they get amplified in optimum level and develop high circulating viral titer [amplified host], therefore are able to spread the infection to naive mosquitoes [7]. Cases are also reported at this stage pigs tend to shed the virus in oronasal secretion and may potentiate the horizontal transmission of JEV infection [8]. Humans and animals like cattle and horses do not develop high viral titers to spread the virus through uninfected mosquito bite and making them ‘dead-end’ hosts, as shown in **Figure 1**. Nonetheless, JEV has an enzootic cycle, due to which the virus can persist in nature to such an extent that it might be next to impossible to eradicate it in the near future. Thus, an effective antiviral therapy and an ideal vaccine against

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0581.v1

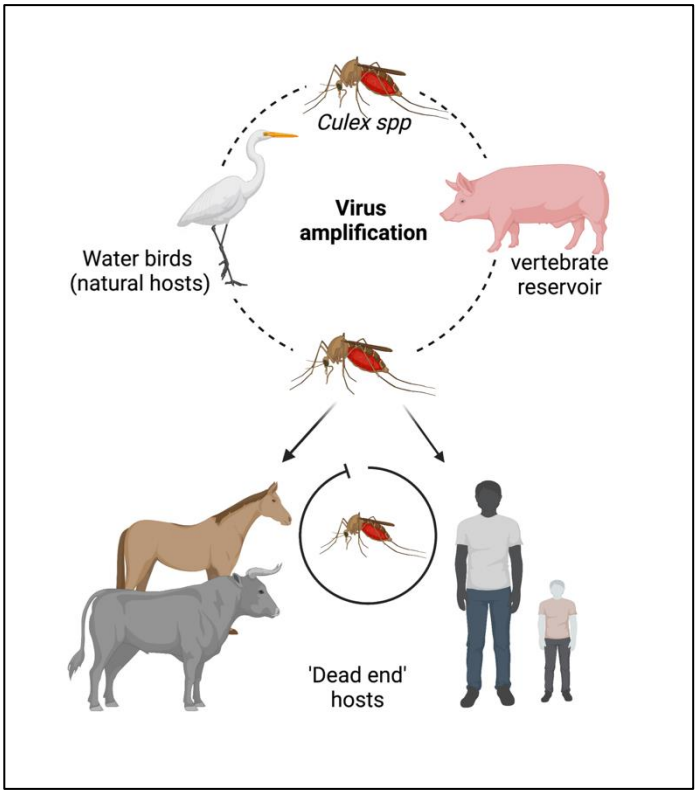

Figure 1: Cycle of Japanese Encephalitis Virus (JEV) infection and amplification*. Long-legged water birds like herons, storks, and ibises are the primary reservoirs and make it as a natural host for JEV. JEV-infected female mosquitoes, especially Culex tritaeniorhynchus transmit the virus from wading water birds to other vertebrate animal species (Pigs, Cattle and other hooved animals) and humans. Pigs (wild or domesticated) acts as a secondary host where virus gets amplified at optimum level and carries the infectious virion from one place to another (vector-free transmission), from where female Culex mosquitos take up the virus and infects to humans by biting them. The infected human becomes 'dead-end' host for the virus as the JEV do not develop a high enough titer in the blood circulation to transmit through feeding mosquitoes.*

JEV is of great importance. JE research has increased rapidly with growing technological advancements, with the primary aim of developing a safe and cost-effective therapeutics (drugs and vaccines) for all age group. This review thoroughly discusses the recent trends in the discovery of JEV-targeted compounds that have the potential to be developed as therapeutic drugs and the efforts that have been done so far towards the therapeutic research against JEV.

2 Epidemiology

The majority of cases of viral encephalitis in the Asian subcontinent are due to JEV [5]. This spans a large region that includes majorly tropical parts of Asia, such as Japan, China, Taiwan, Korea, the Philippines, India and all of Southeastern Asia. Countries with confirmed JE epidemics include India, Nepal, Pakistan, Sri Lanka, Myanmar, Laos, Vietnam, Malaysia, Philippines, Singapore, China, Indonesia, maritime Siberia, Japan, and Korea [9]. Additionally, it has intermittently and infrequently happened in portions of the Western Pacific and northern Australia [5]. Historically, the JEV similar outbreaks had been recorded in Japan in the late 1800s, however, the first confirmed JE case was documented in 1924 in Japan followed by Korea (1933), China (1940), the Philippines (1950), India (1955), and many other Asian countries thereafter [4]. In recent decades, geographical hotspots for JE incidences have shifted considerably from South Asian countries (e.g., Japan, South Korea, and Taiwan) to South East Asian countries like Bangladesh, Cambodia, India, Indonesia, and Pakistan [5], as shown in **Figure 2**. Since the early 1970s, there has been a rise in epidemic activity of JEV in the Indian subcontinent. Afterwards, in late 1990s, the virus persisted to spread in the neighboring territories like Southern Pakistan along with the Kathmandu Valley of Nepal [10].

 doi:10.20944/preprints202212.0581.v1

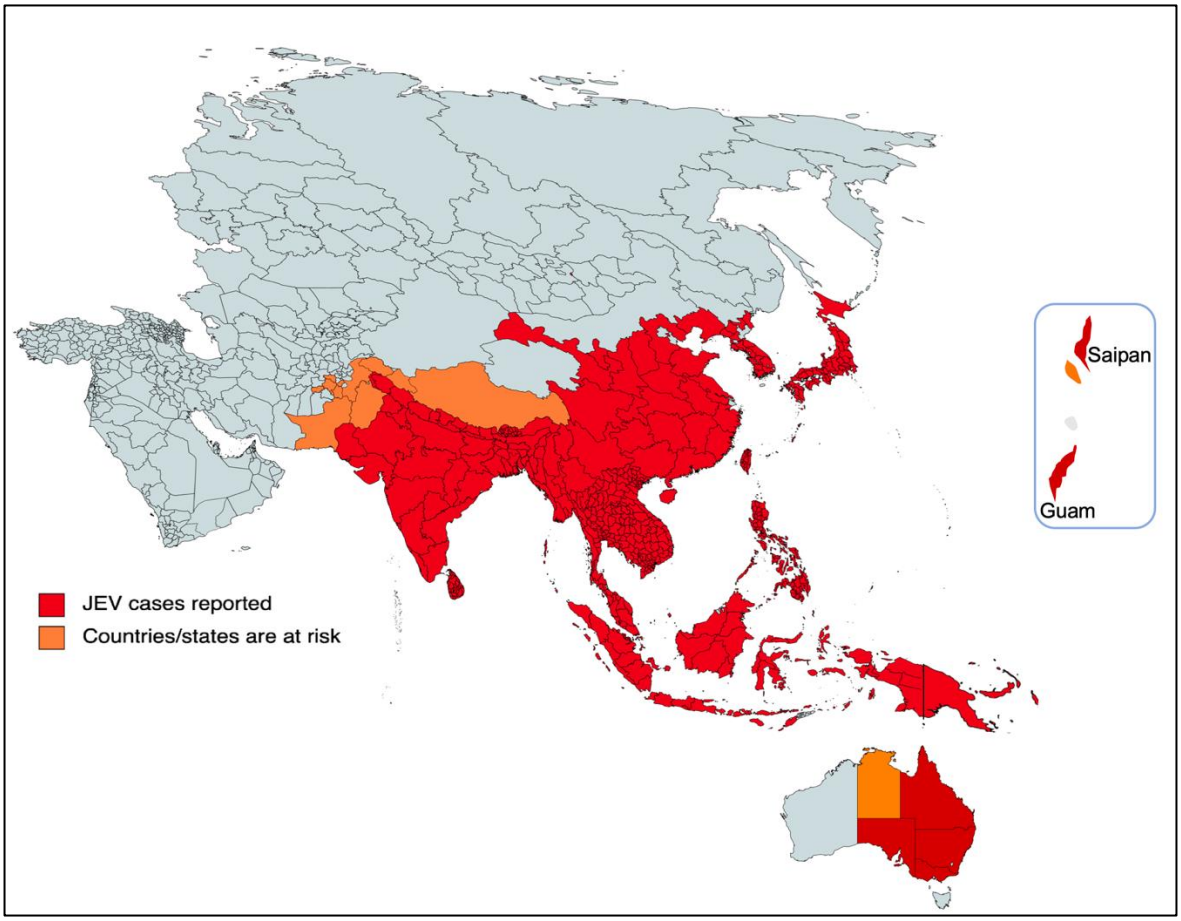

Figure 2: Geographical distribution of JEV. *The red part of focused Indo-Pacific geographical regions and countries indicates where active JEV cases were reported since its outbreak. And, orange color is showing areas which have highest risk of JEV infection in near future. The epidemiological data of JEV was modified from https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis* .

In India, the first JEV evidence was observed back in 1952 and later on it expanded in several states in 1955, with the predominant pediatric cases [10]. The incidence of JEV cases in India have a positive correlation between the disease outbreak and vector density as outbreaks were always preceded by the monsoon season. Indian states like Karnataka and Andhra Pradesh experienced two outbreaks every year from April to July and September to December, however, the outbreak in April-July being quite severe compared to the September-December outbreak [11]. Nonetheless, the cause of seasonal JE severity is still unknown. Amongst the outbreaks in India, the major one that was well documented occurred in the Bankura district of West Bengal resulting in a 42.6% case fatality rate in 1973 [9]. Post-1973 outbreaks, JEV almost covered all parts of the Indian continent and it has been reported at regular intervals. The hot spots for outbreaks are Andhra Pradesh, Assam, Bihar, Haryana, Karnataka, Kerala, Maharashtra, Manipur, Tamil Nadu, Orissa, Uttar Pradesh, and West Bengal. Uttar Pradesh has been under constant surveillance for viral outbreaks since 1978 [11]. Gorakhpur district of Uttar Pradesh witnessed the incidence of the most prolonged epidemic of viral encephalitis between July to November, 2005 [12]. A total of about 5,500 documented cases of viral encephalitis from seven districts of Uttar Pradesh were reported with >1300 fatalities (~23%). Investigation of an encephalitis outbreak in another district of Uttar Pradesh, Lakhimpur-Khiri led to the characterization and identification of

 doi:10.20944/preprints202212.0581.v1

the causal virus homologous to the most common JEV strain GP78 [13-15]. Several reports indicated the expansion of the virus to the newer non-endemic areas, including the northern and northeastern parts of the Indian continent and cases have been reported that signify the virus's spread, including urban areas such as New Delhi, the capital of India. Other massive outbreaks that have been reported include the one in Malkangiri (2012) and another in Manipur (2016) [16].

More recently, JEV infection has been a threat of resurgence that was highlighted by the fairly large epizootic outbreak in Australia in 2022 [17]. In Australia, the JEV case was first identified in 1995 and has remained dormant over the past two decades [18]. In early 2021, it reappeared again and diagnosed in the resident of northern territory in Queensland that resulted in death and it appeared to be a sentinel human case of the recent outbreak in 2022 in the southern Australian states. Afterwards, the JEV was detected in stillbirths, mummified fetuses and newborn piglets from the several commercial piggeries majorly located in the four southern states of Australia (New South Wales, Queensland, South Australia and Victoria), as represented in the **Figure 2**. As of August 01, 2022, there had been 40 confirmed human cases across four states, with five fatalities [19, 20]. The main causative agent of JEV transmission in Australia was the members of the subgroup *Culex sitiens*, particularly *Culex annulirostris* [9]. The other *Culex* species, Culex *quinquefasciatus*, *Culex gelidus*, and *Culex tritaeniorhynchus* were suspected to transmit the disease in Australia [19], although more research and mosquito surveillance need to be carried out to confirm their geographic distribution and abundance in Australia. Nonetheless, the current JEV infection in Southern Australian states poses significant risk to their neighbouring territorial states such as Northern territory and Western Australia.

3 JEV structure and its genome

Japanese Encephalitis Virus is an enveloped, positive-sense single-stranded RNA virus measuring ~ 40-50 nm in diameter with spheroid cubical symmetry. The viral RNA genome (~11 kb) encodes genes for three structural (C: capsid; prM: precursor membrane and E: envelope) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) with the 5'methylated cap and lacking the poly-A tail at 3' end. All ten of these proteins encoded from 3432 amino acids are translated from a single open reading frame of genomic RNA.

The capsid (C) structural protein of JEV dimerizes head-to-tail in an anti-parallel manner. Multiple copies of capsid dimers tend to compose in spherical nucleocapsid, enclosing the viral genomic RNA. Recently, the C protein crystal structure was revealed and it was shown to have α-helixes 1–4 secondary structure, which closely resembles with the capsid protein of DENV, WNV, and ZIKV [21]. Each monomer of the JEV capsid protein consists of four helices: α1 (amino acid 29–38), α2 (amino acid 44–57), α3 (amino acid 63–70), and the longest α4 (amino acid 74–96), connected by short loops. The amino-terminal of α helix-1 forms closed and open confirmation by which it tends to be flexible and allows possible antivirals against this domain. However, the carboxyl-terminal pairing of α helix 4-4 arrangements leads to form a coiled-coil-like structure, which possibly helps in nucleic acid binding.

The viral precursor membrane or pre-membrane (prM) emerges from nascent polyprotein following co-translational cleavage by signal peptide and starts to assemble at viral genomic RNA containing vesicles of the ER. Soon after it is assembled, the vesicle buds from the ER and reaches the Golgi body network, where prM is cleaved by furin enzyme into M protein and forms

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0581.v1

a mature virus particle before its release from the host cell. The exact function of prM/M in JEV is still unknown. However, in other related viruses of the same family like DENV and YFV, it is studied well, where it helps in the assembly of mature viruses and providing the moiety for the proper conformational arrangement of the E protein. Also, the membrane protein of the virus plays a crucial role in the binding of the E protein domain with the host receptor while establishing the infection. After the virion internalization, membrane protein assists in the fusion of viral vesicles to the endosome.

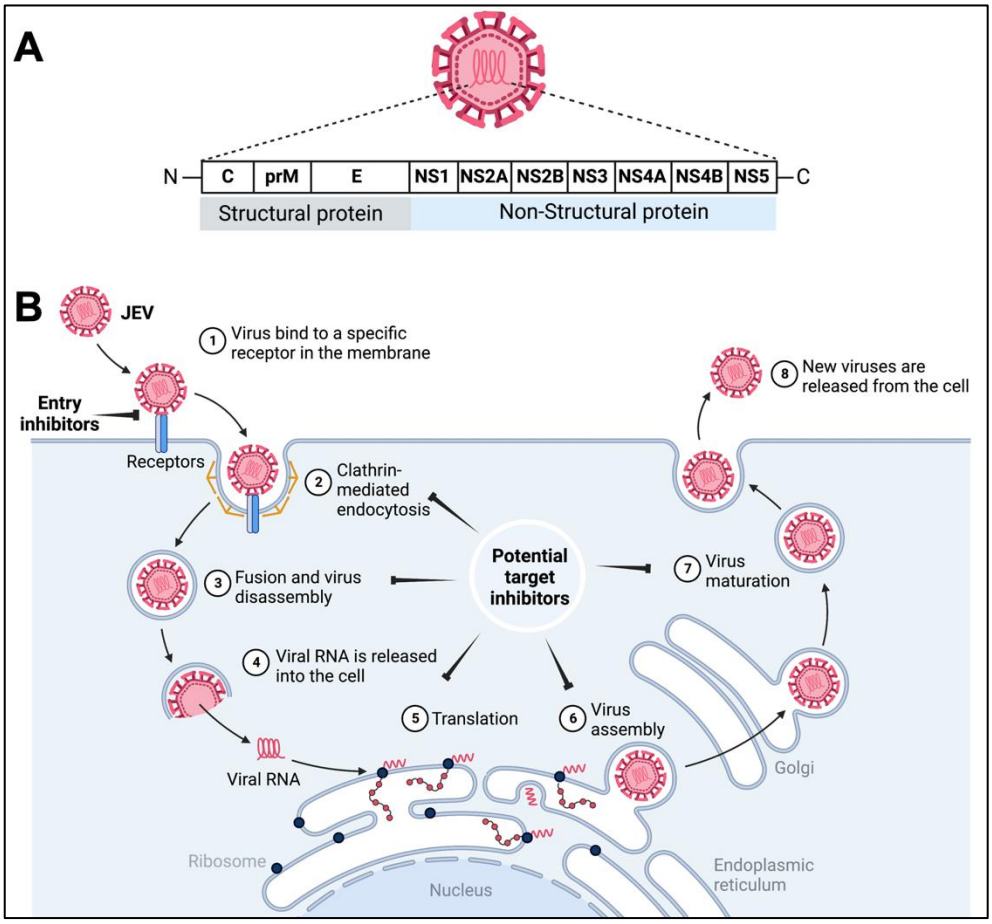

Figure 3: JEV structure, pathogenesis and potential drug targets. *(A) Spherical representative diagram of JEV, comprising of three structural proteins namely capsid (C), precursor membrane (prM) and Envelope (E), and seven non-structural (NS) proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. N and C represents amino- and carboxyl-terminals of the viral polyprotein. (B) JEV pathogenesis start from binding of virion onto the host receptor (DC-SIGN, heparin sulphate, Fc-receptor, etc.) and internalised through Clathrin-mediated endocytosis, thereafter, its fusion with endosome where viral particle changes their confirmation and releases their genomic positive sense single stranded RNA on acidic pH. Soon after, viral RNA starts their translation with the aid of host ribosomal and other essential proteins onto rough endoplasmic reticulum (ER) and makes their structural and non-structural proteins. In cognate, the NS viral proteins help in the making of replication complex and transcribed into complementary negative sense RNA from the genomic positive sense RNA. Also, NS proteins (majorly NS3, NS4B and NS5) form replication complex, and further transcribed into several copies of positive sense genomic RNA which later encapsulated by capsid protein, assembled with prM and E onto ER and assembled virus get matured over trans-Golgi network and finally matured virions releases from host cell through exocytosis. The inhibitory symbols indicate the*

 doi:10.20944/preprints202212.0581.v1

potential drug targets for which development of antivirals efforts have been done so far and continuing research to diminish the JEV infection. Figure was originally created from BioRender.com.

The E of the JEV is an essential protein that covers the virion and is responsible for binding to the host cell receptors and fusion with the host plasma membrane. The E protein is the major protein recognized by virus-neutralizing antibodies. Like other flavivirus E proteins, it has three domains, envelope domain I (EDI), EDII and EDIII. The crystal structure of the E protein revealed that it dimerizes antiparallel head-to-tail like other flavivirus E proteins but with a relatively small interface [22]. The EDI forms the central domain of the envelope with the nine-stranded β-barrel which is located between the extended EDII and globular domain EDIII. EDII is basically formed with two extended loops that extend from EDI and are stabilized by binding with three disulfide bonds and conserving its fusion peptide at the top. Like other flaviviruses, EDIII retains immunoglobulin-like structures at the carboxyl terminus of the ectodomain which tends to bind with host receptors for its virion internalization. With these properties, the EDIII domain could be a potential target for designing antivirals for JEV infection mitigation.

All seven non-structural proteins (NS) are translated and cleaved from the single polyprotein and are majorly involved in the replication and assist in the assembly of new viral particles. The NS1 of JEV is relatively not well studied as other flaviviruses, and its exact function is still unknown. Some studies indicated that JEV NS1 is involved in the replication complex for transcribing the double-stranded intermediate RNA [23]. A few studies have shown that small interfering RNA (siRNA) against the NS1 could inhibit the JEV replication *in vitro* [24]. However, none of the siRNA was carried forward for clinical development. Also, a study by Yen et al., 2015, indicated that NS1 acts as a heterologous epitope and induces a cross-reactive immune response against various pathogens [25]. However, the NS2A protein has direct involvement in the viral RNA genome synthesis and its assembly. The NS2B forms the complex with NS3 and helps in the viral protease activity and NS3 on its own acts as helicase and aid in the function of genome replication. Also, NS3 is believed to have originated from the ER or trans-Golgi body network and works as a reservoir for viral proteins during virion assembly [26]. The NS4 protein is highly hydrophobic and is supposed to participate in making the membrane component. Due to this, it might play a crucial role in virus adaptability in different environments, though the exact function of both NS4A and NS4B is still unclear. The NS5 is a multi-enzymatic protein and a vital component of the viral RNA replication complex. Like other flaviviruses' nonstructural and cellular proteins, the NS5 carries both methyl transferase domain in N-terminus and RNA-dependent RNA polymerase (RdRp) domain in C-terminus [27]. The methyltransferase domain of NS5 is largely responsible for the 5' capping of viral genomic RNA and RdRp domain directly plays a role in RNA replication [28-31].

4 JEV pathogenesis

Viremia must be high enough in reservoir host for a biting naive mosquito to acquire the virus [6]. Once a mosquito sucks blood from an infected host, the virus has to be passed from various physical and physiological barriers before it get replicated [33]. Once virus reaches to the midgut, the viral envelope fuses with the plasma membrane of intestinal epithelial cells and releases its genome into the cytoplasm, if the peritrophic membrane (a potential physical barrier) is not formed yet. Thereafter, the virus gets replicated and virions burst out into the hemocoel from

 doi:10.20944/preprints202212.0581.v1

where it travels to the tracheal system and finally reaches the salivary glands [33]. Eventually, acinar cells of salivary glands become infected with the virus after passing the salivary gland infection barrier and start to shed virions into the saliva and the carrier mosquito ready to infect the subsequent host when it bites.Upon infected mosquito bites, virions finally released to the dermal cells of the healthy human [32], from where the virus causes either latent infection by infecting only mononuclear cells or persistent infection in which it invades the nervous system [34]. Pathogen-associated molecular patterns (PAMP) bind to pathogen recognition receptors (PRR) to induce interferon stimulatory genes (ISGs like PKR, OAS, TRIM21, ISG15, and MX1) *via* the JNK pathway. These interferons induce the antiviral state in the host cell and neighbouring uninfected host cells, but the virus can change it in its favour. The virus invades primarily innate immune cells in the skin like keratinocytes, Langerhans cells, fibroblasts, dermal dendritic cells and endothelial cells [35]. The dendritic cells are the primary target for JEV infection and potential the secretion of pro-inflammatory cytokines such as IL6, IL8, IL12 and TNF-α [36] and attracts other immune cells by which virus gets opportunity to enter the draining lymph nodes and secondary lymphoid organs like the spleen, followed by other peripheral tissues like the kidney, liver, heart, and lungs. The virus replicates in macrophages in tissues and monocytes in the blood [37]. If the immune system can generate a humoral response (IgM) in first 5-days of the virus incubation period (sub-clinical stage) then virus may get cleared before reaching the central nervous system [35]. If not, the most dangerous clinical phase begins and starts to enter the central nervous system after breaching the blood-brain barrier (BBB). There are various ways of breaching the BBB, (a) direct diffusion through endothelial cells, infecting them by replication; (b) Trojan horse mechanism in which the virus enters cerebrospinal fluid (CSF) by colonising in inflammatory cells or blood lymphocytes; (c) chymase release by mast cells or metalloproteases (MMP 2/ MMP 9) which loosens tight junction between endothelial cells [38]; or (d) receptor-mediated endocytosis [34, 35]. All these mechanisms can kill neurons directly by infecting the more causing neuronal bystander death. Virus entry in CSF activates microglial cells, astrocytes, and pericytes due to which they release excessive pro-inflammatory cytokines like COX-2, iNOS, IL-6, TNF-α, which causes inflammation in some parts of the brain (neuroencephalitis) [31]. Studies report that IFN - α (anti-inflammatory cytokine) provides immunity against JEV by inhibiting replication at various stages as well as assembly and release. Its efficacy has been studied *in vitro* but a similar effect was not observed in humans [39,40].

Furthermore, JEV enters neuronal cells using PLVAP and GKN3 receptors [41]. Subsequently, lowers the levels of anti-inflammatory cytokines (IL-10 and IL-4), and also kills neuronal cells [34]. Killing the neurons, stimulates astrocytes and microglial cells to release inflammatory mediators which causes further neuronal cell death [42]. Astrocytes and microglial cells also release chemokines (IP-10, RANTES, IL-8, MCP-1) which facilitate the entry of blood lymphocytes in the CSF, further elevating the infection [42]. The proliferation of neuron progenitor stem cells (NPSC), that are responsible for immune response into CSF, is inhibited due to virus infection. Prolonged inhibition of protein synthesis due to interferons may be lethal to healthy neuronal cells. Studies showed that individuals with a higher concentration of anti-JEV IgM in CSF have more chances of survival without much affecting CNS [43,44].

Interaction of the virus with host receptors includes recognition, attachment, binding, and entry. The process initiates with the association of the virus to attachment factors like heparansulfate proteoglycans which brings it to the vicinity of the target host cell until it finds a

 doi:10.20944/preprints202212.0581.v1

suitable receptor on the plasma membrane. The virus interacts with several host receptors like Heat Shock Protein 70, CD4, α5β3 Integrin, Dendritic cell-specific intercellular adhesion molecule-3- grabbing non-integrin (DC-SIGN), Glucose-regulated protein 78 (GRP78), T-cell immunoglobulin and mucin domain 1 (TIM-1), etc. [35]. After establishing the attachment with the host receptor, the viral particles are internalized by clathrin-dependent and independent endocytosisin fibroblasts, epithelial cells and neuronal cells. Conformational changes in E glycoproteins occur inan endocytic vesicle, which releases hydropathic patches resulting in fusion of the envelope with an endosomal membrane (**Figure 3**). Thereafter, the virus uncoats and releases its single-stranded positive-sense RNA genome in the cytoplasm under the influence of acidic pH [45]. Non-structural proteins of the virus along with host factors and endoplasmic reticulum-associated protein degradation (ERAD) proteins like LC3-1, EDEM 1 and SEI1L interact to form a replication complex at the surface of the endoplasmic reticulum [34, 46, 47]. ssRNA replicates to a double-stranded replicative form, which serves as a template for the synthesis of positive sense mRNA. mRNA is synthesized, and protein is translated and processed by host and viral proteases. Virion assembles in the ER lumen and matures via cleavage of prM to M by host furin at trans-Golgi followed by its release. [35] (**Figure 3**).

5 Clinical manifestations

In general, most JE infections are mild subclinical febrile illness or without any evident symptoms. But, among the patients who develop severe clinical illness (encephalitis), the case fatality rate can be as high as 30%. The patients who survive after being severely infected, about 30-50% of them face behavioral or neurological sequelae such as paralysis, frequent seizures and/or inability to speak [10,48]. The progression of JEV infection is divided into three stages in patients who progress to neurological disease. The first stage, the prodromal phase, is marked by mild fever, chills, muscle pain, meningitis, vomiting, diarrhoea. In the second stage, the acute phase, a person suffers from reduced consciousness, seizures, a parkinsonian syndrome which further manifests into viral encephalitis in which a person may experience tremors, generalized hypertonia, cogwheel rigidity and other abnormalities that may even result in a coma. In some cases, the disease progresses rapidly and maybe lethal. In the third stage, the late phase, the person either recovers or suffers from elongated neurological sequelae [34, 42]. Several parts of the brain such as the hippocampus, thalamus, basal ganglia, parenchyma, cerebral cortex, midbrain, brain stem, temporal lobes, substantia nigra, and anterior horn cells of the spinal cord get affected among which hippocampus is found to be majorly affected. Few histopathological changes observed are perivascular cuffing, vascular leakage, microglial nodule formation, gliomesenchymal nodules, necrolytic lesions, scarred ramified foci, cerebral edema, and congested leptomeninges [34].

6 JEV Genetic Diversity

Sequencing of complete and partial viral genomes has led to the identification of prevalent viral genotypes. Five different JEV genotypes have been identified (GI-GV) so far. The genotypic distribution of JEVs has an observable geographic pattern, with (a) Indonesia- Malaysia region having all 5 isolated genotypes; (b) GI and GII found in the Australia-New Guinea region; (c) GII and GIII prevalent in the Taiwan-Philippines region; (d) Thailand-Cambodia-Vietnam region having GI, GII, and GIII genotypes; (e) Japan-Korea-China region having GI and GIII; and (f)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0581.v1

India-Sri Lanka-Nepal region having GIII. Although several reports suggested that GI is replacing GIII as the dominant genotype in India. Detailed phylogenetic analysis of the present strains suggests the origin of JEV from an ancestor in the Indonesia-Malaysia region and diverged into five different genotypes. The genotypes GIV and GV, which still circulate in the region are older forms and the newer ones which are more recent in evolutionary history, *viz.*, GI, GII and GIII have spread to other parts of the world. A group of GV have been isolated in China and South Korea post- 2008, which indicates that they emerged recently and circulated outside the Indonesia–Malaysia region. Although all JEV genotypes form a single serotype, at least 5 antigenic groups are differentiated by various immunological assays, demonstrating some degree of antigenic variation among circulating JEVs [49]. Thus, the genetic and antigenic heterogeneity of JEV may have a significant impact on JE prevention and control. Genotypes of JEV along with the most affected countries are given in **Figure 4** [50].

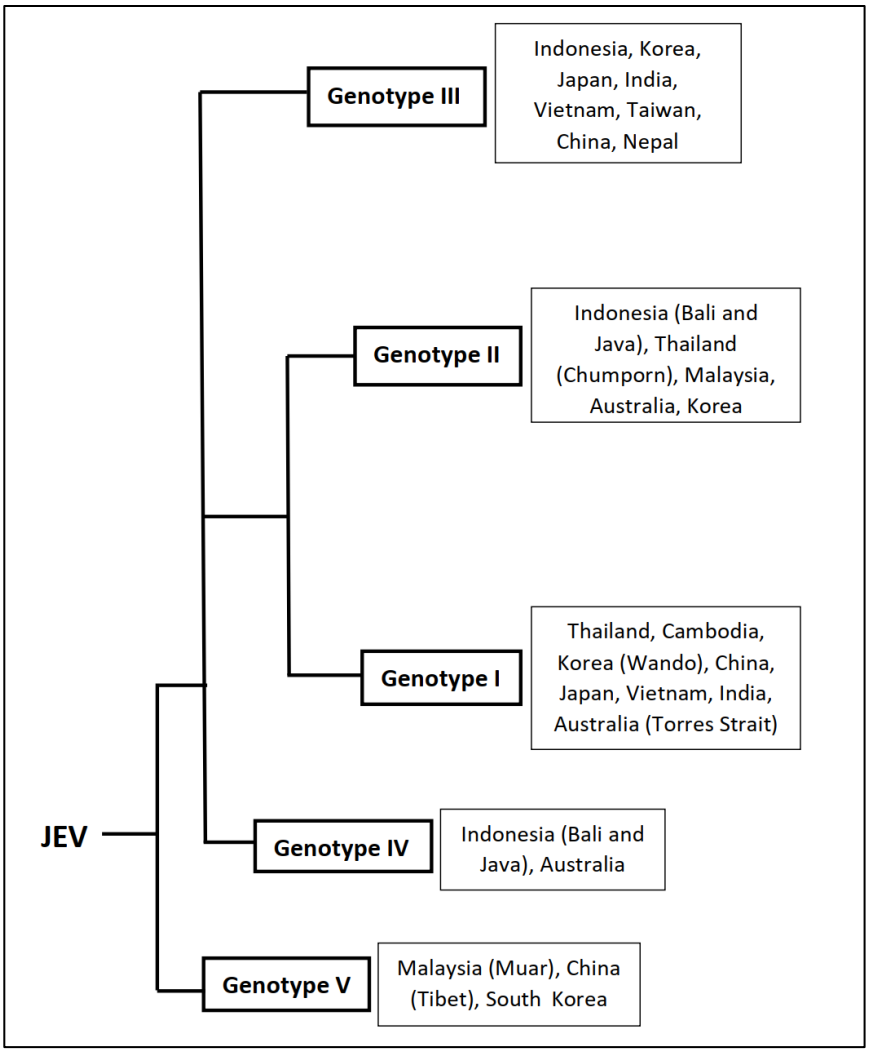

***Figure 4: Genotypes of JEV and most affected countries**. Based on the nucleotide sequences of the C/prM and E protein genes, JEV is classified into five genotypes as Genotype I, II, III, IV and V. A general representation of the origin of various JEV genotypes along with the countries/geographical areas where they are most abundant in or have the greatest impact (in the right side of the respective genotypes). The data represented is adapted from Solomon T et. al, 2003 [50], Gao X et. al, 2015 [49], van den Hurk et al., 2022 [19].*

7 Potential Drug Targets

Various proteins, crucial for viral attachment, replication and maturation, can be considered as potential drug targets. Data states that developing host-related proteins as drug targets can be more advantageous than targeting the viral proteins, as it allows targeting multiple viruses simultaneously [51]. It reduces the chances of drug resistance and maintains drug affectivity, even if the virus is mutated. However, targeting host molecules might be disadvantageous as it may induce various levels of cytotoxicity and side effects at the cellular level [52, 53]. Analysis of the JEV lifecycle at different levels has also identified various levels at which the replication of the

 doi:10.20944/preprints202212.0581.v1

virus can be inhibited *via* targeting different structural and non-structural proteins. Structural proteins like Capsid and Envelope proteins and Non-structural proteins like NS3 and NS5 can be explored as unique drug targets [31].

The C protein dimerizes and the C-terminal of it associates with the viral RNA to form nucleocapsid (NC) [54,55]. Such association allows the stabilization of viral RNA; thus, molecules can be developed inhibiting the dimerization of C protein or the RNA-protein interaction as Anti-JEV drugs [21]. The prM and E proteins, the main constituent of the immature virion, inhibits the premature budding of virus particles [56]. The maturation of virions is a result of the conformational changes of major surface component dimeric E -protein by cellular serine protease furin [56,57]. Studies indicate that, N linked site of domain I of E protein interacts with cellular receptors and can be concluded for the infectivity of the virus [22,58]. Structural analysis has suggested various sites of the target in E protein like β-OG ligand binding pocket, E-protein rafts in mature virus and E homotrimers [56-58].

The proteolytic cleavage of NS2A-NS2B, NS2B-NS3, NS3-NS4A and NS4B-NS5 are essential for the assembly of viral replicase complex [57]. This is processed by a heterodimeric complex of NS2B-NS3. NS3 has a serine protease domain at N-terminal, which is enzymatically inactive, and is active in association with NS2B. NS2B contributes to the essential folding of the NS3 protease site [59, 60]. The C-terminal of protease and helicase domain of NS3 has 7 conserved motifs of NTPase and RNA helicases [59]. NS3 helicases perform vital viral replication functions like DNA duplex resolvation, helping in RNA synthesis initiation, removing protein bound to the genome and secondary structures melting [61,62]. It also has ATPase activity, which regulates ATP-dependent strand separation. Similarly, all NS3 helicases have NTPase activity to hydrolyze nucleoside triphosphate non-specifically to meet the energy requirement of the replication process [62]. NS5 has methyltransferase activity at the N-terminal which regulates the 5’ capping of nascent RNA by methylating the 5’ guanine cap and ribose 2’OH position. Mutation at this site has caused impairment of viral replication [63]. The C-terminal of NS5 has RNA-Dependent RNA polymerase (RdRp) activity which initiates the RNA synthesis in the absence of primers [64]. Absence of RdRp in humans makes NS5 RdRp a very promising drug target. Hence, viral proteins such as NS2B-NS3 protease, NS3 helicase, NS5 methyltransferase and NS5 RdRp might be exploited as potential drug targets for the development of anti-JEV drugs.

8 Anti-JEV drugs

Japanese Encephalitis is one of the most serious infections, and no specific antivirals are available to date. Current therapeutic strategies do not target attenuating the virus but it is the only treatment for various clinical manifestations. Many drugs have been investigated [**Table 1**], but none have been found effective to date, so the journey to find any specific drug against JEV continues.

8.1. Broad spectrum (Non-specific) antiviral molecules used against JEV

Many broad-spectrum antivirals have been studied to inhibit JE. Some of them were identified to inhibit JEV infection actively. Rosmarinic acid has been observed to reduce the viral replication of JEV (GP78) in mice brains [65, 66]. Curcumin, a phytochemical which is also an antioxidant, has shown a reduction in new virus formation via dysregulation of the ubiquitin protease system [31, 67]. A derivative of tetracycline, minocycline has shown a reduction of virus titer, prevention of neuronal apoptosis, and activation of microglial activation *in vitro* and *in vivo* studies [68]. It has also shown effects on the protection of the blood-brain barrier which usually impairs during infection [69, 70]. These all drugs can be tested for their particular activity against JEV. Interferons and interferon inducers like aloe-emodin have also shown inhibitory activity against

 doi:10.20944/preprints202212.0581.v1

JEV infection by creating an antiviral state by triggering an adaptive immune response [71, 72]. Ribavirin, an inhibitor of guanine nucleotide synthesis by targeting inosine monophosphate dehydrogenase, has been tested for JE in children, but it showed an almost negligible effect in treating JEV [73-76]. These several phenomena can be exploited to inhibit the infection of JEV and hence can be explored for an active anti-JEV candidate. Various host factors such as ornithine decarboxylase, histone deacetylases, and HSP70 have been identified to play a crucial role in advancing viral infection which inhibits them with inhibitors. HSP70 due to its upregulation and its function during JEV infection, its inhibitors like apoptozole have also been identified to inhibit JEV *in vitro* [77] (**Table 1**).

8.2. Nucleic acid based anti-JEV molecules

Development in micro-RNA-based studies has allowed exploring nucleic-acid based drug designing against JEV. Targeting the viral genome with miRNA inhibits the virus propagation at transcription and translation levels [102]. Although they lack of specificity with various strains, and require simultaneous administration, have provided a promising outcome in partially or completely inhibiting JEV infection *in vitro* and *in vivo* [30, 64, 78]. Several studies have shown a great and efficient reduction in JEV infection by targeting genes for various essential proteins by miRNA [79]. Besides miRNA, shRNA can also be used to silence a part of the viral genome [80]. Other aspects of nucleic acid-based drugs are morpholino oligomers and peptide nucleic acids (PNAs). PNAs are peptide-like backbones containing nucleic acid derivatives with side chains of heterocyclic bases. Morpholino oligomers are DNA bases attached to methylene morpholine rings backbone linked to phosphorodiamidate groups. They both have the ability to irreversibly bind to complementary sequences with high specificity hence they can be used to inhibit viral replication binding at some sequences of the viral genome [81-83] (**Table 1**).

8.3. Replication cycle-based anti-JEV molecules

Inhibiting viral replication at different stages can be potential target for any drug identification. Initiating from entry to the maturation and release of viral progenies, for every step molecule can be designed which may inhibit the particular step, concluding to inhibit the viral replication. Different approaches have been made to inhibit the attachment and entry of JEV. Proteoglycans like heparin sulfate and chondroitin sulfate, essential cellular receptor, has been identified as a potential target against JEV [30]. Their derivatives have been found to have partial protection against JEV, *in vitro* and *in vivo* [84-86]. Bovine lactoferrin binds to the heparin sulfate receptors and prevents attachment of the virus to the cells [87]. RNA replication is governed by several factors hence many compounds have been tested to target viral replication and have provided effective results in eliminating the infection. MCPIP1 [Monocyte chemoattractant protein 1-induced protein 1] has a nuclease domain which has been observed to express anti-JEV activity *in vitro*. MCPIP1 targets various RNA sites and inhibits replication [88-89]. A phytochemical, pokeweed protein extracted from *Phytolacca americana*, exhibits depurination of viral RNAs. Partial progression of JEV was observed in mice with this protein [90]. Kaempferol, a natural flavanol, has also shown inhibitory activity against JEV by neutralizing the virus *via* binding to the frameshift site of viral RNA [91] [**Table 1**].

Advancements in structural virology and *in silico* methodologies have eased methods to identify new molecules against viral targets, primarily against NS3, NS5 and E proteins due to their essentiality in virus replication and infection. Bortezomib has been identified to target the genome of JEV *in-silico* [92]. Various compounds/drugs have been studied through computational guided drug discovery approach and analysed them *via* advanced high throughput technologies. Although they had different high efficacy levels *in vitro* and *in vivo* many of them failed to show a similar effect in humans or they were detected to be unsuitable in clinical studies [31]. Hence the journey to find some promising drug against JEV continues.

 doi:10.20944/preprints202212.0581.v1

9. JEV Vaccine

Due to the dearth of therapeutics, vaccination is the only reliable means of prevention, other than avoiding mosquito bites 146]. As a part of pre-travel precaution in most countries, multiple doses of JEV vaccines are recommended [147]. Since the 1990s, significant progress has been made in JE surveillance and the implementation of vaccination programs. In 2012, 75% of countries with JE transmission risk had some surveillance programs in place, and 46% had immunization program [148]. Large-scale vaccination of the susceptible human population must be implemented in order to prevent JE. There are several types of JE vaccines, including purified, formalin-inactivated mouse-brain derived, cell-culture derived inactivated, and cell-culture derived live attenuated vaccines. [9]. A brief summary of JEV vaccines are given in **Table 2**

9.1. JE-MB

The first-generation inactivated Nakayama strain JE-MB [marketed as 'JE-VAX' or 'Biken'] vaccine derived from mouse brain was manufactured in Japan and got licensed in 1954 [9]. In Taiwan, the first trials of the crude vaccine were conducted in 1965, where 80% effectiveness was observed. Later, adults from India, Japan, Thailand, and the US reported 80% to 100% seroprotection after receiving a purified vaccination. [149] The Beijing-1 strain, also known as P-1, replaced the Nakayama strain in 1988 after observing its better cross-neutralization and broader coverage on different JEV strains [149]. Based on the JEV Beijing-3 strain, in 1967, a primary hamster kidney [PHK] cell culture-derived inactivated vaccine was produced in China having fewer adverse effects [9]. The vaccination was recommended by the Centers for Disease Control and Prevention (CDC) for age group 1 – 3, in a three-dose regimen 0, 7, and 30 days which showed good neutralizing antibodies in 100% of vaccinee in 6 months. Immunity against JEV was reported to be persisting in some recipients even without the booster for two years, although booster is recommended after one year followed by once every 3 years until age of 10. [9,150]. Later in 2005, the Japanese government retracted this vaccine from their immunization plan when it was temporarily linked to the risk of acute disseminated encephalomyelitis. Though it was uncommon illness but serious when it associated with other hypersensitive responses and become fatal at the rate of 1-17/10,000 vaccinated people. Despite the fact that, the WHO Global Advisory Committee on Vaccine Safety (GACVS) clarify and explained that the JE-MB vaccine had no causal relationship with an elevated risk for acute disseminated encephalomyelitis [151]. The Nakayama strain of JEV had been the predominant strain utilized in JE-MB production throughout Asia since it was identified from a patient's CSF in 1935 and maintained via continual mouse brain passage [152]. However, cell-culture-based vaccinations have mostly supplanted the JE-MB vaccine [153,154].

9.2. JE-VC

JE-VC is a Vero cells culture-derived vaccine made up of inactivated SA 14-14-2 strain of JEV with 0.1% of aluminum hydroxide as an adjuvant [150]. In the United States and most other countries the JE-VC marketed as IXIARO®. However, in Australia and New Zealand, it is sold as JESPECT® and in India its brand name is JEEV® [155]. The JE-VC was initially manufactured by Intercell biomedical, Austria and licensed in 2009 for aged ≥17 years. Later in 2013, it further licensed for aged ≥ 2 months to 16 years [156] and reported 100% seroconversion in vaccinated individuals with the booster dose. But some cases also reported that the decline in seroconversion

 doi:10.20944/preprints202212.0581.v1

rate with time [157,158]. The most common side effects of this vaccine were identified in clinical trials in 13–26% of participants as headache, myalgia, influenza-like sickness, and fatigue in the first week of immunization [150]. The other variant of JE-VC vaccines which are available without using any adjuvant, are made up of inactivated Beijing-1 strain of JEV, being licensed as JEBIK-V and ENCEVAC in Japan in 2009 and 2011, respectively. TC-JEV that was manufactured in Boryung/Korea licensed in 2013 [155].

9.3. JE-CV

IMOJEV is a recombinant chimeric JE vaccine (JE-CV), based on the 17D-204 Yellow Fever Vaccine, where two structural genes (prM and E) were replaced with the JEV prM and E genes of SA 14-14-2 attenuated strain [159]. It was designed by Chamber's group in 1999, and further developed by Guirakhoo's group (Acambis, USA) as a ChimeriVax™-JE vaccine. Thereafter, it was licensed in Europe, USA and Australia with the brand name IMOJEV in 2009, which is manufactured by Sanofi Pasteur [160]. This single-dose live vaccine induces a significant immune response with ~95% seroconversion rate [9] and recommended for its immunization in age group ≥9 months and ≤18 years followed with a booster dose after 1–2 years of priming. Later on, the vaccine was recommended for adults (>18 years) with its single dose followed an optional booster dose after 5 years [149. 161] in JEV endemic areas. Headache, weariness, myalgia, and malaise were the most common side effects observed in its clinical trials in 17–24% of vaccine recipients. Till date, the CV-JE vaccine is approved in 14 countries including Thailand, Australia, Malaysia, Philippines, Hong Kong, and Singapore [162].

9.4. JE-LV

Live attenuated Japanese Encephalitis Vaccine (JE-LV) is made up of attenuated strain of SA 14-14-2 virus and produced from the primary culture of hamster kidney cells [163]. The vaccine was initially developed by CDIBP [Chengdu Institute of Biological Product], China in 1988. This is a single-dose live vaccine, recommended to be administered for children at the age of 8-9 months with a booster dose after 3-12 months. The vaccine has shown 85-95% of efficacy with single as well as double doses in reported trials [149]. According to WHO, neutralizing antibodies are produced in ~90% of vaccine recipients [161] and confer protection against JE infection at least for 5 years. Approximately more than 700 million doses of the JE-LV vaccine have been given out globally since 1988. Although it is an effective vaccine, it has shown various side effects including fever, sleepiness, irritability, nasopharyngitis, gastroenteritis, conjunctivitis and rhinitis in their clinical trials [164]. JE-LV has the largest global production share of almost 50% among all JE Vaccines including its presence in West Pacific regions and Asia [Nepal, Sri Lanka, India and Korea] [164]. Owing its potential effects, it has been used more frequently in JEV endemic areas since 2003 [30].

9.5. JEN-VAC

JEN-VAC is a Vero cell adapted inactivated vaccine developed from Indian Kolar strain (821564XY) which is manufactured and licensed by Indian pharma company, Bharat Biotech International Ltd, since 2014 [165].This vaccine provided >90-96% seroconversion and seroprotection after 28 days of immunization in reported clinical trial which was conducted in 1-50 years aged healthy individuals with the minimal recorded adverse effects like, fever, headache, vomiting, pain at injection site and body ache[165]. The double dose of JENVAC with 24 months

 doi:10.20944/preprints202212.0581.v1

interval shows highest antibody titer and has proven to induce better immunogenicity over live attenuated vaccine made with (SA-14-14-2), hence it is recommended to use for adults as well as children of age groups ≥ 1 year. [166]. JENVAC is a promising vaccine, though not much superior to other alternatives according to the currently available data. [167].

10. Discussion

Despite numerous studies that have improved our comprehension of the virus and how it interacts with the host, JE still poses a serious threat to public health and has the potential to spread globally. Coordinated efforts are required for JE control and management, ranging from mosquito control to the design of effective vaccines and specific antiviral drugs. The majority of JEV outbreaks have taken place in developing countries. Therefore, finding drugs that could reach the underprivileged masses is the obligation of the scientific community, federal governments, and WHO. Anti-JEV drugs developed must be safe for all including children, the elderly, and immuno-compromised people. These drugs must cross the blood-brain barrier (BBB), reach the central nervous system, and remain active even after the initial stage of the infection when the patient begins to show the symptoms of disease and must possess a high genetic barrier to resistance.

Novel antiviral drug development and clinical application must be a significant area of focus in the current scenario. Antivirals with fewer off-target effects include those that inhibit the activity of conserved viral non-structural proteins like protease and polymerase. The availability of JEV NS3 and NS5 crystal structures has made it possible to identify potential inhibitor-binding sites for developing the most effective drugs using structure-guided target-based drug development. [142,168,169,170]. It may be possible to develop antivirals from specific inhibitors targeting host proteins necessary for any stage of the viral life cycle, from attachment and entry to replication and outflow. An active area of research that could produce safe and effective antiviral drugs involves high throughput screening of drug libraries and natural compounds. [141,171]. Also, research focused on Dengue Virus (DENV), Zika Virus (ZIKV) and other similar viruses could be extrapolated to design an effective JEV-specific drug. Studies have shown that several types of phytochemicals, including flavonoids, terpenoids, polysaccharides, alkaloids, thiophenes, lignans, and lectins, have significant antiviral activities. Extensive research is required to fully explore the potential for natural products to be developed as anti-JEV drugs. In addition to having a synergistic antiviral effect, combining different drugs/compounds is an intriguing way to create optimal anti-JE therapies. This strategy allows for a lower dose of each antiviral treatment while maintaining its efficiency and, thus, a lower level of cytotoxicity (unwanted side effects). Different combinations of multiple drugs from various pharmacological categories need to be studied *in vitro* followed by *in vivo* to discover the most effective formulations.

A JEV-specific monoclonal antibody is one example of a prospective treatment. If effective, it might serve as a clinical proof-of-concept for developing treatments for other arboviral encephalitis. This approach has the potential to hasten the development of therapies for more emerging flaviviruses. It is anticipated that novel vaccines with excellent safety profiles and improved immunogenicity will be available in the near future which may restrict JEV infection in new areas. Some examples include the experimental Japanese Encephalitis vaccines that have already been studied in preclinical studies and the next-generation vaccines that could be produced quickly by utilizing adaptable vaccine platforms, similar to the strategy recently used

for the development of COVID-19 vaccine. With highly advanced biomedical science technologies at our disposal, the future holds out hope for establishing specific effective JE treatments and immunization with high coverage rates in the near future and that JE will be an easily controllable disease worldwide.

11. Conflict of Interest

All authors declare to have no conflict of interest

12. Author Contributions

All authors worked on the study conception and design, analysed and interpreted the data. All authors read and approved the final version of the manuscript.

13. References:

1. Burke DS, Leake CJ. Japanese encephalitis. In: Monath TP, editor. The arboviruses: epidemiology and ecology, Vol. 3. Boca Raton: CRC Press; **1988**. pp. 63-92.
2. Simmonds, P.; Becher, P.; Bukh, J.; Gould, E.A.; Meyers, G.; Monath, T.; Muerhoff, S.; Pletnev, A.; Rico-Hesse, R.; Smith, D.B.; et al. ICTV virus taxonomy profile: Flaviviridae. J. Gen. Virol. **2017**, 98, 2–3.
3. Billoir, F.; de Chesse, R.; Tolou, H.; de Micco, P.; Gould, E.A.; de Lamballerie, X. Phylogeny of the genus Flavivirus using complete coding sequences of arthropod-borne viruses and viruses with no known vector. J. Gen. Virol. **2000**, 81, 781–790
4. Tran Minh Quan, Tran ThiNhu Thao, Nguyen ManhDuy, Tran Minh Nhat, Hannah Clapham.Estimates of the global burden of Japanese encephalitis and the impact of vaccination from 2000-2015, *eLife* **2020** 9: e51027.
5. Campbell G, Hills S, Fischer M, et al. Estimated global incidence of Japanese encephalitis. Bull World Health Organ. **2011** 89:766–74.
6. James C Pearce, BSc, Tristan P Learoyd, Dr, Benjamin J Langendorf, Dr, James G Logan, Prof, Japanese encephalitis: the vectors, ecology and potential for expansion, *Journal of Travel Medicine*, May **2018** Volume 25, Issue suppl_1, , Pages S16–S26, https://doi.org/10.1093/jtm/tay009
7. Ricklin ME, García-Nicolás O, Brechbühl D, Python S, Zumkehr B, Nougairede A, Charrel RN, Posthaus H, Oevermann A, Summerfield A. Vector-free transmission and persistence of Japanese encephalitis virus in pigs. Nat Commun. **2016** Feb 23; 7:10832. doi: 10.1038/ncomms10832. PMID: 26902924; PMCID: PMC4766424
8. Chiou, S. S., Chen, J. M., Chen, Y. Y., Chia, M. Y., and Fan, Y. C. The feasibility of field collected pig oronasal secretions as specimens for the virologic surveillance of Japanese encephalitis virus. *PLoS Negl. Trop. Dis.* **2021** 15, 1–12. doi: 10.1371/journal.pntd.0009977.
9. Tiwari S, Singh RK, Tiwari R, Dhole TN. Japanese encephalitis: a review of the Indian perspective. Braz J Infect Dis. **2012** Nov-Dec;16(6):564-73.
10. Kulkarni R, Sapkal GN, Kaushal H, Mourya DT. Japanese Encephalitis: A Brief Review on Indian Perspectives. Open Virol J. **2018** Aug 31; 12:121-130. doi: 10.2174/1874357901812010121. PMID: 30288200; PMCID: PMC6142657.
11. Kanagasabai K, Joshua V, Ravi M, Sabarinathan R, Kirubakaran BK, Ramachandran V, Murhekar MV. Epidemiology of Japanese Encephalitis in India: Analysis of laboratory surveillance data, 2014-2017. J Infect. **2018** Mar;76(3):317-320. doi: 10.1016/j.jinf.2017.09.018. Epub 2017 Sep 29. PMID: 28970044.
12. Jay Prakash P, Kusum V, Vijeta S. Japanese Encephalitis (JE): A curse for people living in Uttar Pradesh, India. J Vaccines Immunol 7 **2021** (1): 036-040. DOI: 10.17352/jvi.000045

13. Tiwari S, Singh RK, Tiwari R, et al. Japanese encephalitis: a review of the Indian perspective. Braz J Infect Dis.**2012**16:564–73.
14. Solomon T. Control of Japanese encephalitis—within our grasp? N Engl J Med. **2006** 355:869–71.
15. Japanese Encephalities: National Center for Vector Borne Diseases Control (NCVBDC) cited **2021** Dec 20
16. Singh LS, Singh HL, Thokchom N, Manojkumar Singh RK. A descriptive study on prevalence pattern of Japanese encephalitis in State of Manipur. Indian J Med Microbiol. **2019** Apr-Jun;37(2):235-240.
17. Australian Government Department of Health and Aged Care. Japanese encephalitis virus (JEV). August 1, **2022** (https://www.health.gov.au/health-alerts/japanese-encephalitis-virus-jev/about#current-status.)
18. van den Hurk, A. F., Pyke, A. T., Mackenzie, J. S., Hall-Mendelin, S., and Ritchie, S. A. Japanese encephalitis virus in Australia: From known known to known unknown. *Trop. Med. Infect. Dis.* **2019** 4. doi: 10.3390/tropicalmed4010038.
19. van den Hurk AF, Skinner E, Ritchie SA, Mackenzie JS. The Emergence of Japanese Encephalitis Virus in Australia in 2022: Existing Knowledge of Mosquito Vectors. Viruses. **2022** Jun 2;14(6):1208. doi: 10.3390/v14061208.
20. Waller, C., Tiemensma, M., Currie, B. J., Williams, D. T., Baird, R. W., and Krause, V. L. Japanese Encephalitis in Australia — A Sentinel Case. *N. Engl. J. Med.* **2022** 387, 661–662. doi: 10.1056/nejmc2207004.
21. Poonsiri T, Wright GSA, Solomon T, et al. Crystal structureof the JapaneseEncephalitis virus capsid protein. Viruses. **2019** 11:623.
22. Luca VC, AbiMansour J, Nelson CA, Fremont DH. Crystal structure of the Japanese encephalitis virus envelope protein. J Virol. **2012** Feb;86(4):2337-46. doi: 10.1128/JVI.06072-11. Epub 2011 Dec 7. PMID: 22156523; PMCID: PMC3302414.
23. David A. Muller, Paul R. Young,Theflavivirus NS1 protein: Molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker Antiviral Research,Volume 98, Issue 2, **2013**,Pages 192-208,ISSN 0166-3542 https://doi.org/10.1016/j.antiviral.2013.03.008.
24. Liu X, Cao S, Zhou R, et al. Inhibition of Japanese encephalitis virus NS1 protein expression in cell by small interfering RNAs. *Virus Genes*. **2006**;33(1):69-75. doi:10.1007/s11262-005-0039-3
25. en LC, Liao JT, Lee HJ, et al. The C Terminus of the Core β-Ladder Domain in Japanese Encephalitis Virus Nonstructural Protein 1 Is Flexible for Accommodation of Heterologous Epitope Fusion. *J Virol*. 2015;90(3):1178-1189. Published **2015** Nov 11. doi:10.1128/JVI.02057-15
26. Eunjung Kim, Jaehwan Nam, Yonggeun Park, Haewol Jo. Analysis of the NS4 Region of Japanese Encephalitis virus K94P05 Isolated from Korea.Journal of bacteriology and virology **1997** ,27(2),197-207.
27. Lu G, Gong P. Crystal Structure of the full-length Japanese encephalitis virus NS5 reveals a conserved methyltransferase-polymerase interface. *PLoSPathog*. **2013**;9(8): e1003549. doi: 10.1371/journal.ppat.1003549
28. Knipe D, Howley P. Fields virology. 6th ed. **2013**.
29. Wang S, Liu Y, Guo J, Wang P, Zhang L, Xiao G, Wang W. Screening of FDA-Approved Drugs for Inhibitors of Japanese Encephalitis Virus Infection. J Virol. **2017** Oct 13;91(21): e01055-17. doi: 10.1128/JVI.01055-17. PMID: 28814523; PMCID: PMC5640845.
30. Vannice, K.S., Hills, S.L., Schwartz, L.M. *et al.* The future of Japanese encephalitis vaccination: expert recommendations for achieving and maintaining optimal JE control. *npj Vaccines* **6**, 82 **2021**. https://doi.org/10.1038/s41541-021-00338-z

31. Ishikawa T, Konishi E. Potential chemotherapeutic targets for Japanese encephalitis: current status of antiviral drug development and future challenges. Expert OpinTher Targets **2015** 19:1379–95.
32. Van den Eynde, C.; Sohier, C.; Matthijs, S.; De Regge, N. Japanese Encephalitis Virus Interaction with Mosquitoes: A Review of Vector Competence, Vector Capacity and Mosquito Immunity. Pathogens **2022**, 11, 317. https:// doi.org/10.3390/pathogens11030317
33. Romoser, W.S.; Turell, M.J.; Lerdthusnee, K.; Neira, M.; Dohm, D.; Ludwig, G.; Wasieloski, L. Pathogenesis of Rift Valley fever virus in mosquitoes: Tracheal conduits & the basal lamina as an extra-cellular barrier. *Infect. Dis. Nat. Mech. Viral Emerg. Persistence* **2005**, *19*, 89–100.
34. Salini Krishnan Unni, Daniel Ruzek, ChintanChattbar, RituMishra, Manish. K. Johri, Sunit K, Singh. JapaneseEncephalitis Virus: from genome to infectome; Institute Pasteur;Microbes and Infection **2011** 13; 312-321; doi:10.1016/j.micinf.2011.01.002
35. Kiran Bala Sharma, SudhanshuVrati, ManjulaKalia. Pathobiology of Japanese Encephalitis Virus Infection; Molecular Aspects of Medicine **2021**; doi: 10.1016/j.mam.2021.100994.
36. Chauhan, S., Rathore, D. K., Sachan, S., Lacroix-Desmazes, S., Gupta, N., Awasthi, A., et al. Japanese Encephalitis Virus Infected Human Monocyte-Derived Dendritic Cells Activate a Transcriptional Network Leading to an Antiviral Inflammatory Response. *Front. Immunol.* **2021** 12, 1–13. doi: 10.3389/fimmu.2021.638694.
37. Kallol Dutta, Pundi. N. Rangarajan, SudhanshuVrati,AnirbanBasu ; Japanese encephalitis : Pathogenesis,prophylactics and therapeutics; Current Science; **2010** Vol. 98, No. 3.
38. Arup Banerjee, AartiTripathi Recent Advancement in understanding Japanese encephalitis; F1000 Research **2019** doi :10.12688/f1000research. 19693.
39. Awanish Kumar, Praveen Sharma, Kamla Kant Shukla, Sanjeev Misra, Kishan Kumar Nyati. Japanese encephalitis virus: Associated immune response and recent progress in vaccinedevelopment; Microbial Pathogenesis **2019** 136; doi: 10.1016/j.micpath.2019.103678.
40. Lin, R.-J., Liao, C.-L., Lin, E., and Lin, Y.-L. Blocking of the Alpha Interferon-Induced Jak-Stat Signaling Pathway by Japanese Encephalitis Virus Infection. *J. Virol.* **2004** 78, 9285–9294. doi: 10.1128/jvi.78.17.9285-9294.2004.
41. . Mukherjee S, Sengupta N, Chaudhuri A, Akbar I, Singh N, Chakraborty S, Suryawanshi AR, Bhattacharyya A, Basu A. PLVAP and GKN3 Are Two Critical Host Cell Receptors Which Facilitate Japanese Encephalitis Virus Entry into Neurons. Sci Rep. **2018** Aug 6;8(1):11784. doi: 10.1038/s41598- 018-30054-z. PMID: 30082709; PMCID: PMC6079088.
42. Debapriya Ghosh, AnirbanBasu. Japanese Encephalitis-A Pathological and Clinical Perspective Diseases **2009** Vol 3, Issue 9; doi: 10.1371/journal.pntd.0000437.
43. Peter M. Winter, Nguyen Minh Dung, Ha Thi Loan, BridgetWills, Le Thi Thu, Deborah House, Nicholas J. White, Jeremy J.Farrar, Anthony Hart, and Tom Solomon. Proinflammatory cytokines and chemokines in Humans with Japanese Encephalitis;The Journal of Infectious Diseases **2004** 190, 1618-26.
44. Sunit Kumar Singh. Molecular pathogenesis of Japanese Encephalitis Virus Infection; Human Emerging and Re-emerging Infections: Viral and parasitic infections **2016**, Volume I, First edition, Pg-117; John Wiley & Sons, Inc
45. Minu Nain, Malik Z. Abdin, Manjula Kalia, and Sudhanshu Vrati,Japanese encephalitis virus invasion of cell: allies andalleys; Reviews in Medical Virology **2015**, 26, 129-141; doi :10.1002/rmv.
46. Sarkar, R., et al. Japanese encephalitis virus capside protein interacts with non-lipidated MAP1LC3 on replication membranes and lipid droplets; J. Gen. Virol **2021** doi:10.1128/JVI.02336-20.

47. Sharma, M., et al., Japanese encephalitis virus replication is negatively regulated by autophagy and occurs on LC3 and EDEM 1 containing membranes. Autophagy **2014** 10 (9), 1637-1651.
48. World Health Organisation https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis
49. Gao, X., Liu, H., Li, M. et al. Insights into the evolutionary history of Japanese encephalitis virus (JEV) based on whole-genome sequences comprising the five genotypes. Virol J **2015** 12, 43
50. Solomon T, Ni H, Beasley DW, Ekkelenkamp M, Cardosa MJ, Barrett AD. Origin and evolution of Japanese encephalitis virus in southeast Asia. J Virol. **2003** Mar;77(5):3091-8. doi: 10.1128/jvi.77.5.3091-3098.2003. PMID: 12584335; PMCID: PMC149749.
51. Badia, R., Garcia-Vidal, E., and Ballana, E. Viral-Host Dependency Factors as Therapeutic Targets to Overcome Antiviral Drug-Resistance: A Focus on Innate Immune Modulation. *Front. Virol.* **2022** 2, 1–17. doi: 10.3389/fviro.2022.935933.
52. Mackenzie JS, Barrett ADT, Deubel V. Japanese Encephalitis and West Nile Viruses. Berlin, Heidelberg: Springer Berlin Heidelberg; **2002**.
53. Kesson AM, Blanden RV, Mullbacher A. The primary in vivo murine cytotoxic T cell response to the flavivirus. West Nile J Gen Virol. **1987** 68:2001–6.
54. Kiermayr S, Kofler RM, Mandl CW, et al. Isolation of capsid protein dimers from the tick-borne encephalitis flavivirusand in vitro assembly of capsid-like particles. J Virol. **2004** 78:8078–84.
55. Wang S-H, Syu W-J, Huang K-J, et al. Intracellular localization and determination of a nuclear localization signal of the core protein of dengue virus. J Gen Virol. **2002** 83:3093–102.
56. Zakaria MK, Carletti T, Marcello A. Cellular targets for the treatment of flavivirus infections. Front Cell Infect Microbiol. **2018** 8:398.
57. Sampath A, Padmanabhan R. Molecular targets for flavivirusdrug discovery. Antiviral Res. **2009** 81:6–15.
58. Perera R, Khaliq M, Kuhn RJ. Closing the door on flaviviruses: entry as a target for antiviral drug design. Antiviral Res.**2008** 80:11–22
59. Lescar J, Luo D, Xu T, et al. Towards the design of antiviral inhibitors against flaviviruses: the case for the multifunctional NS3 protein from Dengue virus as a target. Antiviral Res. **2008** 80:94–101.
60. Luo D, Vasudevan SG, Lescar J. The flavivirus NS2B–NS3 protease–helicase as a target for antiviral drug development. Antiviral Res. **2015** 118:148–58.
61. Wu S-F, Lee C-J, Liao C-L, et al. Antiviral effects of animino sugar derivative on flavivirus infections. J Virol. **2002** 76:3596–604.
62. Gorbalenya AE, Donchenko AP, Koonin EV, et al. N-terminal domains of putative helicases of flavi- and pestiviruses may be serine proteases. Nucleic Acids Res. **1989** 17:3889–97.
63. Dong H, Zhang B, Shi P-Y. Flavivirusmethyltransferase: a novel antiviral target. Antiviral Res. **2008** 80:1–10.
64. Murakami M, Ota T, Nukuzuma S, et al. Inhibitory effect of RNAi on Japanese encephalitis virus replication in vitro and in vivo. MicrobiolImmunol. **2005** 49:1047–56.
65. Swarup V, Ghosh J, Ghosh S, et al. Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murinemodel of Japanese encephalitis. Antimicrob Agents Chemother. **2007** 51:3367–70.
66. Chen C-J, Raung S-L, Kuo M-D, et al. Suppression of Japanese encephalitis virus infection by non-steroidal anti-inflammatory drugs. J Gen Virol. **2002** 83:1897–905.
67. Dutta K, Ghosh D, Basu A. Curcumin protects neuronal cells from japanese encephalitis virus-mediated cell death and also inhibits infective viral particle formation by dysregulation of ubiquitin-proteasome system. J NeuroimmunePharmacol. **2009** 4:328–37.

68. Michaelis, M., Kleinschmidt, M. C., Doerr, H. W., and Cinatl, J. Minocycline inhibits West Nile virus replication and apoptosis in human neuronal cells. *J. Antimicrob. Chemother.* **2007** 60, 981–986. doi: 10.1093/jac/dkm307
69. Mishra MK, Ghosh D, Duseja R, et al. Antioxidant potential of minocycline in Japanese encephalitis virus infection in murine neuroblastoma cells: correlation with membrane fluidity and cell death. Neurochem Int. **2009** 54:464–70.
70. Mishra MK, Dutta K, Saheb SK, et al. Understanding the molecular mechanism of blood–brain barrier damage in anexperimental model of Japanese encephalitis: correlation with minocycline administration as a therapeutic agent. NeurochemInt. **2009** 55:717–23.
71. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol. **2014** 32:513–45.
72. Lin C-W, Wu C-F, Hsiao N-W, et al. Aloe-emodin is an interferon-inducing agent with antiviral activity against Japanese encephalitis virus and enterovirus 71. Int J Antimicrob Agents **2008** 32:355–9
73. Kumar R, Tripathi P, Baranwal M, et al. Randomized, controlledtrial of oral ribavirin for Japanese encephalitis in children in Uttar Pradesh. India Clin Infect Dis. **2009** 48:400–6.
74. Sebastian L, Desai A, Yogeeswari P, et al. Combination of N-methylisatin-β-thiosemicarbazone derivative (SCH16) with ribavirin and mycophenolic acid potentiates the antiviral activity of SCH16 against Japanese encephalitis virus in vitro. Lett ApplMicrobiol. **2012** 55:234–9.
75. Sebastian L, Desai A, Madhusudana SN, et al. Pentoxifyllineinhibits replication of Japanese encephalitis virus: a comparative study with ribavirin. Int J Antimicrob Agents. **2009** 33:168–73.
76. Takhampunya R, Ubol S, Houng H-S, et al. Inhibition of dengue virus replication by mycophenolic acid and ribavirin. J Gen Virol. **2006** 87:1947–52.
77. Yang J, Xu Y, Yan Y, et al. Small molecule inhibitor of ATPase activity of HSP70 as a broad-spectrum inhibitor against flavivirusinfections. ACS Infect Dis. **2020** 6:832–43
78. Anantpadma M, Vrati S. siRNA-mediated suppression of Japanese encephalitis virus replication in cultured cells and mice. J AntimicrobChemother. **2012** 67:444–51.
79. Shen, T., Liu, K., Miao, D., Cao, R., Zhou, B., and Chen, P. Lentivirus-mediated RNA interference against Japanese encephalitis virus infection in vitro and in vivo. *Antiviral Res.* **2014** 108, 56–64. doi: 10.1016/j.antiviral.2014.05.008.
80. Shen T, Liu K, Miao D, et al. Effective inhibition of Japanese encephalitis virus replication byshRNAs targeting various viral genes in vitro and in vivo. Virology. **2014** 454–455:48–59.
81. Yoo J-S, Kim C-M, Kim J-H, et al. Inhibition of Japanese encephalitis virus replication by peptide nucleic acids targetingcis-acting elements on the plus- and minus-strands of viral RNA. Antiviral Res. **2009** 82:122–33.
82. Anantpadma M, Stein DA, Vrati S. Inhibition of Japanese encephalitis virus replication in cultured cells and mice by a peptide-conjugated morpholino oligomer. J AntimicrobChemother. **2010** 65:953–61.
83. Lundin KE, Good L, Stromberg R, et al. Biological Activity and Biotechnological Aspects of Peptide Nucleic Acid. Adv Genet [Internet]. **2006** pp. 1–51.
84. Chen J, Yamada S, Hama Y, et al. Unique heparansulfate from shrimp heads exhibits a strong inhibitory effect on infections by dengue virus and Japanese encephalitis virus. BiochemBiophysRes Commun.**2011** 412:136–42.
85. Kim E, Okumura M, Sawa H, et al. Paradoxical effects of chondroitin Sulfate-E on Japanese encephalitis viral infection. BiochemBiophys Res Commun. **2011** 409:717–22.
86. Lee E, Pavy M, Young N, et al. Antiviral effect of the heparin sulfate mimetic, PI-88, against dengue and encephalitic flaviviruses. Antiviral Res. **2006** 69:31–8.
87. Dutta K, Nazmi A, Basu A. Chemotherapy in Japanese encephalitis: are we there yet? Infect Disord Drug Targets. **2011** 11:300–14.

 doi:10.20944/preprints202212.0581.v1

88. Lin R-J, Chien H-L, Lin S-Y, et al. MCPIP1 ribonuclease exhibits broad-spectrum antiviral effects through viral RNA binding and degradation. Nucleic Acids Res. **2013** 41:3314–26.
89. Falgout B, Markoff L. Evidence that flavivirus NS1-NS2A cleavage is mediated by a membrane-bound host protease in the endoplasmic reticulum. J Virol. **1995** 69:7232–43.
90. Ishag HZA, Li C, Huang L, et al. Inhibition of Japanese encephalitis virus infection in vitro and in vivo by pokeweed antiviral protein. Virus Res. **2013** 171:89–96.
91. Zhang T, Wu Z, Du J, et al. Anti- Japanese-encephalitis-viral effects of kaempferol and daidzin and their RNA-binding characteristics. PLoS ONE. **2012** 7: e30259.
92. Lv B-M, Tong X-Y, Quan Y, et al. Drug repurposing for Japanese encephalitis virus infection by systems biology methods. Molecules **2018** 23:3346.
93. Swarup V, Ghosh J, Mishra MK, et al. Novel strategy for treatment of Japanese encephalitis using arctigenin, a plant lignan. J AntimicrobChemother. **2008** 61:679–88.
94. Cho JY, Kim AR, Yoo ES, et al. Immunomodulatory effect of arctigenin, a lignan compound, on tumour necrosis factor-α and nitric oxide production, and lymphocyte proliferation. J Pharm Pharmacol. **2010** 51:1267–73.
95. Sehgal N, Kumawat KL, Basu A, et al. Fenofibrate reduces mortality and precludes neurological deficits in survivors in murine model of Japanese encephalitis viral infection. PLoSONE. **2012** 7:5427.
96. Swarup V et.al., Antiviral and Anti-Inflammatory Effects of Rosmarinic Acid in an Experimental Murine Model of Japanese Encephalitis. Antimicrob Agents Chemother.**2017** Sep; 51(9): 3367–3370.
97. Saxena SK, Mathur A, Srivastava RC. Inhibition of Japanese encephalitis virus infection bydiethyldithiocarbamate is independent of its antioxidant potential. AntivirChemChemother. **2003** 14:91–8.
98. Kajimura et.al., Protective effect of Astragali Radix by intraperitoneal injection against Japanese encephalitis virus infection in mice. Biol Pharm Bull. **1996** Jun;19(6):855-9.
99. Huang H-N, Rajanbabu V, Pan C-Y, et al. Modulation of the immune-related gene responses to protect mice against Japanese encephalitis virus using the antimicrobial peptide, tilapia hepcidin 1–5. Biomaterials. **2011** 32:6804–14.
100. Harinasuta C, Wasi C, Vithanomsat S. The effect of interferon on Japanese encephalitis virus in vitro. *Southeast Asian J Trop Med Public Health*. **1984**;15(4):564-568.
101. Masood Ahmad Wani et.al. Atorvastatin ameliorates viral burden and neural stem/progenitor cell (NSPC) death in an experimental model of Japanese encephalitis, J Biosci **2020** 45:77
102. XugangWang et.al., Artemisinin inhibits the replication of flaviviruses by promoting the type I interferon production, Antiviral Research **2020** Volume 179, July 2020, 104810
103. Chang C-C, Ou Y-C, Raung S-L, et al. Antiviral effect of dehydroepiandrosterone on Japanese encephalitis virus infection. J Gen Virol. **2005** 86:2513–23.
104. Yang CF, Gopula B, Liang JJ, Li JK, Chen SY, Lee YL, et al. Novel AR-12 derivatives, P12-23 and P12-34, inhibit flavivirus replication by blocking host *de novo* pyrimidine biosynthesis. Emerg Microbes Infect. **2018** 7(1):187.
105. Chen HH, Chen CC, Lin YS, Chang PC, Lu ZY, Lin CF, et al. AR-12 suppresses dengue virus replication by down-regulation of PI3K/AKT and GRP78. Antiviral Res. **2017** 142:158–68.
106. Boldescu V, Behnam MAM, Vasilakis N, et al. Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond. Nat Rev Drug Discov. **2017** 16:565–86.
107. Lu C-Y, Chang Y-C, Hua C-H, et al. Tubacin, an HDAC6 selective inhibitor, reduces the replication of the japanese encephalitis virus via the decrease of viral RNA synthesis. Int J Mol Sci. **2017** 18:954.

 doi:10.20944/preprints202212.0581.v1

108. Guo J, Jia X, Liu Y, Wang S, Cao J, Zhang B, Xiao G, Wang W. Screening of Natural Extracts for Inhibitors against Japanese Encephalitis Virus Infection. Antimicrob Agents Chemother. **2020** Feb 21;64(3): e02373-19.
109. Huang L, Li H, Ye Z, Xu Q, Fu Q, Sun W, Qi W, Yue J. Berbamine inhibits Japanese encephalitis virus (JEV) infection by compromising TPRMLs-mediated endolysosomal trafficking of low-density lipoprotein receptor (LDLR). Emerg Microbes Infect. **2021** Dec;10(1):1257-1271.
110. Warren T.K., Wells J., Panchal R.G., Stuthman K.S., Garza N.L., Van Tongeren S.A., Dong L., Retterer C.J., Eaton B.P., Pegoraro G., Honnold S., Bantia S., Kotian P., Chen X., Taubenheim B.R., Welch L.S., Minning D.M., Babu Y.S., Sheridan W.P., Bavari S. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. *Nature*. **2014** 508:402–405
111. Kumar P, Lee SK, Shankar P, et al. A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. PLoS Med. **2006** 3: e96.
112. Wu Z, Xue Y, Wang B, et al. Broad-spectrum antiviral activity of RNA interference against four genotypes of Japanese Encephalitis Virus based on single MicroRNA polycistrons. PLoS ONE. **2011** 6: e26304.
113. Appaiahgari MB, Vrati S. DNAzyme-mediated inhibition of Japanese encephalitis virus replication in mouse brain. MolTher. **2007** 15:1593–9.
114. Chien Y-J, Chen W-J, Hsu W-L, et al. Bovine lactoferrin inhibits Japanese encephalitis virus by binding to heparansulfate and receptor for low density lipoprotein. Virology. **2008** 379:143–51.
115. Han-Hsiang Chen et.al. Development of antiviral carbon quantum dots that target the Japanese encephalitis virus envelope protein, Jr. Bio. Chem. **2022**, VOLUME 298, ISSUE 6, 101957, JUNE 2022
116. Talarico L, Pujol C, Zibetti R, et al. The antiviral activity of sulfated polysaccharides against dengue virus is dependent on virus serotype and host cell. Antiviral Res. **2005** 66:103–10.
117. Zu X, Liu Y, Wang S, et al. Peptide inhibitor of Japanese encephalitis virus infection targeting envelope protein domain III. Antiviral Res. **2014** 104:7–14
118. Qiu X, Lei Y, Yang P, et al. Structural basis for neutralization of Japanese encephalitis virus by two potent therapeutic antibodies. Nat Microbiol. **2018** 3:287–94
119. Chang S-J, Chang Y-C, Lu K-Z, et al. Antiviral activity of *Isatisindigotica* extract and its derived indirubin against Japanese encephalitis virus. Evid-Based Complement Altern Med ECAM. **2012** 2012: 925830.
120. Johari J, Kianmehr A, Mustafa MR, et al. Antiviral activity of baicalein and quercetin against the Japanese Encephalitis Virus. Int J Mol Sci. **2012** 13:16785–95.
121. Lee CJ, Lin HR, Liao CL, Lin YL. Cholesterol effectively blocks entry of flavivirus. J Virol. (2008) Jul;82(13):6470-80. doi: 10.1128/JVI.00117-08. Epub **2008** Apr 30. PMID: 18448543; PMCID: PMC2447114.
122. Takegami T, Simamura E, Hirai K-I, et al. Inhibitory effect of furanonaphthoquinone derivatives on the replication of Japanese encephalitis virus. Antiviral Res. **1998** 37:37–45.
123. Kim S-J, Park S-N, Oh J-W. Antiviral effect of amphotericin B on japanese encephalitis virus replication. Journal of Microbiology and Biotechnology **2004** Volume 14 Issue 1
124. Fang J, Sun L, Peng G, et al. Identifcation of three antiviral inhibitors against japanese encephalitis virus from library of pharmacologically active compounds 1280. PLoS ONE. **2013** 8: e78425.

 doi:10.20944/preprints202212.0581.v1

125. SabarPambudi, NorihitoKawashita, SupraneePhanthanawiboon, MagotDiataOmokoko, PromsinMasrinoul, Akifumi Yamashita, KriengsakLimkittikul, TeruoYasunaga, Tatsuya Takagi, Kazuyoshi Ikuta, Takeshi Kurosu. A small compound targeting the interaction between nonstructural proteins 2B and 3 inhibits dengue virus replication, Biochemical and Biophysical Research Communications **2013**, Volume 440, Issue 3, Pages 393-398, ISSN 0006-291X
126. Li Z, Brecher M, Deng YQ, Zhang J, Sakamuru S, Liu B, et al. Existing drugs as broad-spectrum and potent inhibitors for Zika virus by targeting NS2B-NS3 interaction. Cell Res. **2017** 27(8):1046–64.
127. Brecher M, Chen H, Li Z, et al. Identifcation and characterization of novel broad-spectrum inhibitors of the flavivirus methyl transferase. ACS Infect Dis. **2015** 1:340–9.
128. Sebastian L, Desai A, Shampur MN, et al. N-methylisatin-beta thiosemicarbazone derivative (SCH 16) is an inhibitor of Japanese encephalitis virus infection *in vitro* and *in vivo*. Virol J. **2008** 5:64.
129. Bhattacharjee A, Chaudhuri R, Dash JJ, Saha M, Choudhury L, Roy S. Pre-treatment with Scopolamine Naturally Suppresses Japanese Encephalitis Viral Load in Embryonated Chick Through Regulation of Multiple Signaling Pathways. ApplBiochemBiotechnol. **2021** Jun;193(6):1654-1674
130. Kumar S, Maurya VK, Kabir R, et al. Antiviral activity of belladonna during Japanese encephalitis virus infection via inhibition of microglia activation and inflammation leading to neuronal cell survival. ACS ChemNeurosci. **2020** 11:3683–96
131. P.N. Nair M. Current Scenario of Antiviral Drugs for Japanese Encephalitis. J Med MicrobiolDiagn, **2014**.
132. Shi Z, Wei J, Deng X, et al. Nitazoxanide inhibits the replication of Japanese encephalitis virus in cultured cells and in a mouse model. Virol J. **2014** 11:10.
133. Rossignol J-F. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. Antiviral Res. **2014** 110:94–103.
134. Fan W, Qian S, Qian P, et al. Antiviral activity of luteolin against Japanese encephalitis virus. Virus Res. **2016** 220:112–6.
135. Li Z, Sakamuru S, Huang R, Brecher M, Koetzner CA, Zhang J, Chen H, Qin CF, Zhang QY, Zhou J, Kramer LD, Xia M, Li H. Erythrosin B is a potent and broad-spectrum orthosteric inhibitor of the flavivirus NS2B-NS3 protease. Antiviral Res. **2018** Feb;150:217-225.
136. Mastrangelo E, Pezzullo M, De Burghgraeve T, et al. Ivermectin is a potent inhibitor of flavivirus replication specifcally targeting NS3 helicase activity: new prospects for an old drug. J AntimicrobChemother. **2012** 67:1884–94.
137. Bhosale S, Kumar A. Screening of phytoconstituents of Andrographispaniculata against various targets of Japanese encephalitis virus: An in-silico and in-vitro target-based approach. Curr Res Pharmacol Drug Discov. **2021** Jul 31; 2:100043.
138. Sebastian L, Madhusudana S, N, Ravi V, Desai A: Mycophenolic Acid Inhibits Replication of Japanese Encephalitis Virus. Chemotherapy **2011** 57:56-61. doi: 10.1159/000321483
139. Gupta SK, Singh S, Nischal A, et al. Molecular docking and simulation studies towards exploring antiviral compounds against envelope protein of Japanese encephalitis virus. Netw Model Anal Health Inform Bioinforma. **2013** 2:231–43.
140. Chen Z, Shao L, Ye J, Li Y, Huang S, Chen H, Cao S. Monoclonal antibodies against NS3 and NS5 proteins of Japanese encephalitis virus. Hybridoma (Larchmt). **2012** Apr;31(2):137-41.

141. Zhe-Rui Zhang, Hong-Qing Zhang, Xiao-Dan Li, Cheng-Lin Deng, Zhen Wang, Jia-Qi Li, Na Li, Qiu-Yan Zhang, Hong-Lei Zhang, Bo Zhang, Han-Qing Ye,Generation and characterization of Japanese encephalitis virus expressing GFP reporter gene for high throughput drug screening, Antiviral Research **2020**, Volume 182,104884,ISSN01663542,https://doi.org/10.1016/j.antiviral.2020.104884.
142. Fang, J., Li, H., Kong, D. *et al.* Structure-based discovery of two antiviral inhibitors targeting the NS3 helicase of Japanese encephalitis virus. *SciRep* **2016** 6, 34550. https://doi.org/10.1038/srep34550
143. Woodman DR, Williams JC. Effects of 2-deoxy-D-glucose and 3 deazauridine individually and in combination on the replication of Japanese B encephalitis virus. Antimicrob Agents Chemother. **1977** Mar;11(3):475-81.
144. Xiao Y, Yang J, Zou L, Wu P, Li W, Yan Y, Li Y, Li S, Song H, Zhong W, Qin Y. Synthesis of 10,10'-bis(trifluoromethyl) marinopyrrole A derivatives and evaluation of their antiviral activities in vitro. Eur J Med Chem. **2022** Aug 5; 238:114436.
145. Ho YJ, Lu JW, Huang YL, Lai ZZ. Palmatine inhibits Zika virus infection by disrupting virus binding, entry, and stability. BiochemBiophys Res Commun. **2019** Oct 22;518(4):732-738
146. Hills SL, Walter EB, Atmar RL, Fischer M. Japanese Encephalitis Vaccine: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep **2019** ;68(No. RR-2):1–33. DOI: http://dx.doi.org/10.15585/mmwr.rr6802a1external icon
147. Batchelor, P., Petersen, K. Japanese encephalitis: a review of clinical guidelines and vaccine availability in Asia. *Trop Dis Travel Med Vaccines* 1, 11 **2015**. https://doi.org/10.1186/s40794-015-0013-6
148. Japanese encephalitis: status of surveillance and immunization in Asia and Western Pacific, 2012. WHO Weekly Epidemiological Record. **2013** 34 (88):357–364
149. Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. Hum Vaccin Immunother. **2017** Jun 3;13(6):1-18. doi: 10.1080/21645515.2017.1285472.
150. Fischer M, Lindsey N, Staples E, Hills S, CDC. Japanese Encephalitis Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. **2010** 59:1–27.
151. Ginsburg, A. S., Meghani, A., Halstead, S. B., & Yaich, M. Use of the live attenuated Japanese Encephalitis vaccine SA 14-14-2 in children: A review of safety and tolerability studies. Human vaccines & immunotherapeutics, **2017** 13(10), 2222–2231.
152. Oya A. Japanese encephalitis vaccine. ActaPediatr JPN **1988** 30:175-84S
153. Centers for Disease Control and Prevention. 2011. Update on Japanese encephalitis vaccine for children in United States, May, 2011. MMWR Morb Mortal Wkly Rep **2011** 60:664e5.
154. Centers for Disease Control and Prevention. Use of Japanese encephalitis vaccine in children: recommendations of the advisory committee on immunization practices, 2013. MMWR Morb Mortal Wkly Rep **2013** 62:898e900.
155. Kumar S, Verma A, Yadav, Dubey, Sumit Kumar Azhar, Esam Ibraheem Maitra, S. S. Dwivedi, Vivek Dhar. Molecular pathogenesis of Japanese encephalitis and possible therapeutic strategies ,Archives of Virology **2022** 1739 1762 167 9 https://doi.org/10.1007/s00705-022-05481-z

156. Hui-Lan Chen, Jia-Kan Chang, Ren-Bin Tang, Current recommendations for the Japanese encephalitis vaccine, Journal of the Chinese Medical Association, Volume 78, Issue5,2015,Pages 271-275, ISSN 1726-4901, https://doi.org/10.1016/j.jcma.**2014**.12.009.
157. Jelinek T. IXIARO® updated: overview of clinical trials and developments with the inactivated vaccine against Japanese encephalitis [published online ahead of print, 2013 May 29]. *Expert Rev Vaccines*. **2013**; doi:10.1586/erv.13.53
158. Taucher C, Barnett ED, Cramer JP, et al. Neutralizing antibody persistence in pediatric travelers from non-JE-endemic countries following vaccination with IXIARO® Japanese encephalitis vaccine: An uncontrolled, open-label phase 3 follow-up study. *Travel Med Infect Dis*. **2020**; 34:101616. doi: 10.1016/j.tmaid.2020.101616
159. Konishi E, Yamaoka M, Khin Sane W, Kurane I, Mason PW. Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding Japanese encephalitis virus pre-membrane and envelope genes. J Virol. **1998** 72(6):4925–30.
160. Appaiahgari, M. B., & Vrati, S. IMOJEV®: A Yellow fever virus-based novel Japanese encephalitis vaccine. Expert Review of Vaccines, **2010**. 9(12), 1371–1384. doi:10.1586/erv.10.139
161. Batchelor P and Petersen K, Tropical Diseases, Travel Medicine and Vaccines **2015** 1:11 DOI 10.1186/s40794-015-0013-6
162. Capeding MR, Alberto ER, Bouckenooghe A, Laot TM, Chansinghakul D, Monfredo C, Machabert T, Feroldi E. Five-Year Antibody Persistence Following a Japanese Encephalitis Chimeric Virus Vaccine (JE-CV) Booster in JE-CV-Primed Children in the Philippines. J Infect Dis. **2018** Jan 30;217(4):567-571. doi: 10.1093/infdis/jix601.
163. Yongxin Yu, Phenotypic and genotypic characteristics of Japanese encephalitis attenuated live vaccine virus SA14-14-2 and their stabilities,Vaccine,Volume 28, Issue 21, **2010** ,Pages 3635-3641,ISSN 0264-410X,https://doi.org/10.1016/j.vaccine.2010.02.105.
164. Kwon HJ, Lee SY, Kim KH, Kim DS, Cha SH, Jo DS, Kang JH. The Immunogenicity and Safety of the Live-attenuated SA 14-14-2 Japanese Encephalitis Vaccine Given with a Two-dose Primary Schedule in Children. J Korean Med Sci. **2015** May;30(5):612-6. doi: 10.3346/jkms.2015.30.5.612.
165. Singh, Anit & Mitra, Monjori & Sampath, Gade & Venugopal, P & Rao, J & Krishnamurthy, B & Gupta, Mukesh & Krishna, S & Sudhakar, B & Rao, N & Kaushik, Yashpal & Gopinathan, K & Hegde, Nagendra & Gore, Milind & Mohan, Krishna & Ella, Krishna. A Japanese Encephalitis Vaccine from India Induces Durable and Cross-protective Immunity Against Temporally and Spatially Wide-ranging Global Field Strains. The Journal of infectious diseases. **2015** 212. 10.1093/infdis/jiv023.
166. Krishna Mohan Vadrevu, Venugopal Potula, Vasant Khalatkar, Niranjana S Mahantshetty, Atish Shah, Raches Ella, Persistence of Immune Responses with an Inactivated Japanese Encephalitis Single-Dose Vaccine, JENVAC and Interchangeability with a Live-Attenuated Vaccine, *The Journal of Infectious Diseases*, Volume 222, Issue 9, 1 November **2020**, Pages 1478–1487
167. Vijay Shankar Balakrishnan, A new universal JE vaccine, Nature India, **2015**, march, doi**:** https://doi.org/10.1038/nindia.2015.37
168. Chuantao Ye, PeiyuBian, Jing Zhang, Han Xiao, Li Zhang, Wei Ye, Yangchao Dong, Yun Zhou, ZhanshengJia, YingfengLei, Structure-based discovery of antiviral inhibitors targeting the E dimer interface of Japanese encephalitis virus, Biochemical and Biophysical Research Communications **2019**, Volume 515, Issue 2, Page366-371, ISSN0006 291X, https://doi.org/10.1016/j.bbrc.2019.05.148.

 doi:10.20944/preprints202212.0581.v1

169. Melissanne de Wispelaere, WenlongLian, SupaneePotisopon, Pi-Chun Li, Jaebong Jang, Scott B. Ficarro, Margaret J. Clark, Xuling Zhu, Jenifer B. Kaplan, Jared D. Pitts, Thomas E. Wales, Jinhua Wang, John R. Engen, Jarrod A. Marto, Nathanael S. Gray, Priscilla L. Yang. Inhibition of Flaviviruses by Targeting a Conserved Pocket on the Viral Envelope Protein,Cell Chemical Biology **2018**, Volume 25,Issue8,Pages1006-1016.e8,ISSN2451-9456, https://doi.org/10.1016/j.chembiol.2018.05.011.

170. Shree V, Navya & Kumar, Anoop & Kant, Kamal. Natural Chemical Entities from Arisaema Genus Might Be a Promising Break-Through Against Japanese Encephalitis Virus Infection: A Molecular Docking and Dynamics Approach. Journal of biomolecular Structure & Dynamics **2020**. 1. 1-13. 10.1080/07391102.2020.1731603.

171. Wang, G., Li, H., Yang, X., Guo, T., Wang, L., Zhao, Z., et al. Guillain–Barré Syndrome Associated with JEV Infection. *N. Engl. J. Med.* **2020** 383, 1188–1190. doi: 10.1056/NEJMc1916977.

172. Solomon T, Dung NM, Wills B, et al. Interferon alfa-2a in Japanese encephalitis randomised double-blind placebo-controlled trial. The Lancet. **2003** 361:821–6. s