Epidemiology, Disease Transmission and Pathogenesis Caused by JE Virus: Its Prevention and Control

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Abstract In the present review article epidemiology of Japanese encephalitis, disease incidences and its control measures have been described in detail. Among important reasons of JE outbreak are registered demographic clustering, ecological imbalance, and insecticide resistance in target mosquito species, poor economy, lack of socio-cultural environment and timely therapeutics. Other complex reasons are negative anthropogenic activities like mixed agro-pig farming practices, paddy farming and sanitation. Water contamination led to the spillovers of the virus from its wildlife reservoir into pig population as well as other vertebrate hosts. This article also explains emergence of new mutant variants/ new genotypes /ecological strains of JE virus and its spread in endemic to non-endemic areas. Due to re-circulation of virus among various hosts and insect vectors, disease is causing very high mortality and morbidity in rural and sub-urban endemic areas. In addition, presence of revertants in overlapping generations of virus joins and re-organizes distanced epidemiology in the area. It has led to induction of high sero-conversion rate in patients. Present review aims to explain all the reasons of JE epidemics, and justify need of proper surveillance, rapid diagnosis, long term safer immunization, vector control and socio-clinical management of JE infection. This article emphasized an urgent need of potential immunization, implementation of planning to improve economic, environmental and socio-cultural conditions. It also strongly indicates need of regular surveillance and immunization of JE affected population to maintain high therapeutic standards to control JE epidemics.

Keywords: JE virus, endemic diseases, epidemiology, disease transmission vector, vertebrate hosts, immunization, eco-17 climate


1. Introduction

Japanese encephalitis is a zoonotic disease caused by RNA virus belonging to family flaviviridae [1]. It is the leading cause of viral encephalitis and is a major public health problem in the Southeast Asian countries. JE is causing high morbidity and mortality in pediatric groups almost every year [2]. Approximately 2 billion people are affected annually in tropical and subtropical countries where Japanese encephalitis (JE) presents a significant risk to humans and animals, particularly in China and India. Most of JE cases are reported annually from the People’s Republic of China (PRC), Korea, Japan, Indonesia, Cambodia, Thailand, Vietnam and Malaysia, and countries belonging to Indian subcontinent, and parts of Oceania with at least 700 million potentially susceptible children. JE virus causes high mortality in various infant groups and imposes lifelong morbidity that convert in post disease fatalities at later stage. Current mortality rate due to JE epidemics is approximately10, 000-18,000 deaths per year. In India, most of the JE incidences are reported from northeastern and southeastern part of the country. The main reasons of JE prevalence and spread are raising vector and vertebrate host population, annual precipitation cycles, and avian migration. Due to spread of natural vector and migration of reservoir hosts and wide increase in human travelling and transportation across the globe has spread the virus to colder climatic regions. Both migration of host and vector population shows scattered occurrence of JE found in different states and regions. In present time virus exists in more than 28 countries in Asia and out of Asian subcontinent. Human travelling, vector mobility and bird migration spread the JE virus and up to long distances in non endemic areas. These are the main reasons that JE virus has extended its geographic ranges to the south pacific region including Australia [3].

Due to demographic, eco-climatic and lack of proper therapeutics, JE epidemics become a regular feature [2]. Its outbreak happens almost every year among children in Southeast Asian countries including India [4]. Most of the JE cases occur in rainy season due to global increase in temperature and rainfall that form enlarged surface water covers that supports mass breeding of vector mosquitoes. There was observed a sharp increase in vector and host population due to development of sub-urban clusters in low land areas in rainy season. It makes an increase in seasonal transmission of JE virus due to fast breeding of mosquito vectors especially in undeveloped rural areas.
Moreover, eco-climate of endemic areas is favoring vector and virus multiplication, infectivity and pathogenicity. Further, perennial water availability in reservoirs and in nearby rice fields are supporting mass breeding of mosquito vectors infected with JE virus. In addition, presence of flood water in rivers, puddles, ponds and vegetation field in endemic area is widely favoring survival of vector population that associates with virus invasion of human population. In dry season irrigation water and small drainages, canals and pond water assist the mosquito vector to maintain infectivity throughout the year. This is the main reason that more JE incidences are occurring in humid climatic areas where human population residing in close vicinity of enlarged water bodies i.e. ponds, rivers, dams, wetlands, irrigation canals and, drainages. These water reservoirs provide an enlarged open surface area for habitation to vector, avian and even mammalian hosts. In true sense, climate, rice fields, land-cover variables and hydrological cycle are assisting the virus to re-circulate in local host population (Figure 1). JE disease outbreak is widely concerned to the precipitation, water availability during summers and hovering population of vector and dwelling hosts (other than man) in the rural, suburban and urban areas.

Furthermore, occurrence of JE is more closely related to temperature than to humidity. However, in the northern region, large epidemics occur during summers whereas in the southern region JE tends to be endemic, but most cases occur throughout the year with a peak rise in the rainy season. Normally, low but continuous incidences of JE occur during summer in South India and in winter in Northern India mainly in Terai region of Nepal and India. Because human society exists in these areas, relies on agro-forestry and agriculture farming that is the only way to earn wages and livelihood, and no supportive industrial development has taken place. It also inter-correlates poor economy, low health regimen and high risk of communicable diseases. Most of the families residing in these areas are of farmers, day wagers, forest dwellers and low income groups having no land, no house and no livelihood. However, due to lack of housing and sizable increase in migrants have made haphazard large clusters of populations in endemic area are massively affected by JE attack repeatedly every year and extremely large number of deaths are reported in these areas. JE virus genotype circulating in this area also causes infection in cold winter and hot summer and its incidences occur throughout the year with a variable mortality. Due to sporadic and clustered population structures, actual JE burden and magnitude of infection cannot be easily estimated.

JE predominantly occur in unvaccinated populations and causes high morbidity in children living in endemic area [5]. To control JE, the World Health Organization has recommended large scale immunization programs in all areas where JE is a public health problem. But from surveys it became clear that only few economically strong Asian countries have reduced the JE load, but poor nations are lagging behind and still struggling for its control. The main reason in such countries is lack of environmental awareness, piling of human and livestock wastes, poor agriculture and farm yard management, low vector control and poor clinical care of patients and vaccine programs. No doubt JE control needs sound awareness among people about eco-climatic, social and clinical care that can assist to cut down disease burden at the national and regional level [6]. Therefore, vaccination can substantially make mortality rate low but risks and effects of regional climate cannot be fully ignored [7]. It is hard truth that JE incidences are not increasing due to poor control measures but also due to sharp increase in vector and host population and changing eco-climatic conditions. Therefore, an overall study of risk factors, control measures, communication and clinical management can minimize JE virus incidences.
The Government of India has initiated an adult JE vaccination program for the first time [9].

Due to climatic reasons new mutations are going on in JE virus genome, which lead to emergence of new environmentally adapted mutant/strains. These are causing very high mortality in rural areas. Further, virus is expanding its boundaries from endemic to non-endemic areas due to availability of large vector population. However, through vector transmission as well as hydrobiological cycles going on in the area, JE virus is largely circulating in the endemic population. Further, genetic adaptabilities developed by the virus against natural immunity of hosts and its conditioning inside vectors are assisting the virus in maintaining high infection rate in early age infants. Its new genotypes are detected in the blood samples of local population and found re-circulate before arrival of rains and filling of water reservoirs with the rain water. JE virus is a climate induced mutable virus which is making essential substitutions in epitopic regions of structural proteins by acquiring changes in the genome that associates with strong antigenicity, immune response in the host and infection rate (Figure 2). Further, it is proved by genetic and molecular analysis that vaccine strains are transforming into infectious strains. It is the main reason that JE became a fast changing viral disease [10]. Further, due to longer eco-climatic association of virus to the amplifying, revertants and human hosts, eradication of JE seems to a very difficult task. In addition, presence of revertants in overlapping generations joins and re-organizes enhancing epidemiology in the area. However, JE virus is extending its course from endemic to epidemic and proliferating and organizing itself in new geographic areas where disease has been deported and recently detected. In the present time JE become a global health issue. Even after control programs launched by CDC and W.H.O. JE infection is controllable but it seems to be very difficult to cut down the JE infection rate to zero. In the present review article actual reasons of virus spread, epidemiological reasons, disease diagnosis and preventive role of different therapeutics in JE control have been elucidated [11].

Figure 2. Inter-relationship of vector susceptibility, disease transmission and mortality and morbidity in endemic area. Climate induced substitutions in epitopic regions of structural proteins in the genome favoring JE virus to maintain disease transmission, mortality and morbidity rate high by repetitive generations of virus inside host and vector population

2. Reasons of Disease Occurrence

Important factors in endemic area are rice field land cover, vector population density, and open surface area of rain water flooded in the nearby area, presence of alternate hosts and lack of personal care [12] and temporal variation in the susceptibility of C. tritaeniorhynchus [13]. Many of these factors are associated with disease emergence, but re-emergence and increase in infectivity is due to change in land use pattern and deforestation, rising population growth and migration, urbanization and increasing global trade [14]. Other reasons of JE outbreak and spread are intensified rice farming; pig rearing and the lack of potential vaccination programs and proper surveillance in these areas have increased the JE outbreak risks. Further, due to vast water logged wetland habitats and their wide use for agriculture purposes in south East Asia and unplanned housing resulted in a sharp increase in both JE virus vector and host population. Excluding vaccination, ecological, geographical and demographic conditions are also responsible for large number of deaths occurring in eastern Uttar Pradesh and Bihar states of India. Further, demographic reasons such as unplanned sub-urban settlements with large number of refugees in clusters, and poverty striven slums become store houses of communicable viruses. Other reasons which have been identified are illegal human trafficking, migration, poor economy, sanitation, medical facilities, housing, health standards, lack of clean drinking water supply, poor vector control and planning and lack of regular immunization are important key factors responsible for outbreak of JE disease in this area. Normally, most JE incidences are observed just after floods, because of very high transmission rate of JE virus and climate favors epidemics. Similarly, mean monthly temperature in April, May and
June rose above 40°C that result in high precipitation and heavy rain falls. In winter irrigated crop land provides sites for residual temperature tolerant mosquito vectors both in hilly and Gangetic Plains of India. Therefore, it become highly essential to understand environmental drivers of JE virus transmission since the enzootic cycle of JE virus is not likely to be totally interrupted. [15]. Further, majority of human Japanese encephalitis JE cases are reported from regions where vector species *Cr. Tritaeniorhynchus* estimated much higher [16].

Climate is a major factor in determining the geographic and temporal distribution of arthropods. More often, characteristic of arthropod life cycles [17] largely determine epidemiology of JE virus in endemic area. Climate plays important role in establishment and multiplication of disease pathogens and survival of transmission vectors in water fed areas. Moreover, JE incidence varies over time, partly due to inter-annual climate variability that effects mosquito vector abundance because it assist as a potential environmental driver and play important role in JE infection. Both temperature and precipitation show increase in JE incidences in rainy months starting from July to September [15]. Both are important factors which broadly change epidemiology of flaviviruses [18]. In addition, incidences of JE virus showed seasonal and regional characteristics, and low immunity demography pattern is largely invaded by the virus more selectively to cause infection [19]. Thus severity of virus infection imposes functional, social and economic impact on aggrieved families [20]. In addition, complex anthropogenic activities like deforestation, drought, forest fires, smog and severe haze, livestock and global climatic changes brought on by El Nino are responsible for increase in vector population. Further, problem of sanitation, contaminated underground water, garbage, wastes and mixed agro-pig farming practices led to the spillovers of the virus from its wildlife reservoir into pig population [8]. No doubt, industrial, agricultural demographic, socio-ecological factors and global climate changes might have altered the bird migration, due to deforestation and development of irrigation projects in natural sites. Further, increasing human settlements, rising population disease transmission vectors, livestock and wild animals in near vicinity of water bodies has invariably led to geographical dispersal of the virus with enhanced epidemic threats [21]. Hence, it is an observable fact that emergence and reemergence of virus disease is associated with complex ecological and molecular factors such as viral recombination and mutation. These are leading causes of formation of more virulent and adaptive strains. In addition, urbanization and human activities creating more permissive environment for vector host interaction and increased air travel and commerce.

From surveillance and disease diagnosis the most dreadful JE serotypes are detected in slums and refugee camps where multiple genotypes of viruses exist due to presence of transmission vectors (mosquitoes) in large numbers. Further, international travels are spreading virus in new non-endemic regions where new acute neurologic infection and complications are on rise. Hence, Travelers to visiting JE endemic areas should be advised to get proper vaccination, to reduce the risk of JE and to maintain personal protective measures to prevent mosquito bites. JE vaccine should give for travelers who might be at greater risk based on the season, location, and duration of their visit and their planned activities [22]. Few transplant-associated cases of viral CNS infection are also noted in few countries [23]. Hence, a large scale surveillance and immunization is warranted to assess the clinical disease burden of flaviviruses in Southeast Asia and rest of the world [24]. Further, to slow down the infection [25] and mortality rates [2] large scale vaccination programs are instantly needed in JE prone areas. Second, it’s effective control can be achieved by controlling susceptible mosquito vector and vertebrate host population in urban and rural areas [26].

### 3. JE Virus Host

*Culex tritaeniorhynchus* is the primary vector of the JE virus which is widely distributed in China, Republic of Korea, India and Nepal. Other flavivirus vectors are *Culex vishnui* group and *Aedes albopictus* [24]. In South India *Culex tritaeniorhynchus* Giles is principal vector of JE virus. Its population growth is supported by rainfall, temperature, precipitation, elevation, land cover and the normalized difference vegetation index (NDVI). Thus, much extended geographical distribution of virus transmission vector species *Culex tritaeniorhynchus* Giles and *Culex vishnui* group (*C. tritaeniorhynchus*) and their mass breeding throughout year are responsible for disease incidence in endemic and epidemic areas. [27]. These mosquito species infect infants and children following a bite either during day time or night time. Thus JE flaviviruses are transmitted either by mosquito bites or due to infected underground drinking water but it never transmitted by humans [28]. Mosquitoes imbibe blood from two-three distinct hosts but virus is largely harmful to man. *Culex tritaeniorhynchus* also feed mainly (56.6%) on cattle and goats. Vectors use mixed blood meals mostly (96.7%) from cattle and goats and show epidemiologic implications of multiple feeding in the transmission of JE virus [29]. Other mosquito vector species noted from the disease areas (s) are *Culex tritaeniorhynchus* (39%), *Culex sitiens* group (11%), *Culex* (Culex) species (35%), and <1% each of *Aedes. albopictus*, *Aedes oaxkleyi*, *Aedes saipanensis*, *Culex annulirostris marianae*, and *Culex fuscatus*. But from the national and international data *Culex tritaeniorhynchus* was found to a major vector which transmit JE virus and responsible for disease outbreak [30]. But high cattle density, its number decides mosquito population of *Culex. gelidus*, not of the *Culex vishnui* subgroup [31] and *Culex tritaeniorhynchus* and *Culex bitaeniorhynchus*, [32]. Wild females of *Culex tritaeniorhynchus* for JE virus were found highly infectious in rural areas in wet, cool or hot climate. More importantly, temporal and spatial changes in the competency of the vector appeared to influence the JEV infection rate in vector [33]. Furthermore, because of their ecology and strong likelihood that global warming may significantly increase the potential for disease emergence and/ or spread [14]. However, in endemic areas, strong seasonality in JE occurrence is observed which is maintained through the year due to abundance of virus, vector and host population mainly in Tarai region of Nepal and Northern India [34]. JE virus is maintained and transmitted among diverse host population by vector
mosquitoes, but ecological factors are responsible for circulation of virus in ecosystem and high morbidity in various infant groups in endemic area [31]. Pigs are natural reservoir hosts while wading birds, egret, herons, swine are amplifying hosts and are real carriers of virus [35]. More specifically, in endemic areas a high seroconversion rate is noted among pigs [5]. Thus host density, its distribution and vector abundance influences Japanese encephalitis (JE) [31]. Though, JE is regarded as a disease of children in the endemic areas but in the newly invaded areas, it affects both the adults and children because of the absence of protective antibodies. Thus, risk of JE increases as the number of vector mosquitoes increases [36] while lethality due to developing mutations in JE virus and expansion of JE virus in endemic area depends on annual water cycle, irrigated rice field and pig farming [36].

4. Climate and Virus Circulation: Of Acute Encephalitis Syndrome in India

Occurrence of Japanese encephalitis incidence depends on mode of transmission and vector density in relation to urban pig holdings [37] but regional eco-climate conditions also play major role in infectivity of host. In addition, wet humid climate, annual precipitation cycle and expanding human population, resurging vector population, changed landscape [38] and formation of climate induced virus genotypes, host and vector density are key factors for emergence and re-emergence of arboviral diseases in Southeast Asia [17]. These are considered as main drivers of the Japanese encephalitis virus ecology and genetic stability, seroconversion and virus invasion of host [39]. This complex association of weather and anthropogenic factors is responsible for transmission of Japanese encephalitis (Table 1) [13] yearly outbreak of disease [40] in endemic areas including India, Nepal and China [41]. Due to increasing human movements, virus has been transmitted to new sites where it has been slowly establishing itself. This is the main reason that previously JE disease was thought as neglected tropical diseases of Asia, Oceania [42], but recently it has been reached in colder regions of the world due to human travels [43]. It has been carried to North America by migratory birds which are potential amplifying hosts of JE virus [44]. Disease is appeared as pig-associated viral zoonoses in Laos [45], China [46], Africa, and the Americas [47]. JE is also prevalent in coastal and island countries of South Pacific [48], Australia. [49], Japan [50], Korea, Vietnam, Thailand, Taiwan, Nepal, India and China [51]. Its different mutant types are molecularly characterized in resident pigs and susceptible mosquitoes which rely for blood feeding on huge pig farms and human population[52]. It is true that regional disparities attribute disease burden such as unsafe water and poor sanitation [51]. But presence of large number of wild migratory and resident water birds and piggeries in middle of the poor human habitation settlements in Eastern and Northern India creates more infectious environment in rainy season that supports massive transmission of JE virus [53]. Further, both ecological and molecular shifts and high precipitation play important role in transmission of Japanese encephalitis virus (JE) and show a complex effect on antibody-positive rate in vertebrate hosts like pigs [54]. However, precipitation data collected by satellites has also assisted to analyze increasing arbovirus activity in the tropics [55] (Table 1).

Table 1. Major eco-climatic factors responsible for Japanese encephalitis outbreak and its expansion to non-endemic areas

<table>
<thead>
<tr>
<th>Natural</th>
<th>Year 1980-2010</th>
<th>Transmission rate</th>
<th>Disease incidence</th>
<th>Mortality rate</th>
<th>Morbidity rate</th>
<th>Net survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation</td>
<td>3.5-23.7</td>
<td>2.2-22.75</td>
<td>1.94-6.14</td>
<td>79.2-95.7</td>
<td>0.65-2.51</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>17.3-43.5</td>
<td>4.05-26.15</td>
<td>2.74-7.64</td>
<td>79.2-95.8</td>
<td>0.61-2.12</td>
<td></td>
</tr>
<tr>
<td>Humidity</td>
<td>18.4-39.3</td>
<td>3.25-21.66</td>
<td>1.94-6.86</td>
<td>78.2-95.5</td>
<td>0.65-2.51</td>
<td></td>
</tr>
<tr>
<td>Landscape</td>
<td>1.23%</td>
<td>23.14</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Floods</td>
<td>&lt;43.2%</td>
<td>39.07</td>
<td>1.94-6.14</td>
<td>80.1-95.7</td>
<td>0.65-2.54</td>
<td></td>
</tr>
<tr>
<td>Wetlands</td>
<td>2.34-17.6%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ground water</td>
<td>2.14 times (50.718)</td>
<td>49.21</td>
<td>9.21</td>
<td>81.2-97.3</td>
<td>0.125-1.04</td>
<td></td>
</tr>
<tr>
<td>Carbon emissions</td>
<td>6% by 2000</td>
<td>VIN</td>
<td>VIN</td>
<td>VIN</td>
<td>VIN</td>
<td></td>
</tr>
<tr>
<td>Agricultural</td>
<td>7.8-43.2%</td>
<td>5.3-37.2%</td>
<td>1.94-8.23</td>
<td>82.2-97.7</td>
<td>0.125-1.00</td>
<td></td>
</tr>
<tr>
<td>Forestry</td>
<td>&lt;17.65%</td>
<td>&lt;18.65%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Vector population</td>
<td>26.7-121.3/m2</td>
<td>&lt;69.11%</td>
<td>1.94-8.49</td>
<td>88.2-98.03</td>
<td>0.125-1.00</td>
<td></td>
</tr>
</tbody>
</table>

| Anthropogenic | | | | | |
| Population size | <1.34% | NA | NA | Na | Na |
| Human traveling | 16.9% | 14.23% | 1.96-6.23 | 81.2-97.3 | 0.125-1.00 |
| Human settlement | 23.42% | 22.81% | 1.96-7.10 | 80.1-95.7 | 0.125-1.00 |
| Slums | 22.76% | 22.14% | 9.21 | 81.2-96.5 | 0.125-1.00 |
| Refugee camps | 26.32% | 25.11% | 9.21 | 88.2-98.03 | 0.125-1.00 |
| Tribals | 19.23% | 18.15% | 2.74-7.64 | 81.2-97.3 | 0.125-1.00 |
| Pig Farming | 3.65 times (86.505) | <70.9% | 2.93-8.29 | 88.2-96.4 | 0.125-1.00 |
| Paddy Farming | 8.8-47.3% | 6.7-43.2% | 2.04-8.23 | 88.2-96.4 | 0.125-1.00 |
| Foulery farms | 4.7-31.66% | N | N | p=78.3 | 0.125-1.00 |
| Livestock | 13.65 | 12.71 | 1.94-8.66 | p=76.4 | 0.125-1.00 |
| Human wastes | 77.65 | <76.2% | 2.74-7.64 | P=71.2 | 0.125-1.00 |
| Pest resurgence | SI | SI | SI | CR 88.2-98.03 | 0.125-0.623 |
| Education | 37.0-86.55% | N | N | N | UPD |
| Therapeutic | NIl-4.3% | 1.23% | 1.06-8.66 | 99.7% | 0.125-0.241*** |

Data is based on reports submitted during 1980-2010. ** Man made wet lands-farm ponds, stock ponds and tanks, irrigation channels, canals, ditches and rice fields. NA=data not available, N=negligible and indirectly associated, C=cumulative, p=permanent and stable, UPD=un-noticed public domain. SI=significant increase, CR=cumulative rate. ***Almost very low survival rate, VIN=visible increase not estimated, VID=visible decrease not estimated. Total survival is indicating natural immune protection. # There is a difference in cases reported by hospitals and natural disease occurrence. Hence, natural mortality was estimated by using hospital data+ unreported cases where patients could not reach to the hospital and died without medical care.
5. Disease Status and Genotypic Variations v/s Infectivity

The viral encephalitis epidemic was occurred during 2000 and gripped seven districts of Assam and a similar epidemic was also happened in Gorakhpur region of Uttar Pradesh in 2005 and gripped eight districts. Recent reports show presence of various JE virus genotypes which are circulating in much extended geographic ranges from Southeast Asia to Australia. Mutations and eco-climatic factor/s are responsible for emergence of JE Genotypes [56,57]. These also have raised significant conversion of low virulent virus to high virulent virus and significant increase in lethality. Moreover, climate induced minor significant differences are noted in JE virus genome mostly due to incorporation of point mutations. This seems to be major reason of epidemiological changes in JE virus in South East Asia [58]. Furthermore, parental strains are diverging very fast and latest highly dreadful strains/variants of JE virus are coming up with high lethality. It is also proved from phylogenetic studies that at least three genotypes of JE virus are circulating in Malaysia, India [59], and China [60], two in both Japan [58] and Australia [61,62]. By enlarge five genotypes G1-GV, of Japanese encephalitis virus has been identified from distinct geographical distribution with varied epidemiology [56,63]. It proves that virus shows extensive genetic diversity among JE virus isolates in different climatic conditions. Recently JE threat is also noted in adults of Japan in spite of the fact that disease is of pediatric groups. Further from genealogical studies pertain to changes in parental sequences and its further modifications led to evolution of new JE virus genome. Therefore, it is more speculative that JE virus was originated in the Indo-Malaysian region from where it has spread in all surrounding countries by migratory birds and international human travelling and new virus has been arrived in different parts of the world. Due to acquiring new genetic and eco-climatic adaptations virus has achieved high survival rate in different climates and establishing itself in non-endemic areas. However, genetic divergence and acquiring new ecological adaptations virus shows its likely emergence in sub-tropical climate and spread to temperate climate regions. Few accessory factors are which are natural assisting the virus is vast floral and faunal diversity, precipitation and annual water availability in this region.

Complete nucleotide and amino acid sequences of different Japanese encephalitis virus strain showed intra-group divergence in ~10-20 of nucleotide sequences that all isolates fell into 5 different genotypes which are circulating in South East Asia, Australia, Europe and USA. These climate-induced differences in genotypes among JE isolates have been identified in temperate countries that from genotypes of sub-tropical countries such as India, Nepal, Bangladesh, Bhutan, and Tibet. However, high nucleotide homologies obtained among isolates collected from different countries show significant variations in genotypes (Figure 4). On the basis of sequence homologies two genotypes III and I were found to be very closer because they bore near about similar point mutations. Similarly, phylogenetic analysis of different JE virus isolates shows differences in the genome which fell in to different clusters, because of different epitopic determinants in antigenic sites. However, intergroup genetic distances were larger and are diverging more (Figure 4). Contrary to this, isolates from the same region have shown clear significant molecular variations which have identified different from parental strains because of genetic differences. When compared both inter and intra-group clusters showed a net difference in epitopic determinants and such variations lead to sever pathogenesis (Figure 4). Further, origin of virulent strains of JE is through recombination between far distantly placed virus isolates, whose clustering shows antigenic cross reactivity, displayed high infection rate. This is most possible associating reason of rising neurovirulence and infection rate in the human population.

Japanese encephalitis virus (JEV) genotype III (GI1) has been responsible for human diseases but recently JE virus genotype I (GI) has been isolated from mosquitoes collected from many countries, but it has not been isolated from JE patients from China [64]. Virus is spreading not only in geographic ranges but it is spreading into new host ranges more similar to West Nile virus (WNV), Toscana virus, and enterovirus 71 (EV71) [64]. Emerging infections also result from opportunistic spread of viruses into known niches, often resulting from attenuated host resistance to infection. In India currently disease is reported from the states of Andhra Pradesh, Assam, Bihar, Goa, Haryana, Karnataka, Kerala, Maharashtra, Manipur, Tamil Nadu, Uttar Pradesh, West Bengal and Nagaland and Union Territory of Delhi. Till 2007 103389 AES/ JE cases and 33729 deaths (CFR 32.62%) are reported since 1978. Government of India launched vaccination campaign in highly endemic states of Assam, Karnataka, West Bengal and Uttar Pradesh in 2006 and in Andhra Pradesh, Bihar, Haryana, Maharashtra, Tamil Nadu in 2007 and 2008 respectively which has resulted in reduced incidence of JE in these states [65] (Table 2). India still needs large scale immunization and surveillance to minimize the JE related deaths and morbidities.

6. Control Measures

6.1. Surveillance

There is an urgent need of hospital-based surveillance and immunization in endemic areas [66] to cut down JE disease burden and mortality rate [67]. A real time data obtained from surveillance may clear reasons of re-emergence of Japanese encephalitis epidemics [68-70], rate of virus infection in mosquito vectors, amplifying hosts and humans [71]. However, epidemiological outcomes can establish association of viruses to CNS related syndromes and pathogenesis [72]. Therefore, for finding exact figure of infectivity, both immunization and post immunization successes are to be added to find rate prevention of natural infection [73]. Thus, an updated epidemiological data can answer real cause of high incidences, etiologies, sero-conversion rate [74,75] and post immunization outcomes in patients [76]. Good surveillance data will also assist in finding reasons of emergence and re-emergence, prevalence of human vector borne diseases, vector population dynamics and its transmission potential [77]. Similarly, genomic sequencing
data of virus and its vector, may clear long association of mosquitoses and infecting virus [78].

Hence, for making immunization programs more successful, new surveillance standards and advanced diagnostics are to be developed to fight against the severity of JE disease [79]. Moreover, for starting timely therapeutics of JE patients, disease identification is highly important. Therefore, new potential biomarkers are needed for identification of genotype based disease incidences occurring in different countries [80,81]. New sophisticated biomarkers can ably provide rapid disease diagnosis that will assist in timely clinical care of JE infected patients [82]. It will lower down mortality and infection rate, as well as provide prevention of disease [83]. Therefore, for establishing facts on occurrence of disease, proper identification of virus strain, infectivity and morbidity index should be established by using sophisticated diagnosis methods, techniques tools, and more efficient surveillance data [84]. In addition, important prevention strategies such as vaccination, vector control and health education are required to achieve good success [85].

Comparative study of genomic data will make disclosure of facts about virus epidemiology, genetics and ecological reasons. It will also find changes in virus genome and genotype distribution in different climatic zones [86]. It will also assist in identification of microclimatic variants of JE virus in local regions and may provide clear directions on new clinical challenges generated in patients [82]. It will also assist in finding recent epidemiological shifts made by JE virus genotypes in new climate regions [18]. In China age and latitude were identified as important risk factors required strengthening the surveillance system for JE cases at risk in the prevalent areas [87]. Similar risk factors were also noted in Nepal and has launched an extensive laboratory-based JE surveillance in 2004. It leads to a remarkable reduction in disease burden after mass immunizations from 2005 to 2010 [18]. Similarly, in India disease surveillance was done in Kushinagar district, Uttar Pradesh [88] where JE epidemic mostly falls in peak monsoon months of July to August hence. A large population section is living in a hope to have an effective monsoon months of July to August hence. A large zones [86]. It will also assist in identification of microclimatic variants of JE virus in local regions and may provide clear directions on new clinical challenges generated in patients [82]. It will also assist in finding recent epidemiological shifts made by JE virus genotypes in new climate regions [18]. In China age and latitude were identified as important risk factors required strengthening the surveillance system for JE cases at risk in the prevalent areas [87]. Similar risk factors were also noted in Nepal and has launched an extensive laboratory-based JE surveillance in 2004. It leads to a remarkable reduction in disease burden after mass immunizations from 2005 to 2010 [18]. Similarly, in India disease surveillance was done in Kushinagar district, Uttar Pradesh [88] where JE epidemic mostly falls in peak monsoon months of July to August hence. A large population section is living in a hope to have an effective vector control and immunization program. To make JE control more successful, good surveillance system, and more prompt referral system is essentially needed for good supportive treatment of the patients [35]. Therefore, Centre for Disease Control and Prevention has implemented worldwide Japanese encephalitis surveillance and immunization program [89]. An unpredictable epidemiology makes its prevention an important part of its control [90]. Hence, other than vaccination issues like hygiene, environment, education, economy, are widely concerned to JE control [91].

6.2. JE Diagnosis

6.2.1. Disease Symptoms

After transmission of virus to a healthy human being, it passes an incubation period of 5 to 15 days and then enters the bloodstream. It travels to various glands, multiplies, transfers and settles in the brain, where it cultures enormously and attack the CNS. Parallel to this, virus starts replication in Langerhens, dendretic and skin cells and migrates to draining lymph nodes [92]. Now viral infectious proteins and disjunctive power of body cells and other factors play important role in development of an early immune response. As soon as the infection reaches to secondary lymphoid tissue viremia spreads to visceral organs e.g. liver, kidney and spleen. Virus escapes from blood, avoids targeting immune system, and transported to brain and spinal cord via a hematogenous route. It crosses the blood brain barrier by passive transport after active replication in epithelial cells or by infected inflammatory cells. Virus infects bilateral thalami, brain stem, hippocampoi and brain cortices [93]. Virus imposes acute encephalitic syndrome and severely infect tissue covering of the brain and spinal cord (the meninges) becomes infected and swollen.

At this stage, JE virus infected patient, develop a stiff and painful neck. By day two or three, the patient feels fever, severe headache, nausea, and vomiting. Other major symptoms, mostly observed in JE patients are swelling in the brain, loss of balance and coordination, paralysis of some muscle groups, tremors, seizures, and lapses in consciousness. The patient looks a stiff mask-like appearance of the face that also becomes dehydrated and loses weight due to extremely high fevers. At 5 day post-infection, patients' body cells show robust expression of pro-inflammatory cytokines and chemokines with increased number of infiltrating inflammatory cells into the brain. Further, infiltration of leucocytes towards site of infection increased with a marked up-regulation in expression of genes relevant to infiltration. Macrophage receptor CLECSA/DAP-12 signaling has shown the involvement in this robust neuroinflammation. [94]. At 6 day post-infection if no suitable treatment is being made available 100 % mortality was observed. Symptoms get worse with the rising body temperature, and brain dysfunction carries patient in coma and lead to death in 7-14 days. Hence, early recognition of these signs may help clinicians to manage morbidity in JE patients [95].

Those patients who have recovered display permanent neuronal disabilities due to brain damage and show lifelong neurological defects such as deafness, mental retardation, emotional liability and hemiparesis. Other symptoms which have been noted in pediatric groups are significantly higher percentage of neck rigidity than adult patients. More specifically, JE virus genotypes III infected patients show more severe neuro-physiological disturbances like consciousness impairment, meningeal signs, rigidity, hemiparesis, tetraperesis, convulsive seizures, abnormal behavior, hemorrhagic fever and febrile illnesses [96]. But full bloom JE patients show bizarre movements, auscultative behavior and euphoria without fever and altered sensorium. As soon as body temperature rises, virus massively attacks CNS and patients show psychological disturbances, higher cerebral dysfunction, speech disorders (dysphonia, dysarthria, dysphasias, apraxia and agnosia), extra pyramidal, features, and hypothalamic disturbances, cranial nerves including pupils and fundi and seizures. Other important non-neurological features of prognostic importance are included abnormal breathing, abrupt onset of fever, headache, dystonias and various movement disorders, opsoclonus and gaze palsyse are clearly visualized in acute encephalitic syndrome (AES). JE Virus imposes permanent irreversible changes like residual neuropsychiatric and neurological features in
the survivors [97]. Children infected with JE virus showed acute limb paralysis or neck stiffness and later on they become encephalopathic and show multiple neurological manifestations like thalamic abnormalities [98].

A large number of patients of JE are left with several minor or gross residual neuropsychiatric and neurological features after the acute phase. In this series hospital discharged patients showed more neurological deficits and become physically disable and need constant care by family members. These patients also show cognitive difficulties [99] which are intermittently improved with the time and patients eventually returned to their normal life. Some of them were left with non-disabling residual neurological signs even after 14 years, but they could not return to their livelihood [100]. Dystonia and decelerate rigidity are major effects appeared in patients with, paralytic features and seizures. Based on clinical diagnosis JE virus imposes seizures like other viruses which depend on the area of brain involved. But no comprehensive data regarding late unprovoked seizures in different viral encephalitis is being made available. Therefore, a more authentic and valid diagnosis is needed when separate the disease from other viral diseases. Hence, prospective studies are required to document the risk of late unprovoked seizures and epilepsy following viral encephalitis due to different viruses as well as to determine the clinical characteristics, course, and outcome of post-encephalitic epilepsy in affected patients [101]. In addition, due to lack of proper and timely diagnosis of JE virus and extra delay in treatment very high mortality in infants has been observed [102]. Therefore, early recognition of clinical features of JE disease helps in disease management [95] and to cut down mortality rate in infants [103].

6.3 Serological Analysis

The JE epidemic is confirmed by epidemiological, clinical and entomological data collected from the disease covered area [104]. But severity of infection and morbidity risk can be analyzed only by applying serological tests in patient’s blood serum. Serological analysis decides prevalence of virus in population groups living in endemic area. However, it provides an authentic report on disease incidence and fatality rate. By enlarge in endemic areas there was no difference obtained in case incidence among children or adults [104]. But recovery rate in adults was higher due to an upgraded immune defense. At the same time infants lack this property and face fatal consequences in large numbers. Moreover, very low recovery rate was observed in infants. Presence of virus in the patents was confirmed by serological tests heam-agglutination inhibition and neutralization tests. A serum bilirubin level in pediatric groups was found normal, while it was disturbed in adults infected with JE virus. Similarly, a significant elevation in level of serum proteins, number of WBCs, and aspartate transaminase activity was found in CSF of adult patients in comparison to non-diseased (P<0.05) [71]. Most possible it occurs due to annual and summertime precipitation, and the seroconversion rate to JE virus. It shows much complex alterations in serum parameters, and displays both positive and inverse effects which depend on the biology of vector and hosts and eco-climate of the regions [54]. More important is an acute CSF sample was found to be more sensitive and specific than an acute serum sample [105]. Hence, both serological and molecular diagnosis of Japanese encephalitis is most essential to reveal severe pathogenic effects generated by the virus in pediatric age groups with Acute Encephalitis Syndrome [71]. These standard analyses can answer increasing public health problems in JE affected areas [106].

6.4. Biomarkers

For routine confirmation of JE virus in blood serum and CSF, ELISA is most commonly used to quantify level of IgM antibodies secreted in response to virus attack and invasion on body cells [107]. However, measurement of CSF IgM antibodies levels by ELISA [106] can clearly display altered immune status in JE patients [107]. It also makes clear release of protective antibodies, immune responses and infection status in patients [107,108]. It is also used to measure sero-prevalence of JE in pigs, ducks, and horses, wild birds [109] and other alternate hosts [110]. Seroprevalence of JE virus in wild birds [109] and human [111] is detected by focus reduction neutralization test [109]. Similarly, identification of flavivirus infection of West Nile virus and JE in human cases could be detected by serological differentiation tests [112,113]. Moreover, level of infectivity and virus load in children can be detected by IgM antibody assay (Tang et al, 2012) [19] and ELISA-array [114]. Therefore, presence of IgM antibody above normal level in serum shows positive sign of JE and it also recognizes status of Japanese encephalitis and Japanese encephalitis virus in patients [115]. However, an increased neutralization antibody titer and presence of JE virus nucleic acid in CSF shows rising phase of Japanese Encephalitis. However, serological reports can investigate both occurrence of disease, and success rate of vaccines [116].

But it is not possible to know structural and molecular changes occur at genome and protein level by using such routine methods. Hence, development of validated biomarkers for more authentic and fast diagnosis of JE disease is highly needful. Hence, to improve JE diagnosis and to accelerate therapeutic measures for JE patients upgraded biomarkers are to be needed. Moreover, immuno-histochemical and neuroimaging biomarkers could facilitate more accurate diagnosis of JE and could monitor the efficacies of disease modifying and caring therapies. These biomarkers could disclose disease progression by identifying significant changes occur in body fluids, cells and tissues. Similarly, measurement of virus neutralizing antibodies, cytokines, interleukins, complements, intracellular viral proteins, pro-inflammatory cytokines and cellular differentiation (CD) protein levels in CSF will help to recognize virus generated abnormalities in the patients. New potential biomarkers such as brain mapping, immunotyping, expression of non-structural viral proteins, active caspase-3 activity, reactive oxygen species and nitrogen species and levels of stress-associated signaling molecules can explore cytopathological effects of JE virus. In addition, systematic mRNA profiling, DNA and protein microarrays could help to confirm the disease at an earlier stage. Further, antigenic comparisons in newly emerging JE virus mutants also help to detect environment specific
alterations in JE virus genotypes causing high pathogenesis. More exceptionally, molecular methods can detect circulating genotypes of Japanese encephalitis virus in mosquitoes [117] and swine herds [115].

Hence, molecular, genetical, immunological and ecological analysis is used to detect JE virus genotype based infectivity in particular geographical and eco-climatic zone. This data could assist to correlate altered virus genetics with the mortality, morbidity and expansion of endemicity of JE virus (Figure 3). Possibly it may be due to presence of single, or more than one genotype present in the endemic area [118,119]. Thus changing clinico-laboratory profile of encephalitis patients can be prepared by using multivariate data on molecular and ecological aspects of virus [95]. Similarly, real-time fluorogenic reverse transcription polymerase chain reaction assay in specific detection of virus [120] and differentiation of serotypes in Japanese encephalitis virus, and West Nile virus. For this purpose combined reverse-transcription loop-mediated isothermal amplification assay is also conducted [121]. All these methods also assist in proper diagnosis of JE and post vaccination effects in patients [122]. Similarly, a taqman-based real-time PCR with primers and probe designed identify NS1 gene and its associating genes. These are also used to confirm the virus serotype in JE infected children [123,124]. Furthermore, comparison of reverse transcription loop-mediated isothermal amplification, conventional PCR and real-time PCR could assist in deciding disease status, virus genotypes and transmission of Japanese encephalitis virus in endemic and non-endemic population [64,125]. Similarly, both recombination and positive selection could be identified in complete genome sequences of Japanese encephalitis virus [126]. More over full genome micro-arrays could help in establishing emergence of JE virus genotypes and, its dominance in different parts of the world [127]. Similarly, a novel biosensor, based on serum antibody immobilization is made for rapid detection of viral antigens [128]. In addition, more powerful molecular biology tools are also used to detect virus antigens in CSF [129]. Moreover, an effective and differential diagnosis is always required for confirming the presence of pathogen and its generated effects inside host body in endemic areas. Moreover, structural differences at the level of prM Proteins and its regulatory genes could assist in finding most prevalent genotypes in endemic area [130].

**Figure 3.** Figure showing phase wise expansion of JE virus disease out of endemic zonographic ranges. Expansion is equivalent to level of variations obtained in JEV genotypes. Mass scale genome sequences and their comparisons have given three different levels of climate induced genomic variations (0.1-3.0% or more) that has created five different genotypes GI, GII, GIII, GIV, GV which are circulating in different regions with different adaptabilities. These are point mutations which have made virus antigens more lethal that result in very high mortality. % variations acquired by the virus have increased the antigenicity, immune response infection rate in hosts

7. Immunization

JE is a vaccine-preventable disease; hence for its control large scale immunization should be launched to achieve considerable reduction in morbidity and mortality [131,132]. A potential vaccine inhibits chances of acute encephalitis and rapidly restores consciousness in patients [133]. Therefore, an overall assessment of required doses should be quantified based on population- studies before immunization to reduce the disease burden and to achieve the higher post immunization targets on JE control [99]. With this, partial effects of vaccination, immune protection cover, its success rate, and epidemiological status may assist in control of JE virus in a predetermined time frame [134]. It also implies to determine schedule and dose regimens recommended for routine vaccination to achieve the targets [135]. Therefore, JE vaccination is strongly recommended to those who visit the JE virus endemic regions having very high transmission rate, especially in the rainy season [36]. Vaccination is essentially required for persons who remain engage in extensive outdoor activity in rural areas where JE endemicty is very high. Similarly, health-care providers and international travelers should have appropriate JE preventive vaccines and health guidance before traveling...
Further, understanding the spatial dynamics of the JE risk factors may be useful in providing important information to immunization program. Further, understanding the spatial dynamics of JE risk factors may be useful in providing important information on success of immunization programs going on in different countries [15]. Because JE incidence varies widely over time and partly due to inter-annual climate variability, it affects mosquito vector abundance. Sometime it becomes more complex in climatically favorable conditions after vaccination as vaccine strain reversible associate to new genotype. Therefore, estimate of JE reduction through vaccination should be prepared by taking account of climate inter-annual variability and molecular mechanism of JE vaccine attenuation. Because both are very critical points in JE vaccine quality controls [138]. Further, JE virus surveillance is must to monitor the dynamics of JE virus strains within the region [32] for making successful immunization and to decease JE incidences [68].

Recent progress in the development of live attenuated vaccines has given hope for an effective vaccine, which is both easy to use and inexpensive for large scale programs [139]. But delayed and partial immunization is responsible for occurrence of large number of deaths every year. [140]. Immunization with denatured vaccine is a crime and severe negligence that costs many lives. Hence, vaccines should be properly stored. Moreover, partial coverage of the vaccine leads to emergence of mutated strains of JE virus. Considering this fact, it is highly useful to understand the JE virus genotype distribution, its molecular epidemiological in serum/ cerebrospinal fluid (CSF) samples [58]. Therefore, a complete data on disease related effects, seasonality and transmission of JE virus will assist in evaluation of vaccination and reduction in JE incidence in climate ridden endemic areas [7]. From the surveillance data it becomes clear that JE vaccine can improve the condition of patient within 2 weeks prior to the onset of illness. Hence, it is highly needful to incorporate new potential JE vaccines into immunization programs in all areas where JE is a major health problem [141]. But the main hindrance is underdevelopment and inefficiency of medical services. Further, international report on JE virus transmission gave alarm to the few countries about possible risk of JE virus epidemics far greater than the data reported by government hospitals. There is fear that only hospitals registered cases were counted, but deaths occurred out of the hospitals are mind blowing. Hence, both JE surveillance data (hospital data) and social cum public data should be included to assess the lethality and requirement placed for JE immunization program. It essentially needs increased awareness of JE disease burden at the national and regional levels. Hence, there is an urgent need of WHO prequalified pediatric JE vaccines and international support for surveillance and vaccine introduction in countries with limited resources. Further, local centers should abide by disease control and prevention needs to improve immunization coverage and the efficiency of JE vaccine needs to be re-evaluated in a population at risk of disease [68].

7.1. Use of Virus Neutralizing Antibodies

Neutralizing antibodies generated in response to virus antigens provide protection against JE virus. These antibodies play important role in determining viral pathogenicity and cellular profusion [142]. These easily recognize viral surface antigens that can assist to know spread of virus during acute infection and in defense making against re-infection. More often, virus neutralizing antibodies prevent the virus from entering the cells, while viral agglutination (IgM) reduces the number of infectious agents to attach with the blood cells. These antibodies also agglutinate viral particles and function as an opsonizing agent and facilitate Fc or k3b receptor mediated phagocytosis of the viral particles. Such neutralizing antibodies also limit dissemination of infection and mediate humoral immune defense by binding to flavivirus pathogenic antigens. Contrary to this, passive transfer of these polyAbs or mAbs against yellow fever virus pathogenic antigen/proteins could not successfully protect mice against lethal challenge [143], while virus antigen specific antibodies synthesized inside host operate through receptor binding did complete neutralization of virus attack. However, mAbs generated against NS1 are non-neutralizing but are proved highly effective against virus attack [128,144,145]. These also display strong complement fixing activity [146], and show heavy complement mediated cytolysis of infected cells and show antibody dependent cytoxicity (ADCC).

The principal target for the neutralizing antibodies is the E proteins [147], a subset of prM or any other virus associated protein [148,149,150]. These are proved highly protective to normal body cells [146,151,152]. Hence, both Poly Abs and mAbs inhibit viral attachment to host cells or block viral penetration by binding to epitopes that are necessary to mediate fusion of viral envelope with plasma membrane. In addition cross-protective antibodies produced against improved epitope sequences of common determinants of E and M proteins have shown some good results. Antibodies synthesized against these proteins may ably suppress the viral replication [153] and proliferation inside host [154]. However, persistence of neutralizing antibody [155] after a prolonged exposure of virus also helps to detect viral diseases for long term in human population [156]. But pre-existing neutralizing antibodies increased the symptomatic disease without induction by vaccine dose [157]. Mice with different susceptibility to Japanese encephalitis virus infection show selective neutralizing antibody response [158], but also showed Flavivirus-induced antibody cross-reactivity [159]. Similar, virus specific neutralizing immune responses against JE virus is also obtained by using chimeric peptides for immunization [160,161]. Flaviviruses, mainly neurotropic viruses such as JE virus and tick borne encephalitis virus persist for longer period inside host neurons [162,163], and evolve specific tactics to evade both innate and adaptive immune responses [144]. Therefore, more than the virus and host cell interactions, proper identification of neurovirulent pathogenic peptides is highly essential for generation of more appropriate mAbs.

JE virus peptides, recombinant proteins and mutant proteins are used to generate strong JE virus neutralizing antibodies which may respond more effectively and efficiently against virus generated lethal challenge in experimental animals. Thus, infectious protein derived peptides are of immense therapeutic importance not only to generate neutralizing antibodies for protection against
JE virus but also for nullifying the effect of viral antigens. In addition, B cells play critical role in dissemination of infection [144], by secretion of important bio-molecules such as interferons. Similarly, antibody molecules and complement proteins, T and natural killer cells contribute to the control and elimination of viral infections [164,144]. Further, protective immunity is also generated by synthesizing monoclonal antibodies against non-structural proteins which could protect passively immunized animals from lethal challenge of viruses [144]. It is also induced by both DNA based [165] and virus-vectored immunization with prM and E proteins that may elicit long-lived protective immunity against the flavivirus [166,152]. Thus both natural antibodies and complement can operate a joint attack and [167,168], curve on neuro-invasiveness [169] and acute necrosis of B cells in patients [170]. Moreover, flavivirus cross-neutralizing monoclonal antibody that recognizes a novel epitope within the fusion loop of E protein are used to control JE virus generated neurovireulence [171]. These antibodies in combination to CD8+ T cells play pivotal role in recovery from infection in a murine model of Japanese encephalitis [172]. Further, to seek cross protection against lethal virus challenge in mice recombinant E protein domain III of Japanese encephalitis virus showed proper immune responses against infection [3]. More-exceptionally, pre-existing Japanese encephalitis virus neutralizing antibodies and cell culture based vaccines [173] showed better protection in infants and juveniles of man [156]. These also reduce prevalence of flavivirus infection [174].

7.2. Use of vaccines

Viral proteins elicit pathogenesis in host by attaching themselves to the healthy cells by receptor binding and establish virus attachment and entry into the host. Therefore, to obstruct the infection at initial stage vaccination of infants and children is highly essential. Though, numerous options of JE treatment are available but vaccines are only hope for prevention of Japanese encephalitis. These have shown better efficacy and high safety profile in JE affected patients than any other treatment. Therefore, a potential vaccine prepared against proper genotypes would prove more effective rather than vaccines imported from other countries because such vaccines will show inhibition power to neutralized antigenic epitopes of virus genotype. Hence, vaccination is only reliable method to control JE in comparison to any drug treatment because of its target specificity. This is only tool that can successfully stop and eliminate the JE disease/infection in rural and sub-urban areas. It is highly safer method for sustainable control of virus and last hope to cure the death striven patients. Hence, more stable vaccines/antibodies are to be provided for generation of long-term immunity in un-diseased population. Administration of vaccine enhances immunity level in infants and children of various age groups and provides extra safe cover for their survivorship during post vaccination phase. No doubt an effective vaccination can curb the JE virus generated disease and pathogenesis in millions of patients [175]. Therefore, an appropriate and timely vaccination can reduce the disease burden of disease and significantly cut down the rate of JE virus generated pathogenesis and mortalities in diseased and un-diseased population [176]. It is safer treatment that provides wider protection to travelers mainly to outdoor health workers, surveyors and researchers who get extensive viral exposure in rural areas during their stay in endemic region. It is also recommended for expatriates and migrants living in endemic areas throughout a transmission season or longer. Further, regular vaccination can successfully break the annual cycle of disease occurrence in endemic areas. Hence, for fast control, dismantling of annual cycle of virus complete elimination of susceptible mosquitoes is highly important. JE virus transmission can be fully controlled by killing vector (Culex sp.) population during breeding period in paddy field or in any other small habitat. It can significantly lower down JE incidences. There are reports that virus transmitting mosquito species have acquired pesticide resistance and show host range differences. Hence, both vaccination and mosquito control are most essential strategies for the prevention of JE epidemics.

Due to better protection efficacy, vaccines are considered as necessary tool to control JE, but it is a bitter truth that vaccines are not reached/available to the poor countries in Asia. Therefore, for emerging vector-borne flavivirus diseases vaccines are only solution to combat the epidemics [177]. Initially an effective attenuated vaccine was prepared in 1941, which was recommended for routine use in endemic countries in 1950s [170]. It has shown good results and found highly successful against JE control in Japan, Korea, and Taiwan. But due to some inadequate disease surveillance, inconsistent vaccine supply, lack of guidance and support for immunization, and limited advocacy have made obstacles and hampered the JE eradication program in the past [178]. After seeing large number of fatalities occurred in 2006-2007 in northern part of the country Indian government has launched large scale vaccination in endemic states [179] to control Japanese encephalitis outbreak [180]. More often, only two types of JE vaccines XIARO- IC51 and SA 13-14-2 are currently in use in several JE endemic countries within the Asia-Pacific region. These are W.H.O recognized vaccines which are internationally available in the market. These vaccines have significantly cut down cumulative attacking rate of encephalitis up to 51 per 100,000 in the placebo group and 5 per 100,000 in each vaccine group. To fulfill this aim, new single-dose compatible vaccines have been generated and these are used for routine immunization of various infant groups by W.H.O. Recently, Global Vaccine Safety Initiative (GVSI), has prepared a blueprint plan to ensure the safety of vaccines to be used worldwide over the next eight years and beyond. The GVSI is supporting the Global Vaccine Action Plan which is a roadmap for vaccination campaigns and aimed at preventing millions of deaths from disease worldwide. The period 2010-2020 has been declared the Decade of Vaccines. Based on preparation method and different substrates are used for preparation of JE vaccines. Few important vaccines are used for control of JE syndrome are

7.3. Use of Formalin-inactivated JE Vaccine

A first generation formalin-inactivated JE vaccine was derived from mouse brain in China in early fifties. It is based either on the Nakayama strain, which were isolated
in Japan in 1935, or on the Bejing-1 strain [181,182,183]. Viet Nam also produced, mouse brain-derived, inactivated Japanese encephalitis vaccine which is used in control JE in Northern Viet Nam [184]. It is routinely used for immunization in Japan and several Asian countries and is commercially available in the international market [185]. It was largely generated by Japan and its other manufacturers are South Korea, Taiwan, Thailand and Vietnam. Currently, vaccine is used in China, India, Japan, South Korea, Sri Lanka, Thailand and Viet Nam. Its three doses (0.5 ml each) are required to immunize children on days 0, 7 and 30, followed by a booster at 1 year and thereafter at intervals of 3 years. Later on, a formalin-inactivated vaccine was prepared from primary hamster kidney cell cultures in China which is regular in use since 1968. It is principle vaccine of China which is more immunogenic than any other vaccine [185]. It is a live vaccine that shows an overall anti-viral efficacy between 76-95% in primary immunization of infants and provides wider protection. It shows stable phenotypic and genetic characteristics. There was found no reversion to neurovirulence of the vaccine strain in animal models as well as in patients. It is unable to cause suckling mice or weanling mice disease [186,187] (Table 2). Live attenuated vaccine shows cytokine and chemokine responses to Japanese encephalitis infection in human population [118]. It is true that even after large scale vaccination JE cases are still reported every year. Therefore, for boosting immunity and providing wider protection cover, a regular vaccination should be maintained [5] by using low cost potential JE vaccines in endemic population [188].

<table>
<thead>
<tr>
<th>District</th>
<th>Area</th>
<th>Population</th>
<th>Density</th>
<th>Literacy rate</th>
<th>Population growth*</th>
<th>Annual precipitation</th>
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<tr>
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<tr>
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<td>17.69%</td>
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<td>75.16 per cent</td>
<td>18.94%</td>
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<td>Deoria</td>
<td>2,535 km²</td>
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<td>14.23%</td>
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<tr>
<td>Padauna</td>
<td>2,535 km²</td>
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<td>N</td>
<td>63%</td>
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<td>983 mm</td>
</tr>
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</table>

Population growth rate is based on 2001-2011 census. N represents data not available.

7.4. Use of Chimeri-Vax-JE or IMOJEV Vaccine

ChimeriVax™-JE vaccine was developed by UK/USA based company Acambis by growing on Vero cells. It was prepared, tested and is passing through licensing stage in Australia [189]. It was prepared by using SA14-14-2 JE virus strain and its eight passages were done in primary kidney cells of dog, cultivated in Vero cells and formulated with 0.1 % Aluminium hydroxide. Vero cells maintained in serum free medium are used as main substrate of this vaccine [190]. This vaccine is under trial based on insertion of sequences which are responsible for virulence and pathogenesis. Therefore, to reconstruct ChimeriVax™-JE and JE-VAX® four major genotypes were identified by neutralization assays and passive protection studies in mice. However, greatest protection against JE virus could be generated by using strains of genotypes II and III, although some protection was also afforded against genotypes IV strains and I. Further, attenuation of chimeric virus (YF-JE chimeric virus) was made by modifying at least three of the six amino acids in YF-Virus [203]. This live, attenuated SA 14-14-2 vaccine was used for immunization to control Japanese encephalitis in Nepal [197].

Further, for achieving greatest protection Chimeric virus vaccines were developed by fusion of two viral proteins by making site directed mutations, specifically based on insertion of sequences which are responsible for virulence and pathogenesis. Therefore, to reconstruct ChimeriVax™-JE and JE-VAX® four major genotypes were identified by neutralization assays and passive protection studies in mice. However, greatest protection against JE virus could be generated by using strains of genotypes II and III, although some protection was also afforded against genotypes IV strains and I. Further, attenuation of chimeric virus (YF-JE chimeric virus) was made by modifying at least three of the six amino acids in E protein [198], due to which it was assigned a name as ChimeriVax-JETM. It elicits JE virus neutralizing antibody responses as well as protection against nasal and intra-cerebral virus challenge in rhesus monkeys [199,200,201]. The Chimeric virus was shown no replication in mosquitoes which were fed the Chimerivax-JE vaccine [202]. It has shown superiority over JE-VAX® [203] as it shows larger protection against Japanese encephalitis virus and decline the pathological symptoms in [198] lethally challenged mice [204]. In addition, mouse brain derived vaccine is replaced by Japanese encephalitis chimeric virus vaccine (JECV) [186]. A single dose of JE-CV elicited rapid sero-conversion in a higher proportion of vaccine than the current vaccines.
Similarly, a IMOJEV ®--a recombinant Japanese encephalitis chimeric vaccine (JE-CV) [186] was developed on the basis of clinical reports [205]. Similarly, another vaccine was tried to construct by using replication-defective canary poxvirus (ALVAC). It was prepared by using attenuated vaccinia virus strain NYVAC in a vector that express the pr-M, E, NS1 and NS2a genes from JE virus. The vaccine candidates were found to be well tolerated but their immunogenicity was found too weak, especially in non-vaccinia immune volunteers, to warrant further development [14]. The vaccine was tested in human adult volunteers in the USA, where it showed good safety and immunogenicity. It has shown 94% efficacy in phase II trials and it raised protective neutralizing antibody levels after a single dose [186,201]. The vaccine is undergoing Phase III clinical trials in the USA and Australia for immunization [183,184,206,207] whereas a parallel pediatric development program has been launched in Thailand by Sanofi Pasteur [208]. Recently Central Research Institute, Kasuali, has succeeded to develop a Japanese encephalitis vaccine by using mouse brain killed virus strain. Its 3 doses are required to generate immune protection in children against JE virus (Table 2). Besides this, a tissue culture vaccine is also in developing phase and waiting for clinical trials for standardization and commercialization. In addition several other vaccines are in their later stage of development and are under trial [176,209].

Mainly, safety of vaccine is assigned by using viremia, clinical signs and neuropathological changes occur in patients. It is also determined based on effect of vaccine to generate an immune response that must be protective against virus challenge. Further, post vaccination effects such as reactinogeneity and persistence of immune response are also considered for evaluation of vaccine efficacy. It is true for vaccines that it must show a moderate to severe hypersensitivity type reactions mainly neurological adverse effects in patients. There is no reduction in sero-conversion rates when other childhood vaccines are given simultaneously. In addition, vaccine administration in patients shows local and systemic side effects like urticaria and angioedema (1 case per 1000 vaccinations) [181]. Therefore, for improving the efficiency of flavivirus vaccines and its adverse effects molecularly mixed vaccines are designed and prepared. These are recognized as second-generation vaccines. Second generation vaccines are non-mouse brain derived JE vaccines developed by using genetically engineered virus proteins. These are based on promising approach and are considered to be the best JE vaccines of the future [210]. A live attenuated genetically engineered (Chimeri Vax JE vaccine) virus was made by replacing the genes encoding the two structural proteins (prM and E) of yellow fever virus (YF), 17 D vaccine strain with corresponding genes of an attenuated vaccine strain (SA14-2-14) of JE virus [211,212].

7.5. Use of XIARO- IC51

XIARO or IC51 vaccine was developed by Austrian Biotech Company Intercell Biomedical Ltd. It induces neutralizing antibody titers and shows wider protection against JE virus infection [161]. It requires only 2 doses for complete immunization, and is currently licensed in the U.S., Europe, UK, Canada and Australia [213]. It was prepared by using purified formalin inactivated whole virus [214,215,216]. Its two trials have been completed and vaccine is licensed by the US FDA for adults [217] (US-FDA). Both XIARO- IC51 and SA 13-14-2 JE vaccines have different sero-conversion rates and neutralization antibody titers. Further, a Vero cell-derived purified inactivated JE vaccine was developed by Chengdu Institute of Biological Products in China in 1988 [218]. Later on it was made by several other manufacturers [219], either by using the virulent Nakayama strain, or by attenuated SA14-14-2 JEV strain [220,201]. It is a live attenuated vaccine which is produced by using a stable neuro-attenuated strain of the JE virus (SA-14-14-2) and is licensed in China and several Asian countries. It was extensively used in India from 2006 to 2008 for immunization of children [221]. It was prepared after 11 passages done in weaning mice followed by 100 passages in primary hamster kidney cells [222], lyophilized, and administered to children at one year of age and again at two years [223]. Although this vaccine is not WHO prequalified at this time, still efforts are being made to bring the production and quality control to international standards. First time, it was administered in 200 000 children in southern China where it showed 99-100% sero-conversion rate in non-immune subjects and provided protective efficacy over 5 years [224]. Its single dose can stimulate adequate immune response in the recipient of the vaccine [225] and showed 96.2% efficacy [222]. Its post vaccination studies showed 90% neutralizing antibody persistence at 4 years and 64% at 5 years after a single-dose of the vaccine [201,226,227,186]. In present time, more than 30 million doses of the live SA14-2-14 vaccine are distributed annually in southern and western China. In India from May 2006 it was administered in 9.3 million children in 11 districts scattered among 4 states where JE was considered as highly endemic [209]. After vaccination few adverse events with severe symptoms and mortalities were also reported, but most of the immunized cases were found survived [228]. Though cell culture-based vaccines show excellent tolerability but are not be used in the population living in endemic areas where the risk of infection is extremely high, but these are only useful for travelers and military personnel [229].

7.6. Use of Green Cross Vaccine

Later on Green Cross vaccine was developed by Green Cross Vaccine Company but it was not licensed in the United Kingdom. Similarly, a Semple vaccine from phenolized sheep-brain was developed in Thailand and is used for vaccination children aged between 1-6 years [230]. It spectacularly reduced the rate of neurological complications in patients up to 8.31 cases per 1000 persons vaccinated (1:120) and successfully cut down the number of susceptible children after the second dose [231]. Generally, 3 doses of this vaccine are prescribed for immunization and third dose is given at 6 month interval after second dose [232]. However, for protection from JE virus infection both inactivated and the attenuated vaccines were used for immunization purpose. The development of a Swine vaccine against JE virus is on high priority as it could help to prevent epidemics in humans (Table 2). Similarly, both LAV SA-14-14-2 virus
and inactivated P3 strain (IPV) vaccine showed protective efficacy against JE virus genotype I.

7.7. Use of DNA Vaccines

To fight against flavivirus infection DNA vaccines are generated by using virus DNA encoding antigenic proteins, the recipients take up the DNA and encoded protein antigen is expressed leading to both humoral and cell mediated immune response. DNA integrates into the chromosomal DNA and viral antigen is expressed in the injection area. DNA vaccines have more advantage as encoded protein is expressed in the host in its natural form and there occurs no destruction or modification. The immune response is accurately directed to the antigen exactly as it is expired by the pathogen. DNA vaccines show prolonged expression of the antigen, which generates specific immunological memory. These have more advantage over peptide antigens as they do not require handling and storage of the plasmid DNA. As it is well known fact that natural infection also generates immunity but DNA vaccines much ably generate immunity much faster and initiate strong immune response upon administration [233]. However, plasmid-based DNA immunization provides titer 1:600 while formalin-inactivated vaccine provides 1:1000 [234]. Besides this, oral immunization of mice with live JE virus also generates a protective immune response [235].

7.8. Use of Peptide Vaccines

Peptide vaccines are safe, easy to store and can be carried to tropical regions for immunization by storing them in sealed dry ice packets. Peptide vaccines were found non-infectious after use in comparison to DNA vaccine. Therefore, multipurpose peptide with several different epitopes is used for production of high titers of JE virus neutralizing antibodies. However, for active immunization, many natural and recombinant peptides have been used to generate potential vaccines. There are few demerits of peptide vaccines as they show poor immunogenicity because short amino acid sequences are not highly immunogenic and fruitful changes are not possible in the peptide molecule to elicit an immune response. For production of antibodies complete viral protein or its formed peptides having small segments of proteins or antigenic determinants or epitopes are used to elicit the immune response. Besides this use of non-infection, sub-viral extracellular particle (Eps) is an inexpensive and safety strategy for the production of protein based flavivirus vaccines.

For making recombinant vaccines, molecular clones of YFV (Yellow fever Virus) and Japanese encephalitis virus were used and tested for neurovirulence in mouse model and immune protection coverage before going for vaccination trials. Six amino acid changes were made in E protein (E107, E138, E176, E279, E315 and E439) and three in the non-structural genes were associated with the attenuation [237]. Thus, both non-structural protein prM and E protein contain antigens conferring protective humoral and cellular immunity against JE virus. It is a safer and cheaper vaccine, which has demonstrated 88-96% effectiveness in the large-scale trials. Vaccine showed systemic reactionogenicity and no neurotoxicity. This vaccine was administered in children in Nepal where it showed 96% efficacy. It is licensed for use in Nepal, Sri Lanka and China. A Chimeric T helper-B cell peptide vaccine for Japanese encephalitis virus was indigenously developed in 2008 by Indian Scientists at National Institute of Virology, Pune, by M. M. Gore and his team and got an U.S Patent [238].

Furthermore, peptides mainly synthetic require mixing of large amount of adjuvant, which is considered much undesirable and unsafe. Further, chemical conjugation of peptides is a very difficult task and these are not much reproducible because linker may remain immunogenic is un-ensured. Peptide vaccines are devoid of any T cell epitopes and amino acid sequences do not show immune suppression, molecular mimicry like DNA sequences. DNA vaccines enhance infection induced antibody responses differently. Maximum neutralization titer a peptide vaccine shows 1:20 against JE virus [239], while it is much higher in case of DNA vaccines. Though peptide vaccines are easy to develop but these are temperature sensitive and denature at normal temperature and are of no use. Furthermore, stability of single chain proteins could be optimized by using new linker molecules [240] or by generating more appropriate short antigen epitopes with defined structure and function to elicit strong antigenicity [241]. Rod shaped virus particles (VLPs) were made by assembling the JGMV and CV peptides and are used to raise antibodies or vaccines [239]. There is a possibility that peptides derived from E protein may have better possibilities to generate VLPs and fusion proteins (JGMV peptide) which can be used to obtain higher titers of neutralizing antibodies. However, for production of prM and envelope E protein mammalian cells are used to yield more amount of these proteins. Recently, insect cell expression system with Sf9 cells to generate viral antigens such as JE virus envelopes proteins. These cells could produce 10-100 fold larger amount of envelop proteins than mammalian cells [242]. Besides this, simple feed batch cultivation of recombinant E. coli cells resulted in high yield of recombinant JE virus proteins [243]. These multivalent subunit peptide vaccines showed better cellular and humoral immunity rather than simple vaccines. These are also developed by constructing synthetic peptides that may contain both immunodominant B cells and T cell epitopes. To generate an appropriate and desirable CTL response, the vaccine must be developed intra-cellular so that the peptides can be processed and presented together with class I MHC molecules. These can be developed by preparing solid matrix antibody antigen complexes by attaching monoclonal antibodies to particulate solid matrices. Besides this, immunestimulating complexes of liposome are having membrane proteins from many pathogens are developed for immunization purposes.

8. Virus Specific Chemotherapy

There should be a hunt for searching more effective drugs to expedite the therapeutics of JE patients [244]. These new potential therapeutic agents are widely concerned to antibiotics such as minocycline, short interfering RNA, arctigenin, rosmarinic acid, DNAzymes...
etc. DNAzymes also potentially work against JE virus generated infection and inhibit the replication and proliferation of Japanese encephalitis virus [245]. These result in a sharp reduction in JE virus titer in host brain, which may extend the lifespan, and recovery of infected patient. There is a need to develop virus replication inhibitor drugs for more efficacious therapeutics other than DNAzymes. However, new innovations are highly demanded to make virus specific chemotherapy, and its lethal side effects should be confirmed in animal models. In addition there is an utmost need for a catch-up program for implementation of drug regimens more selectively against communicable lethal viruses [246]. For chemotherapeutics interferon alpha was found most promising drug in small open trials, but a recent double-blind placebo controlled trial showed that it did not affect the outcome in children affected with JE [247]. Moreover, virus specific chemotherapy should be used to manage inhibition of virus replication that may result in inhibition of infection at an earlier stage. Further, diverse mimimotope of active antigens that can mimic the JE virus neutralizing antigen activity can be generated. In addition, many JE virus infectious mutant clones can be generated by insertion of short introns or cloning into artificial chromosomal systems to have more potential vaccine candidates [248]. Few other chemical agents like Nitric oxide (NO) has been shown to suppress JE virus RNA synthesis, viral protein accumulation, and virus release from infected cells [249,250]. Similarly, various types of cytokines, complement proteins, enzymes, antibodies and passive transfer of activated CTLs, T cells, B cells and NK cells can be used to destruct viral infection. In addition, other strategies like RNA silencing and interference, activation of complement, use of recombinant virus antigens, VLPs and fusion proteins are also used for therapeutic purposes. In addition, cellular inhibitors of JE virus that may bind to surface receptors in cell membrane glycol-proteins and glycol-lipids are searched to keep out infectious agents out of the cells or its binding to host cell membrane molecules is obstructed by any other agents. Hence, strong membrane receptor inhibitors may be of high therapeutic use.

9. Control of Disease Transmission and Epidemics

An overall comparison of molecular data available on serotypes and mutant strains clearly shows that JE virus has made significant changes in key genomic sites mainly those regions which determine antigenicity of virus. (epitopic regions). Further genetic, ecological and bioinformatics analysis of epidemiological data showed different patterns of transmission, host selection, endemicity and infection rate, circulation of different genotypes, and evolution of various mutas strains of flaviviruses in different eco-climatic zones. However, genomic variations and genetic distance analysis also made clear that JE virus was originated in the Indonesia – Malaysia region from an ancestral virus, from which different genotypes GV was diverged, followed by GIIH, GII and GI [2] (Table 3). Genotype IV appears to be confined to the Indonesia-Malaysian region as GIV. It was isolated from mosquitoes in China and other South Korea [242] while GI-III virus is circulating throughout Asia and Australia in a variety of hosts. Moreover, GIV isolates collected from Java, Mali and Sumatra showed six amino acid substitutions within the E protein (Table 3). However, microevolution lead to incorporation of new lethal amino acid sequences in ecological and vaccine strains of JE virus. It has significantly increased the severity of pathological effects in patients manifold. Besides this, several amino acids within the E protein of the Indonesian isolates were found to be under directional evolution and/or co-evolution. Such naturally occurring evolution is likely to affect the disease profile and the vaccine efficacy against circulating JE virus genotype I in Eastern [59] and G III in Northern part of Asia [244]. Thus, different JE genotypes showed close genetic relationship and display very little differences in their antigenic sites. These were found much larger in potential to cause acute encephalitis syndrome in infant groups. More often, molecular epidemiological studies have confirmed the presence of different subgroups of JE virus genotypes of which I, II, III are largely circulating in different Southeast Asian countries. These viruses are chronologically related to one another and more frequently cause JE disease. Further, JE virus has recently changed from genotype III (G III) to GII(GI) and imposes life threatening situations for both human and animal populations in Asia countries [251] (Table 3). However, advent of heterotypic genotypes has reduced the vaccine efficacy and raise emergence of antibody resistant mutants after incorporation of new lethal and neuroviralgenes which are circulating in the endemic population of this region. This is the main reason despite the availability of vaccines JE related mortality rate is still high in India and its neighboring countries [252]. It indicates failure of vaccines or its partial working action. Therefore, a vaccine must possess enough potential to work against JE virus during initial phase of virus infections and might show long lasting efficacy after administering a single dose [253]. However, a protein that contains the critical neutralizing antibody determinants [236,254] elicits high protective immune response and can be used for vaccination purpose against JE virus. Therefore, before preparing an appropriate vaccine, possible mutations, its most possible effects on antigenic amino acid sites mainly on epitopes of virus proteins should be assessed in Silico and in experimental animals and correlated with neuro-virulence and pathogenesis. Thus it is assumed that vaccine strain should be more stable and its viral proteins with single and multiple antigenic determinants must be tested for its susceptibility for any future conformational change. Antigenic sites of these proteins are highly susceptible to single site mutations which make significant changes in protein structure. It also alters binding to receptors that is most commonly occurred in the flaviviruses. Further roles of amino acid substitutions in JE virus strains in the attenuation process and neurovirulence must be tested in different animal models and in human hosts to have an appropriate vaccine design. Moreover, favorable climate exists in South East Asia, which is assisting the JE virus in multiplication cycling and circulation during summer and rainy season. Further, rapid globalization and gradual shifts in global climate have changed the virus infectivity pattern from endemic to epidemic and virus is showing its presence in newer areas
More specifically, subtropical eco-climatic region shows togetherness of so many hosts of JE virus and other flaviviruses both wild and domesticated animals. These are working as hotspots for the emergence and evolution of new JE virus strains. More often, open farming of pigs, poultry birds, rabbits and goats in the middle of human habitation are responsible for rapid amplification of virus or creation of new amino acid substitutions in virus E protein. These are responsible for origin of new mutants and serotypes in local habitations. However, beside encephalitis virus, some other viruses like FMDV (Foot and Mouth Disease Virus) also showed outbreaks in the endemic areas in the same season but they show lesser mortality rate than JE virus. This confirms inter-relationship between both the viruses. In nature there might be some viruses which may infect both animals and plants, and inoculate ordinarily in plant hosts and maintain their replication rate very high. Most possibly they may also find its way inside a suitable animal host. Now it has been cleared from epidemiological surveillance data that in recent years virus has not only enhanced lethality but also expanded its geographic range from Indonesia to Australia.

In addition, both climate and enzootic biology showed that viral proteins are highly susceptible to changing conditions of climate and making host-immune interactions more vulnerable in the endemic areas. These are responsible for both summertime and annual precipitation and seroconversion rate in JE virus infection [254]. Therefore virus infection is directly correlated with various climatic factors and presence of virulent proteins [10]. Besides this, other important factors, which are responsible for re-emergence of JE virus, are geographic and epidemiological location, physical environment, presence of uncontrolled virus vectors, clinical patterns followed, and presence of alternate vertebrate hosts, circulating JE virus serotypes, international travels and ongoing rapid climatic changes [15]. Further, disturbances in ecological niche of water birds, their forced migration to non-endemic areas lead to wider spread of JE V in ecological niche of water birds, their forced migration to non-endemic areas lead to wider spread of JE virus and other flaviviruses both wild and domesticated animals. These are working as hotspots for the emergence and evolution of new JE virus strains. More often, open farming of pigs, poultry birds, rabbits and goats in the middle of human habitation are responsible for rapid amplification of virus or creation of new amino acid substitutions in virus E protein. These are responsible for origin of new mutants and serotypes in local habitations. However, beside encephalitis virus, some other viruses like FMDV (Foot and Mouth Disease Virus) also showed outbreaks in the endemic areas in the same season but they show lesser mortality rate than JE virus. This confirms inter-relationship between both the viruses. In nature there might be some viruses which may infect both animals and plants, and inoculate ordinarily in plant hosts and maintain their replication rate very high. Most possibly they may also find its way inside a suitable animal host. Now it has been cleared from epidemiological surveillance data that in recent years virus has not only enhanced lethality but also expanded its geographic range from Indonesia to Australia.

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**Table 3. Japanese encephalitis virus genotypes isolated from different parts of South East Asia and other countries**

<table>
<thead>
<tr>
<th>Genotype Location</th>
<th>Epitope</th>
<th>Method used</th>
<th>JEV surveillance</th>
<th>Lethality reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Japan</td>
<td>E 327 Gln 20.2-21.2 8.5-9.9</td>
<td>Multiplex RT-PCR</td>
<td>Mosquito based</td>
<td>High</td>
</tr>
<tr>
<td>G1 China, Korea, Cambodia, Thailand, Australia</td>
<td>prM and E glycoprotein genes</td>
<td>RT-PCR</td>
<td>Mosquito based</td>
<td>High</td>
</tr>
<tr>
<td>G1II Australia Malaysia, Indonesia</td>
<td>prM and E glycoprotein genes</td>
<td>RT-PCR</td>
<td>Pigs</td>
<td>High</td>
</tr>
<tr>
<td>GIIII Taiwan</td>
<td>E protein</td>
<td>Multiplex RT-PCR</td>
<td>Mosquito-based</td>
<td>High</td>
</tr>
<tr>
<td>GIII-III Asia and Australia</td>
<td>E176, 177,227, 244, 264</td>
<td>Amino acid sequencing</td>
<td>Mosquito based</td>
<td>Higher</td>
</tr>
<tr>
<td>GIII West Bengal India</td>
<td>Envelope proteins</td>
<td>Mac-ELISA and RT-PCR</td>
<td>Human</td>
<td>Higher</td>
</tr>
<tr>
<td>GIII Northern India</td>
<td>E 176, 177,227,244,264,279</td>
<td>Gene sequencing</td>
<td>Human</td>
<td>Highest</td>
</tr>
<tr>
<td>GIII Chongqing, China</td>
<td>CQ11-66 strain, E- genes</td>
<td>Nested PCR</td>
<td>Human</td>
<td>Highest</td>
</tr>
<tr>
<td>GIII Shanghai, China, Wuhan, Nepal, Taiwan, Japan, Philippines, Korea, Sichuan, Yunnan</td>
<td>Envelope proteins</td>
<td>RT-PCR</td>
<td>Human</td>
<td>Highest</td>
</tr>
<tr>
<td>GIIV Indonesia-Malaysia region, Indonesia</td>
<td>Six AA substitutions</td>
<td>Amino acid sequencing</td>
<td>Mosquito based</td>
<td>Higher</td>
</tr>
<tr>
<td>GIV Malaysia China and South Korea</td>
<td>Six AA substitutions</td>
<td>Amino acid sequencing</td>
<td>Mosquito based</td>
<td>Higher</td>
</tr>
<tr>
<td>GIV Singapore, Australia</td>
<td>Structural genes</td>
<td>Nucleotide and AA sequencing</td>
<td>Mosquito based</td>
<td>Mild</td>
</tr>
<tr>
<td>GIV Japan</td>
<td>Structural genes</td>
<td>Nucleotide sequencing</td>
<td>Mosquito based</td>
<td>Mild</td>
</tr>
</tbody>
</table>

In recent years virus has not only enhanced lethality but also expanded its geographic range from Indonesia to Australia. In addition, both climate and enzootic biology showed that viral proteins are highly susceptible to changing conditions of climate and making host-immune interactions more vulnerable in the endemic areas. These are responsible for both summertime and annual precipitation and seroconversion rate in JE virus infection [254]. Therefore virus infection is directly correlated with various climatic factors and presence of virulent proteins [10]. Besides this, other important factors, which are responsible for re-emergence of JE virus, are geographic and epidemiological location, physical environment, presence of uncontrolled virus vectors, clinical patterns followed, and presence of alternate vertebrate hosts, circulating JE virus serotypes, international travels and ongoing rapid climatic changes [15]. Further, disturbances in ecological niche of water birds, their forced migration to non-endemic areas lead to wider spread of JE virus and other flaviviruses both wild and domesticated animals. These are working as hotspots for the emergence and evolution of new JE virus strains. More often, open farming of pigs, poultry birds, rabbits and goats in the middle of human habitation are responsible for rapid amplification of virus or creation of new amino acid substitutions in virus E protein. These are responsible for origin of new mutants and serotypes in local habitations. However, beside encephalitis virus, some other viruses like FMDV (Foot and Mouth Disease Virus) also showed outbreaks in the endemic areas in the same season but they show lesser mortality rate than JE virus. This confirms inter-relationship between both the viruses. In nature there might be some viruses which may infect both animals and plants, and inoculate ordinarily in plant hosts and maintain their replication rate very high. Most possibly they may also find its way inside a suitable animal host. Now it has been cleared from epidemiological surveillance data that in recent years virus has not only enhanced lethality but also expanded its geographic range from Indonesia to Australia.

Most of the JE cases and positive serotypes are circulating in clusters in Tarai region of India and Nepal, and also in other similar eco-climatic zones. However, a spatial heterogeneity was observed between Japanese encephalitis virus induced destruction and environmental variables [255]. It is highly noticeable that positive mutants of JE virus circulate more and more in rainy season, because large scale transmission facilitated by mass breeding of mosquito vector that result in a sharp increase in MIR (minimum infection rates). This MIR is significantly, decline in winter and summer season [15]. Further, re-emergence of genotype V in Asia has increased the risk of JE virus transmission, mortalities and morbidities more alarming. This is one of the major reasons that JE virus transmission occurs in rural agro-forestry areas, [256] but recently its transmission has been also identified in urban areas such as Delhi [257]. However, to control the disease transmission virus vector also identified in urban areas such as Delhi [257]. Further, strong efforts should be made to enhance awareness about vector control, sanitation and surveillance, through environmental education programs to be launched at national and international level. There are integrated demographic, ecological and economic reasons of virus transmission which are reversing lethality and expanding disease incidences in peripheral endemic areas (Figure 4).
9.1. Vector Control

Climate is a major factor in determining the geographic and temporal distribution of arthropods. The characteristics of arthropod life cycles are associated with consequent dispersal patterns of arboviruses, their evolution, and efficiency to establish the disease in vertebrate hosts [17]. A diverse group of mosquito vectors are transmitting different JE virus genotypes which are circulating in human population and other vertebrate hosts. Thus JE virus genotypes are establishing themselves in different epidemiological locations i.e. endemic to epidemic area. Virus genotypes are circulating in human population by making most favorable changes in their antigenic/epitopic regions. These JE genotypes are also circulating by interchanging of virus among hosts by overlapping population of infected mosquitoes. Though virus invades reservoir hosts but evoke pathogenesis in human as they are main target group. Further, hydrobiology of endemic area contains 150 freshwater both small and large lakes in 16 districts of eastern U.P and all are under endemicity. This area also hold with more than 12 major feeder canals, 3 open dams which are working as open pastures for breeding of mosquitoes. Naturally, this open water range enormously expands in rainy season because of heavy torrent rains received by the area almost every year. Again there is great misery that low land (tarai) seeps in large collection of flood water, full of garbage, human and animal wastes, fertilizers and other chemicals. All this aid the large vegetation cover to flourish and support large plankton and fish population to feed and attract migratory birds in large numbers. Parallel to this, breeding of mosquitoes; get induced in adjoining paddy fields with supported supplementation of different fertilizers. In addition, new migrants received by the water reservoirs aid mass breeding of mosquito which are responsible for virus multiplication and transmission. Once there area get rains, a rapid increase occurs in vector population, amplifying hosts and migratory birds. It is remarkable that freshwater bodies also exist in urban area but occurrence of JE cases is lesser, because of prophylactic and sanitation measures are maintained. More or less people use various tools and means for their protection from mosquito bites in urban areas. Contrary to this > 90% of rural people do not use medicated net, or any mesh, or any repellent to keep away the blood sucking JE vectors during day and night time. Reasons are not biological but pure economic as a large section of population cannot afford mosquito nets, potable drinking water and medical expenses because of poverty strive. This is one of the major factors which are responsible for occurrence of large number of JE cases in

Figure 4. (a) showing reasons of high transmission, lethality and expansion of disease area and incidences, (b) inter-relationships of genomic variations, micro niche and % susceptibility of host and virus genetics and (c) formation of different JEV genotypes GI, GII, GIII, GIV, GV from parental stock
poor rural areas. If basic amenities such as mosquito nets, repellents, fumigants, pesticides are being made available JE infection level will be significantly cut down. Further, vector control will cut down JE affected cases and will work as an alternative control of JE disease. Hence, it is highly important to generate awareness among dwellers and common people in urban, suburban and rural areas to essentially manage personal protection measures to prevent mosquito bites and follow prophylactic measures to avert effect of JE (Figure 5).

Figure 5. Tripartite arrangement for control of JE virus infection in endemic-eco-climatic zone

9.2. Cultural Control of Virus

The emergence/re-emergence of JE is associated with complex factors, such as viral recombination and mutation, leading to more virulent and adaptive strains. Furthermore, urbanization and human activities are creating more permissive environment for vector-host interaction, and increased air travel and commerce. There is a common habit among rural poor that they do not bear full sleeve shirts and remain naked in upper bears during the day as well as night time. Otherwise people can skip the day light mosquito bites by using thick and full sleeve cloths. To minimize the mosquito bites larger portion of body should be covered by using soft, airy and thick cloths. It is very hard to find safety without any protective cover to keep away the hovering mosquito swarms in rural areas. There is a close relationship among JE mosquito vector, amplifying hosts and carrier of JE that are birds. It is very difficult to control arrival of water birds, and water borne JE outbreak because of failure of control strategies. But shifting of dirty piggeries away from the middle of human vicinities is possible. It is a common social practice in this area that a larger population of infected pigs is available in human surroundings. Unfortunately government has not been implemented any scheme to shift the pig houses from the rural human settlements. These are major social and administrative negligencies, which are responsible for outbreak of disease in many in endemic areas such as Nepal and India since the early 1980s. Since then, it has spread from its origins in lowland plains to the Kathmandu Valley as well as in hill and mountain districts. A complete ban on pig and paddy farming is economically possible in Nepal and Tarai region of U.P. and Bihar. Therefore, without having cultural renaissance of public, maintainance of long term vaccination programmes and clinical improvements JE control seems to be impossible. Hence, for controlling JE, all risk factors including poverty, physical protection, close proximity to pigs, rice paddy fields and water birds, and vector population are to be controlled. To fight against the rising JE incidences CDC has recommended large scale vaccination of children vaccine for children for disease control and Prevention [258] Due to geographical, demographic, presence of vector and various hosts and climatic reasons viral encephalitis is endemic in 8 districts of Nepal, 24 districts in Shanxi Province of China 20 districts of India [258,259].

9.3. Water Management and Clean Agriculture

However, for JE control, flood water and irrigation management are important in Tarai regions of India and Nepal. If irrigation of field is avoided mainly in months of March, April and May it will lead to a significant cut down in vector population in Tarai regions. Moreover, a break in water cycle will break mosquito breeding which can indirectly manage JE virus transmission and infection rate very low. This is the time when due to temperature extremes both vector and non human-host population goes down. Very clearly, water does make revival of vector population and virus infection. However, artificial application of water to the land or soil can be reduced by adopting shifting agronomic practices. It will need improved varieties of agricultural crops, maintenance of landscapes, and re-vegetation of disturbed soils during periods of early summer. There should be landscape designing to flow out large portion of water through drainages. It will also help in natural or artificial removal of surface and sub-surface water from endemic areas in rainy months. Open water area in terraced rice fields (rice paddies), should not be over flooded, it must be drained out to control mass breeding of mosquitoes (Table 2). In addition, new varieties of paddy are to be adopted that can grow in short supply of water.

From various studies it become clear that to control water borne JE epidemics [261] and its expansion, [262,263] safe cleaner water supply should be provided through pipe line for community use. Further, testing of
JE virus in water supply should make more feasible to screen presence of virus pathogens and associating microbial contamination by using W.H.O recommended kits. Similarly, testing of chemical contaminants in water can limit adverse health concerns as it reports and direct use of water resources. Inadequate water, sanitation and hygiene continue to pose a major threat to human health. These risk factors contribute to millions of unnecessary deaths each year, including 1.8 million diarrheal related deaths in children less than 5 years of age. Further, occurrence of water borne JE cases can be controlled by making clean potable drinking water supply. Water should be treated at larger scale, distributed or either treated at local level. Moreover, by following Integrated vector management approach with AWDI and use of larvivorous fish in water ponds can reduce vector population, and can reduce the transmission level and JE burden in rural areas [264]. Hence, drinking-water quality improvement should be based on WHO Guidelines (IWA Bonn Charter) that recommend pro-active efforts to reduce risks and prevent contamination before water reaches the consumer. This can be achieved by shifting emphasis of drinking-water quality management to a holistic risk-based approach that covers the catchment-to-consumer (Table 2). Such an approach is called WSPs whose widespread implementation can reduce the global disease burden mainly attributed due to poor drinking-water and inadequate sanitation and hygiene. Therefore, if water management and socio-cultural methods are applied, these can automatically cut down >60% of JE cases. However, vector control in summer & winter season can cut down the disease burden. Furthermore, vaccination & disease surveillance would provide a direction to work for eradication of JE.

9.4. Socio-clinical Management

For achieving higher success not only to control JE but its other associating endemic diseases a platform should be made to ascertain the participation of politicians, economists, scientists, technologists, agriculturists, clinicians, researchers, local administration, regional civic bodies and people to find good solutions. There should be a holistic long term plan for eradication of disease. No doubt a progressive human and environmental relationship is to be established through national and international coordination for abatement of JE. It needs a fast action, new prevention strategies, improved health policies, long term funding, and adequate vaccine supply [265]. Further, to ensure fruitful outcomes of JE control societies should promote cleanliness drives in sub-urban and rural areas. Community centers should be established to function at three different levels i.e. clinical care of patients, immunization and rehabilitation of survivors. Each community centre might have at least 10 medical personnel’s with modern diagnostic facilities. These centers should also organize people’s awareness programs about vector control and sanitation. For having good out comes local population should be educated for making them aware of clean habitations, use of safe potable water, vector and host control. A community center may not have a peripheral area of not more than 20 km squares and it must be established in disease prone areas where population density and JE incidences is observed much higher than non diseased areas. Further, a transparent association and coordination is to be required among three different ministries i.e. Agriculture, Health and urban and rural planning to take the joint responsibility of JE control. There must be a joint action plant to be formed by including local administration, central government and W.H.O. to ensure large scale immunization, vector control and clean water supply in these areas. Further, to rehabilitate Acute Encephalitis Syndrome (AES) survivors long term clinical cares should be ascertained because poor people cannot afford treatment related expenses. Therefore, rationalization of initial expensive hospital treatments and financial aids to JE survivors will provide a high success rate [20]. Further, countries with limited resources should provide first priority in international support for surveillance and vaccination [6].

Further, to achieve fast control in endemic diseases like JE new alternatives of occupational farm yard practices should be searched. Therefore, piggeries and poultry farms should be replaced or shifted out of the endemic area. Further, to cut down the incidence rate of viral encephalitis special attention should be given on cleaner husbandry and a fast control of migratory water birds. Pig farming between the human habitations should be ceased immediately [266]. Other factors which are related JE virus infection in human are lack of cultural attributes i.e. type of living, sanitation standards, vector control, healthy animal breeding houses, proper surveillance, routine immunization and Coordinated Disease Controlling System and Net working in rural areas. Therefore, launching of sanitation and education programs is highly needful for JE vulnerable population [267]. Another part of JE control is related to risk management in JE virus infected human travelers. There should be total ban on intruders without vaccination. Hence, travelling advice, clinical consultations, quarantine and management of safe visits should be chalked out. There must be a prompted reassessment of vaccination, recommendations should made internationally to include a greater number of travelers and dwelling human population. [90] Further, for socio-clinical management of JE important parameters such as age, gender, economic class, and community and ecological should be included to find most affected or vulnerable class from surveillance data. Such studies could assist in finding vocational distribution of JE cases, its regional and seasonal distribution. These could also infer realistic post immunization successes of vaccine and also adjudge relationships among vectors, hosts, and JE virus isolates circulating in endemic areas [115]. Other important reasons such as economic breezes and non-policy management seem to be major controlling factor for infectious diseases. It is true that endemic areas in Southeast Asia are centered with poor; all around there is a lack of sanitation, hospitals, and clinical care and control of vector population (Figure 5). If cultural and socio-economic conditions are improved these could assist in JE control more positively. Although, JE is endemic mainly in tropical climate areas, but its existence and proliferation going on in temperate and cold climates mainly in plains, hills and valleys. No doubt management of cultural environment and socio-clinical ecology of the region will shorten the geographical range of endemic JE because ecologial poor may not necessarily poor in culture.
10. Conclusion

JE is a vector borne fatal disease whose outbreak occurs almost every year in Southeast Asian countries including India, Nepal and China. It is regularly spreading its extent due to demographic, environmental and therapeutic reasons. JE outbreak occurs almost every year among children in Southeast Asian countries. In India, infection rate is high that thousands of JE incidences/cases are reported from northeastern and southeast part of the country almost every year. Severity of infection and fatalities were observed very high in monsoon months in paddy growing districts of eastern Uttar Pradesh. New climate induced genotypes are detected in the blood samples of local population which re-circulate before arrival of rains and filling of water reservoirs with the rain water. The main cause of evolution of new strains is substitution mutations which are going on in the virus. These new environmentally adapted mutant strains accelerate the infection rate and cause very high mortality in rural areas of Northeastern parts of India [268]. Major reasons are presence of vector population and high annual precipitation cycles, demographic patters, alternate hosts, JE infected human population scattered in different states and regions [269]. Further, due to longer eco-climatic association of virus, presence of amplifying, revertants & human hosts eradication of JE seems to a very difficult task. In addition, presence of revertants in overlapping generations joins and re-organizes distanced epidemiology in the area. A complex process of anthropogenic driven deforestation, climatic changes brought on by El Nino-related drought, forest fire and severe haze, and ecological factors of mixed agro-pig farming practices and design of pig-sties led to the spillovers of the virus from its wildlife reservoir into pig population. In India, in last 10 years JE incidences have been alarmingly increased due to rising vector and vertebrate host population in paddy growing areas. Due to evolution of climate induced new mutant variants/strains of JE virus rate of mortality and morbidity has been increased in rural and sub-urban areas. These mutant variants/new genotypes are detected in the blood samples of local population which re-circulate before arrival of rains and filling of water reservoirs with the rain water. It has lead to induction of high sero-conversion rate in patients. Pig farming between the human habitations should be ceased immediately and these should be shifted far away from human habitations.

Beside, vaccine based therapeutics, both surveillance and diagnostic standards should be maintained for timely detection of disease and providing therapeutics to the patients. In addition, for over all control of JE as well as other communicable diseases prevailing in the same endemic region long term planning be needed with a JE control and warning organization. More attention should be paid for cultural control of virus applying cleanly drives, livestock and pig farm shifting, waste disposal, supply of safe drinking water, high standard medical facilities, bio-informatics facilities and proper and timely vaccination. Vaccination of travelers is highly essential because they remain at greater risk of JE virus infection. Hence, visits should be planned only after having proper vaccine administration based on the season, location, and duration of visit. Further, researches on molecular detection and genotyping of Japanese encephalitis virus in mosquitoes and host population, agronomic shifts and crop system replacement are essentially required. There must be a complete ban on entry of refugees in slums because these are store houses of viruses. However, to control the disease transmission by the vector must be minimized either by direct control of pesticides or by using repellent coated mosquito nets, clothes, safe window mesh and regular treatment of water storage tanks. Further, efforts should be made to control the JE infection below risk level by applying for good surveillance, regular potential immunization and JE awareness at national and international level. There is an utmost need to improve poor economy and socio cultural environment to manage the disease below risk level. Therefore, for total control of JE epidemic, a proper planning and its implementation by applying good surveillance standards, fast immune and serological diagnosis of disease, launching of sentinel programs are much needed for large scale immunization [270,271]. In addition, a plan should fulfill the development of clean and hygienic human habitats by control migration of rural people to urban areas, improvement of slums and dismantling refugee camps check illegal cross broader illegal human trafficking. For estimation of distribution of Japanese encephalitis virus in Asia ecological niche modeling and disease incidence and virus endemic to epidemic modeling is highly needful [272]. Though it is true that JE infection rate cannot become zero because of fast changing virus but a continuous and timely potential vaccine and cultural control can cut down the mortality and morbidity rate and maintain very low infection rate.

Conflict of Interests

Author has no conflict of interests. The author alone is responsible for the content and writing of the paper.

Abbreviations

JEV – Japanese Encephalitis virus, NS-Non structural proteins, CSF-Cerebrospinal fluid, VLPs, Virus like particles VAPs virus associating proteins, MABs-monoclonal antibodies, WNV-West Nile virus, NLP-nucleocapsid-like particles, FMDV- Foot and Mouth Disease Virus, JGMV-Johnson Grass Mosaic Virus, TCR-T Cell Receptor, CTL Cytotoxic Lymphocytes, CNS-Central nervous system.

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