American Journal of Infectious Diseases and Microbiology, 2015, Vol. 3, No. 1, 38-64
Available online at http://pubs.sciepub.com/ajidm/3/1/6

DOI:10.12691/ajidm-3-1-6

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

Ravi Kant Upadhyay*

Department of Zoology, D D U Gorakhpur University, Gorakhpur, U.P. India
*Corresponding author: rkupadhya@yahoo.com

Received December 24, 2014; Revised January 21, 2015; Accepted February 26, 2015

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 vey 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*

Cite This Article: Ravi Kant Upadhyay, "Epidemiology, Disease Transmission and Pathogenesis Caused by JE Virus: Its Prevention and Control." *American Journal of Infectious Diseases and Microbiology*, vol. 3, no. 1 (2015): 38-64. doi: 10.12691/ajidm-3-1-6.

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.

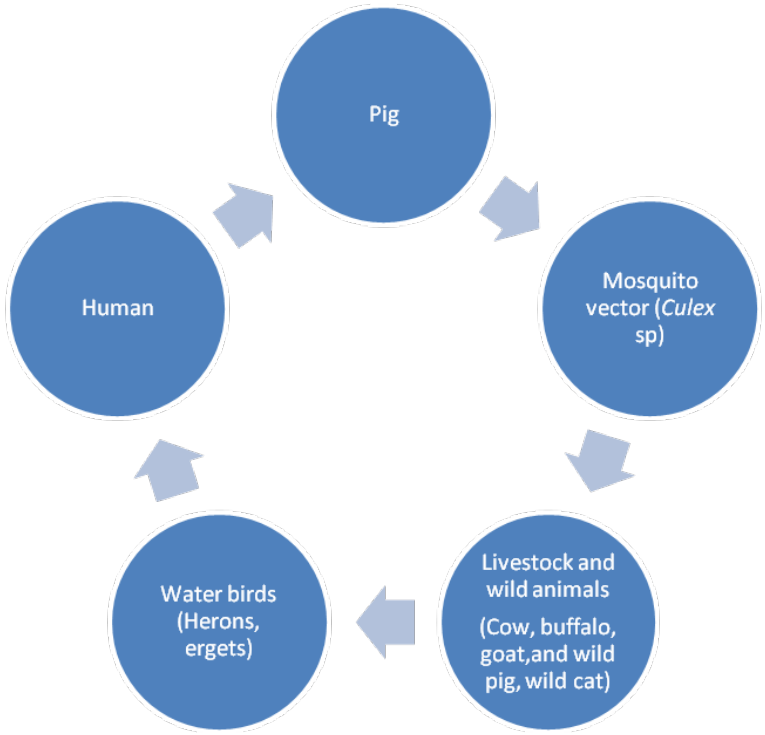

Figure 1. Circulation of JE virus in vertebrate hosts and mosquito vector in endemic area

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 Tarai 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 regimens 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

[8].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].

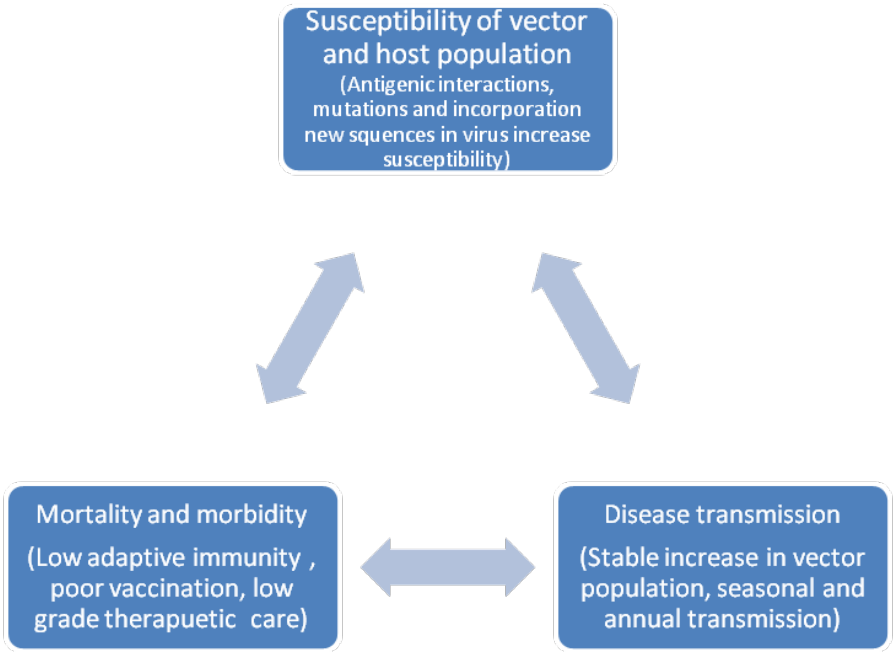

Figure 2. Inter-relationship of vector susceptibility, disease transmission and mortality and morbidity in endemic area. Climate induced substitutions in epitpoic regions of structural proteins in the genome favoring JE virus to maintain disease transmission, mortality and morbidity rate high by repetitive 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 40^0c 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 Plaines 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 *Cx. 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 EI 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 *Adedes 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 oakleyi*, *Aedes saipanensis*, *Culex annulirostris marianae*, and *Culex fuscanus*. But from the national and international data *Culex tritaeniorhychus* 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 sero-conversion 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

Natural	Year 1980-2010	Transmission rate	Disease incidence	Mortality rate	Morbidity rate	Net survival
Precipitation	980-1387*	3.5-23.7	2.95-22.75	1.94-6.14	79.2-94.7	0.65-2.54
Temperature	31.2-34.2	17.3-43.5	4.05-26.15	2.74-7.64	79.2-95.8	0.61-2.12
Humidity	69.8-76.3	18.4-39.3	3.25-21.66	1.94-6.86	78.2-95..5	0.65-2.51
Landscape	6%-28%	1.23%	23.14	NA	NA	NA
Floods	Almost every year	<43.2%	39.07	1.94-6.14	80.1-95.7	0.65-2.54
Wetlands	125	2.34-17.65%	NA	NA	NA	NA
Ground water	2.34-7.23 meters	2.14 times (50.718)	49.21	9.21	81.2-97.3	0.125-1.04
Carbon emissions	6% by 2008	VIN	VIN	VIN	VIN	VID
Agricultural (n)	11.2%	7.8-43.2%	5.3-37.2%	1.94-8.23	82.2-97.7	0.125-1.00
Forestry	23.0-16.5%	<17.65%	<18.65%	NA	NA	NA
Vector population	26.7-121.3/m2#	3.44 times (81.528)	<69.11%	1.94-8.49	88.2-98.03	0.125-1.00
Anthropogenic						
Population size	110-2,399/ km^2	<1.34%	NA	NA	Na	NA
Human traveling	11230-42370	16.54%	14.23%	1.96-6.23	81.2-97.3	0.125-1.00
Human settlement	12.37%	23.42%	22.81%	1.96-7.10	80.1-95.7	0.125-1.00
Slums	230%	22.76%	22.14%	9.21	81.2-96.5	0.125-1.00
Refugee camps	110%	26.32%	25.11%	9.21	88.2-98.03	0.125-1.00
Tribals	7.01-2.175	19.23%	18.15%	2.74-7.64	81.2-97.3	0.125-1.00
Pig Farming	2.3-39.2%	3.65 times (86.505)	<70.9%	2.93-8.29	88.2-96.4	0.125-1.00
Paddy Farming	38.6-67.23%	8.8-47.3%	6.7-43.2%	2.04-8.23	88.2-96.4	0.125-1.00
Poultry farms	4.37-31.86%	C* 34.7%	N	N	p*78.3	0.125-1.00
Livestock	16.43-34.65%	13.65	12.71	1.94-6.86	p*76.4	0.125-1.00
Human wastes	56.8 times	77.65	<76.2%	2.74-7.64	P*71.2	0.125-1.00
Pest resurgence	13.4	SI	SI	SI	CR 88.2-98.03	0.125-0.623
Education	37.0-86.55%	N	N	N	N	UPD
Therapeutic	Nil-4.35%	1.23%	2.3	1.0-6.86	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 but not estimated, VID=visible decreased but 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 (GIII) 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 mosquitoes 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 have 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 un-predictable 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, hippocampi 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 CLEC5A/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, hemiperesis, 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 palsies are clearly visualized in acute encephalitic syndrome (AES). JE Virus imposes permanent irreversible changes like residual neuropsychiatic and neurological features in

the survivors [97]. Children infected with JE virus showed acute limb paralysis or neck stiffness and later on they become enephalopathic 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 sero-conversion 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].

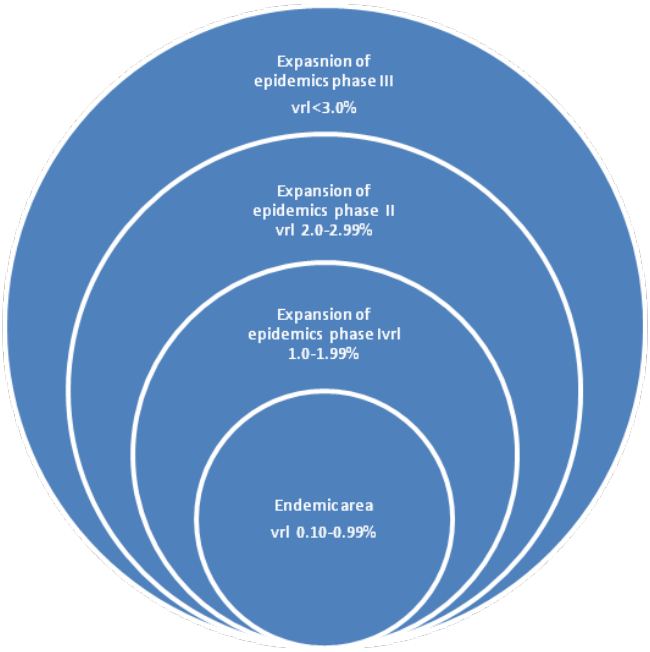

Figure 3. Figure showing phase vise expansion of JE virus disease out of endemic zoographic ranges. Expansion is equivalent to level of variations obtained in JEV genotypes. Mass scale genome sequences and their comparisons have given three different levels of climate induced genomic variations (0.1-3.0% or more) that has created five different genotypes GI, GII, GIII, GIV, G V 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 endemicity is very high. Similarly, health-care providers and international travelers should have appropriate JE preventive vaccines and health guidance before traveling

[136,137]. 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 pathogenecity 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 cytotoxicity (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 neurovirulence [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 Beijing-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 2. Demographic data on JE prone endemic area of eastern Uttar Pradesh India

District	Area	Population	Density	Literacy rate	Population growth*	Annual precipitation
Siddharthnagar	2,752 km^2	2,553,526	882/km^2	67.81 per cent%	25.17%	897.8 mm.
Kushinagar	2,873.5 km^2	3,560,830	1,200/km^2 U4.87 per cent	67.66 per cent	23.08%	904 mm
Maharajganj	2,934.1 km^2	2,665,292	910/km^2	64.3	22.61%	1166 mm
Basti	1,036 km^2 (	114,651	110/km^2	59.5%	13%	904 mm
Santkabir nagar	1,659.15 km^2	1,714,300	1,041 /km^2	69.01	20.71%	808.
Gorakhpur	7,483.8 km^2	4,436,275	1,336 /km^2	73.25%	17.69%.	803.5 mm
Mau	1,713 km^2	2,205,170	1,300/km^2	75.16 per cent	18.94%	904 mm
Deoria	2,535 km^2	3,098,637	1,200/km^2	73.53	14.23%	864.38 mm
Padrauna	2,535 km2	N	N	63%	N	1,018 mm
Azamgarh	3,054 km^2	4,616,509	1,500/km^2	72.69%	17.17%	1,110 mm
Ghazipur	3,384 km^2	3,622,727	1,100/km^2	74.27%.	19.26%	1034 mm
Jaunpur	4,038 km^2	4,476,072	1,100/km^2	73.66%	14.89	987 mm
Varanasi	4,535 km^2	3,676,841	2,399/km^2	77.05%	17.32%.	1608.9mm
Ballia	3168	3,223,642	1,081/km^2	86.65%	11.00%	983 mm

Population growth rate is based on 2001-2011census. 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 and its 2 X 6 microgram dose regimen is used to safe vaccination of children [191]. It has shown very high sero-conversion rate and shows better safety in mice and non human primates after immunization [192]. In man, it provides better preclinical safety, immunogenicity and protective efficacy than any other vaccine. Further, Biken strain based vaccine named as JE-Vax, was developed in United States, Centers for Disease Control and Prevention and is widely used in western countries [193]. Similarly, a recombinant chimeric Japanese encephalitis virus/tick borne encephalitis virus vaccine was generated by attenuation [194]. For this purpose Japanese encephalitis virus vaccine strain SA14-14-2 was used as backbone which was found highly immunogenic and protective against JE virus infection in mice and nonhuman primates [195]. This freeze-dried, cell culture-derived Japanese encephalitis vaccine (inactivated) has shown superior immunogenicity [196]. 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 reactinogenecity 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 vaccines) [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

IXIARO 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 spectrally 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 P_3 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 [236]. Before preparing a peptide vaccine it is very essential to identify the peptide sequences that trigger a protective 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 reactiongenicity 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 envelop E protein mammalian cells are used to yield more amount of these proteins. Recently, insect cell expression system with Sf_9 cells to generate viral antigens such as JE virus envelops 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, immune-stimulating 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 mimmotopes 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 bio-informatics analysis of epidemiological data showed different patterns of transmission, host selection, endemicity and infection rate, circulation of different genotypes, and evolution of various mutant 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 GIII, 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 GI(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 neurovirulent genes 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

(Figure 3) [253]. 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.

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

Genotype Location	Epitope	Method used	JEV surveillance	Lethality reported
G I Japan	E 327 Glu 20.2-21.2 8.5-9.9	Multiplex RT-PCR	Mosquito based	High
G IChina, Korea, Cambodia , Thailand, Australia	prM and E glycoprotein genes	RT-PCR	Mosquito based	High
G II Australia Malaysia, Indonesia	prM and E glycoprotein genes	RT-PCR	Pigs	High
GIII Taiwan	E protein	Multiplex RT-PCR	Mosquito-based	High
GI-III Asia and Australia	E176, 177,227, 244, 264	Amino acid sequencing	Mosquito based	Higher
G III West Bengal India	Envelope proteins	Mac-ELISA and RT-PCR	Human	Higher
G III Northern India	E 176, 177,227,244,264, 279	Gene sequencing	Human	Highest
GIII Chongqing, China	CQ11-66 strain, E- genes	Nested PCR	Human	Highest
GIII Shanghai, China, Wuhan, Nepal, Taiwan, Japan, Phillipines, Korea, Sichuan, Yunan	Envelope proteins	RT-PCR	Human	Highest
GIV Indonesia-Malaysia region, Indonesia	Six AA substitutions	Amino acid sequencing	Mosquito based	Higher
GV Malaysia China and South Korea	Six AA substitutions	Amino acid sequencing	Mosquito based	Higher
G V Singapore, Australia	Structural genes	Nucleotide and AA sequencing	Mosquito based	Mild
G V Japan	Structural genes	Nucleotide sequencing	Mosquito based	Mild

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 genotype in new locations (Figure 3). Meanwhile, locally adapted mutant strains are finding its way through virus vectors and different types of migratory water birds to newer areas. These infected water birds are working as flying cargos of JE virus, which can land anywhere in similar eco-climatic areas and virus genotypes, are easily transferred/distributed by the mosquitoes/vectors to the human through occasional bites. Further, in new locations virus has started establishing accordingly by making most required necessary changes in epitopic region, which govern the neurovirulence. Therefore, strength of vaccines should be improved for disease control and prevention, with wider immunization coverage and control of secondary hosts of JE virus. It must be re-evaluated from time to time to lower down the risk for occurrence and reemergence of JE virus in rural pockets. Similarly, human migration is also supporting the expansion of endemicity.

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 alarmingly. 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 must be minimized either by using broad spectrum pesticides or by using repellent coated mosquito nets, clothes, safe window mesh and regular treatment of water storage tanks. 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).

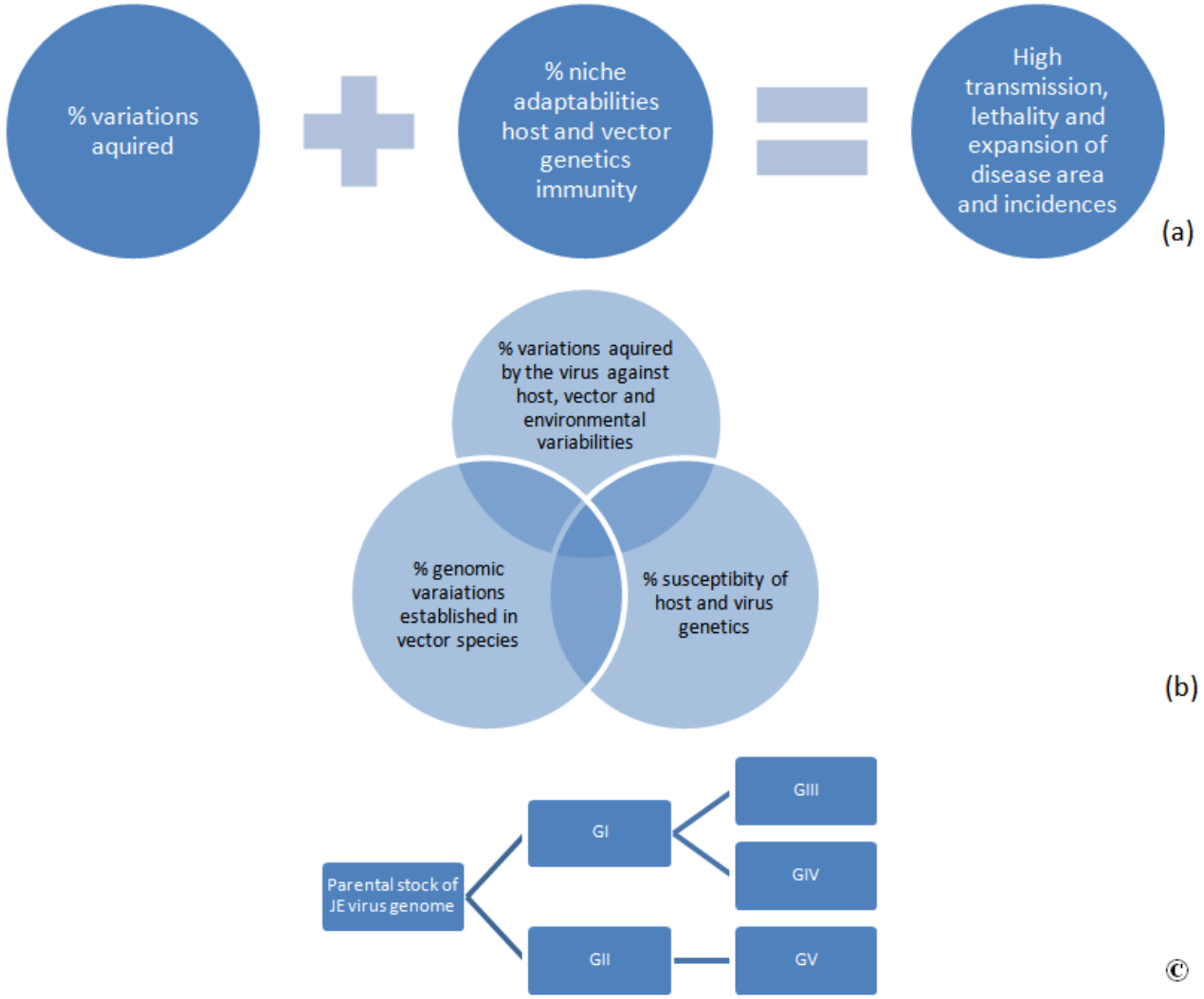

Figure 4. (a) showing reasons of high transmission, lethality and expansion of disease area and incidences, (b) inter-relationships of genomic variations, micro niche and % susceptibility of host and virus genetics and (c) formation of different JEV genotypes GI, GII, GIII, GIV, G V from parental stock

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 breading 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

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).

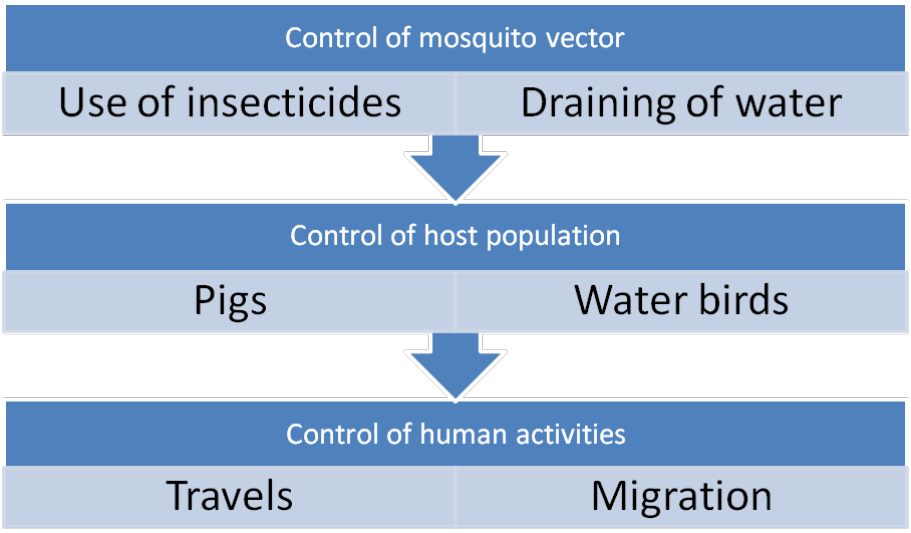

Figure 5. Tripartite arrangement for control of JE virus infection in endemic-eco-climatic zone

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, maintainace 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 diagnotistic 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 about clean habitations, use of safe potable water, vector and host control. A community centre 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 ecological 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 EI 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.

References

[1] Go, Y.Y., Balasuriya, U.B., Lee, C.K., (2014).Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. *Clin Exp Vaccine Res*.; Vol. 3 no.1, pp. 58-77.

[2] Pujhari, S.K., Prabhakar, S., Ratho, R.K., Modi, M., Sharma, M., Mishra, B., (2011).A novel mutation (S227T) in domain II of the envelope gene of Japanese encephalitis virus circulating in North India. *Epidemiol Infect*; Vol. 139, no.6, pp.849-56.

[3] Li, S., Fang, M., Zhou, B., Ni, H., Shen, Q., Zhang, H., Han, Y., Yin, J., Chang, W., Xu, G., Cao, G., (2011). Simultaneous detection and differentiation of dengue virus serotypes 1-4, Japanese encephalitis virus, and West Nile virus by a combined reverse-transcription loop-mediated isothermal amplification assay. *Virol J*; Vol. 8, pp.360.

[4] Kabilan, L., (2004).Control of Japanese encephalitis in India: a reality. *Indian J Pediatr* ; Vol. 71, no.8, pp.707-12.

[5] Arai, S., Matsunaga, Y., Takasaki, T., Tanaka-Taya, K., Taniguchi, K., Okabe, N., Kurane, I., (2008). Vaccine Preventable Diseases Surveillance Program of Japan. japanese encephalitis: surveillance and elimination effort in Japan from 1982 to 2004. *Jpn J Infect Dis* ; Vol. 61, no.5, pp.333-8.

[6] Centers for Disease Control and Prevention (CDC). (2013).Use of Japanese encephalitis vaccine in children: recommendations of the advisory on immunization practices, *MMWR Morb Mortal Wkly Rep.* Vol. 62, no. 45, pp.898-900,

[7] Impoinvil, D.E., Ooi, M.H., Diggle, P.J., Caminade, C., Cardosa, M.J., Morse, A.P., Baylis, M., Solomon, T., (2013).The effect of vaccination coverage and climate on Japanese encephalitis in Sarawak, Malaysia. *PLoS Negl Trop Dis* ; Vol. 7, no.8, pp.2334.

[8] Chua, K.B., (2010).Risk factors, prevention and communication strategy during Nipah virus outbreak in Malaysia. *Malays J Pathol.* Vol. 32, no.2, pp.75-80.

[9] Borah, J., Dutta, P., Khan, S.A., Mahanta, J., (2013).Association of weather and anthropogenic factors for transmission of Japanese encephalitis in an endemic area of India. *Ecohealth*; Vol. 10, no. 2, pp.129-36.

[10] Rodhain, F., (2010). Japanese encephalitis: a fast-changing viral disease. *Bull Soc Pathol Exot* ; Vol. 103, no. 3, pp. 135-54.

[11] Gould, E.A., Solomon, T., Mackenzie, J.S., (2008).Does antiviral therapy have a role in the control of Japanese encephalitis? *Antiviral Res*; Vol. 78, no. 1, pp. 140-9.

[12] Witt, C.J., Richards, A.L., Masuoka, P.M., Foley, D.H., Buczak, A.L., Musila, L.A., 2011The AFHSC-Division of GEIS Operations Predictive Surveillance Program: a multidisciplinary approach for the early detection and response to disease outbreaks. *BMC Public Health.* Vol. 11 no.2, pp.S10.

[13] Samuel, P.P., Muniaraj, M., Thenmozhi, V., Tyagi, B.K., (2013). Entomo-virological study of a suspected Japanese encephalitis outbreak in Muzaffarpur district, Bihar, India. *Indian J Med Res* ; Vol. 137, no. 5, pp.991-2.

[14] Mackenzie, J.S., Williams, D.T., (2009).The zoonotic flaviviruses of southern, south-eastern and eastern Asia, and Australasia: the potential for emergent viruses. *Zoonoses Public Health*; Vol.56, no.6-7, pp.338-56.

[15] Impoinvil, D.E., Solomon, T., Schluter, W.W., Rayamajhi, A., Bichha, R.P., Shakya, G., Caminade C., Baylis, M., (2011).The spatial heterogeneity between Japanese encephalitis incidence distribution and environmental variables in Nepal. *PLoS One.* Vol.6, no.7, pp. e22192,

[16] Miller, R.H., Masuoka, P., Klein, T.A., Kim, H.C., Somer, T., Grieco, J., (2012). Ecological niche modeling to estimate the distribution of Japanese encephalitis virus in Asia. *PLoS Negl Trop Dis* ; Vol. 6, no.6, pp. 1678.

[17] Dash, A.P., Bhatia, R., Sunyoto, T., Mourya,D.T., (2013).Emerging and re-emerging arboviral diseases in Southeast Asia. *J Vector Borne Dis*: Vol.50, no.2, pp.77-84.

[18] Dumre, S.P., Shakya, G., Na-Bangchang, K., Eursitthichai, V., Rudi Grams, H., Upreti, S.R., Ghimire, P., KC, K., Nisalak, A., Gibbons, R.V., Fernandez, S., (2013).Dengue virus and Japanese encephalitis virus epidemiological shifts in Nepal: a case of opposing trends. *Am J Trop Med Hyg*; Vol. 88, no.4, pp.677-80.

[19] Tang, X.Y., Kang, K., Shang, S.Y., Xu, C., Li, X.L., Ma, H.X., Chen, H.M., Xu, B.L., (2012). Epidemic characteristics and viral antibody level among healthy people of Japanese B encephalitis in Henan province in 2010. *Zhonghua Yu Fang Yi Xue Za Zhi*; Vol.46, no.10, pp.888-91.

[20] Griffiths, M.J., Lemon, J.V., Rayamajhi, A., Poudel, P., Shrestha, P., Srivastav, V., Kneen,R., (2013). Medina-Lara,A., Singh, R.R., Solomon,T., The functional, social and economic impact of acute encephalitis syndrome in Nepal--a longitudinal follow-up study, *PLoS Negl Trop Dis.* Vol.7, no. (9), pp.e2383.

[21] Gould, E.A., Solomon, T., Mackenzie, J.S., (2008).Does antiviral therapy have a role in the control of Japanese encephalitis? *Antiviral Res*; Vol.78, no.1, pp.140-9.

[22] Centers for Disease Control and Prevention (CDC). (2013).Use of Japanese encephalitis vaccine in children: recommendations of the advisory committee on immunization practices, 2013. *MMWR Morb Mortal Wkly Rep* ; Vol. 62, no.45, pp.898-900.

[23] Tyler, K.L., (2009). Emerging viral infections of the central nervous system: part 1. *Arch Neurol.*; Vol. 66, no.8, pp.939-48.

[24] Hiscox, A., Winter, C.H., Vongphrachanh, P., Sisouk, T., Somoulay, V., Phompida, S., Kaul,S., Sananikhom, P., Nguyen, T.Y., Paul, R.E., Brey, P., Bryant, J.E., (2010).Serological investigations of flavivirus prevalence in Khammouane Province, Lao People's Democratic Republic, 2007-2008. *Am J Trop Med Hyg*, vol.83. no.5,pp.1166-9.

[25] Erlanger, T.E., Weiss, S., Keiser, J., Utzinger, J., Weidenmayer, K., (2009). Past, present, and future of Japanese encephalitis, Emerg Infect Dis, vol.15, no.1, pp.1-7.

[26] Nett, R.J., Campbell, G.L., Reisen, W.K., (2009).Potential for the emergence of Japanese encephalitis virus in California. *Vector Borne Zoonotic Dis* ; Vol. 9, no. 5, pp.511-7.

[27] Masuoka, P., Klein, T.A., Kim, H.C., Claborn, D.M., Achee, N., Andre, R., Chamberlin J., Small, J., Anyamba, A., Lee, D.K., Yi, S.H., Sardelis, M., Ju, Y.R., Grieco, J., (2010). Modeling the distribution of *Culex tritaeniorhynchus* to predict Japanese encephalitis distribution in the Republic of Korea, *Geospat Health.* Vol.5, no.1, pp. 45-57.

[28] Verma, R., Praharaj, H.N., (2012). Bilateral facial palsy as a manifestation of Japanese encephalitis. *BMJ Case Rep*.

[29] Arunachalam, N., Samuel, P.P., Hiriyan, J., Rajendran, R., Dash, A.P., (2005).Short report: observations on the multiple feeding behavior of *Culex tritaeniorhynchus* (Diptera: culicidae), the vector of Japanese encephalitis in Kerala in southern India. *Am J Trop Med Hyg*, Vol.72, no.2, pp.198-200.

[30] Mitchell, C.J., Savage, H.M., Smith, G.C., Flood, S.P., Castro, L.T., Roppul, M., (1993). Japanese encephalitis on Saipan: a survey of suspected mosquito vectors. *Am J Trop Med Hyg* ; Vol. 48, no. 4, pp.585-90.

[31] Hasegawa, M., Tuno, N., Yen, N.T., Nam, V.S., Takagi, M., (2008).Influence of the distribution of host species on adult abundance of Japanese encephalitis vectors *Culex vishnui* subgroup and *Culex gelidus* in a rice-cultivating village in northern Vietnam. *Am J Trop Med Hyg* ; Vol. 78, no.1, pp.159-68.

[32] Takhampunya, R., Kim, H.C., Tippayachai, B., Kengluecha, A., Klein, T.A., Lee, W.J., Grieco, J., Evans, B.P., (2011). Emergence of Japanese encephalitis virus genotype V in the Republic of Korea. *Virol J*; Vol. 8, pp.449.

[33] Samuel, P.P., Arunachalam, N., Rajendran, R., Leo, S.V., Ayanar, K., Balasubramaniam, R., Tyagi, B.K., (2010). Temporal variation in the susceptibility of Culex tritaeniorhynchus (Diptera: Culicidae) to Japanese encephalitis virus in an endemic area of Tamil Nadu, South India. *Vector Borne Zoonotic Dis* ; Vol. 10, no.10, pp.1003-8.

[34] Partridge, J., Ghimire, P., Sedai, T., Bista, M.B., (2007).Banerjee, M., Endemic Japanese encephalitis in the Kathmandu valley, Nepal. *Am J Trop Med Hyg*; Vol. 77, no.6, pp.1146-9.

[35] Gupta, N., Chatterjee, K., Karmakar, S., Jain, S.K., Venkatesh, S., Lal, S., Bellary, (2008).India achieves negligible case fatality due to Japanese encephalitis despite no vaccination: an outbreak investigation in 2004. *Indian J Pediatr*. Vol. 75, no. 1, pp.31-7.

[36] Oya, A., (2007).Kurane IJapanese encephalitis for a reference to international travelers. *J Travel Med.*; Vol. 14, no. 4, pp. 259-68.

[37] Lindahl, J.F., Ståhl, K., Chirico, J., Boqvist, S., Thu, H.T., Magnusson, U., (2013).Circulation of Japanese encephalitis virus in pigs and mosquito vectors within Can Tho city, Vietnam. *PLoS Negl Trop Dis*; Vol. 7, no. 4, pp. 153.

[38] Joshi, R., Kalantri, S.P., Reingold, A., Colford Jr.J.M., (2012). Changing landscape of acute encephalitis syndrome in India: a systematic review. *Natl Med J India*; Vol. 25, no. 4, pp. 212-20.

[39] Le Flohic, G., Porphyre, V., Barbazan, P., Gonzalez, J.P., (2013).Review of climate, landscape, and viral genetics as drivers of the Japanese encephalitis virus ecology. *PLoS Negl Trop Dis* ; Vol. 7, no. 9 pp. e2208.

[40] Borah, J., Dutta, P., Khan, S.A., Mahanta, J., (2013).Association of weather and anthropogenic factors for transmission of Japanese encephalitis in an endemic area of India. *Ecohealth*; Vol.10, no. 2. Pp.129-36.

[41] Feng, Y., Fu, S., Zhang, H., Petersen, L.R., Zhang, B., Gao, X., Yang, W., Zhang, Y., Dao, B., Li, K., Li, N., Yin, Z., Liu, Y., Nasci, R., Wang, H., Liang, G., (2013). High incidence of Japanese encephalitis, southern China. *Emerg Infect Dis*; Vol. 19, no. 4, pp.672-3.

[42] Kline, K., McCarthy, J.S., Pearson, M., Loukas, A., Hotez, P.J., (2013). Neglected tropical diseases of Oceania: review of their prevalence, distribution, and opportunities for control. *PLoS Negl Trop Dis*; Vol. 7, no. 1, pp.:e1755.

[43] Spissu, N., Panichi G., Montisci, A., Fiore, F., (2013). West Nile Virus outbreak in Sardinia, Italy, in 2011. *J Infect Dev Ctries*; Vol. 7, no. 1, pp.6-9.

[44] Nemeth N., Bosco-Lauth A., Oesterle P., Kohler D., Bowen R.. (2012).North American birds as potential amplifying hosts of Japanese encephalitis virus. *Am J Trop Med Hyg*; Vol. 87, no.4, pp. 760-7.

[45] Conlan J.V., Vongxay K., Jarman R.G., Gibbons R.V., Lunt R.A., Fenwick S., Thompson R.C., Blacksell.S.D. (2012). Serologic study of pig-associated viral zoonoses in Laos. *Am J Trop Med Hyg*; Vol. 86, no. 6, pp. 1077-84.

[46] Yeh J.Y., Park J.Y., Ostlund E.N. (2011). Serologic evidence of West Nile Virus in wild ducks captured in major inland resting sites for migratory waterfowl in South Korea. *Vet Microbiol*; Vol.154, no.1-2, pp. 96-103.

[47] May F.J., Davis C.T., Tesh R.B., Barrett A.D.. (2011). Phylogeography of West Nile virus: from the cradle of evolution in Africa to Eurasia, Australia, and the Americas. *J Virol* ; Vol.85, no. 6, pp. 2964-74.

[48] Ciota A.T., Koch E.M., Willsey G.G., Davis L.J., Jerzak G.V., Ehrbar D.J., Wilke C.O., Kramer L.D. (2011). Temporal and spatial alterations in mutant swarm size of St. Louis encephalitis virus in mosquito hosts. *Infect Genet Evol*; Vol. 11, no.2, pp. 460-8.

[49] van den Hurk, A.F. Hall-Mendelin S., Johansen C.A., Warrilow D., Ritchie S.A.. (2012). Evolution of mosquito-based arbovirus surveillance systems in Australia. *J Biomed Biotechnol*; 2012:325659.

[50] Konishi E., Kitai Y., Nishimura K., Harada S.. (2012)Follow-up survey of Japanese encephalitis virus infection in Kumamoto Prefecture, South-West Japan: status during 2009-2011. *Jpn J Infect Dis*; Vol. 65, no. 5, pp. 448-50.

[51] Carlton E.J., Liang S., McDowell J.Z., H. Li, Luo W., Remais J.V.. (2012). Regional disparities in the burden of disease attributable to unsafe water and poor sanitation in China. *Bull World Health Organ*; Vol. 90, no.8, pp. 578-87.

[52] Teng M., Luo J., Fan J.M., Chen L., Wang X.T., Yao W., Wang C.Q., Zhang G.P.. (2013).Molecular characterization of Japanese encephalitis viruses circulating in pigs and mosquitoes on pig farms in the Chinese province of Henan.*Virus Genes*;Vol. 46, no.1,pp.170-4.

[53] Mishra N., Kalaiyarasu S., Nagarajan S., Rao M.V., George A., Sridevi R., Behera S.P., Dubey S.C., McCracken T., Newman S.H.. (2012). Serological evidence of West Nile virus infection in wild migratory and resident water birds in Eastern and Northern India. *Comp Immunol Microbiol Infect Dis* ; Vol. 35, no.6, pp.591-8.

[54] Kurane I., Shibasaki K., Kotaki A., Hijioka Y., Takasaki T.. (2013).The effect of precipitation on the transmission of Japanese encephalitis (JE) virus in nature: a complex effect on antibody-positive rate to JE virus in sentinel pigs. *Int J Environ Res Public Health*; Vol. 10, no. 5, pp. 1831-44.

[55] Schuster G., Ebert E.E., Stevenson M.A., Corner R.J., Johansen C.A.. (2011). Application of satellite precipitation data to analyse and model arbovirus activity in the tropics. *Int J Health Geogr*; Vol.10, pp. 8.

[56] Schuh J. A., Melissa J.. Ward, Andrew J., Leigh Brown, and Alan D.T. Barrett, (2013).Phylogeography of Japanese Encephalitis Virus: Genotype Is Associated with Climate *PLoS Negl Trop Dis*, vol. 7, no. 8, pp. e2411.

[57] Li M.H., Fu S.H., Chen W.X., Wang H.Y., Guo Y.H., (2011).Genotype v Japanese encephalitis virus is emerging, *PLoS Neglected Trop D* vol.5, pp. e1231.

[58] Morita K., (2009).Molecular epidemiology of Japanese encephalitis in East Asia. *Vaccine*, 27, pp. 7131-7132.

[59] Sarkar A., Banik A., Pathak B.K., Mukhopadhyay S.K., and Chatterjee S., (2013). Envelope protein gene based molecular characterization of Japanese encephalitis virus clinical isolates from West Bengal, India: a comparative approach with respect to SA14-14-2 live attenuated vaccine strain, *BMC Infect Dis. Vol.*13, no. 1, pp. 368.

[60] Li-Juan Xu, Ruixi Liu, Sheng Ye, Hua Ling and Chao-Min Zhu (2013).Genomic analysis of a newly isolated of Japanese encephalitis virus strain, CQ11-66, from a pediatric patient in China. Virology Journal, vol.10, no. 101.

[61] A., Pyke, D Williams, Nisbet D., (2001).The appearance of a second genotype of Japanese encephalitis virus in the Australasian region. *Am J Trop Med Hyg* vol.65, pp.747-753.

[62] Wang H, Li Y, Liang X, Liang G, (2009).Japanese encephalitis in mainland china. *Japan J Infect Dis*, vol. 62, pp. 331-336.

[63] Tiwari S.C., Thenmozhi V., Arunachalam N., Samuel P.P, Tyagi B.K., (2008). Desiccated vector mosquitoes used for surveillance of Japanese encephalitis virus activity in endemic southern India. Trop. Med Int Health, Vol. 13, no. 2, pp. 289-290.

[64] Wang L., Fu S., Zhang H., Ye X., Yu D., Deng Z., Yuan J., Zhai Y., Li M., Lv Z., Chen W., Jiang H., Gao X., Cao Y., Wang H., Tang Q., Liang G.. (2010). Identification and isolation of Genotype-I Japanese encephalitis virus from encephalitis patients. *Virol J*; Vol.7, pp.345.

[65] Dhillon G.P., Raina V.K. (2008).Epidemiology of Japanese encephalitis in context with Indian scenario. *J Indian Med Assoc* ; Vol.106, no.10, pp.660-3.

[66] Alera M.T., Velasco J.M., Ypil-Cardenas, C.A. Jarman R.G., Nisalak A.N., Thaisomboonsuk B., Gibbons R.V., Dimaano E.M., Yoon I.K. (2013).Hospital-based surveillance of Japanese encephalitis at a tertiary hospital in Manila. *Southeast Asian J Trop Med Public Health*.;Vol. 44, no.5, pp. 791-8.

[67] Yen N.T., Duffy M.R., Hong N.M., Hien N.T., Fischer M., Hills S.L. 2010).Surveillance for Japanese encephalitis in Vietnam, 1998-2007. *Am J Trop Med Hyg.*;Vol. 83, no.4, pp.816-9.

[68] Hu Q., Chen B., Zhu Z., J Tian., Zhou Y., Zhang X., Zheng X.. (2013).Recurrence of Japanese encephalitis epidemic in Wuhan, China, 2009-2010. *PLoS One*; Vol. 8, no. 1, pp. e52687.

[69] Wang X.X., Jiang J.X., Wang F., Zhang X.S., Yu D.S., Wang P., Zhao H., Li H. (2012). Analysis on epidemiology and the main clinical symptoms of viral encephalitis in Gansu, 2009-2011. *Zhonghua Yu Fang Yi Xue Za Zhi*; Vol 46, no.12, pp.1099-102.

[70] Zhang J.K., Lin S.H., Zhou X.Y., Chen D.L., Fu S.H., Liu X.C., Yuan W., Cao Y.O., Wang H.Y., Li W., Hu M., Liang G.D.. (2012). Japanese encephalitis virus with genotype I is predominant in Sichuan Province. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*; Vol.26, no.4, pp.241-5.

[71] Borah J., Dutta P., Khan S.A., Mahanta J.. (2012). Epidemiological concordance of Japanese encephalitis virus infection among mosquito vectors, amplifying hosts and humans in India. *Epidemiol Infect*; Vol.141, no.1, pp.74-80.

[72] Hoshino A., Saitoh M., Oka A., Okumura A., Kubota M., Saito Y., Takanashi J., Hirose S., Yamagata T., Yamanouchi H., Mizuguchi M.. (2011). Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. *Brain Dev*; Vol. 34, no.5, pp.337-43.

[73] Baba K., Okuno Y., Tanaka-Taya K., Okabe N.. (2011).Immunization coverage and natural infection rates of vaccine-preventable diseases among children by questionnaire survey in 2005 in Japan. *Vaccine*; Vol.29, no.16, pp.3089-92.

[74] Okabe N., Y Tada., Yasui Y.. (2011). Epidemiology of acute encephalitis or encephalopathy in Japan. *Nihon Rinsho* ; Vol. 69, no.3, pp.411-6.

[75] García-Bocanegra I., Busquets N., Napp S., Alba S., Zorrilla I., Villalba R., Arenas (2011). A Sero survey of West Nile virus and other flaviviruses of the Japanese encephalitis antigenic complex in birds from Andalusia, southern Spain. *Vector Borne Zoonotic Dis*; Vol. 11, no.8, pp.1107-13.

[76] Nishiyama I., Ohtsuka Y., Tsuda T., Kobayashi K., Inoue H., Narahara K., Shiraga H., Kimura T., Ogawa M., Terasaki T., Ono H., Takata T.. (2011).An epidemiological study of children with status epilepticus in Okayama, Japan: incidence, etiologies, and outcomes. *Epilepsy Res*; Vol.96, no. 1-2, pp.89-95.

[77] Rascalou G., Pontier D., Menu F., Gourbière S.. (2012).Emergence and prevalence of human vector-borne diseases in sink vector populations. *PLoS One*; Vol. 7, no.5, :e36858.

[78] Coffey L.L., Page B.L., Greninger A.L., Herring B.L., Russell R.C., Doggett S.L., Haniotis J., Wang C., Deng X., Delwart E.L.., (2014).Enhanced arbovirus surveillance with deep sequencing: Identification of novel rhabdoviruses and bunyaviruses in Australian mosquitoes. *Virology*; Vol. 448, pp.146-58.

[79] Upadhyay R.K., (2013).Japanese Encephalitis virus generated neurovirulence, antigenicity and host immune responses, *ISRN Virology*, Vol. 2013, ID830396.

[80] Wang S., Luo X., Wei W., Zheng Y., Dou Y., Cai X.. (2013).Calculation of evolutionary correlation between individual genes and full-length genome: a method useful for choosing phylogenetic markers for molecular epidemiology. *PLoS One*.;Vol. 8, no.12:e81106.

[81] Upadhyay R.K. (2013). Biomarkers in Japanese encephalitis: a review. *Biomed Res Int*. 2013:591290.

[82] Koga M.. (2013).Bickerstaff brainstem encephalitis: epidemiology, diagnosis, and therapy. *Nihon Rinsho* ; Vol. 71, no.5, pp.898-903.

[83] Sambri V., Capobianchi M., Charrel R, Fyodorova M., Gaibani P., Gould E., Niedrig M., Papa A., Pierro A., Rossini G., Varani S.,

Vocale C., Landini M.P., (2013). West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention. *Clin Microbiol Infect*;Vol.19, no.8, pp.699-704.

[84] Saxena S.K., Mishra N., Saxena R., Singh M., Mathur A.. (2009).Trend of Japanese encephalitis in North India: evidence from thirty-eight acute encephalitis cases and appraisal of niceties. *J Infect Dev Ctries* ; Vol.3, no.7, pp.517-30.

[85] OlsenS.J., Supawat K., Campbell A.P., Anantapreecha S., LiamsuwanS., Tunlayadechanont S., Visudtibhan A., Lupthikulthum S., (2010). Japanese encephalitis virus remains an important cause of encephalitis in Thailand. *Int J Infect Dis*; Vol.14, no.10,:e888-92.

[86] Pradier S., Lecollinet S., Leblond A. (2012).West Nile virus epidemiology and factors triggering change in its distribution in Europe. *Rev Sci Tech*;Vol. 31, no.3, pp.829-44.

[87] An Z.J., Ma Y.J., Bo F., Huang H., Zhang S.T. (2007). Analysis on surveillance data regarding the level of Japanese encephalitis antibody in Heilongjiang. province, 2006. *Zhonghua Liu Xing Bing Xue Za Zhi* ; Vol. 28, no.12, pp.1186-9.

[88] Kakkar M., Rogawski E.T., Abbas S.S., Chaturvedi S., Dhole T.N., Hossain S.S., Krishnan S.K.. (2013). Acute encephalitis syndrome surveillance, Kushinagar district, Uttar Pradesh, India, 2011-2012. *Emerg Infect Dis*; Vol.19, no.9, pp.1361-7.

[89] Centers for Disease Control and Prevention (CDC). (2012).Japanese encephalitis surveillance and immunization--Asia and the Western Pacific, MMWR Morb Mortal Wkly Rep: Vol. 62, no.33, pp. 658-62.

[90] MayerC.A., Neilson A.A. (2010).. Japanese encephalitis - prevention in travelers. *Aust Fam Physician :* Vol. 39, no.6, pp.389-94.

[91] Misra U.K., Kalita J. (2010). Spectrum of movement disorders in encephalitis. *J Neurol*; Vol. 257, no. 12, pp.2052-8.

[92] Slater-Handshy T., Droll D.A., Fan X., Di Bisceglie, A.M. and Chambers T.J. (2004).HCV E2 glycoprotein: mutagenesis of N-linked glycosylation sites and its effects on E2 expression and processing. *Virology*. Vol. 319, no. 1, pp. 36-48.

[93] Ayukawa R, Fujimoto H, Ayabe M, Shoji H, Matsui R, Iwata Y, Fukuda H, Ochi K, Noda K, Ono Y, Sakai K, Takehisa Y, Yasui K. (2004).An unexpected outbreak of Japanese encephalitis in the Chugoku district of Japan, 2002, Jpn J Infect Dis. Vol.57, no. 2, pp. 63-6.

[94] Gupta N., Lomash V., Rao P.V., (2010). Expression profile of Japanese encephalitis virus induced neuroinflammation and its implication in disease severity. *J Clin Virol*; Vol.49, no.1, pp.4-10.

[95] Bhatt G.C., Bondre V.P., Sapkal G.N., Sharma T., Kumar S., Gore M.M., Kushwaha K.P., Rathi A.K.. (2012). Changing clinico-laboratory profile of encephalitis patients in the eastern Uttar Pradesh region of India. *Trop Doct* ; Vol. 42, no.2, pp.106-8.

[96] Buchy P., VoV.L., Bui K.T., Trinh T.X., Glaziou P., Le T.T., Le V.L., Bui T.C.. (2005).Secondary dengue virus type 4 infections in Vietnam. *Southeast Asian J Trop Med Public Health*. ; Vol. 36, no.1, pp.178-85.

[97] Sarkari N.B., Thacker A.K., Barthwal S.P., Mishra V.K., Prapann S., Srivastava D., Sarkari M., (2012). Japanese encephalitis (JE). Part I: clinical profile of 1,282 adult acute cases of four epidemics. *J Neurol*; Vol. 259, no. 1, pp.47-57.

[98] Dung N.M., Turtle L., Chong W.K., Mai N.T., Thao T.T., Thuy T.T., Kneen R., Phu N.H., Wills B., Farrar J., Das K., Solomon T., (2009). An evaluation of the usefulness of neuroimaging for the diagnosis of Japanese encephalitis. *J Neurol*; Vol. 256, no. 12, pp.2052-60.

[99] Hossain M.J., Gurley E.S., Montgomery S., Petersen L., Sejvar J., Fischer M., Panella A., Powers A.M., Nahar N., Uddin A.K., Rahman M.E., Ekram A.R., Luby S.P., Breiman R.F.. (2010).Hospital-based surveillance for Japanese encephalitis at four sites in Bangladesh, 2003-2005. *Am J Trop Med Hyg* ; Vol. 82, no.2, pp.344-9,

[100] Sarkari N.B., Thacker A.K., Barthwal S.P., Mishra V.K., Prapann S., Srivastava D., Sarkari M., (2012). Japanese encephalitis (JE) part II: 14 years' follow-up of survivors. *J Neurol*; Vol. 259, no. 1, pp.58-69.

[101] Misra U.K., Tan C.T., Kalita J.. (2008). Viral encephalitis and epilepsy. *Epilepsia*; Vol.49, Suppl 6, pp.13-8.

[102] Gunasekaran P., Kaveri K., Arunagiri K., Mohana S., Kiruba R., Kumar V.S., Padmapriya P., Suresh Babu B.V., Sheriff. A.K. Japanese encephalitis in Tamil Nadu (2007-2009). (2012). *Indian J Med Res*; Vol. 135, no.5, pp.680-2.

[103] Michael B.D., Solomon T.. (2012). Seizures and encephalitis: clinical features, management, and potential pathophysiologic mechanisms. *Epilepsia*; Vol. 53 Suppl 4, pp.63-71.

[104] Kaur R., Agarwal C.S., Das D.. (2002).An investigation into the JE epidemic of 2000 in Upper Assam--a perspective study. *J Commun Dis*; Vol. 34, no.2, pp.135-45.

[105] T. Solomon, Thao T.T., Lewthwaite P., Ooi M.H., Kneen R., Dung N.M., N. White. (2008).A cohort study to assess the new WHO Japanese encephalitis surveillance standards. *Bull World Health Organ*; Vol. 86, no.3, pp.178-86.

[106] Sarkar A, Taraphdar D., Mukhopadhyay S.K., Chakrabarti S., Chatterjee S., (2011). Serological and molecular diagnosis of Japanese encephalitis reveals and increasing public health problem in the state of West Bengal, India, *Trans R Soc Trop Med Hyg.* Vol.106, no.1, pp. 15-9.

[107] Handique S.K., Das R.R., Saharia B., Das P., Buragohain R., P. Saikia. (2008). Coinfection of Japanese encephalitis with neurocysticercosis: an imaging study. *AJNR Am J Neuroradiol*; Vol. 29, no.1, pp.170-5.

[108] Natividad F.F., Daroy M.L., Alonzo M.T., Matias, R.R. Suarez L.A., Inoue S., (2006). Use of IgM-capture ELISA for confirmation of Japanese encephalitis infections in the Philippines. *Southeast Asian J Trop Med Public Health*; Vol. 37, no. 3, pp.136-9.

[109] MurataR., HashiguchiK., YoshiiK., KariwaH., NakajimaK., Ivanov L.I., LeonovaG.N., Takashima I. (2011). Seroprevalence of West Nile virus in wild birds in far eastern Russia using a focus reduction neutralization test. *Am J Trop Med Hyg* ; Vol.84, no. 3, pp.461-5.

[110] Pant. G.R. (2006).A serological survey of pigs, horses, and ducks in Nepal for evidence of infection with Japanese encephalitis virus. *Ann N Y Acad Sci*; Vol. 1081, pp.124-9.

[111] Zuo S., Zhao Q., Guo X., Zhou H., Cao W., J. Zhang. (2014). Detection of Quang Binh virus from mosquitoes in China. *Virus Res*; Vol. 180, pp.31-8.

[112] Stiasny K., Aberle S.W., Heinz F.X.. Retrospective identification of human cases of West Nile virus infection in Austria (2009 to 2010) by serological differentiation from Usutu and other flavivirus infections. *Euro Surveill* ; Vol. 18, no.43, pp.20614, (2013).

[113] Chheng K., Carter M.J., Emary K., Chanpheaktra N., Moore C.E., Stoesser N., Putchhat H., et al.,, (2013). A prospective study of the causes of febrile illness requiring hospitalization in children in Cambodia. *PLoS One* ; Vol.8 no.4, :pp. e60634.

[114] Kang X., Li Y., Fan L., Lin F., Wei J., Zhu X., Hu Y., Li J., Chang G., Zhu Q., Liu H., Yang Y.. (2012). Development of an ELISA-array for simultaneous detection of five encephalitis viruses. *Virol J*; Vol. 9, pp.56.

[115] Zheng Y., Li M., Wang H., Liang G.., (2012). Japanese encephalitis and Japanese encephalitis virus in mainland China. *Rev Med Virol* ; Vol. 22, no.5, pp.301-22.

[116] Prasad S.R., Kumar V., Marwaha R.K., Batra K.L., Rath R.K., Pal S.R., (1993). An epidemic of encephalitis in Haryana : serological evidence of Japanese encephalitis in a few patients, *Indian Pediatric*, : Vol. 30, no.7, pp.905-10.

[117] Seo H.J., Kim H.C., Klein T.A., Ramey A.M., Lee J.H., Kyung S.G., Park J.Y., Cho Y.S., Cho I.S., Yeh J.Y., (2013).Molecular detection and genotyping of Japanese encephalitis virus in mosquitoes during a 2010 outbreak in the Republic of Korea, *PLoS One*, vol.8, no.2, pp.e55165.

[118] Zhang L., Luan R.S., Jiang F., Rui L.P., Liu M., Li Y.X., Yin Z.D., Luo H.M.. (2012).Epidemiological characteristics of Japanese encephalitis in Guizhou Province, China, 1971-2009, *Biomed Environ Sci.* Vol,25, no.3, pp.297-304.

[119] Sarkar A., Taraphdar D., Mukhopadhyay S.K., Chakrabarti S., Chatterjee S.. (2012). Molecular evidence for the occurrence of Japanese encephalitis virus genotype I and III infection associated with acute encephalitis in patients of West Bengal, India, 2010. *Virol J* ; Vol. 9, pp.271.

[120] Buitrago D., Rocha A., Tena-Tomás, C. Vigo M., Agüero M., Jiménez-Clavero M.A.. (2012). Real-time fluorogenic reverse transcription polymerase chain reaction assay for the specific detection of Bagaza virus. *J Vet Diagn Invest*; Vol. 24, no.5, pp.959-63.

[121] Li S.H., Li X.F., Zhao H., Jiang T., Deng Y.Q., Yu X.D., Zhu Q.Y., Qin E.D., Qin. C.F. (2011). Cross protection against lethal West Nile virus challenge in mice immunized with recombinant E

protein domain III of Japanese encephalitis virus. *Immunol Lett*; Vol.138, no. 2, pp.156-60.

[122] De Filette, M., Ulbert S., Diamond, M. Sanders N.N.. (2012). Recent progress in West Nile virus diagnosis and vaccination. *Vet Res*; Vol.43, pp.16.

[123] Paticheep S., Kongsaengdao S.. (2011).Viral infection of central nervous system in children: one year prospective study. *J Med Assoc Thai*; Vol. 94, no.7pp,S24-31.

[124] C.Song, C. Zhu, C. Zhang, S. Cui. "Detection of porcine parvovirus using a taqman-based real-time pcr with primers and probe designed for the NS1 gene". *Virol J* ; Vol.7, pp.353, 2010.

[125] Chen M.J., Lin C.Y., Wu Y.T., Wu P.C., Lung S.C., Su. H.J. (2012).Effects of extreme precipitation to the distribution of infectious diseases in Taiwan, 1994-2008. *PLoS One*; Vol. 7, no.6, :e34651.,

[126] Carney J., Daly J.M., Nisalak A., Solomon T.. (2012). Recombination and positive selection identified in complete genome sequences of Japanese encephalitis virus. *Arch Virol*; Vol.157, no. 1, pp.75-83.

[127] Pan X.L., Liu H., Wang H.Y., Fu S.H., Liu H.Z., Zhang H.L., Li M.H.. et al, (2011).Emergence of genotype I of Japanese encephalitis virus as the dominant genotype in Asia. *J Virol*. Vol. 85, no.19, pp. 9847-53.

[128] Huy T.Q., Hanh N.T., Thuy N.T., Chung P.V., Nga P.T., Tuan M.A.. (2011). A novel biosensor based on serum antibody immobilization for rapid detection of viral antigens. *Talanta* ; Vol. 86, pp.271-7.

[129] Li X.L., Fu S.H., Liu W.B., Wang H.Y., Lu Z., Tong S.X., Li Z.X., Nasci R.S., Kosoy O., Cui Y., Liang G.D.. (2013). West Nile virus infection in Xinjiang, China. *Vector Borne Zoonotic Dis*; Vol. 13, no. 2, pp. 131-3.

[130] Ali A., Igarashi A., Paneru L.R., Hasebe F., Morita K., Takagi M., Suwonkerd W., Tsuda Y., Wada Y.. (1995).Characterization of two Japanese encephalitis virus strains isolated in Thailand. *Arch Virol.* : Vol. 140, no. 9, pp.1557-75.

[131] Saxena V., Dhole T.N.. (2008). Preventive strategies for frequent outbreaks of Japanese encephalitis in Northern India. *J Biosci*; Vol. 33, no.4, pp. 505-14.

[132] Touch S., Hills S., Sokhal B., Samnang C., Sovann L., Khieu V., Soeung S.C., Toda K., Robinson J., Grundy J.., (2009). Epidemiology and burden of disease from Japanese encephalitis in Cambodia: results from two years of sentinel surveillance. *Trop Med Int Health* ; Vol. 14, no.11, pp.1365-73.

[133] Jia N., Zhao Q.M., Guo X.F., Cheng J.X., Wu C., Zuo S.Q., Dai P.F., Zhao J.Y., Zhang J.S.. (2011). Encephalitis temporally associated with live attenuated Japanese encephalitis vaccine: four case reports. *BMC Infect Dis*; Vol.11, no.344.

[134] Satou K., Nishiura H., (2007).Evidence of the partial effects of inactivated Japanese encephalitis vaccination: analysis of previous outbreaks in Japan from 1953 to 1960. *Ann Epidemiol*; Vol. 17, no.4, pp.271-7.

[135] Okabe, N. (2005). Background of recent JE vaccine issues. *Uirusu*; Vol. 55, no.2, pp.303-6.

[136] Centers for Disease Control and Prevention (CDC), (2005).Japanese encephalitis in a U.S. traveler returning from Thailand, 2004 *MMWR Morb Mortal Wkly Rep.* Vol.54, no.5, pp.123-5.

[137] Werlinrud A.M., Christiansen C.B., Koch A., (2011). Japanese encephalitis in a Danish short-term traveler to Cambodia. *J Travel Med*; Vol.18, no.6, pp.411-3.

[138] Ye Q., Li, X.F. Zhao, H. Li S.H., Deng Y.Q., Cao R.Y., Song K.Y., et al,. (2012). A single nucleotide mutation in NS2A of Japanese encephalitis-live vaccine virus (SA14-14-2) ablates NS1' formation and contributes to attenuation. *J Gen Virol* ; Vol. 93, no. Pt 9, pp.1959-64.

[139] M.Diagana, P.M. Preux, M. Dumas. (2007). Japanese encephalitis revisited. *J Neurol Sci*; Vol.262, no.1-2, pp.165-70.

[140] Gulati B.R., Singha H., Singh B.K., Virmani N., Kumar S., Singh R.K., (2012).Isolation and genetic characterization of Japanese encephalitis virus from equines in India. *J Vet Sci*; Vol.13, no.2, pp.111-8.

[141] Centers for Disease Control and Prevention (CDC).Japanese encephalitis surveillance and immunization--Asia and the Western Pacific, (2012).MMWR Morb Mortal Wkly Rep. Vol.62, no.33, pp.658-62.

[142] Heinz F. X., Berger R., Tuma W., Kunz C., (1983). A topological and functional model of epitopes on the structural glycoprotein of tick-borne encephalitis virus defined by monoclonal antibodies. *Virology*, pp. v. 126, pp. 525-37,

[143] Schlesinger J.J., Chapman, S. (1995).Neutralizing F (ab′) 2 fragments of protective monoclonal antibodies to yellow fever virus (YF) envelope protein fail to protect mice against lethal YF encephalitis. *J. Gen. Virol*, v. 76, pp. 217-20.

[144] Henchal E.A., Henchal L.S., Schlesinger J.J., (1988). Synergistic interactions of anti-NS1 monoclonal antibodies protect passively immunized mice from lethal challenge with dengue 2 virus. *J. Gen. Virol*, v. 69: pp. 2101-7.

[145] Brinton M.A, Arnheiter H., Haller O., (1982).Interferon independence of genetically controlled resistance to flaviviruses. *Infect. Immun*, v. 36, pp. 284-288.

[146] E. A. Gould A. Buckley, (1989).Antibody dependent enhancement of yellow fever and Japanese Encephalitis Virus neurovirulence. *J. Gen. Virology*. v. 70: pp. 1605-1608.

[147] Saini M., Vrati, S. A (2003). Japanese encephalitis virus peptide present on Johnson Grass Mosaic virus like particles induces virus neutralizing antibodies and protects mice against lethal challenge, *Journal of Virology*, pp. 3847-3494.

[148] Kaufman B.M., Summers P.L., Dubois D.R. *et al*. (1989).Monoclonal antibodies for dengue virus prM glycoprotein protect mice against lethal dengue infection. *Am. J. Trop. Med. Hyg*, vol. 41, pp. 576-80.

[149] Kimura-Kuroda J, Yasui K.. (1988).Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies, *J. Immunol.*, vol.141, pp. 3606-10.

[150] Mason P.W., (1989).Maturation of Japanese encephalitis virus glycoproteins produced by infected mammalian and mosquito cells. *Virology*, v. 169 no. (2), pp. 354-64.

[151] Falgout B., Bray M., Schlesinger J.J., Lai C.J., (1990). Immunization of mice with recombinant vaccine virus expressing authentic dengue virus non-structural protein NS1 protects against lethal dengue virus encephalitis. *J. Virol*, v. 64, pp. 4356-63.

[152] Putnak J.R., Schlesinger J.J., (1990).Protection of mice against yellow fever virus encephalitis by immunization with a vaccinia virus recombinant encoding the yellow fever virus non-structural proteins, NS1, NS2a and NS2b. *J. Gen. Virol*, 71, pp. 1697-702.

[153] Nathanson N., Cole G.A., (1971).Immunosuppression: a means to assess the role of the immune response in acute virus infections. *Fed. Proc*, vol.. 30, pp. 1822-30.

[154] Diamond S. M., (2003).Evasion of innate and adaptive immunity by flaviviruses. *Immunology and Cell Biology*, vol. 81. pp. 196-206.

[155] Nikolay B., Diallo M., Boye C.S., Sall A.A.. (2011).Vector Borne Zoonotic *Dis.*Vol. 11, no.11, pp. 1417-23.

[156] Anderson K.B., Gibbons R.V., Thomas S.J., Rothman A.L., Nisalak A., Berkelman R.L., Libraty D.H., Endy T.P., (2011).Preexisting Japanese encephalitis virus neutralizing antibodies and increased symptomatic dengue illness in a school-based cohort in Thailand. *PLoS Negl Trop Dis*, vol. 5 no. 10, pp. e1311.

[157] Holzman H., Heinz F.X., Mandl C.W., Guitakhoo F., Kunz C., (1990).A single amino acid substitution in envelope protein E of tick-borne encephalistis virus leads to attenuation in the mouse model. *J. Virology*, vol. 64, pp. 5156-9.

[158] Wang K., Deubel V., (2011).Mice with different susceptibility to Japanese encephalitis virus infection show selective neutralizing antibody response and myeloid cell infectivity. *PLoS One*, vol. 6 no. 9, pp. e24744.

[159] Mansfield K.L., Horton D. L., Johnson N., Li L., Barrett A.D., Smith D.J., Galbraith S.E., T. Solomon, A.R. Fooks, (2011). Flavivirus-induced antibody cross-reactivity. *J Gen Virol*, v. 92 no. (Pt 12): pp. 2821-9.

[160] Gangwar R.S., Shil P., Sapkal G.N., Khan S.A., Gore M.M., (2011). Induction of virus-specific neutralizing immune response against West Nile and Japanese encephalitis viruses by chimeric peptides representing T-helper and B-cell epitopes. *Virus Res,*. vol. 163 no. 1, pp. 40-50.

[161] Van Gessel Y., Klade C.S., Putnak R., Formica A., Krasaesub S., Spruth M., Cena B., Tungtaeng A. Gettayacamin, M., Dewasthaly S.. (2011).Correlation of protection against Japanese encephalitis virus and JE vaccine (IXIARO(®)) induced neutralizing antibody titers. *Vaccine*, vol.29, no.35, pp.5925-31.

[162] Ogawa M,, Okubo H,, Tsuji Y., Yasui N., Someda K., (1973).Chronic progressive encephalitis occurring 13 years after Russian spring summer encephalitis. *J. Neurol. Sci*, vol. 19, pp. 363-73.

[163] Chastel C.. (2011). Asymptomatic infections in man: a Trojan horse for the introduction and spread of mosquito-borne arboviruses in non-endemic areas? *Bull Soc Pathol Exot*, vol. 104, no.3, pp.213-9.

[164] Samuel, C. E. (1991).Antiviral actions of interferon: Interferon-regulatedcellular proteins and their surprisingly selective antiviral activities, *Virology*, pp. 183, 1-11.

[165] Ling Yi Lin, Li-Kuang Chen, Ching-Len Liao, Chia-Tsui Yeh, Hsien-Yuan Chiang Shiou-Hwa Ma, Jin-Ling Chen, Yue-Ling Huang, Shih-Shun Chen, (1998).Protective Immunity in Mice Virus Nonstructural Protein NS1 Elicits DNA Immunization with Japanese Encephalitis, *J. Viro*, vol. 72 no. 1, pp. 191.

[166] Colombage G., Hall R., Pavy M., Lobigs M., (1998).DNA-based and alphavirus-vectored immunisation with prM and E proteins elicits long-lived and protective immunity against the flavivirus, Murray Valley encephalitis virus. *Virology*, vol. 250, pp. 151-63.

[167] Ochsenbein A. F., Zinkernagel R.M., (2000). Natural antibodies and complement link innate and acquired immunity. *Immunol. Today*, vol. 21: pp. 624-30.

[168] Volanakis J.. (2002).The role of complement in innate and adaptive immunity. *Curr. Top. Microbiol. Immunol*. Vol, 266: pp. 41-56.

[169] Nathanson, N., Cole G.A., (1971).Immunosuppression: a means to assess the role of the immune response in acute virus infections. *Fed. Proc*, vol. 30, pp. 1822-30.

[170] Monath T. P., McLean R. G., Cropp, C. P. G. Parham L, Lazuick J. S., Calisher. C. H. (1981). Diagnosis of eastern equine encephalomyelitis by immunofluorescent staining of brain tissue, *Am J Vet Res,*. Vol, 42 no. 8, pp. 1418-1421.

[171] Deng Y.Q., Dai J.X., Ji G.H., Jiang T., Wang H.J., Yang H.O., Tan W.L., Liu R., Yu M., Ge B.X., et al (2011).A broadly flavivirus cross-neutralizing monoclonal antibody that recognizes a novel epitope within the fusion loop of E protein. *PLoS One*, vol.6, no.1, pp.e16059.

[172] Larena M., Regner M., Lee E., Lobigs M.. (2011).Pivotal role of antibody and subsidiary contribution of CD8+ T cells to recovery from infection in a murine model of Japanese encephalitis. *J Virol, vol.*85, no.1, pp.5446-55.

[173] Liu K., Yao Z., Zhang L., Li J., Xing L., Wang X., (2012).MDCK cell-cultured influenza virus vaccine protects mice from lethal challenge with different influenza viruses. *Appl Microbiol Biotechnol.* 2012 Jun; vol.94, no.5, pp.1173-9.

[174] Venturi G., Marchi A., Fiorentini C., Ramadani N., Quaglio G., Kalaveshi A., Bertinato L., Putoto G., Benedetti E., Rezza G., Ciufolini M.G.. (2011).Prevalence of antibodies to phleboviruses and flaviviruses in Peja, Kosovo. *Clin Microbiol Infect*, Vol. 17, no.8, pp.1180-2.

[175] Dubischar-Kastner K., Eder S., Buerger V., Gartner-Woelfl G., Kaltenboeck A., Schuller E., Tauber E., Klade C., (2010). Long-term immunity and immune response to a booster dose following vaccination with the inactivated Japanese encephalitis vaccine IXIARO, IC51. *Vaccine*, Vol, 19; no. 28, pp. 5197-202,

[176] Halstead S.B., Thomas S. J.. (2011). New Japanese encephalitis vaccines: alternatives to production in mouse brain. *Expert review of vaccines*, vol. 10, no.3, pp. 355-64.

[177] Gubler D.J.. (2011). Emerging vector-borne flavivirus diseases: are vaccines the solution? *Expert Rev Vaccines*, vol. 10, no.5, pp.563-5.

[178] W.H.O 2005-2010, (2009) World Health Organization Department of Immunization, Vaccines and Biologicals. Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies. http://whqlibdoc.who.int/hq/2006/WHO_IVB_05.19_eng.pdf,).

[179] Verma R., (2012).Japanese encephalitis vaccine: need of the hour in endemic states of India. *Hum Vaccin Immunother*, vol.8, no.4, pp.491-3.

[180] Kay M.. (2013). Encephalitis claims 13 more lives as Indian government launches vaccine against one cause of the outbreak. *BMJ*, vol. 347, pp.:f6175.

[181] Ohtaki E., Matsuishi T., Hirano Y., Maekawa K., (1995). Acute disseminated encephalomyelitis after treatment with Japanese B encephalitis vaccine (Nakayama-Yoken and Beijing strains). *J Neurol Neurosurg Psychiatry*, vol.. 59: pp. 316-317.

[182] Hombach J., Solomon T., Kurane I., Jacobson J,, Wood D., (2005). Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2-3 September, 2004, *Vaccine*, vol.. 23, no.45, pp. 5205-5211

[183] Hoke C.H., Nisalak A., Sangawhipa N., et al. (1988).Protection against Japanese encephalitis by inactivated vaccines. *N Engl J Med*, vol. 319 no. 10, pp. 608-614.

[184] MarksF., NguyenT.T., TranN.D., NguyenM.H., Vu H.H., MeyerC.G., YouY.A., KoningsF., LiuW., WierzbaT.F., Xu Z.Y., (2012).Effectiveness of the Viet Nam produced, mouse brain-derived, inactivated Japanese encephalitis vaccine in Northern Viet Nam, *PLoS Negl Trop Dis*., Vol. 6, no.12, pp. :e1952.

[185] Halstead S.B., Tsai, T.F. (2004). Japanese Encephalitis Vaccines, In: Plotkin SA, Orenstein WA. *Vaccines*, 4th ed, Philadelphia: Saunders; 1).

[186] Torresi, J, McCarthy K, Feroldi E., Méric, C. (2010). Immunogenicity, safety and tolerability in adults of a new single-dose, live-attenuated vaccine against Japanese encephalitis: Randomised controlled phase 3 trials. *Vaccine.* vol. 23; no. 28 (50), pp. 7993-8000.

[187] Konishi E., Ajiro N., Nukuzuma C., (2003).Comparison of protective efficacies of plasmid DNAs encoding Japanese encephalitis virus proteins that induce neutralizing antibody of cytotxoic T lymphocytes in mice, *Vaccine*, vol. 21, no. 25-26, pp. 3675-3683.

[188] Bharati K., Vrati. S., (2006).Japanese encephalitis: development of new candidate vaccines. *Expert Rev Anti Infect Ther*. Vol.4, no.2, pp.313-24.

[189] Monath T. P., McCarthy K., Bedford P., et al. (2002). Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections, *Vaccine*, vol. 20 no. 7-8, pp. 1004-1018.

[190] Montagnon B. J., Fanget B., Nicolas A.J., (1981).The large-scale cultivation of VERO cells in micro-carrier culture for virus vaccine production: preliminary results for killed poliovirus vaccine, *Dev Biol Stand*, vol. 47: pp. 55-64.

[191] Schuller E., Jilma B,, Voicu V., et al., (2008).Long-term immunogenicity of the new Vero cell-derived, inactivated Japanese encephalitis virus vaccine IC51 six and 12 month results of a multicenter follow-up phase 3 study. *Vaccine*, vol. 26. no. 34, pp. 4382-4386.

[192] Schuller E., Klade C.S., Wolfl G., Kaltenbock A., Tauber S., E., (2009).Comparison of a single, high-dose vaccination regimen to the standard regimen for Dewasthaly the investigational Japanese encephalitis vaccine, IC51: a randomized, observer-blind, controlled Phase 3 study. *Vaccine*, v. 27 no. 15, pp. 2188-2193.

[193] Centers for Disease Control and Prevention. (2009).ACIP provisional recommendations. http://www.cdc.gov/vaccines/recs/provisional/#acip,

[194] Wang, H.J.. Li, X. F.Q. Ye, Li, S.H. Deng Y.Q., Zhao H., Xu Y.P., Ma J., Qin E.D., Qin C.F.. (2014).Recombinant chimeric Japanese encephalitis virus/tick-borne encephalitis virus is attenuated and protective in mice, *Vaccine*, vol.32, no.8, pp.949-56.

[195] Li X.F., Deng Y.Q., Yang H.Q., Zhao, H. Jiang, T., Yu X.D., Li S.H., et al, (2013).A chimeric dengue virus vaccine using Japanese encephalitis virus vaccine strain SA14-14-2 as backbone is immunogenic and protective against either parental virus in mice and nonhuman primates. *J Virol*. Vol. 87, no.24, pp.13694-705.

[196] Kikukawa A., GomiY., AkechiM., OnishiT., ManabeS., NamazueJ., FukeI., IshikawaT., OkunoY., Ueda, S. (2012).Superior immunogenicity of a freeze-dried, cell culture-derived Japanese encephalitis vaccine (inactivated), *Vaccine*, *vol.*30, no.13, pp.2329-35.

[197] Upreti S.R., Janusz K.B., Schluter W.W., Bichha R.P., Shakya G., Biggerstaff, B.J., Shrestha M.M., et al., (2013). Estimation of the impact of a Japanese encephalitis immunization program with live, attenuated SA 14-14-2 vaccine in Nepal. *Am J Trop Med Hyg*; Vol.88, no.3, pp.464-8.

[198] Arroyo J., Guirakhoo, F., Fenner S., Zhang Z.X., Monath T. P., Chambers T. J., (2001).Molecular basis for attenuation of neurovirulence of a yellow fever virus/Japanese encephalitis chimera vaccine (Chimeri Vax-JE), *J. Virology*, vol. 75, pp. 934-942.

[199] Bray, M., Zhao B. T, Markoff L, Eckels K. H, Chanock R. M.,. Lai, C.J Mice immunized with recombinant vaccinia virus expressing dengue virus 4 structural proteins with or without NS1 are protected against fatal dengue virus encephalitis. *Journal of Virology*, vol. 63, pp.2853-2856.

[200] Ling Yi Lin, Li-Kuang Chen, Ching-Len Liao, Chia-Tsui Yeh, Hsien-Yuan Chiang Shiou-Hwa Ma, Jin-Ling Chen, Yue-Ling Huang, Shih-Shun Chen, (1998).Protective Immunity in Mice

Virus Nonstructural Protein NS1 Elicits DNA Immunization with Japanese Encephalitis, *J. Viro*, vol. 72, no. 1, pp. 191.

[201] Nasveld P.E., Ebringer A., Elmes N, Bennett S., Yoksan S., Aaskov J., McCarthy K., et al, (2010). Long term immunity to live attenuated Japanese encephalitis chimeric virus vaccine: randomized, double-blind, 5-year phase II study in healthy adults. *Human vaccines.* vol. 6, no.12, pp. 1038-46,

[202] Bhatt T.R., Crabtree M.B., Guirakhoo F., Monath T.P. , Miller, B.R. (2000).Growth characteristics of the chimeric Japanese encephalitis virus vaccine candidate, ChimeriVax-JE (YF/JE SA14--14--2), in *Culex tritaeniorhynchus, Aedes albopictus*, and *Aedes aegypti* mosquitoes, Am J Trop Med Hyg. Vol.62, no.(4), pp.480-4.

[203] David W.C., Beasley C., Todd D., Jose Estrada-Franco, Roberto Navarro-Lopez, Arturo Campomanes-Cortes, Tesh B R., Weaver, S. C., Barrett Alan D.T., (2004).Genome Sequence and Attenuating Mutations in West Nile Virus Isolate from Mexico. *Infect Dis*, vol. 10, no.12, pp. 2221-2224.

[204] Liu P., Lu Hao, Shuang Li, Gregory Moureau, Yong-Qiang Deng, Yongyue Wang, Lijiao Zhang, Tao Jiang, Xavier de Lamballerie, Cheng-Feng Qin, A. E. Gould, Su. Jingliang, F. Gao, (2012). Genomic and antigenic characterization of the newly-emerging Chinese duck egg-drop syndrome flavivirus: genomic comparison with Tembusu and Sitiawan viruses, *J Gen Virol, vir.0.043554-0;*

[205] Appaiahgari M.B., Vrati S., (2012).Clinical development of IMOJEV ®--a recombinant Japanese encephalitis chimeric vaccine (JE-CV), *Expert Opin Biol Ther*. Vol.12, no.9, pp.1251-63.

[206] Tauber E., Kollaritsch H,, Von F.. Sonnenburg, et al, (2008).Randomized, double-blind, placebo-controlled phase 3 trial of the safety and tolerability of IC51, an inactivated Japanese encephalitis vaccine, *J Infect Dis*, vol. 198, no.4, pp. 493-499.

[207] U.S. Public Health Service, (1988).Centers for Disease Control, National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories. Washington, DC : U.S. Public Health Service.

[208] Sanofi Pasteur. http://www.sanofipasteur.com/sanofi-pasteur2/front/ index.jsp?siteCodepSP _CORP&code Rubriquep 34, (2009).

[209] WHO SEARO. Thailand 2007 EPI Fact Sheet, Data as reported in the 2007 WHO/UNICEF Joint Reporting Form on Immunization, (2008).

[210] Gromeier M., Wimmer E., Gorbalenya A. E., (1999).Genetics, pathogenesis and evolution of picornaviruses, in *Origin and Evolution of Viruses*, E. Domingo, R. G. Webster, and J. J. Holland, Eds., pp. 287-343, Academic Press, London, UK, pp. 287-343.

[211] Guy B., Guirakhoo F., Barban V., Higgs S, Monath T.P., Lang J., (2009).Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses. *Vaccine*, vol. 28, no. 3, pp. 632-649.

[212] D Yang, XF Li, Q Ye, HJ Wang, YQ Deng, SY Zhu, Y Zhang, SH Li, CF Qin., (2014). Characterization of live-attenuated Japanese encephalitis vaccine virus SA14-14-2. Vaccine. Vol.32, no. 23, 2675-81.

[213] Intercell. Japanese encephalitis vaccine. http://www.intercell.com/main/forvaccperts/products/japanese-encephalitis-vaccine/. Accessed 26 August (2009).

[214] Dubischar-Kastner K., Eder S., Buerger V., Gartner-Woelfl G., Kaltenboeck A., Schuller E., Tauber E., Klade C., (2010). Long-term immunity and immune response to a booster dose following vaccination with the inactivated Japanese encephalitis vaccine IXIARO, IC51, *Vaccine*, vol. 19; no. 28, pp. 5197-202.

[215] Eder S., Dubischar-Kastner K., Firbas C., Jelinek T., Jilma B., Kaltenboeck A., Knappik M., Kollaritsch H., Kundi M., Paulke-Korinek M., Schuller E., Klade C.S., (2011).Long term immunity following a booster dose of the inactivated Japanese Encephalitis vaccine IXIARO®, IC51. *Vaccine*, vol. 21, no.29 (14): pp. 2607-12.

[216] Kaltenbock, A. Dubischar-Kastner K, Eder G., et al. (2009). Safety and immunogenicity of concomitant vaccination with the cell-culture based Japanese Encephalitis vaccine IC51 and the hepatitis A vaccine HAVRIX1440 in healthy subjects: a single-blind, randomized, controlled phase 3 study. *Vaccine*, vol. 27, no. 33, pp. 4483-4489.

[217] U.S. Public Health Service, (1988)Centers for Disease Control, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. Washington, DC: U.S. Public Health Service.

[218] Eckels K.H., Yu Y.X., Dubois D.R, Marchette N.J, Trent D.W, Johnson A.J., (1988).Japanese encephalitis virus live-attenuated vaccine, Chinese strain SA14-14-2; adaptation to primary canine kidney cell cultures and preparation of a vaccine for human use. *Vaccine*, vol. 6, no.6, pp. 513-518.

[219] W.H.O 2005-2010, World Health Organization Department of Immunization, Vaccines and Biologicals. Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies, http://whqlibdoc.who.int/hq/2006/WHO_IVB_05.19_eng.pdf, (2009).

[220] Hennessy S., Liu Z., Tsai T.F., et al, (1996).Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. *Lancet*, vol. 347, no. 9015, pp. 1583-1586.

[221] Xin Y.Y., Ming Z. C., Peng G. Y.. L. H. Min, (1988).Safety of a live attenuated Japanese encephalitis virus vaccine (SA 14-14-2) for children, *Am. J. Trop. Med. Hyg*, vol. 39, pp. 214-217.

[222] Beasley D.W., Lewthwaite P., T. Solomon, (2008). Current use and development of vaccines for Japanese encephalitis. *Expert Opin Biol Ther*, vol. 8, no. 1, pp. 95-106.

[223] Chokephaibulkit K., Sirivichayakul C., Thisyakorn U., Sabchareon A., Pancharoen C, Bouckenooghe A, Gailhardou S, Boaz M, Feroldi E, (2010).Safety and immunogenicity of a single administration of live-attenuated Japanese encephalitis vaccine in previously primed 2- to 5-year-olds and naive 12- to 24-month-olds: multicenter randomized controlled trial, *The Pediatric infectious disease journal*, vol. 29, no. 12, pp. 1111-7.

[224] Ohrr H., Tandan J.B., Sohn Y.M., Shin S.H., Pradhan D.P, Halstead S.B., (2005). Effect of single dose of SA 14-14-2 vaccine 1 year after immunization in Nepalese children with Japanese encephalitis: a case-control study, *Lancet*, vol. 366, no. 9494, pp. 1375-1378.

[225] Tandan J.B., Ohrr H., Sohn Y.M., et al., (2007).Single dose of SA 14-14-2 vaccine provides long-term protection against Japanese encephalitis: a case-control study in Nepalese children 5 years after immunization, *Vaccine*, vol. 25, no. 27, pp. 5041-5045.

[226] Bista M., Banerjee M.K., Shin S.H., et al., (2001).Efficacy of single-dose SA 14-14-2 vaccine against Japanese encephalitis: a case control study, *Lancet*, vol. 358, no. 9284. pp. 791-5.

[227] Nimmannitya S,. Hutamai S., Kalayanarooj S., Rojanasuphot S., (1995).A field study on Nakayama and Beijing strains of Japanese encephalitis vaccines, *Southeast Asian J. Trop. Med. Public Health*, vol. 26, no. 4, pp. 689-693.

[228] World Health Organization (WHO). (2006). Position paper—Japanese encephalitis vaccines, *Weekly Epidemiological Record*, vol. 81, no.34/35, pp. 331-340.

[229] Kollaritsch H., Paulke-Korinek M., Dubischar-Kastner K.. (2009). IC51 Japanese encephalitis vaccine. *Expert Opin Biol Ther*; vol.9, no.7, pp.921-31.

[230] Nitatpattana N., Dubot-Pérès, A, Gouilh. M.A., Souris M., Barbazan P., Yoksan S., Lamballerie X. de, Gonzalez J.P., (2008).Change in Japanese encephalitis virus distribution, Thailand, *Emerg. Infect. Dis*, vol. 14, no. 11, pp. 1762-1765.

[231] Konishi E., Yamoka M., Win K.S., Kurane I., Mason P.W., (1998). Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding Japanese encephalitis virus premembrane and envelope gens. *J Virol*, vol.72, pp. 4925-4930.

[232] Swaddiwuthipong W., WenigerB.G., WattanasriS., M., WarrellJ., (1988).A high rate of neurological complications following Semple anti-rabies vaccine. *Trans R Soc Trop Med Hyg*, vol. 82, no. 3, pp. 472-5.

[233] Miura K., Onodera T., Nishida A., Goto N., and Fujisaki Y., (1990).A single gene controls resistance to Japanese encephalitis virus in mice, *Archives of Virology*, vol. 112, no. 3-4, pp. 261-270.

[234] Kaur R.. Sachdeva G., Vrati S., (2002).Plasmid DNA immunization against jpanese encephalitis virus; immunogenicity of membrane anchored and secretary envelope protein, *J. Infect. Dis*, vol. 185, pp. 1-12.

[235] Ramkrishna C. R., Desai S. K., Shanker S. K., Chandramukhi S. K., Ravi R., (1999).Oral immunization of mice with live Japanese encephalitis virus induces a protective immune response. *Vaccine*, vol. 17, pp. 3102-3108.

[236] Kolaskar A.S., Kulkarne-Kale U.. (1999).Prediction of three dimensional structure and mapping of conformational epitopes of envelope glycoprotein of Japanese encephalitis virus. *Virology*. vol. 261, pp. 31-42.

[237] Ni H., Burns N. J., Chang G.J., Zhang M.J., Wills M.R., Trent, D.W., Sanders P.G., Barrett A.D., (1994). Comparison of nucleotide and deduced amino acid sequence of the 50 non-coding region and structural protein genes of the wild-type Japanese encephalitis virus strain SA14 and its attenuated vaccine derivatives. *J Gen Virol*, vol. 75, pp. 1505-1510.

[238] Patent (patent no. 7,425,335), (2008).Chimeric T helper-B cell peptide vaccine for Japanese encephalitis virus, Gore; M. M. (Pune, IN), Kolaskar; Ashok (Pune, IN), Dewasthaly; S. S. (Pune, IN), Kulkarani-Kale; Urmila D. (Pune, IN), Sawant; Sangeeta (Pune, IN).

[239] Mason P.W., (1989).Maturation of Japanese encephalitis virus glycoproteins produced by infected mammalian and mosquito cells. *Virology*, vol. 169, no. 2, pp. 354-64.

[240] Robinson C. R., Saurer R. T., 1998).Optimizing the stability of single chain proteins by linker length and composition mutagenesis. *Proc. Natl. Acad. Sci. USA*, vol. 95, pp.5929-5934.

[241] Regenmortel M. H. V., (2000).Molecular design versus empirical discovery in peptide based vaccine coming to terms with fuzzy recognition sites and ill defined structure-function relationships in Immunology. *Vaccine*,. vol. 18, pp. 216-22.

[242] M, Kuwahara, Konishi E., (2010).Evaluation of extracellular subviral particles of dengue virus type 2 and Japanese encephalitis virus produced by *Spodoptera frugiperda* cells for use as vaccine and diagnostic antigens. *Clin Vaccine Immunol*, vol. 17, pp.1560-6.

[243] Tripathi N.K., Shukla J., Biswas K.C., Lakshmana Rao P.V., (2010).Development of a simple fed-batch process for the high-yield production of recombinant Japanese encephalitis virus protein. *Appl. Microb.Biotechnol*, vol. 86, no.6, pp. 1795-803.

[244] DuttaP., Khan S.A., KhanA.M., Borah J., Sarmah C.K., Mahanta J., (2011). The effect of insecticide-treated mosquito nets (ITMNs) on Japanese encephalitis virus seroconversion in pigs and humans. *Am J Trop Med Hyg*, vol.84, no.3, pp.466-72.

[245] Appaighari M. B., Vrati S., (2007). DNAzyme-mediated inhibition of Japanese Encephalitis virus replication in mouse brain. *Molecular Therapy*, vol. 15, no. 9, pp. 1593-1599.

[246] Wong S.C., Ooi M.H., AbdullahA.R., Wong S.Y., Krishnan S., Tio P.H., Pek P.C., Lai B.F., Mohan, A., Muhi J., Kiyu A., Arif M.T., Cardosa M.J., (2008).A decade of Japanese encephalitis surveillance in Sarawak, Malaysia: 1997-2006. *Trop Med Int Health*., vol. 13, no.1, pp.52-5,

[247] T.Solomon, Recent Advances in Japanese encephalitis, *J. Neurovirol*; vol.9, no.2, pp. 274-83, (2003).

[248] Chiou S.S., Chen W.J., Phenotypic changes in the Japanese encephalitis virus after one passage in Neuro-2a cells: generation of attenuated strains of the virus, Vaccine, vol.26, no. 1, pp.15-23, (2007).

[249] Yun S. I., Kim S.Y., Rice C. M., Lee Y.M., (2003).Development and application of a reverse genetics system for Japanese encephalitis virus. *J. Virol*, vol. 77, pp. 6450-6465.

[250] Lin Y.L., Huang Y.L., Ma S.H. *et al*, (1997).Inhibition of Japanese encephalitis virus infection by nitric oxide: antiviral effect of nitric oxide on RNA virus replication. *J. Virol*, vol. 71, pp. 5227-35.

[251] Chen Y.Y., Lin J.W., Fan Y.C., Chiou S.S., (2012).Detection and differentiation of genotype I and III Japanese encephalitis virus in mosquitoes by multiplex reverse transcriptase-polymerase chain reaction. *Transbound Emerg Dis. vol*.61, no. 1, pp.37-43.

[252] Endy T. P. and Nisalak A,, (2002).Japanese enchphalitis virus:ecology and epidemiology, Current Topics in Microbiology and Immunology, vol. 267, pp. 11-48.

[253] Guirakhoo F., Zhang Z.X., Chambers T. J., Delagrave S., Arroyo J., Barrett A.D., Monath T.P., (1999). Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis, *Virology*. vol.257, no.2, pp.363-72.

[254] Monath T. P., Soike K., Levenbook I., Zhang Z.X., Arroyo J., Delagrave S., Myers G., BarrettA.D., Shope R.E., M.Ratterree, Chambers T.J., Guirakhoo F., (1999).Recombinant, chimaeric live, attenuated vaccine (ChimeriVax) incorporating the envelope genes of Japanese encephalitis (SA14-14-2) virus and the capsid and nonstructural genes of yellow fever (17D) virus is safe, immunogenic and protective in non-human primates. *Vaccine*. vol.17, no.15-16, pp.1869-82.

[255] Wu X., Zhou T., O'Dell S., Wyatt R. T., Kwong P. D., and Mascola J. R., (2009).Mechanism of human immunodeficiency virus type 1 resistance to monoclonal antibody b12 that effectively targets the site of CD4 attachment, *Journal of Virology*, vol. 83, no. 21,pp. 10892-10907.

[256] Upadhyayula S. M., Rao Mutheneni S., Nayanoori H. K., Natarajan A., and Goswami P., (2012).Impact of weather variables on mosquitoes infected with Japanese encephalitis virus in Kurnool district, Andhra Pradesh, *Asian Pacific Journal of Tropical Medicine*, vol. 5, no. 5, pp. 337-341,

[257] Centers for Disease Control and Prevention (CDC), (2013).Japanese encephalitis surveillance and immunization—Asia and the Western pacific, *MMWR Morbidity and Mortality Weekly Report*, vol. 62, no. 33, pp. 658-662.

[258] Update on Japanese encephalitis vaccine for children: United States, 2011. Centers for Disease Control and Prevention (CDC). Update on Japanese encephalitis vaccine for children: United States, May 2011. *MMWR Morb Mortal Wkly Rep*, vol.60, no.20, 664-5, (2011).

[259] Kumari R., Kumar K., Rawat A., Singh G., Yadav N. K., and Chauhan L. S., (2013).First indigenous transmission of Japanese Encephalitis in urban areas of National Capital Territory of Delhi, India, *Tropical Medicine & International Health*, vol. 18, no. 6, pp. 743-749.

[260] Zhang D.L., Ma L.B., Fu S.H., Wang L.H., Wang H.Y., Zhang M., Wei Z.G., et al., (2008). Clinical characteristics and laboratory assay of adult Japanese encephalitis patients in an outbreak in Yuncheng, Shanxi Province, 2006, *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*, vol.22, no.2, pp.95-7.

[261] Rathi A.K., Kushwaha K.P., Singh Y.D., Singh J., Sirohi R., Singh R.K., Singh U.K., (1993).JE virus encephalitis: 1988 epidemic at Gorakhpur. Indian Pediatr. Vol.30, no. 3, pp.325-333.

[262] Mackenzie J. S., Poidinger M., Lindsay M.D., Hall R.A., Sammels L.M., (1994).Molecular epidemiology and evolution of mosquito-borne flaviviruses and alphaviruses enzootic in Australia. Virus genes, Vol. 11, no.2-3, 225-37.

[263] Risebro H.L., Doria M.F., Andersson Y., Medema G., Osborn K., Schlosser O., Hunter P.R., (2007).Fault tree analysis of the causes of water borne outbreaks, *J Water Health*, Vol.5, no.1, pp.1-18.

[264] Keiser, J., Maltese M.F., Erlanger T.E.,. Bos R, Tanner, M., Singer B.H., Utzinger J., (2005). Effect of irrigated rice agriculture on Japanese encephalitis, including challenges and opportunities for integrated vector management. *Acta Trop*, vol.95, no.1, pp.40-57.

[265] AleraM.T., VelascoJ.M., Ypil-CardenasC.A., JarmanR.G., NisalakA.N., ThaisomboonsukB., GibbonsR.V., Dimaano E.M., Yoon I.K., (2013). Hospital-based surveillance of Japanese encephalitis at a tertiary hospital in Manila, *Southeast Asian J Trop Med Public Health*, vol. 44, no.5, pp.791-8.

[266] Ting S.H., Tan H.C., Wong W.K., Ng M.L., Chan S.H., Ooi E.E., (2004). Seroepidemiology of neutralizing antibodies to Japanese encephalitis virus in Singapore: continued transmission despite abolishment of pig farming?, *Acta Trop*, vol.92, no.3, pp. 187-91,

[267] DhakalS., StephenC., AleA., Joshi D.D., (2012).Knowledge and practices of pig farmers regarding Japanese encephalitis in Kathmandu, Nepal. *Zoonoses Public Health*. vol.59, no.8, pp.568-74.

[268] Upadhyay R. K., (2014).Evolution of new variants/mutants of JE virus, its effect on neurovirulence, antigenicity, host immune responses and disease transmission in endemic areas. Journal of Viruses, Vol. 2014, pp.1-35, ID 516904.

[269] Turtle L., Griffiths M.J., T. Solomon. (2012). Encephalitis caused by flaviviruses. *QJM* ; Vol.105, no.3, pp.219-23,

[270] Yeh J.Y., Lee J.H., Park J.Y., Seo HJ, Moon JS, Cho I.S., Kim H.P, et al, (2012).A diagnostic algorithm to serologically differentiate West Nile virus from Japanese encephalitis virus infections and its validation in field surveillance of poultry and horses, *Vector Borne Zoonotic Dis.* Vol.12, no.5, 372-9.

[271] Yeh J.Y., Lee J.H., Seo H.J., Park J.Y., J.S. Moon, Cho, I.S., Moon JS, (2010).Fast duplex one-step reverse transcriptase PCR for rapid differential detection of West Nile and Japanese, encephalitis viruses, *J Clin Microbiol.* Vol.48, no.11, pp.4010-4.

[272] Miller R.H., Masuoka, P. Klein T.A., Kim H.C., Somer T., Grieco J.. (2012). Ecological niche modeling to estimate the distribution of Japanese encephalitis virus in Asia. *PLoS Negl Trop Dis* ; Vol. 6, no.6, pp.1678.