REVIEW AND LITERATURE
2. REVIEW OF LITERATURE

2.1 JAPANESE ENCEPHALITIS VIRUS

Japanese Encephalitis Virus (JEV) is a mosquito borne virus, causes life threatening viral disease Japanese Encephalitis (JE). The virus belongs to the member of the genus *Flavivirus* under the family *Flaviviridae* (Lindenbach and Rice 2001). JE is endemic to a large portion of Asia and South East Asia. Now the disease is not only confined into the endemic areas, but also has been spreading into the non-endemic zones around the world. It is a neurotropic inflammatory killer disease, affecting the central nervous system especially the cerebrum, cerebellum and spinal cord. JE is becoming an internationally important emerging arboviral infection. The disease causes significant morbidity as well as mortality. Every year, approximately 30,000-50,000 cases with a mortality rate of about 25% are reported from all over the world; nearly 30-50% of the cases survive with persistent neurological and/or psychological sequelae (Sarkar et al., 2012a). The morbidity and mortality especially in children and young adults (aged 2-15 years) are very high (Chatterjee et al., 2004), although elderly people are not spared, if affected. Approximately 3 billion people are at risk in the JE endemic zone. Outbreaks due to JEV in Nepal and India between 2005 and 2007 have resulted 11,000 cases and more than 2000 deaths. JE is transmitted by *Culex* mosquitoes. Expansion of rice irrigation into the semi-arid regions of South East Asia has impact on disease burden as the *Culex* mosquitoes breed in the paddy field.
2.1.1 DISTRIBUTION

2.1.1.1 GLOBAL SCENARIO

Outbreak of encephalitis presumably caused by JEV infection were recognized in Japan as early as 1871 (Burke and Morath, 2001). In 1924, a severe epidemic with more than 6000 cases was recorded in Japan and a filterable agent was determined to be responsible for this epidemics. In 1934, a filterable agent from the brain of a human was transmitted to reproduce the encephalitis in intracerebrally inoculated monkeys. JE was first isolated in Japan in 1935 (Sarkar et al., 2012a). This was the Nakayama strain of JEV, isolated from the brain of a human which was used as an inactivated vaccine previously. In 1938, the virus was first isolated from its vector *Culex tritaeniorhynchus* (Hurk et al., 2009). In Russia, JE first occurred in 1938. In the Philippines, first reports of JE cases occurred in the early 1950s (Erlanger et al., 2009). In 1949, large epidemics were reported from South Korea for the first time (Solomon et al., 2000). Epidemics in northern Vietnam followed in 1965 (currently 1000–3000 cases nationally a year), and in Chiang Mai in northern Thailand in 1969 (currently 1500–2500 cases nationally a year) (Solomon et al., 2000). In Burma, Bangladesh and south-western Nepal region, JE cases were first recorded in the late 1970s. Sri Lanka experienced its first epidemic with 410 cases and 75 deaths in 1985. JE cases were also reported from Pakistan (Igarashi et al., 1994). In the western Pacific islands, the disease has occurred in Guam in 1947 (Solomon et al., 2000) and Saipan in 1990 (Mitchell et al., 1993). JE is endemic in Malaysia. From this region, the virus was first isolated in the 1960s and approximately 100 cases are recorded every year. JE is endemic in Indonesia also; 1000–2500 cases of encephalitis are reported annually, although in most cases, the aetiological agent was not confirmed (Wuryadi et al., 1989). In 1995, the first case of JE occurred in the Badu Island and other island of Australian Torres Straits, and was reported for the first time from north Queensland in 1998 (Solomon et al.,
2000). Repeated epidemics at intervals were observed in South East Asia (Japan, Philippines, Korea, Thailand, Taiwan, China, India) have been documented (Richman et al., 1997). In the Asia-Pacific region, it is the most important cause of viral encephalitis, accounting for more than 50,000 cases with 10,000 deaths annually (WHO 2007).

Fig A: Geographical distribution of Japanese Encephalitis Virus (Hurk et al., 2009).

2.1.1.2 INDIAN SCENARIO

During the last 3 decades, JEV is one of the major public health problems in India. In India JE was first recognized in 1955 from cases of encephalitis admitted to the Christian Medical College and Hospital, Vellore (Tamilnadu) (Namachivayam and Umayal 1982). In 1958, this virus was isolated from wild-caught mosquitoes in the same area and was isolated from brain tissue of human cases (Reuben and Gajanana, 1997). Until 1970, JE cases were reported from South India mainly (Namachivayam and Umayal 1982). A total of about 65
cases were reported from South-India between the year 1955 and 1966 (Carey et al., 1968). The first major outbreak of JE involving more than 700 cases and 300 deaths occurred in Burdwan and Bankura districts of West Bengal in 1973. The recurrence of JE epidemic in the district of Bankura and Burdwan in the year 1976 (Banerjee et al., 1976; Rajagopalan and Panicker 1978) with 307 cases and 126 deaths (Vaughn and Hoke 1992) and again in the year 1987 and 1988, in the district of Burdwan, (Mukhopadhyay et al., 1990) have been well documented. From 1978 to 2007, 103,389 cases and 33,729 deaths due to JEV infection were recorded from various parts of India including the states of Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa and Union territories of Goa and Pondicherry (Dutta et al., 2010; Kabilan et al., 2004). Presently, JE is not only endemic in many areas but it is also spreading to naive non-endemic areas. Outbreaks of JE have been reported from Lakhimpur district of Assam from July-August, 1989. The virus had affected 90 villages of the district, covering a population of approximately 36,000 and 50% case fatality rate (Vajpayee et al 1992). Epidemic of JE has occurred in Andhra Pradesh during October-November, 1999 affecting 15 out of 23 districts with 873 cases and 178 deaths (Rao et al 2000). In November, 1999, three cases of JE were reported for the first time from two villages in Tamil Nadu (Victor et al 2000). In July 2003, outbreak occurred in Warangal and Karim Nagar districts of Andhra Pradesh (Das et al 2004). Later, several outbreaks were reported from Assam in three consecutive years from August 2000-2002 (Phukan et al 2004). Diagnosis was confirmed in 53.7% patients with ratios of 1.8:1 and 1.4:1 for male to female and pediatric to adult patients respectively. Most of the cases were pediatrics at the age of 7 to 12 years (34.2%). In 1988, a serious outbreak occurred in Gorakhpur region (UP) (Rathi et al 1993). In 2005, one of the largest outbreaks of JE was reported from Gorakhpur and adjoining districts. More than 1380 children died and about 5800
were admitted due to JEV infection (Kanojia 2007). In India, children are mainly affected by the virus. The morbidity rate ranged from 0.30 to 1.5 per 1,00,000 population whereas the case fatality rate has ranged from 18 to 60 per cent. About 30-50% of the recovered patients suffer from neurological deficits (Reuben and Gajanana 1997).

![Figure B: JE affected states and districts of India.](source: NVBDCP, Ministry of Health and Family Welfare, Govt. of India.)

### 2.1.2 ORIGIN & EVOLUTION

Since the emergence of JEV in Japan in 1870s, it has spread in most of the part of Asia and has become one of the major public health problems worldwide. Currently five genotypes of JEV are circulating all over the world (Nga et al., 2004). JEV is believed to be originated from the Indonesia-Malaysia region from an ancestral virus which is common to JEV and Murray Valley Encephalitis virus (MVEV) (Solomon et al., 2003). From this ancestral virus, JEV genotype IV (GIV), and genotype V (GV) diverged, followed by the genotype I (GI),
genotypes II (GII) and genotypes III (GIII). GIV is confined to the Indonesia-Malaysia region, whereas more recent genotypes (GI, GII, and GIII) have spread to other areas of South-East Asia. In 1952, only a single GV isolate Muar strain was isolated in Singapore from a brain of a patient who originated in Muar, Malaysia (Mohammed et al., 2011). Recent evolutionary analysis suggested that the Muar strain (GV), is the oldest JEV lineage from which the other four genotypes diverged about 450 years ago.

GI and GIII caused outbreaks in epidemic regions, whereas GII and GIV of JEV are associated with endemic disease (Mohammed et al., 2011). GI includes isolates from Southeast Asia, Australia, Korea, Vietnam, and Japan from 1967 to till date (Nga et al., 2004); GII includes isolates from Korea, southern Thailand, Malaysia, Indonesia, Papua New Guinea and Australia (Williams et al., 2000); GIII were isolated from Southeast Asia, Japan, China, Korea, Taiwan and the Central Asia sub-continent from 1935 to till date (Schuh et al., 2010); GIV include the isolates only from Indonesia between 1980 and 1981 and GV isolate is the Muar strain (Williams et al., 2000; Uchil & Satchidanandam, 2001; Pyke et al., 2001). The most of the JEV isolates that have been studied belong to GIII (Williams et al., 2000). Nucleotide sequence analysis of PrM or E gene of JEV revealed that all the isolates are grouped in distinct clusters within each genotype, but between the different clusters no clear distinct geographical boundaries were noticed (Nga et al., 2004).

In past few decades, JE has spread into the new territories. It might be due to the bird (act as a reservoir) migration, new irrigation projects and increasing animal husbandry (Innis, 1995; Nga et al., 2004). Moreover, different genotypes of JEV had introduced in some regions. Nucleotide sequencing and phylogenetic analyses of the PrM and E genes of JEV, isolated from both pigs and mosquitoes in Thailand, Combodia and Korea (TS00 and TS4152, respectively) clustered in GI (Pyke et al., 2001). All previous Australasian JE virus isolates
belong to GII (Williams et al., 2000), along with Malaysian and Indonesian isolates. JEV GII includes strains that have been isolated sporadically between 1951 and 1999 in Korea, southern Thailand, Malaysia, Indonesia, Papua New Guinea and northern Australia (Schuh et al., 2010). Genetic characterization of the Bennett strain (Korea, before 1951) revealed that GII, mainly associated with endemic disease, has been circulating for at least 19 years longer than previously thought. Prior to this study, GII genotype had never been isolated from Southern Thailand. The Bennett strain was either imported from nearby Japan or it is more likely that the circulation of this strain coincided with a GII epidemic that quickly became extinct or GII became endemic in Korea for a period of time and then disappeared. New strains, which were isolated, were closely related to GI of the Thai strains and had been shown to be genetically and antigenically different from those isolated in previous decades (Ma et al., 2003; Nam et al., 1996; Takegami et al., 2000). Between 1930 and 1990, GIII was predominant in USSR, Korea and Japan. In Japan, nucleotide analysis of 23 isolates showed that all strains isolated before 1991 belonged to GIII genotype, while those isolated between 1994 and 2001 belonged to GI (Ma et al., 2003). It has been found that, the isolated GI Japanese strains showed a close genetic relationship with Korean and Malaysian strains. In another study, it was found that the strains isolated from Vietnam and Japan between 1986 and 1990, were clustered in GIII, but the strains isolated between 1995 and 2002 grouped within GI and became dominant though previously this genotype was unreported in Vietnam (Nga et al., 2004). In addition, in this study, strains isolated from 1995-2002 were more closely related to those isolated in the 1990s than to the older GI strain.

In India, both GI and GIII JEV strains were isolated from the acute encephalitis syndrome (AES) cases in the Gorakhpur region, Uttar Pradesh in the year 2009 (Fulmali et al., 2011). Several outbreaks have been documented in this region since 1978 (Mathur et al., 1982)
and is highly JE endemic region. GI JEV isolates from India share close genetic relationship with GI strains from Japan and Korea. The exact reason although remain unclear, but migratory birds might played an important role for transmission of the disease (Huang et al., 2010).

Figure C: The geographical distribution and spread of JEV genotypes. Countries are grouped into geographical regions: A, Indonesia (excluding New Guinea) and Malaysia; B, Australia and New Guinea; C, Taiwan and the Philippines; D, Thailand, Cambodia, and Vietnam; E, Japan, Korea, and China; F, India, Sri Lanka, and Nepal. Region A contains all genotypes of JEV, including the oldest. [Source: Saxena et al., 2011; Solomon et al., 2003]

2.1.3 CLINICAL MANIFESTATION

The onset of symptoms in JE infection is characterized by sudden onset of high fever with diarrhoea and rigors. This is followed by violent headache, vomiting and a reduced level of consciousness with convulsions and altered sensorium (Miyake 1964). Convulsions occur in up to 85% of JE infected children (Kumar et al., 1990; Dickerson et al., 1952) and 10% of
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adults (Poneprasert et al., 1989). After a short febrile illness, acute flaccid paralysis in one or more limbs had been reported, despite a normal level of consciousness. Weaknesses occur mainly in the legs and are asymmetric. Thirty percent of such patients develop encephalitis with reduced level of consciousness but in most acute flaccid paralysis is the only feature (Kumar et al., 1991). Although encephalitis is a prominent feature in JEV infection, only one in 200 develop severe psycho-neurological sequelae like convulsion, motor abnormalities, speech disorder, emotional disturbance, hearing deficit etc. Cerebrospinal fluid (CSF) findings include moderately elevated protein level and pressure with normal sugar level. Moderate leukocytes count with relative lymphopenia is observed in peripheral blood.

The incubation period of JE infection is 6-16 days. It can divide into three phases:

a) Prodromal phase, which last for 1-6 days. High fever associated with vomiting, malaise, anorexia, headache, abdominal pain and diarrhoea (in children mainly) are the main symptoms at this stage.

b) Acute encephalitis stage, which include the signs involving the central nervous system (CNS).

c) Late convalescent stage: when fever subsides, reduce acute inflammation and neurological complications begin to develop. The cerebral complications rise even if death does not occur persists, those who survive.

2.1.4 PATHOGENESIS

In human, infections due to JEV cause a broad range of clinical manifestation; from subclinical asymptomatic infection to acute and lethal meningoencephalitis (Solomon et al., 2002). The rate of disease development due to JEV infection, ranges from 1:50 to 1:1000,
remaining are subclinical or lead to mild illness (Vaughn and Hoke 1992). JEV is transmitted to the human by the vector mosquitoes. In mosquitoes, after ingestion of blood meal containing virus, the virus first infect the posterior midgut followed by the replication in the anterior midgut epithelial cells (Hurk et al., 2009). In the next stage, multiplication of the virus occur in the fat body cells, adjacent to the midgut followed by the infection of the fat body cells in the hemocoel. After the release of the virus into the haemolymph, it invades the salivary gland; ultimately the virus is secreted in saliva during refeeding the susceptible vertebrate host (Hurk et al., 2009). The time gap between the ingestion of the blood meal and the secretion of the virus from the salivary gland plays an important role in disease transmission. Increased temperature shortens the incubation period and increase the virus transmission rate in nature.

In humans, after the entry of JEV through the bite of infected mosquitoes, primary multiplication of the virus occurs in extra neural local sites, Langerhans dendritic cells in the skin (Vaughn and Hoke, 1992). Then the virus reaches to the local lymph nodes. At this stage, the early immune responses have already developed. But it is not sufficient to counter the virus. So the virus spreads to the secondary lymphoid organ and then the efferent lymphatics carry the virus through the thoracic duct into the blood stream. Subsequently viremia develops, leading to inflammatory changes in the heart, lungs, liver and reticulo-endothelial system resulting in the prodromal stage. The incubation period of the virus ranges from 4 to 14 days to develop the symptoms. Normally the infection is either subclinical or mild with non-specific prodrome and it is cleared before neuro-invasion occurs. Circulating antibody may limit this process. In susceptible individuals, the virus invades the CNS probably by crossing the blood brain barrier (BBB) by passive transport across the endothelium, by active replication in endothelial cells, or by a ‘Trojan horse’ mechanism in which the virus is carried into the brain by infected inflammatory cells (Diamond et al., 2003; Johnson 1987). Normally after three
days of post inoculation, virus migration from peripheral blood to CNS occurs (Kalita et al., 2003), resulting encephalopathy. Proinflammatory mediators released by activated microglia induce neuronal death in JE.

2.1.5 HISTOPATHOLOGY

Usually grey matter of the nervous system is involved in JE infection. The thalamus and brain stem, mainly the substantia nigra and pons are mostly affected due to JEV infection (partial hypothalamic, thalamus) (Kalita and Misra 2000). Pathologic changes occur mainly in the grey matter and predominantly affect the diencephalon, mesencephalon, brain stem and cerebellar Purkinje cells, involving both thalami and the substantia nigra the most severely. Invasion of the neuron by JEV is followed by perivascular cuffing, infiltration of inflammatory cells (T cells and macrophages) into the parenchyma and phagocytosis of the infected cells (Miyake 1964; Johnson et al., 1985). A greater number of CD4+ cells are seen in the CNS than in the blood. Infected neurons contain antigen in their cell bodies, axons and dendrites which suggest that the virus spread from one neuron to other. The white matters usually appear normal. In patients that die rapidly, there may be no histological signs of inflammation but viral antigens in the neurons were confirmed by histochemical studies.

2.1.6 ENZOOONOTIC CYCLE

2.1.6.1 VECTORS OF JEV

JEV is transmitted normally by the bite of infected mosquitoes (Richman et al., 1997), mainly by the Culex mosquitoes. Cx. tritaeniorhynchus is the predominant species for transmission of the disease in India (Kanojia et al., 2003). It breeds normally in the stagnant water (such as paddy field). Cx. pseudovishnui, Cx. Vishnui, Cx. whitmorei, Cx. gelidus, Cx.
epidesmus, Anopheles subpictus, An. peditaeniatus and Mansonia uniformis also have the role to transmit the disease in India. Cx. gelidus is the most important vector of JE in Sri Lanka, Thailand, Malaysia, Vietnam (Kanojia et al., 2003) and Indonesia (Richman et al., 1997). Cx. tritaeniorhynchus displays intraspecific variation in susceptibility to JEV infection. The vector is more susceptible with Japanese strains than the strains from Taiwan and Pakistan (Takahashi 1982), although the genetic basis for this difference in Cx. tritaeniorhynchus susceptibility has not been established. Cx. tritaeniorhynchus mainly feed on the cattle (Reuben et al., 1992), but as the bovines do not produce sufficient viremia to infect mosquitoes, the transmission of JEV does not occur through them (Mwandawiro et al., 2000; Ilkal et al., 1988). In a study from India, it has been found that highest percentage of pig feeding was observed in Culex tritaeniorhynchus (40%) and Culex vishnui (35.3%) in comparison to other prevalent species. In contrast, Culex pseudovishnui, another member of this group was not attracted to pigs (0.4%). In Asia, humans account for only a small proportion (less than 5%) of blood meals for most Culex vectors of JEV. Few human feeds were recorded for Culex tritaeniorhynchus (0.4%), Culex vishnui (0.4%) and Culex pseudovishnui (0.8%), indicating their occasional contact with human hosts.

Depends on geographical and temporal variations, mosquito infection rates affect. Climatic conditions, agricultural practice, mosquitos’ population and the presence of the susceptible amplifying hosts are the factors for JEV infections in that area (Hurk et al., 2009). In temperate region, JE is transmitted in the late summer and early fall, but in tropical region, like in India, the disease transmission rate increase in the monsoon and post monsoon period and in some region throughout the year (Saxena & Dhole 2008). In Northern parts of India and Nepal, transmission occurs from June to November, and in Southern India and Sri Lanka epidemics are found from September to January (ENVIS News Letter, 2006).
2.1.6.2 HOST

Although many animals like cattle, dogs, goats and rodents can be infected by this virus, only those which develop high viremia are important in the natural cycle. Pigs are the main amplifying host (Richman et al., 1997) as they develop high and persistent levels of viremia because of their high body temperature and high body surface areas. Not only that, they are often keep close to the humans (Richman et al., 1997). Ardeid birds are also involved for the maintaining and amplifying JEV in the environment, and spread it to the new geographical areas (Rodrigues et al., 1981). In India, one study amongst the wild birds showed neutralizing antibodies particularly in two species of birds belonging to the family Ardeidae, *Ardeola grayii* (pond heron) and *Bubulcus ibis* (cattle egret) (Rodrigues et al., 1981). Studies by Buescher and colleagues (Buescher et al. 1959; Scherer et al., 1959) established the role of ardeid in the ecology of JEV. During a five-year study period, neutralizing and hemagglutination inhibiting antibodies of JEV were detected from the black-crowned night heron (*Nycticorax nycticorax*), plumed egret (*Egretta intermedia*), and little egret (*Egretta garzetta*). Because of their close association with humans and varying levels of seroprevalence,
pigeons, sparrows, ducks, and chickens have been implicated in natural transmission cycles of JEV, although their actual role in these cycles has not been clearly understood (Hurk et al., 2009). Due to the brief viremia stage and low viral titres in human, it is the accidental dead end host of the virus and the transmission does not occur from them (Richman et al., 1997).

2.1.7 SOCIO-ECONOMIC IMPACT

Socio-economic status is also an important factor for JE manifestation. In a population-based case-control study in central China, it was found that the risk of JE is associated with lower family income, lower parental education, and living in houses near the periphery of villages and poor quality of houses (Luo et al., 1995). It was suggested that those parents, had high family income and higher level of education, might be more conscious about having their children vaccinated properly.

A study at Bellary, Karnataka showed that low socio economic status and living in unhygienic conditions near rice fields contributed to the high incidence of JE (Anuradha et al. 2011). In West Bengal, the economy is mainly dependent on agriculture. A study at this state by Badari revealed that those with primary education or below and possessed domestic animals like pigs and were more affected by the virus. High incidence of the disease was observed among the economically backward classes. Besides cultivation, to raise the economic status, the population of this community lived in close association with pigs which are known to be the amplifying host for JEV.

2.1.8 MOLECULAR BIOLOGY OF JEV

JEV particles are 50 nm in diameter and have a spherical nucleocapsid surrounded by a lipid bilayer containing two viral surface proteins, PrM/M and E (Konishi et al., 1992).
Fig. E: Japanese Encephalitis Virus genome and proteins. The details are described below.

[Source:http://www.ias.ac.in/meetings/annmeet/70am_talks/svrati/img6.html]

The genome is a positive sense, single stranded RNA molecule of approximately 11,000 nucleotides. This molecule contains 5' and 3' untranslated regions (UTRs) and a single ORF that encodes a 3432 amino acid polyprotein, which is co- and post-translationally cleaved by viral and host proteases into three structural proteins: the capsid protein (C), the precursor (prM) of the membrane protein, and the envelope protein (E), as well as seven non-structural proteins (NS): NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Schuh et al., 2010). The structural proteins are included into the mature virions whereas the nonstructural proteins play an important role in viral replication and polypeptide processing.

The C protein (12 to 14 kDa) is positively charged and forms the structural component of the virus nucleocapsid (Kaur and Vrati, 2003). During viral assembly and budding into the intracellular membrane compartment, the heterodimer PrM protein present on the surface of the intracellular immature virion is acquired with the E protein. During export of this intracellular immature virion into the extracellular environment, this PrM protein is cleaved by
a cellular protease to generate M protein, which is present on the surface of the extracellular mature virions.

**2.1.9 REPLICATION CYCLE**

The first step of the replication cycle is binding or attachment to the cell surface of the molecule for the entry of the virus. Glycosaminoglycans, mainly the highly sulphated form of glycosaminoglycans on the cell surface play an important role in JEV entry (Liu et al., 2004). This 74 kDa molecule act as a potential candidate for cellular receptor of JEV.

JEV inter into the cell by clathrin (receptor) mediated endocytosis (step 2) (Nawa et al., 2003). The viral envelops are fused with cellular membranes of endosomal vesicles in low pH dependent manner, which allows the viral core to be released into the cytoplasm. The viral RNA is then uncoated in next step (step 3) and enter into the cytoplasm. To provide the necessary proteins for viral replication and mRNA synthesis, the viral RNA is translated (step 4). The single ORF of the viral genome is translated into a large polyprotein which is co or post translationally processed into mature proteins by cellular protease and viral seine protease NS3 along with NS2B (Chambers et al., 1990). Replication of the RNA genome occurs in cytoplasmic replication complexes which are localised at the perinuclear membranes (step 5) (Westaway et al., 2003). These complexes contain the genomic RNA and viral replicases consisting of the viral non-structural proteins in association with cellular proteins. In first step of genome replication, the minus strand viral RNA is synthesized which serve as templates for the synthesis of multiple copies of RNAs.

All of the viral non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A and NS5) are involved in RNA replication. NS1 has an essential role in RNA amplification. NS3 is a multifunctional protein shown to have protease, RNA helicase and nucleotide triphosphate
activities. The small hydrophobic proteins NS2A and NS4A also localize in the replication complex, which is located in virus induced membrane structures. NS4A has been shown to genetically interact with NS1; it has potential role as a replicase component in RNA replication. NS5 have both RNA dependent RNA polymerase and methyltransferase activity.

Progeny virions are assembled by encapsidating the genomic RNA into the core cells of capsid proteins, enveloped by two viral surface protein (PrM and E)- embedded host derived lipid membranes. The morphogenesis takes place by budding through intracellular membranes into cytoplasmic vesicles (step 6) (Heinz and Allison 2003). Intracellular viral particles appear to be present within the lumen of an intracellular membrane structure, which are then transported to the plasma membrane via the cellular secretory pathway (step 7).

During the transport to the plasma membrane, the intracellular viral particles undergo the maturation process. Glycans on the PrM and E proteins are modified by trimming and terminal addition of sugar residues (Chambers et al., 1990b). The N terminal portion of the PrM is cleaved by furin or related protease in the trans-golgi apparatus (Heinz et al., 1994); this cleavage is prevented by elevation of the pH in acidic intracellular compartments (Heinz et al., 1994; Heinz and Allison 2003). This PrM cleavage is generally considered to distinguish extracellular viral particles from intracellular viral particles (Shapiro et al., 1972) and is required for the generation of highly infectious viruses (Allison et al., 1995). The uncleaved PrM interacts with E proteins, forms a fusion inactive PrM/E heterodimer, and keeps the E protein from undergoing an acid induced conformational change during the transport to the plasma membrane (Heinz et al., 1994; Heinz and Allison 2003).

Fusion of the vesicles with the plasma membrane releases a large amount of progeny virions into the extracellular compartment (step 8) (Mason 1989). In addition to infectious viral particles, non-infectious viral like particles known as slowly sedimenting hemagglutinin
(SHA), are produced from virus infected cells which consist of mainly E and M proteins with some PrM. The expression of PrM and E proteins in JEV has been shown to be sufficient for production of virus like particles, which elicit the protective immunity (Konisha and Mason 1993).

Fig. F: Overview of the JEV replication cycle.

[Source: Molecular Biology of Flavivirus, edited by Kalipzky M and Borowshi P]

2.1.10 CURRENT VACCINES AGAINST JE

Human vaccination is the most effective measure to control the virus. There are three types of inactivated vaccines and one type of live attenuated vaccine currently used in the world; mouse brain-derived, purified vaccine, which is based on either the Nakayama-NIH or Beijing-1 [P-1] strains, primary hamster kidney (PHK) cell-derived vaccine purified vaccine based on the Beijing-3 [P-3] strain, Vero-cell derived purified vaccine based on the P-1, P-3 or

The first generation of JE vaccine included inactivated mouse-brain derived vaccine and inactivated vaccine cultivated on PHK cells. Initially the crude formalin inactivated mouse brain derived JE vaccine was tested in Japan in 1930s which was produced from Nakayama and Beijing 1 strain (Dutta et al., 2010) and got the licence in 1954. It was available internationally under the Biken label (distributed by Sanofi Pasteur as JE-Vax) (Halsted and Thomas 2010). These were later licensed to be produced in the United States also. This vaccine is also independently produced in China, India, Thailand and Taiwan. Central Research Institute, Kasauli was the manufacturer in India (Appaiahgari et al., 2006). It was available in lyophilized form in which gelatin and sodium glutamate is used as stabilizers and thimerosal as preservative (Bharati and Vrati 2006). An efficacy of 91% is reported in a study from Thailand with >65,000 children (Hoke et al. 1988).

Although safe and effective, this vaccine has some common allergic side effects like erythema, swelling, tenderness, fever, headache, malaise and dizziness and also in some cases neurological complication in 1–2 cases per 1,000,000 vaccine recipients (Plesner et al., 2003). Although the exact reasons of these are not known, but gelatine and residual murine protein might be the reason. These vaccines are expensive, difficult to develop and require multiple doses. This vaccine could not provide long term immunity. Although this was recommended by WHO, but due to these difficulties new type of vaccine developed. BIKEN ceased the production of JE-VAX in the year 2005 (Halsted and Thomas 2010).

In China, an inactivated vaccine produced from the Beijing-P3 strain and passaged in PHK cell line was developed and is in use since 1967. It has relatively low side effects and is
easy to manufacture. In an extensive randomized field trial in China, its efficacy was found to range between 76-90% (Tsai et al., 1999; Saxena and Dhole 2008). Although the vaccine efficacy was reported 80% for single dose and 98% with two doses (Hennessy et al. 1996) but the drawback of this vaccine was the PHK substrate and was not approved by WHO for human vaccine production (Saxena and Dhole 2008).

The development and licensure of second-generation vaccine i.e. non-mouse brain-derived JE vaccines is important news for nations looking for options to protect travellers, expatriate workers, and military personnel. African green monkey kidney Vero-cell derived purified vaccine based on the P-1, P-3 or SA14-14-2 strains and PHK cell derived live attenuated vaccine based on the SA14-14-2 strain of the JE virus belong to the second generation vaccine. These vaccines are highly acceptable in endemic countries because of their improved safety profile and lower dosage requirements (Xin et al., 1988).

A live attenuated vaccine SA14–14-2, manufactured by Chengdu Institute of Biological Products, has progressively been introduced into China. Several studies have demonstrated an excellent immune response after a single dose of SA 14-14-2 vaccine, with neutralizing antibody responses produced in 85%-100% of non-immune children (Halsted and Tsai 1999; Sohn et al., 1999). This vaccine has an excellent safety profile and its efficacy in large scale trials (involving 1200,000 children) is also very high. The vaccine was alum adjuvanted and did not contain any stabilizers additive which could cause allergic responses (Kumar et al., 2009; Tsai et al., 1998). Recently, the vaccine has been licensed for use, and millions of doses have been administered in Nepal, India, Sri Lanka, and South Korea (Solomon 2006).

WHO’s Global Advisory Committee on Vaccine Safety has reviewed the SA 14-14-2 vaccine and acknowledged its excellent safety and efficacy profile (Burke and Monath, 2001). PATH has negotiated concessional prices for the use of SA-14–14-2 in India, Sri Lanka, and
Nepal for public health preventive programs (PATH, 2009). The SA14–14-2 vaccine has recently been incorporated into the national program for immunizations in India.

The IC51 vaccine (IXIARO; in Australia and New Zealand, JESPECT) is a purified, formalin-inactivated, whole virus JE vaccine developed by Intercell AG (Halsted and Thomas, 2010). In the year 2009, this product got the licence to be used in the United States, Australia, and Europe (Halsted and Thomas 2010). The vaccine was developed at the Walter Reed Army Institute of Research (Silver Spring, MD) (Putnak et al., 1996). The vaccine is based on a SA14–14-2 virus strain passaged 8 times in primary dog kidney cells, cultivated in Vero cells, and formulated with 0.1% aluminum hydroxide (Eckels et al., 1988). Vero cells maintained in serum-free medium were selected as the manufacturing cell substrate. The absence of serum, additional stabilizers or additives made it a safer vaccine. Multinational phase 3 immunogenicity trials demonstrated that the IC51 vaccine was well tolerated and elicited noninferior immune responses, compared with control (JE-VAX) (Tauber et al., 2007).

A single dose recombinant vaccine ChimeriVax JE (IMOJEV) was developed from cell culture. This live, attenuated virus was prepared by replacing the genes encoding two structural proteins (prM and E) of yellow fever virus (YF) 17D vaccine strain with the corresponding genes of an attenuated vaccine strain SA14-14-2 of JE (Chambers et al., 1999; Guirakhoo et al., 1999). The prM and E proteins contain critical antigens conferring protective humoral and cellular immunity against JE (Konishi et al., 1998). It appears safer than yellow fever 17D vaccine but has a similar profile of immunogenicity and protective efficacy. Like this, a number of other viruses like poxviruses (Kanesa-Thasan et al., 2000; Konishi et al 1994), adenoviruses (Appaihagari et al 2006), have been used for the production of JE vaccines.

In India, a Vero cell-culture derived formaldehyde inactivated JE vaccine using P 20778 strain (Indian isolate) has been developed, which developed high titers of anti-JE
antibodies in mice and sera from the immunized mice efficiently neutralized different JEV strains with different efficacies (Appaiahgari and Vrati 2004). The virus could be inactivated at 22°C and was preferred because it was faster and did not require any cold room storage.

In the United States, two JE vaccines are licensed in the United States; the inactivated mouse brain–derived vaccine (JE-VAX [JE-MB]) and an inactivated Vero cell culture-derived vaccine IXIARO (CDC, 2010). IC51 or IXIARO has been licensed for use in the United States, Europe, and Australia, and ChimeriVax-JE or IMOJEV has recently filed for licensure in Australia and in Thailand. These together with the inexpensive Chinese SA14–14-2 live attenuated vaccines are available in some Asian countries and change the vaccine landscape.

Effective vaccine against JEV is now available and has been used partially in some districts of West Bengal. Even then every year sporadic cases are continuously being reported from the vaccinated districts. From 2003-08, a total of 204 JE cases and 25 deaths due to JEV infection from these districts have been reported (Health West Bengal, 2009). These cases were mainly from the districts of Burdwan, Birbhum, Hooghly, Midnapore (West) and Purulia. There are significantly higher incidences of fever cases in the young and young adult age group. For this purpose, isolation, identification and molecular characterization of the presently circulating strain along with the random titration of the respective antibody in the population, at present, is highly necessary in this region.
2.2 CHIKUNGUNYA VIRUS

Chikungunya virus (CHIKV) is an emerging mosquito borne virus, caused recent outbreaks associated with severe morbidity. It is an enveloped, positive strand RNA virus belonging to the genus *Alphavirus* of the *Togaviridae* family ([Mavalankar et al., 2008](#)). Traditionally, CHIKV causes a febrile illness similar to that seen in dengue virus (DENV) infections. Fever, headache and prolonged arthralgia are the common features of Chikungunya fever. While the acute febrile phase of the illness normally resolves within a few days, but the pain associated with CHIKV infection of the joints, mainly in the peripheral small joints typically persists for weeks or months causing serious economic and social impact on both the individual and the affected communities. During the 2005–2007 explosive epidemics on the Indian Ocean islands and in India, anecdotal cases of CHIKV-associated deaths, encephalitis and neonatal infections were reported ([Power and Logue 2007](#)). About 1.4 million people were affected in India during that time and both *Aedes aegypti* and *Aedes albopictus* have been found to be responsible for this disease.

2.2.1 EPIDEMIOLOGY AND NATURAL HISTORY

Although several Chikungunya outbreaks have been recorded from middle of eighteen centuries, but initially all these were mistakenly documented as Dengue fever. In the year 1779, outbreaks of febrile disease in the city of Batavia (current Jakarta, Indonesia) and in the city of Cairo were mistakenly cited as the first documentation of dengue fever. But now it is now believed to be an outbreak of chikungunya fever ([Carey et al., 1971](#)). In 1820s, an epidemic of acute, self-limiting febrile illness with Chikungunya like symptoms was reported from Africa, West Indies and India.
CHIKV was first isolated from the serum of a febrile human in Makonde Plateau in Tanzania (formerly Tanganyika) in 1953 during an epidemic of dengue-like illness (Robinson, 1955; Ross, 1956). After that, epidemics were reported from Philippines in 1954, 1956, and 1968 (Macasaet 1970) and in south Sumatra, Java, Timor, Sulawesi, the Moluccas Islands Senegal, Ivory Coast, Indonesia, Kenya and Re'union islands between 1982 and 2006 (Power et al., 2007).

In Asia, the virus was first isolated in 1958 in Bangkok (Hammon and Sather, 1964). Other Asian and southeast Asian countries like Myanmar, Philippines, Malaysia, Pakistan and the Pacific islands were also affected (Jupp and McIntosh, 1988).

The first outbreak in India was recorded in Kolkata in 1963 (Shah et al., 1964). In 1964, the epidemics were recorded in the east coast areas like Chennai, Pondicherry, Vellore, Vishakapatnam (Rao, 1965). In 1965, in Nagpur 40–70% was affected in certain wards and mortality was negligible (Arankalle et al., 2007). Sporadic cases were recorded in Barsi, Maharashtra in 1973. The strains causing the epidemics in India between 1963 and 1973 belonged to the Asian subgroup (Arankalle et al., 2007).

Chikungunya fever, a previously neglected arboviral disease, has caught new attention as it reemerged around the globe. The epidemic in 2005 in the Indian Ocean region, probably emerged first in Kenya (Pialoux et al., 2007), then reached to the Comoros (January, 2005) and Seychelles (March, 2005), followed by Mauritius. The virus reached Reunion in the month of March in 2005 (Pialoux et al., 2007). The disease continued to spread in many parts of south-east Asia, involving Malaysia in late 2006 (AbuBakar et al., 2007), Singapore in early 2008 (Leo et al., 2009; Ng et al., 2009), Thailand from August 2008 (Thavara et al., 2009; Theamboonlers et al., 2009), Indonesia in 2009 (Laras et al., 2005) and Myanmar in 2010 (Ng et al., 2010).
In Italy, an outbreak of more than 200 confirmed cases in the summer of 2007 (Rezza et al., 2007) created tremors as the disease was no longer restricted to developing, tropical countries (Enserink, 2007). Cases were further reported from UK, Belgium, Germany, Czech Republic, Norway, Italy, Spain and France, Hong Kong, Canada, Taiwan, Sri Lanka and the USA. These cases were directly associated with the travelers from India and islands of the Indian Ocean, which were affected (Warner et al., 2006).

In India, Andhra Pradesh (AP) was the first state to report the re-emergence of the disease in December 2005 (Saxena et al., 2006). Over 80,000 patients were suspected from this state. Since then, about 1,80,000 cases have occurred in India (Ravi, 2006). About 1.39 million people were affected in 2006, 60,000 in 2007, 95,000 in 2008 and about 67,000 in 2009 (Ministry of Health and Family Welfare, 2010).

Fig. G: Countries with reported Human CHIKV Infections till May 2012

[Source: Geographic Distribution of Chikungunya Virus, CDC (http://www.cdc.gov/chikungunya/map/index.html)]
2.2.2 ORIGIN AND EVOLUTION

For CHIKV, three lineages with distinct genotypic and antigenic characteristics have been identified: East-central-southern African (ECSA) phylogroup, Asian phylogroup and the West African phylogroup (Pialoux et al., 2007). Recent findings suggest that the virus evolution also plays a key role in the epidemiology of the disease. The virus is known to have emerged in Africa and subsequently introduced in Asia (Powers et al., 2000). Two distinct CHIKV lineages were delineated, one containing all isolates from western Africa and the other consist of all Southern and East African strains and the isolates from Asia. The Asian genotype of CHIKV had never been reported in Africa. These suggest that in Asia, an African strain of CHIKV was independently evolved for several centuries, resulting in the Asian genotype (Ng et al., 2010). The ancestor of all of the CHIKV strains is estimated to have emerged between 150 and 1350 years ago (Powers et al., 2000). The Asian genotypes evolved from its African ancestors about 50–310 years ago and the west and east African lineages diverged approximately 100–840 years ago (Powers et al., 2000). This study also reported that in early 1900, the ECSA and Asian lineages could have diverged from a common ancestor.

Genetic analyses of the virus strongly suggest that the current pandemic started following a chikungunya fever outbreak in Kenya in 2004 (Powers and Logue 2007). The causative virus belongs to a new lineage of the East-central-southern African (ECSA) genotype (Parola et al., 2006; Schuffenecker et al., 2006). Whole genome and E1 gene-based molecular clock analyses revealed that the Yawat strain and current pandemic viruses could have diverged from an East/ Central African ancestor of CHIKV between 1960 and 1980 (Cherian et al., 2009). Cherian and his colleagues also suggested that Uganda-82 like strain was most likely the earliest ancestor of current pandemic viruses and originated between 1991 and 2004, probably in 1998. The phylogenetic analyses revealed that Chikungunya outbreaks in the
Indian Ocean Islands and India were caused by the two separate sub-lineages of CHIKV strains within a large monophyletic clade of the ECSA genotype (Hapuarachchi et al., 2010; Yergolkar et al., 2006). All the isolates of ECSA genotype from the south and south-east Asian countries and Italy were clustered within the Indian sub-lineage. Both of these sub-lineages evolved from Kenyan isolates which indicates that the recent pandemic virus belong to a central/east African.

2.2.3 CLINICAL MANIFESTATION

CHIKV is one of the major viral pathogen throughout the world which causes Chikungunya infection. The clinical manifestation include acute febrile phase of the illness with rigor, a petechial or maculopopular rash which lasts only 3-7 days (Mohan 2006; Simon et al., 2008; Sudeep and Parashar 2008), followed by a prolonged arthralgia that affects the joints of the extremities. Although Chikungunya infection is not a killer disease, but high morbidity rate and prolonged polyarthralgia causes substantial socioeconomic impact in affected countries (WHO, 2009). In susceptible populations, both the genders are equally affected. The pain associated with CHIKV infection of the joints persists for weeks or months depending on their age (Mohan 2006).

The incubation period of Chikungunya disease is from 2-10 days (Richman et al., 1997). At the acute phase, the symptoms of the disease include fever up to 104 °F (Deller & Russell, 1968) with rigor, a petechial or maculopopular rash, which appear on 2–5 days post infection and may last up to 10 days (Taraphdar et al., 2012). This is distributed mainly on the face, limbs and trunk of the body (Deller & Russell, 1968). The most severe clinical manifestations in CHIKV infections are severe joint pain and polyarthralgia, which can last for
years in some cases, depending on their age (Deller & Russell, 1968; Fourie & Morrison, 1979). Various studies documented that this arthritis mimics rheumatoid arthritic syndrome (Bouquillard et al., 2001; Fourie & Morrison, 1979). Studies by S.P. Manimunda and his colleagues showed that in 36% of patients the chronic arthritis following CHIKV infection behaved clinically as RA (Manimunda et al., 2010). In the acute stage, the arthritis due to CHIKV was symmetrical in nature, but in chronic stage arthritis was asymmetrical and even a mono arthritis. Headache, retro-orbital pain, photophobia, lumbar back pain, chills, weakness, malaise, nausea, vomiting and myalgia may also be associated (Brighton, 1981; McGill, 1995; Calisher, 1999). Typically, the fever lasts for two days and then ends abruptly. The chronic phase of disease has not been extensively investigated in contrast to acute stage. CHIKV antigens and viral replication have been detected in satellite cells, but not in muscle fibers (Ozden et al., 2007). These findings suggested that CHIKV might infect human muscle satellite cells, which are potential virus reservoirs and causing the chronic muscle pain (Laksmi et al., 2008; Lemant et al., 2008).

Neurological complications due to CHIKV have been reported from various parts of world. A hospital based surveillance on the island of Re’union in the Indian Ocean in 2005 revealed many atypical cases amongst the CHIKV infected individuals (Economopoulou et al., 2009). About 10% of the affected patients were died.

In India, the first large series of neurological complications like encephalitis, myopathy, neuropathy, myelopathy and myeloneuropathy due to CHIKV infection was reported from Maharastra in 2006 (Chandak et al., 2010). In the same year, neurological complications like altered level of consciousness in form of confusion, disorientation, drowsiness and delirium were observed amongst the hospitalized patients in Kota, India (Rampal et al., 2007). About 1/3rd of the Chikungunya affected individuals showed the complications at the CNS levels in
the form of encephalitis, encephalomyelitis and optic neuritis and some of them were died also. Studies at Bellary, India, revealed acute CNS infections in children with a febrile illness (<2 weeks' duration) with altered mental status and seizures (Lewthwaite et al., 2009). In Andaman and Necobar Island, four cases of acute flaccid paralysis due to CHIKV were reported (Singh et al., 2008). All had progressive weakness of all four limbs which was ascending and symmetrical. Flaccid limb weakness following CHIKV infection was reported from India in the 1964 epidemic. In Ahmedabad, India increased mortality rate associated with Chikungunya epidemic during August-November 2006 was also been reported (Mavalankar et al., 2008).

2.2.4 PATHOGENESIS

Transmission of CHIKV occurs through a bite by the infected vector mosquitoes. Following transmission, CHIKV replicates in the skin and then disseminates to the liver and joints, through the blood (Talarmin et al., 2007). The incubation period is 2–4 days and is followed by a sudden onset of clinical disease with no prodromal phase. The acute phase of the disease lasts for 3–10 days with high fever, rigors, headache, photophobia and a petechial or maculopapular rash, but the convalescent phase usually lasts from weeks to months with accompanying joint pain and swelling of joints; sometimes it may last for a year or more (Schwartz and Albert 2010). The main target cell of CHIKV is the fibroblast (Couderc and Lecuit 2009). In joints, CHIKV is detected in the connective tissue located beneath the synovial wall, and in the skin CHIKV is found in the deep dermis. Both joint and muscle connective tissues contains a high amount of nociceptive nerve-endings (Stecco et al., 2007) that may account for the muscle and joint pain. The pain triggered by joint mobilisation may also result from the infection of musculo-tendinous insertions surrounding them.
CHIKV gets access to the CNS via the Virchow-Robin spaces and choroid plexuses. Choroid plexus epithelial cells may also secondarily infected via their apical surface, and then reach the leptomeninges and ependymal cells and thus amplifying viral titres in the CSF. However, CHIKV does not infect the brain microvessels and parenchyma and does not induce detectable tissue alteration at the brain parenchyma level.

2.2.5 CHIKV TRANSMISSION CYCLES

2.2.5.1 VECTORS

In Asia and the Indian Ocean region the main CHIKV vectors are *Aedes aegypti* and *Aedes albopictus* (Delatte et al., 2008). CHIKV appears to be enzootic across tropical regions of Africa and Asia. CHIKV in West and Central Africa is believed to be maintained in a sylvatic cycle involving wild non-human primates and forest-dwelling *Aedes spp.* mosquitoes like *Aedes albopictus, Aedes furcifer, Aedes taylori* (Diallo et al., 1999; McIntosh et al., 1977). In rural regions, the outbreaks are dependent upon the sylvatic mosquito densities, which increase with periods of heavy rainfall (Lumsden, 1955). *Aedes aegypti* has been found to be the most significant vector in Asia (Powers and Logue 2007). These mosquitoes are urban and peridomestic, anthropophilic mosquitoes that maintain close associations with humans and thus, are likely responsible for regional large outbreaks.

Although *Aedes albopictus* has been considered an accessory vector, some recent urban outbreaks in Indian Ocean, parts of India and in Southeast Asia, have been vectored primarily by this mosquito (Peterson et al., 2010). This may be due to that, *Aedes albopictus* is a competent vector for Dengue and other arboviral infection (Schwartz and Albert 2010) and replacing the *Aedes aegypti* in some places (including China, the Seychelles, and Hawaii).
Review of literature

(Lamballerie et al., 2008; Gould and Higgs 2009; Pialoux et al., 2007). Aedes albopictus has a wide geographical distribution and is native to Southeast Asia and has colonized both tropical and temperate regions. It is particularly resilient, and can survive in both rural and urban environments (Pialoux et al., 2007). The mosquito’s eggs are highly resistant and can remain viable throughout the dry season, giving rise to larvae and adults the following rainy season. Originating from Asia, and initially sylvatic, A albopictus has spread due to urbanisation. A albopictus has been imported through the trade in used tyres and in ornamental plants (Knudsen 1995). Due to its aggressive, diurnal nature the infectivity by this vector is high, which is one of the reasons of rapid spreading of this disease all over the world.

![Diagram of CHIKV cycles](source: Peterson et al., 2010)

Fig. H: (A) CHIKV in Africa is maintained in a sylvatic cycle involving forest dwelling Aedes spp. mosquitoes and nonhuman primates. (B) In urban settings, CHIKV circulates in a man-mosquito-man cycle vectored principally by the Aedes aegypti mosquito.

[Source: Peterson et al., 2010]

2.2.5.2 HOST

Human beings serve as the reservoir for chikungunya virus during epidemic periods. Outside these periods the main reservoirs are monkeys, rodents, birds, and other unidentified vertebrates. Outbreaks might occur in monkeys when herd immunity is low; the animals
develop viraemia but no pronounced physical manifestations (Inoue et al., 2003; Wolfe et al., 2001).

### 2.2.6 MOLECULAR BIOLOGY OF CHIKV

CHIKV, an arbovirus belonging to the genus Alphavirus (*Togaviridae* family), has a single-stranded RNA genome, a 60–70 nm diameter capsid and a phospholipid envelope. Eighteen glycoprotein spikes extend from the virion surface in a T=4 lattice, giving the virion a total diameter of 69 nm. The flower-like spikes composed of E1 protein and E2 dimer; these are anchored with the membrane by conventional membrane-spanning anchors at the C-terminal regions (Harisson 1986, Sc). The nucleocapsid arranged in icosahedral T=4 symmetry in pentamer and hexamer capsid protein clusters. The positively charged outer rim of the capsomer is oriented towards the membrane and the highly charged N terminal domain binds to the viral RNA. The nucleocapsid complexes the 42S genomic RNA.

The genome, which is 11 to 12 kb in length, is organized with nonstructural proteins (nsP1-4) at the 5'-end and structural proteins (Capsid-E3-E2-6k-E1) at the 3'-end excluding the 5' cap nucleotide, an 1-poly(A) tract and the 3' poly(A) tail (CHIKV S27 strain) (Pialoux et al., 2007). The 5' end of the genome has a 7-methylguanosine cap, while the 3' end is polyadenylated. The polyprotein of the structural gene are generated by translation of a subgenomic mRNA, which is further processed to produce a capsid protein, two major envelope surface glycoproteins (E1 and E2) as well as two small polypeptides, E3 and 6K (Weaver et al., 2005). Conserved sequences at the junction between non structural and structural domains and in 5' and 3' terminal nonstructural regions serve as promoter. E1 and E2 are post-translationally modified in the endoplasmic reticulum and Golgi apparatus (Power et al., 2007) before being transported to the plasma membrane (Schlesinger & Schlesinger, 1986) where they maintain a
close association with each other, forming a trimeric heterodimer spike structure (Anthony & Brown, 1991; Paredes et al., 1993). The two domains on the E2 proteins are associated mainly with antibody mediated viral neutralization and the E1 glycoprotein contains the viral hemagglutinin, fusion protein and cross reactive neutralizing epitopes (Richman et al., 1997). The C-terminal domain of the 6K polypeptide acts as a signal sequence for the translocation of E1 (Richman et al., 1997).

Fig. I. Organization of the CHIKV genome and gene products.

[Source: Solignat et al., 2009]

2.2.7 REPLICATION CYCLE

CHIKV attach to the cells via envelope glycoproteins E1 and E2. After binding to the cell membrane, virions are taken up in the endocytic vesicles (Cheng et al., 1995; Strauss and Strauss 1994). Virions spike, mainly through the E1 fusion promoting protein induce bridging of virion and vesicle membranes with release of the nucleocapsid. Acidic conditions in the
vesicle lead to shrinkage of the capsid, exposure of a ribosomal binding domain, and releasing RNA from the capsid matrix. In the cytoplasm, positive sense viral genome functions as messenger RNA. Nonstructural proteins are translated as polyprotein, mainly as P123 moiety when translation is terminated by an UAG codon between nsP3 and nsP4 or when replaced by a sense codon as P1234 polyprotein.

After infection by the virus, RNA replicase complexes are assembled on modified endosomal and lysosomal membranes (Froshauer et al., 1988). The partially purified replicase complexes are membrane associated (Ranki & Kiiariainen 1979) and contain viral nonstructural proteins nsP1, nsP2, nsP3, and nsP4, as well as a cellular 120-kDa protein and other cellular proteins (Barton et al., 1991). These are capable of limited RNA synthesis. These complexes are formed early in infection and are stable throughout the infection cycle. Cellular proteins are also thought to be required for RNA synthesis, possibly as components of the replicase complexes. One or more of the structural proteins may interact in some way with the RNA replication machinery to regulate RNA synthesis.

RNA is replicated with the production of negative sense RNA templates, which are transcribed either to full-length genomic positive sense RNA or, when initiated at the internal junction site, to 26S sub genomic RNA. The 5' ends of both are capped and methylated. Genomic RNA can bind to replicase, producing more negative strand templates; to ribosomes, where translation leads to additional nonstructural proteins; or, after they become available, to capsid proteins for arrangement into nucleocapsid (Richman et al., 1997). Subgenomic RNA is co-translationally processed with fast auto proteolytic release of capsid protein. This cleavage exposes an N-terminal signal sequence that facilitates transport and insertion of the PE2 polyprotein (containing E2) into the ER membrane. Subsequent signals on PE2 lead to insertion of the 6K protein and translocation and cleavage of the E1. The envelop proteins are
modified in the ER lumen by addition of the carbohydrate chains and fatty acids and further modified during transport through the Golgi apparatus. PE2 and E1 dimers are formed in the ER; during transport through the Golgi apparatus, PE2 is cleaved to form E2 and E3. The envelop spikes are transported to the cell membrane, where the E2 cytoplasmic domain associates with the nucleocapsid C-terminal domain prior to viral budding.

Fig. J: Summary of the CHIKV replication cycle.

[Source: Solignat et al., 2009]

2.2.8 VACCINE AND ANTIVIRAL DEVELOPMENT

CHIKV vaccine is highly desirable as CHIKV has high infection rates during outbreaks, the geographical distribution of this virus is extensive and the morbidity associated with the disease is high. CHIKV from the original outbreak (Mason & Haddow, 1957) was inactivated by formalin. The preparation harvested from green monkey kidney cells was found
to induce high levels of antibodies. It developed good protection in monkeys after challenge with four strains of CHIKV (Harrison et al., 1967). Potency tests were conducted in mice and were followed up with a human volunteer study. No adverse events were noted in any volunteers and all subjects developed significant levels of neutralizing antibody by day 42 (Harrison et al., 1971).

The development of a second generation CHIKV vaccine CHIK 181/clone 25 began using the 15561 strain as seed material. This was developed by series of 18 plaque-to-plaque passages in MRC-5 cells of the starting virus, which had been passaged 11 times in GMKC culture (Levitt et al., 1986). The vaccine was examined in both Ae. aegypti and Ae. albopictus, the two mosquitoes known to be competent urban vectors of the virus (Banerjee et al., 1988; Turell et al., 1992). While the vaccine was able to replicate in both species and could be transmitted by the mosquitoes that received the virus via intrathoracic inoculation, there was no evidence of reversion to virulence. Unfortunately, limited resources and lack of commercial potential (prior to the 2005–2007 outbreaks) restricted the scope of additional testing; however, overall, the vaccine appeared to be promising with further safety, immunogenicity and duration of immunity studies being warranted.

The latest development in vaccine production was a Material Transfer Agreement on September 6th 2006 signed by the United States Army Medical Research Institute for Infectious Diseases (USAMRIID) and the French National Institute of Health and Medical Research (Inserm) Transfert, Inserm’s technology-transfer organization (Powers and Logue 2007). This agreement allowed the transfer of records of previous clinical studies and supplies of the vaccine and seed stock from which it was made for further development of the vaccine, including additional clinical trials in affected areas. (Press Release – Embassy of the United States of America, Paris, France. September 14th 2006).
As CHIKV characteristically induces a non-fatal, self-limiting disease, treatment has historically been entirely supportive for the symptoms. With the primary signs including fever and joint pain with swelling, analgesics, antipyretics and anti-inflammatory agents are the most appropriate treatment. Commonly, aspirin was used, but due to the reports of possible haemorrhagic manifestations, other options (Tesh, 1982) including acetaminophen, ibuprofen, steroid therapy and indomethacin is preferred over aspirin. Paracetamol or non-steroidal anti-inflammatory drugs can be used (Mohan et al., 2010). Compounds including ribavirin, sulfated polysaccharides (iotacarrageenan, fucoidan and dextran sulfate), 6-azauridine, glycyrrhizin and interferon-a have been evaluated for their ability to inhibit replication of CHIKV in cell culture (Power and Logue 2007). With the exception of the polysaccharides, all were found to have both potent and selective antiviral activity (Briolant et al., 2004). The scope of the outbreaks from 2005-2007 has sparked much needed interest by researchers in the pharmaceutical industry in developing viable antiviral agents against virus.

The possible reasons for the re-emergence of chikungunya in India are mostly obscure. Re-emergence of viral infections is mainly due to a variety of social, environmental, behavioral and biological changes. Phylogenetic trees corroborated historical evidence that the virus originated in Africa and subsequently was introduced into Asia. In West Bengal, after its re-emergence, it has already been spread all over the state with a huge number of cases. Encephalopathy due to CHIKV is not now very uncommon. So constant monitoring on the circulating strain is required to understand if any mutation has occurred in the virus that has facilitated the spread of this virus in this state of West Bengal and the involvement of the virus to cause encephalopathy, if any.