7. SUMMARY AND CONCLUSION

7.1 Japanese Encephalitis Virus

1) JE is still a threat to the paediatric age group in West Bengal. Out of 828 samples tested, 291 samples (35.1%) were positive to JEV infection.

2) Yearly distribution of JE cases from 2005-2011 showed that initially in the year 2005, JE cases were much higher, but suddenly it declined in the year 2006. Again from the year 2007, it is in the increasing state.

3) The highest number of JE cases was recorded in the year 2011.

4) JE vaccines were given to the children belonging to the age group of 1-15 years in some JE endemic districts of West Bengal from 2006-2009. In spite of vaccination, JE cases were reported from the paediatric-adolescent age group from the vaccine covered districts in the year 2010 and 2011.

5) The maximum numbers of positive cases were found from the districts of Malda, Midnapore [East], Jalpaiguri, Nadia and Murshidabad, where no vaccination has yet been initiated.

6) In 2011, JE cases were reported from the district of Jalpaiguri and Coochbehar, which are located in the hilly and cold climatic regions. Migratory birds, which also act as a reservoir for the virus, might play an important role in the transmission of the disease.

7) The highest numbers of positive cases have been recorded in the age group 11-20 years, followed by 0-10 years,
8) Male individuals were more affected than the females.

9) JE risk was significantly associated with the rural residents, living in close proximity to irrigated rice fields (preferred breeding place for vector mosquito of JE) and pig-rearing places.

10) Most of the positive cases (56%) belonged to the uneducated group. Within the literates, 90.2% of the positive cases had the lower educational level like primary, lower secondary and secondary.

11) Maximum number of JE positive cases (65%) belonged to the low income group (income level ≤ 5000/ month).

12) Hemagglutination Inhibition (HAI) study shows that the JEV is in circulation in the districts of Birbhum, Burdwan and Hooghly, which were vaccinated from 2006-2009. But the detection of HAI antibody level in those districts was not up to the mark and was not potent enough to protect the total population against the JEV infection.

13) Most of the JE positive cases (88.3%) found in the monsoon and post-monsoon period. The highest number of JE cases was recorded in the month of September; followed by October and November.

14) Phylogenetic analysis based on the complete gene sequences of the two isolates from West Bengal i.e. IND-WB-JE1 and IND-WB-JE2 indicated almost close clustering of the West Bengal isolates in genotype III. Phylogenetically these isolates showed maximum homology (99% nucleotide similarity) with the first isolated strain of India
i.e. Vellore P20778, obtained from a patient from Vellore (south India), during 1958 and with H225 strain, isolated in the year 2009 from a horse in Haryana (North India).

15) In IND-WB-2 isolate, a non-synonymous unique mutation G9645T was found in the NS5 gene, which corroborates A688S change in the polypeptide.
7.2.1 Monotypic infection due to CHIKV

1) After the re-emergence of the CHIKV in 2006, all the districts of the Southern part of West Bengal, India were affected by the year 2008.

2) In 2009, CHIKV-positive cases were recorded from North Bengal, i.e. from Malda and Dakshin Dinajpur.

3) In the year 2011, CHIKV infection was also recorded from the district of Jalpaiguri, located at the northern region of this state.

4) CHIKV-positive cases have significantly increased from 7.01% to 47.1% during the study period i.e. from 2006-2011.

5) Although all age groups were affected by CHIKV, slightly higher numbers of positive cases were observed in the age group of 31–40 years (28.1%) followed by 41–50 years (20.5%).

6) Females were more affected than the males.

7) The highest number of CHIKV-positive cases was recorded in the monsoon and post monsoon period.

8) A total of 16 isolates were achieved by tissue culture system from 2006-2011. Molecular characterization showed that these isolates belonged to East-Central-South African origin. The percentage nucleotide sequence identity of these 16 West Bengal isolates with other Indian isolates, belonged to the ECSA genotype is 99%.
9) These West Bengal ECSA isolates showed 95% nucleotide sequence identity with the Asian genotypes (IND-63-WB1 and IND-73-MH5) and 86% nucleotide sequence identity with the African genotypes (strain 37997).

10) In our study, the IND-11-WBST1, IND-11-WBST2 and IND-11-WBST3 isolates contain both E1-226A and E1-K211E mutations, which are highly conserved in the ECSA-CHIKV strains, circulated in the Asian urban areas where *Aedes aegypti* were abundant. These were isolated in the year 2011 from Kolkata.

11) The IND-11-WBST1 isolate also contained E1-K211V and E2-V264A variations. These were first reported from France in September 2010, which was an autochthonous imported case from Rajasthan, India. In India, the same was reported from Tamil Nadu, Andhra Pradesh and Delhi in 2010.

12) The remaining 13 strains, which contained E1-211K and E1-A226V mutation, were isolated from rural/semi urban areas of West Bengal from 2006-2010, where *Aedes albopictus* and *Aedes aegypti* are abundant. No genetic variability for the structural gene was observed within each other of these 13 strains.

13) E2-G60D and E2-I211T mutations were found in all the isolates of West Bengal. The E2-G60D mutation can alone determine the CHIKV infectivity for both *Aedes albopictus* and *Aedes aegypti*, but only moderately regulate the effect of the E1-A226V mutation in *Aedes albopictus*. The E2-G60D and E2-I211T mutation together can increase the infectivity of the CHIKV with A226V mutation in *Aedes albopictus*. 
14) In the E3 region of the IND-11-WBST1, IND-11-WBST2 and IND-11-WBST3 isolates contained E3-V303I and E3-P320S mutation, which were unique and were not reported earlier.

7.2.2 Dual infection due to CHIKV and DENV

1) Dual infection by both CHIKV and DENV were recorded in the year 2010 and 2011. During this time, a total of 115 patients were affected by both the infection.

2) Like CHIKV cases, highest number of dual infected cases was found in the age group of 31–40 years (31.3%).

3) The female/male ratio was 1.8:1. Females were much more affected than males.

4) Urban/semi-urban areas were more affected by both CHIKV and DENV.

5) Dual-infected cases attained its peak in the month of October.

6) Four districts of West Bengal i.e. Kolkata, Nadia, North 24 parganas and South 24 parganas were affected by both the viruses. These four districts were also affected by the monotypic infection of CHIKV.

7) In case of dual infection, ELISA result revealed that the OD value of the Chikungunya IgM antibody was at least four times higher than the OD value of the dengue IgM antibody. This result amply justifies that the CHIKV infection took place in the later phase of biphasic manifestation of fever, which was found in all the dual infected cases.

8) No haemorrhagic manifestation was observed.
7.2.3 CNS manifestation in CHIKV infection

1) Four samples were identified as CHIKV infection among the acute encephalitic syndrome cases, referred to ICMR Virus Unit, Kolkata. All these were identified during the year 2010-11.

2) The case—1 and case—2 had the acute encephalitis syndrome; case—3 was the case of acute disseminated encephalomyelitis whereas the case—4 had the symptoms of meningo encephalopathy with bulbar involvement.

3) Both blood and CSF specimens of all the samples were tested for the detection of CHIKV etiology in them by ELISA and RTPCR method. All the four patients’ samples were confirmed by ELISA and RTPCR method.

4) In all four cases CSF report showed lymphocyte pleocytosis with moderately raised CSF protein (range 66-135 mg/dl) which indicated the cases of viral encephalitis.
Future steps to be taken:

1) To avoid the risk of JE, these rural residents should take personal protection from the vector mosquitoes by controlling of larva of the vector. On the other hand, to overcome the risk of JE, rural residents should introduce either rearing of pigs in the modern farms, far from their housing or they may start rearing of cattle (considered as dead-end host with low viremia) instead of pigs as an additional income source to reduce the chances of disease transmission. Socio-economic and environmental conditions highly influence the JE incidences in West Bengal and the affected populations, whether educated or uneducated, were not aware of the disease and its preventive measures. Therefore, enmass awareness about the severity of the disease and its post recovery complications in the rural areas of this state is extremely necessary. Also implementation of vaccination in some districts of West Bengal could not control the disease burden in those areas. Therefore, full coverage of vaccine among the young and young adult is highly recommended in the state of West Bengal. Adult vaccination programme should start.

2) CHIKV is rapidly spreading in the state of West Bengal with high morbidity, suppressing the dengue activity. The co circulation of CHIKV and DENV simultaneously may provide a suitable environment to allow genetic re-assortment of DENV and CHIKV, resulting in the evolution of new strain, which if spill over the population will create a great public health problem. CNS manifestation due to CHIKV infection is also an alarming situation. The changing pattern needs detail molecular study which is under investigation. Personal protection from the vector mosquitoes and control of larva of the vector should be taken.