MOLECULAR CHARACTERIZATION OF SOME *Chikungunya virus* ISOLATES FROM 2007/08 OUTBREAK IN SRI LANKA

A PROJECT REPORT PRESENTED BY

H. M. ISHARA PETHUM HERATH

to the Board of Study in Biochemistry and Molecular Biology of the

POSTGRADUATE INSTITUTE OF SCIENCE

in partial fulfilment of the requirement
for the award of the degree of

MASTER OF SCIENCE IN EXPERIMENTAL BIOTECHNOLOGY

of the

UNIVERSITY OF PERADENIYA
SRI LANKA
2010

ABSTRACT

MOLECULAR CHARACTERIZATION OF SOME *Chikungunya virus* ISOLATES FROM 2007/08 OUTBREAK IN SRI LANKA

H. M. ISHARA PETHUM HERATH

Postgraduate Institute of Science

University of Peradeniya

Peradeniya

Sri Lanka.

Chikungunya virus (CHIKV) is an insect-borne virus which causes the disease known as Chikungunya viral fever. The disease was first described by Marion Robinson and W.H.R. Lumsden in 1955, following an outbreak in 1952 on the Makonde Plateau in Tanzania. The virus was first isolated in 1953 by R.W Ross during an epidemic in Newala district in Tanzania, East Africa. CHIKV was first detected Sri Lanka in 1965 and it resulted in an explosive outbreak of this debilitating disease attacking a large number of people. The second major Chikungunya outbreak in Sri Lanka was reported in 2006/2007. However, very little was known about the origin and phylogenetics of CHIKV in Sri Lanka.

The objective of this study was to determine the possible origins of CHIKV in Sri Lanka and to infer its transmission patterns. This study was expected to set up basic DNA sequence data which would help in the identification of mutations in the genome of the virus currently circulating in Sri Lanka and detection of novel strains of the virus in the future. Serum samples were collected from Genetech Molecular Diagnostics, Colombo 08, Sri Lanka. Most of the samples were collected during the 2007/ 2008 Chikungunya epidemic and few samples from 2009. These serum samples are confirmed for the presence of the Chikungunya virus by an RT-PCR assay.

Twenty nine serum samples were processed and RNA was extracted by using a silica-guanidium thiocyanate viral RNA extraction method. Viral RNA was amplified by RT-PCR using primers specific for the E1 gene. Amplified RT-PCR products were purified and sequenced using a ABI Prism 310 genetic analyzer. Bidirectional cycle sequencing was performed in order to confirm the sequences. The sequences were analyzed and compared with the other geographical isolates in the ICVTdB to find the phylogenetic relationships between the global isolates and Sri Lankan isolates.

After aligning only the Sri Lankan CHIKV sequences, fifteen differences in the nucleic acid sequences and two differences in amino acid sequences were observed. From the sequence alignment, it was found that several Sri Lankan isolates shared identical sequences. These isolates had at least one nucleotide change in the E1 gene. Although the Chikungunya disease itself arrived in Sri Lanka from Southern India, the evolutionary origins of the Chikungunya virus in Sri Lanka points to the Central/East/South African genotypes. All the Sri Lankan isolates clustered with the Central/East/South African genotypes.

The genetic distance among the Sri Lankan isolates were a low value of 0.010. This is an indication of the high level of genetic homogeneity among the Sri Lankan isolates. Genetic distance among the isolates within a cluster consisting of the Sri Lankan individuals and the isolates from the Central-East/South African genotype was 0.013. This low genetic distance is indicative of the high level of genetic similarity between the Sri Lankan isolates and the reference isolates from the Central-East/South African genotype.

The E1 partial sequences (295bp) were compared with the available sequences in NCBI data. They showed the highest similarity to the Indian isolates in the database, especially from the Kerala region. This supports the epidemiological evidence for the arrival of the Chikungunya disease from South India first to the north western region of Sri Lanka before spreading throughout the country.