THE QUASISPECIES OF EV-A71 IN HAND, FOOT AND MOUTH DISEASE (HFMD) PATIENTS DURING THE 2003 SIBU, SARAWAK OUTBREAK

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DEDICATION

This thesis is a dedication to..

♥ Parents who encourage and siblings who support
♥ The family members and friends who love asking, "When are you going to further your study?" and followed by "When are you going to graduate?"

This is it. Alhamdulillah.
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- My supervisor, Assoc. Prof. Dr. David Perera for guiding and helping me to find my direction in this journey.

Thank you. Terima kasih.
Hand, Foot and Mouth Disease (HFMD) is a common childhood illness. Viruses from the enterovirus genus, in particular enterovirus 71 (EV-A71) and other species A enteroviruses, are the most common viruses responsible for HFMD infection.

Similar to other RNA viruses, EV-A71 also exist as quasispecies. Quasispecies describe a cloud of viral population in which mutation and selection pressures are at a balance. This occurs when viruses are adapting to environmental changes.

In this study, we determined the quasispecies population of EV-A71 genogroup B5 viruses, obtained during a HFMD outbreak in Sibu, Sarawak in 2003. Ten patients with multiple swabs sample with confirmed EV-A71 genogroup B5 were selected for the study. Partial VP1 gene sequence from two separate swab samples from each of the selected patients were cloned. A total of twenty randomly selected colonies with the correct inserts were selected from each sample and subjected to sequencing. These cloned sequences were then compared to a parental consensus sequence generated directly from PCR of the primary clinical sample. Sequence differences observed between cloned product and parental PCR product suggest quasispecies diversity of EV-A71 for that sample.

The three sample types studied included throat, rectal and vesicle swab samples. Ten percent of the cloned-derived colonies were identified with nucleotide change. About a third of the nucleotide changes led to a silent mutation during protein translation. No correlation was observed between quasispecies diversity and the phenotype of the virus as determined by clinical presentation of the patient.
Although the cloned-derived colonies in the vesicles swab samples are variants instead of clonal, the percentage of the cloned-derived colonies with amino acid change is only 6.7% which is the lowest compared to the other two sample types.
ABSTRAK

Penyakit Tangan, Kaki dan Mulut (HFMD) adalah penyakit biasa di zaman kanak-kanak. Virus dari genus enterovirus, terutamanya enterovirus 71 (EV-A71) dan spesies lain enterovirus A, adalah virus yang paling biasa menjadi penyebab kepada jangkitan HFMD.

Sama seperti virus-virus RNA yang lain, EV-A71 juga wujud sebagai kuasispesies. Kuasispesies menggambarkan satu kelompok populasi virus di mana mutasi dan tekanan pemilihan berada dalam keadaan seimbang. Ini terjadi apabila virus menyesuaikan diri dengan perubahan persekitaran.


Walaupun koloni di dalam sampel vesikel adalah varian dan bukannya klonal, peratusan koloni dengan perubahan asid amino hanya 6.7% sahaja iaitu yang terendah berbanding dua lagi jenis sampel.
## TABLE OF CONTENTS

Dedication .................................................................................................................. ii

Acknowledgements .................................................................................................. iii

Abstract ....................................................................................................................... iv

Abstrak ....................................................................................................................... vi

Table of contents ....................................................................................................... viii

List of tables ............................................................................................................... xv

List of figures .............................................................................................................. xvii

Abbreviations ............................................................................................................. xxi

Chapter 1: Literature Review ..................................................................................... 1

1.1 Picornaviridae ..................................................................................................... 1

1.2 Viruses causing Hand, Foot and Mouth Disease (HFMD) .................................... 5

1.3 HFMD .................................................................................................................. 7

1.4 HFMD outbreak in Sibu, Sarawak ....................................................................... 10

1.4.1 Sarawak state .................................................................................................. 10

1.4.2 Sibu division .................................................................................................. 14

1.4.3 HFMD outbreaks ............................................................................................ 14

1.5 Enterovirus ......................................................................................................... 16

1.5.1 Enterovirus structure .................................................................................... 18

1.5.2 Enterovirus genome ...................................................................................... 18

1.5.3 Viral proteins ................................................................................................ 21

1.5.3.1 Structural proteins ................................................................................... 21

1.5.3.2 Non structural proteins ........................................................................... 22
1.5.4 Enterovirus 71 (EV-A71) ................................................................................ 23
1.6 Quasispecies ................................................................................................. 31
  1.6.1 Definition ............................................................................................... 31
  1.6.2 Mechanism of quasispecies occurrence ............................................... 31
  1.6.3 Quasispecies and persistence ............................................................... 34
  1.6.4 Quasispecies and pathogenicity ............................................................ 34
Chapter 2 : Statement Of The Research Problem ............................................. 36
Chapter 3 : Materials And Methods ................................................................. 38
  3.1 Project outline .......................................................................................... 38
  3.2 Primer design ......................................................................................... 42
  3.3 Nucleic acid extraction .......................................................................... 44
  3.4 Optimizing the PCR protocol .................................................................. 45
    3.4.1 PCR optimization for cloning work .................................................. 46
      3.4.1.1 PCR with Taq DNA polymerase .............................................. 46
      3.4.1.2 PCR with Pfx50 DNA polymerase ........................................ 47
    3.4.2 PCR optimization for sequencing work ............................................ 47
      3.4.2.1 Purification of PCR products .................................................. 48
      3.4.2.2 Random primer for RT-PCR................................................... 49
  3.5 Cloning of primary samples .................................................................... 49
    3.5.1 Reverse Transcriptase PCR (RT-PCR) ............................................. 50
    3.5.2 PCR .................................................................................................. 51
      3.5.2.1 Optimised nested PCR for amplification of target partial VP1 gene for quasispecies determination ......................................................... 51
      3.5.2.1.1 First PCR ........................................................................... 54
3.7.1.2 Sequencing........................................................................................................... 69
3.8 Data analysis .................................................................................................................. 69
Chapter 4 : Results And Discussion.................................................................................. 71
4.1 In-house nested RT-PCR to amplify portion of VP1 gene............................................. 71
4.2 Optimizing the nested PCR protocol ........................................................................... 73
4.3 Amplification of the VP1 gene for sequencing............................................................... 82
4.4 Cloning of primary samples ......................................................................................... 87
  4.4.1 Amplification of partial VP1 for cloning ............................................................... 87
  4.4.2 Screening of transformants .................................................................................... 91
    4.4.2.1 Patient 1 ........................................................................................................... 91
      4.4.2.1.1 Sample 11874............................................................................................. 91
      4.4.2.1.2 Sample 11875............................................................................................. 91
    4.4.2.2 Patient 2 ........................................................................................................... 93
      4.4.2.2.1 Sample 11840............................................................................................. 93
      4.4.2.2.2 Sample 11841............................................................................................. 93
    4.4.2.3 Patient 3 ........................................................................................................... 95
      4.4.2.3.1 Sample 11949............................................................................................. 95
      4.4.2.3.2 Sample 11955............................................................................................. 95
    4.4.2.4 Patient 4 ........................................................................................................... 97
      4.4.2.4.1 Sample 10900............................................................................................. 97
      4.4.2.4.2 Sample 10901............................................................................................. 97
    4.4.2.5 Patient 5 ........................................................................................................... 99
      4.4.2.5.1 Sample 12679............................................................................................. 99
      4.4.2.5.2 Sample 12681............................................................................................. 99
4.4.2.6 Patient 6 ........................................................................................................ 101
  4.4.2.6.1 Sample 12868 ...................................................................................... 101
  4.4.2.6.2 Sample 12871 ...................................................................................... 101
4.4.2.7 Patient 7 ........................................................................................................ 103
  4.4.2.7.1 Sample 10416 ...................................................................................... 103
  4.4.2.7.2 Sample 10419 ...................................................................................... 103
4.4.2.8 Patient 8 ........................................................................................................ 105
  4.4.2.8.1 Sample 10286 ...................................................................................... 105
  4.4.2.8.2 Sample 10294 ...................................................................................... 105
4.4.2.9 Patient 9 ........................................................................................................ 107
  4.4.2.9.1 Sample 10243 ...................................................................................... 107
  4.4.2.9.2 Sample 10249 ...................................................................................... 107
4.4.2.10 Patient 10 ................................................................................................. 109
  4.4.2.10.1 Sample 10366 .................................................................................... 109
  4.4.2.10.2 Sample 10368 .................................................................................... 109
4.5 Isolation of recombinant plasmid DNA from selected transformants ............... 111
4.6 Investigation for quasispecies sequences ............................................................ 114
  4.6.1 Sequencing of parental virus .......................................................................... 114
  4.6.2 Sequencing and comparison of cloned-derived sequences to their respective parental viral sequence ......................................................................................... 118
    4.6.2.1 Samples from patient 1 ........................................................................... 118
      4.6.2.1.1 Sample 11874 .................................................................................. 118
      4.6.2.1.2 Sample 11875 ................................................................................ 122
    4.6.2.2 Samples from patient 2 ........................................................................... 125
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6.2.2.1 Sample 11840</td>
<td>125</td>
</tr>
<tr>
<td>4.6.2.2.2 Sample 11841</td>
<td>128</td>
</tr>
<tr>
<td>4.6.2.3 Samples from patient 3</td>
<td>131</td>
</tr>
<tr>
<td>4.6.2.3.1 Sample 11949</td>
<td>131</td>
</tr>
<tr>
<td>4.6.2.3.2 Sample 11955</td>
<td>135</td>
</tr>
<tr>
<td>4.6.2.4 Samples from patient 4</td>
<td>138</td>
</tr>
<tr>
<td>4.6.2.4.1 Sample 10900</td>
<td>138</td>
</tr>
<tr>
<td>4.6.2.4.2 Sample 10901</td>
<td>142</td>
</tr>
<tr>
<td>4.6.2.5 Samples from patient 5</td>
<td>146</td>
</tr>
<tr>
<td>4.6.2.5.1 Sample 12679</td>
<td>146</td>
</tr>
<tr>
<td>4.6.2.5.2 Sample 12681</td>
<td>149</td>
</tr>
<tr>
<td>4.6.2.6 Samples from patient 6</td>
<td>153</td>
</tr>
<tr>
<td>4.6.2.6.1 Sample 12868</td>
<td>153</td>
</tr>
<tr>
<td>4.6.2.6.2 Sample 12871</td>
<td>157</td>
</tr>
<tr>
<td>4.6.2.7 Samples from patient 7</td>
<td>161</td>
</tr>
<tr>
<td>4.6.2.7.1 Sample 10416</td>
<td>161</td>
</tr>
<tr>
<td>4.6.2.7.2 Sample 10419</td>
<td>165</td>
</tr>
<tr>
<td>4.6.2.8 Samples from patient 8</td>
<td>169</td>
</tr>
<tr>
<td>4.6.2.8.1 Sample 10286</td>
<td>169</td>
</tr>
<tr>
<td>4.6.2.8.2 Sample 10294</td>
<td>173</td>
</tr>
<tr>
<td>4.6.2.9 Samples from patient 9</td>
<td>177</td>
</tr>
<tr>
<td>4.6.2.9.1 Sample 10243</td>
<td>177</td>
</tr>
<tr>
<td>4.6.2.9.2 Sample 10249</td>
<td>181</td>
</tr>
<tr>
<td>4.6.2.10 Samples from patient 10</td>
<td>185</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1.1 Serotypes of the enterovirus A genus.............................................................. 25
Table 1.2 Clinical findings from some of the EV-A71 outbreaks ........................................ 30
Table 3.1 Selected study samples......................................................................................... 40
Table 3.2 List of PCR primers ............................................................................................. 43
Table 4.1 Summary of nucleotide differences observed in cloned-derived sequences of sample
11874 when compared to the parental virus sequence ......................................................... 121
Table 4.2 Summary of nucleotide differences observed in cloned-derived sequences of sample
11949 when compared to the parental virus sequence .......................................................... 134
Table 4.3 Summary of nucleotide differences observed in cloned-derived sequences of sample
10900 when compared to the parental virus sequence ......................................................... 141
Table 4.4 Summary of nucleotide differences observed in cloned-derived sequences of sample
10901 when compared to the parental virus sequence ......................................................... 145
Table 4.5 Summary of nucleotide differences observed in cloned-derived sequences of sample
12681 when compared to the parental virus sequence ......................................................... 152
Table 4.6 Summary of nucleotide differences observed in cloned-derived sequences of sample
12868 when compared to the parental virus sequence ......................................................... 156
Table 4.7 Summary of nucleotide differences observed in cloned-derived sequences of sample
12871 when compared to the parental virus sequence ......................................................... 160
Table 4.8 Summary of nucleotide differences observed in cloned-derived sequences of sample
10416 when compared to the parental virus sequence ......................................................... 164
Table 4.9 Summary of nucleotide differences observed in cloned-derived sequences of sample
10419 when compared to the parental virus sequence ......................................................... 168
Table 4.10 Summary of nucleotide differences observed in cloned-derived sequences of sample 10286 when compared to the parental virus sequence.......................... 172

Table 4.11 Summary of nucleotide differences observed in cloned-derived sequences of sample 10294 when compared to the parental virus sequence.......................... 176

Table 4.12 Summary of nucleotide differences observed in cloned-derived sequences of sample 10243 when compared to the parental virus sequence.......................... 180

Table 4.13 Summary of nucleotide differences observed in cloned-derived sequences of sample 10249 when compared to the parental virus sequence.......................... 184

Table 4.14 Summary of nucleotide differences observed in cloned-derived sequences of sample 10366 when compared to the parental virus sequence.......................... 188

Table 4.15 Summary of nucleotide differences observed in cloned-derived sequences of sample 10368 when compared to the parental virus sequence.......................... 192

Table 4.16 List of primers for nucleotide change confirmation DNA sequencing .................. 194

Table 4.17 Comparison of mutation types within the virus population of the same patient.. 199

Table 4.18 Fraction of sample type................................................................. 201

Table 4.19 Number of colonies with amino acid change by sample type.......................... 201

Table 4.20 Fraction of mutation type by sample type........................................... 201
LIST OF FIGURES

Figure 1.1 Genera assignment in Picornaviridae family ................................................................. 4
Figure 1.2 Blisters on the palm ........................................................................................................ 8
Figure 1.3 Blisters on the soles ......................................................................................................... 8
Figure 1.4 Map of Malaysia ............................................................................................................ 12
Figure 1.5 Map of Sarawak ............................................................................................................ 13
Figure 1.6 Genome organization of enteroviruses ......................................................................... 20
Figure 3.1 Flowchart of the general outline of this quasispecies study ......................................... 41
Figure 3.2 Typical nested PCR ........................................................................................................ 53
Figure 4.1 Product of nested RT-PCR primer test ........................................................................ 72
Figure 4.2 Optimization of nested PCR done with Taq DNA Polymerase using dilutions of the
first PCR reaction ......................................................................................................................... 74
Figure 4.3 Dilution of nested PCR done with Pfx50 DNA polymerase ........................................ 74
Figure 4.4 Nested PCR done on Sample 4 with 2 µL diluted first PCR product ........................... 75
Figure 4.5 Nested PCR done on Sample 4 with 5 µL diluted first PCR product ........................... 76
Figure 4.6 Nested PCR done on Sample 4 with purified first PCR product ................................. 79
Figure 4.7 Nested PCR done on Sample 4 with gel extracted first PCR product ...................... 80
Figure 4.8 Optimization with random hexamer ............................................................................ 81
Figure 4.9 Nested RT-PCR to amplify partial VP1 region of EV-A71 isolates that were used
for DNA sequencing .................................................................................................................... 83-86
Figure 4.10 Nested RT-PCR to amplify partial VP1 region of EV-A71 isolates that were used
for cloning purposes .................................................................................................................... 88-90
Figure 4.11 Colony PCR done on samples 11874 and 11875 ......................................................... 92
Figure 4.12 Colony PCR done on samples 11840 and 11841............................................. 94
Figure 4.13 Colony PCR done on samples 11949 and 11955............................................. 96
Figure 4.14 Colony PCR done on samples 10900 and 10901............................................. 98
Figure 4.15 Colony PCR done on samples 12679 and 12681............................................. 100
Figure 4.16 Colony PCR done on samples 12868 and 12871............................................. 102
Figure 4.17 Colony PCR done on samples 10416 and 10419............................................. 104
Figure 4.18 Colony PCR done on samples 10286 and 10294............................................. 106
Figure 4.19 Colony PCR done on samples 10243 and 10249............................................. 108
Figure 4.20 Colony PCR done on samples 10366 and 10368............................................. 110
Figure 4.21 Plasmid DNA of some the transformants for patient 6................................. 113
Figure 4.22 Nucleotide sequence alignment report of all 20 viruses............................. 115-116
Figure 4.23 Amino acid sequence alignment report of all 20 viruses............................. 117
Figure 4.24 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 11874................................................................. 119-120
Figure 4.25 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 11875................................................................. 123-124
Figure 4.26 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 11840................................................................. 126-127
Figure 4.27 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 11841................................................................. 129-130
Figure 4.28 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 11949................................................................. 132-133
Figure 4.29 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 11955................................................................. 136-137
Figure 4.30 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10900 .......................................................... 139-140
Figure 4.31 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10901 .......................................................... 143-144
Figure 4.32 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 12679 .......................................................... 147-148
Figure 4.33 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 12681 .......................................................... 150-151
Figure 4.34 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 12868 .......................................................... 154-155
Figure 4.35 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 12871 .......................................................... 158-159
Figure 4.36 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10416 .......................................................... 162-163
Figure 4.37 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10419 .......................................................... 166-167
Figure 4.38 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10286 .......................................................... 170-171
Figure 4.39 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10294 .......................................................... 174-175
Figure 4.40 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10243 .......................................................... 178-179
Figure 4.41 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10249 .......................................................... 182-183
Figure 4.42 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10366. ................................................................................................................................. 186-187

Figure 4.43 Nucleotide sequence alignment of cloned-derived and parent sequences of sample 10368. ................................................................................................................................. 190-191

Figure 4.44 Alignment report for the protein sequences with missense and nonsense mutations. ................................................................................................................................. 196

Figure 4.45 Alignment report for the protein sequences with frame shift mutations. ......... 197
ABBREVIATIONS

µg  
µL  
bp  
BSA  
cDNA  
Cl  
CsCl  
CSF  
CV-A16  
DNA  
dNTP  
DTT  
EDTA  
EV-A71  
g  
HCl  
HFMD  
IHCM  
kb  
KCl  
LB  
M  
MgCl₂  
MgSO₄  
mL  
mM  
M-MuLV  
Mᵣ  
mRNA  
NaCl  
NaI  
NaOH  
ng  
ORF  
PCR  
RNA  
rpm  
RT-PCR  
SDS  
SOB  
SOC  
TAE  
TBE  
U
UHQ: ultra-high quality
UNIMAS: Universiti Malaysia Sarawak
UTR: untranslated region
UV: ultra violet
V: voltage
VP: viral protein
VPg: genome-linked protein
w/v: weight over volume
CHAPTER 1 : LITERATURE REVIEW

1.1 Picornaviridae

The name is derived from the word *pico* meaning small and RNA, hence *Picornaviridae* means small RNA virus.

Members of the family *Picornaviridae* are small, isometric, non enveloped, single stranded, linear, and have a positive sense RNA genome (Stanway, 1990; Prescott *et. al.*, 1993; Hyypiä *et. al.*, 1997; van Regenmortel *et. al.*, 2000) which belongs to Group IV in the Baltimore viral classification system.

The virion is between 20 to 28 to 30 to 35 nm in diameter (Stanway, 1990; Murphy, 1996) with a genome size between 7.0 to 7.2 and 8.4 to 8.5 kilo base (kb) (Stanway, 1990; van Regenmortel *et. al.*, 2000). The relative molecular weight (M_r) is between 8 to 9 x 10^6 Daltons and the buoyant density is between 1.33 to 1.45 g/cm^3 in caesium chloride (CsCl) (Rueckert, 1996; Murphy, 1996).

*Picornaviridae* has a capsid made up of 60 identical protomers arranged in icosahedral symmetry (Murphy, 1996) around the core of a single stranded RNA (Hyypiä *et. al.*, 1997; van Regenmortel *et. al.*, 2000). Each protomer contains one copy of four structural proteins, VP1 to VP4 (Stanway, 1990; Murphy, 1996; Hyypiä *et. al.*, 1997; van Regenmortel *et. al.*, 2000). The proteins VP1, VP2 and VP3 - also known as 1D, 1B and 1C respectively - are the surface proteins while VP4 - also known as 1A - is an internal structural protein. The nucleic acid contains a single long open reading frame (ORF) (Murphy, 1996; van Regenmortel *et. al.*, 2000) The ORF encodes for the polyprotein precursor to structural (VP1, VP2, VP3, VP4) and non structural proteins (2A, 2B, 2C, 3A, 3B, 3C, 3D) A poly A tail is
located after the 3' untranslated region (3' UTR) and a small protein called VPg is linked covalently to the 5' untranslated region (5' UTR) (Stanway, 1990; Rueckert, 1996; van Regenmortel et al., 2000). Both UTRs contain regions of secondary structure, essential in genome function (van Regenmortel et al., 2000). It has no peptomer and looks almost like a sphere.

There are six genera with sixteen species in the *Picornaviridae* family. The genera are divided based on their physiochemical properties such as acid stability and buoyant density (Stanway, 1990). The genera are enterovirus (8 species), rhinovirus (2 species), cardiavirus (2 species), aphthovirus (2 species), hepatovirus (1 species) (Hyypiä et al., 1997; Pringle, 1999; van Regenmortel et al., 2000; Chapman and Tracy, 2002) and parechovirus (1 species) (Pringle, 1999; van Regenmortel et al., 2000; Chapman and Tracy, 2002).

A revision done of the *Picornaviridae* family abolished all the existing species (serotypes) and replaced it by species consisting of groups of related serotypes (clusters). There are nine genera and twenty species in the *Picornaviridae* family after the revision (Pringle, 1999). One species was reassigned to hepatovirus genus (van Regenmortel et al., 2000) and three new genera were established. These new genera are erbovirus (1 species), kobuvirus (1 species) and teschovirus (1 species) (Pringle, 1999; Chapman and Tracy, 2002). Earlier, there was also genus echovirus in *Picornaviridae* family (Hyypiä et al., 1997). However, this virus were later to be shown as misidentified and reassigned to other genera (van Regenmortel et al., 2000).

In the latest release of virus taxonomy by ICTV, *Picornaviridae* family is divided into twenty six genera with forty six species. Rhinovirus was removed from the family and eighteen new genera were added. The new genera are sapelovirus which has three species, avihepatovirus, aquamavirus, avisivirus, cosavirus, dicipivirus, gallivirus, hunnivirus,