CONTENTS

Acknowledgements	c
Abstract in Thai	e
Abstract in English	h
List of Tables	n
List of Figures	р
List of Abbreviations	r
List of Symbols	t
List of Glossary	u
Chapter 1 Introduction	1
Chapter 2 Literature review	4
2.1 General characteristics and genome structure of rotaviruses	5
2.2 Classification of rotaviruses	9
2.3 Molecular epidemiology of rotaviruses	11
2.3.1 Molecular epidemiology of human rotaviruses	12
2.3.2 Molecular epidemiology of porcine rotaviruses	16
2.4 Evidences for interspecies transmission of rotaviruses	19
2.5 Mechanisms of genetic variation of rotaviruses	22
2.5.1 Genetic reassortment or antigenic shift	22
2.5.2 Point mutations or antigenic drift	23
2.5.3 Genetic rearrangement or intragenic recombination	23
2.6 Rotavirus vaccines	24
Chapter 3 Objectives	26

Chapter 4 Materials and methods	27
4.1 Specimen collection	27
4.2 Stool sample preparation and screening for group A rotavirus	27
by reverse transcription (RT) and multiplex polymerase	
chain reaction (multiplex PCR)	
4.2.1 Sample preparation	27
4.2.2 Viral RNA extraction	28
4.2.3 Reverse transcription (RT) reaction	28
4.2.4 Detection of group A rotaviruses	29
4.3 Molecular genetic characterizations of group A rotaviruses	30
4.3.1 G genotyping	30
4.3.2 P genotyping	38
4.3.3 Newly designed P[13] primer	45
4.3.4 Genetic characterization of unusual rotavirus strains	46
4.4 Nucleotide sequence analysis of rotavirus nontypeable strains	54
4.4.1 Purification of PCR product	54
4.4.2 Nucleotide sequencing	55
4.4.3 Sequence analysis and construction of phylogenetic tree	55
Chapter 5 Results	64
5.1 Prevalence of human and porcine group A rotaviruses	64
5.2 Molecular characterization of group A rotaviruses	64
5.2.1 Identification of G and P genotypes of human and	64
porcine rotaviruses by multiplex RT-PCR	
5.2.2 Identification of P genotypes of porcine rotaviruses	71
by P[13] specific primer	
5.2.3 Identification of G and P genotypes of nontypeable	75
strains by nucleotide sequence analysis	
5.2.4 Distribution of G and P genotype combinations of	78
human and porcine rotaviruses	
5.2.5 Phylogenetic analysis of VP7 and VP4 gene sequences	81
of human and porcine rotaviruses	

G9P[19] unusual human and porcine rotavirus strains	
Chapter 6 Discussion	102
Chapter 7 Summary	109
References	111
Appendix	137
Appendix A	138
Curriculum Vitae	140
A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A A	

5.2.6 Genetic characterization and phylogenetic analysis of

91

LIST OF TABLES

Table 2.1 The rotavirus genome segments, encoded proteins, and functions	7
Table 2.2 Percentage of nucleotide identity cut-off values for defining the	11
genotypes of 11 rotavirus gene segments	
A818186	
Table 4.1 Oligonucleotide primers for G genotyping of group A rotaviruses	33
Table 4.2 Oligonucleotide primers for P genotyping of group A rotaviruses	40
Table 4.3 Oligonucleotide primers for full-length genotyping of52	2
VP4, VP6, VP7, NSP4, and NSP5 genes of group A rotaviruses	
Table 4.4 The GenBank accession numbers of VP7 gene of group A	57
rotavirus reference strains	
Table 4.5 The GenBank accession numbers of VP4 gene of group A	59
rotavirus reference strains	
Table 4.6 The GenBank accession numbers of VP6 gene of group A	61
rotavirus reference strains	
Table 4.7 The GenBank accession numbers of NSP4 gene of group A	62
rotavirus reference strains	
Table 4.8 The GenBank accession numbers of NSP5 gene of group A	63
rotavirus reference strains	
Copyright [©] by Chiang Mai Universit	Y
Table 5.1 The G and P genotypes of human group A rotaviruses as	70
determined by RT-multiplex PCR	
Table 5.2 The G and P genotypes of porcine group A rotaviruses as	70
determined by RT-multiplex PCR	
Table 5.3 The representative of P genotypes of nontypeable porcine	71
rotavirus strains identified by nucleotide sequence analysis	
Table 5.4 The P genotypes of nontypeable porcine rotavirus strains	72
identified by specific primer for P[13] and P[23] genotypes	

Table 5.5 The G genotypes of nontypeable human rotavirus strains as	76
identified by nucleotide sequence analysis	
Table 5.6 The P genotypes of nontypeable human rotavirus strains as	76
identified by nucleotide sequence analysis	
Table 5.7 The G genotypes of nontypeable porcine rotavirus strains as	77
identified by nucleotide sequence analysis	
Table 5.8 Distribution of G and P genotype combinations of human rotaviruses	80

Table 5.9 Distribution of G and P genotype combinations of porcine rotaviruses80



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF FIGURES

Figure 2.1 Electron micrograph of intact rotavirus particles	6
Figure 2.2 A schematic representation of the rotavirus triple-layered	6
particle and cut-away view of the rotavirus structure	
21818126	
Figure 4.1 Diagram illustrating the amplification of VP7 gene and	36
G genotyping of human group A rotaviruses by multiplex PCR	
Figure 4.2 Diagram illustrating the amplification of VP7 gene and	37
G genotyping of porcine group A rotaviruses by multiplex PCR	
Figure 4.3 Diagram illustrating the amplification of VP4 gene and	43
P genotyping of human group A rotaviruses by multiplex PCR	
Figure 4.4 Diagram illustrating the amplification of VP4 gene and	44
P genotyping of porcine group A rotaviruses by multiplex PCR	
TAL HULLAN	
Figure 5.1 Agarose gel electrophoresis demonstrates the PCR product sizes	66
of G genotypes of human rotaviruses in comparison with the	
reference strains	
Figure 5.2 Agarose gel electrophoresis demonstrates the PCR product sizes	67
of P genotypes of human rotaviruses in comparison with the	
reference strains	
Figure 5.3 Agarose gel electrophoresis demonstrates the PCR product sizes	68
of G genotypes of porcine rotaviruses in comparison with the	
reference strains	
Figure 5.4 Agarose gel electrophoresis demonstrates the PCR product sizes	69
of P genotypes of porcine rotaviruses in comparison with the	
reference strains	
Figure 5.5 The newly designed primer for P[13], namely P[13]F	73

Figure 5.6 Agarose gel electrophoresis demonstrates the PCR product sizes	74
of P[13] genotype in comparison with the reference strains	
Figure 5.7 The phylogenetic analysis of partial nucleotide sequences of the	82
VP7 gene of selected human and porcine rotavirus strains	
Figure 5.8 The phylogenetic analysis of partial nucleotide sequences of the	88
VP4 gene of selected human and porcine rotavirus strains	
Figure 5.9 The phylogenetic analysis of full-length nucleotide sequences of	93
the VP7 gene of selected human and porcine rotavirus strains	
Figure 5.10 The phylogenetic analysis of full-length nucleotide sequences of	95
the VP4 gene of selected human and porcine rotavirus strains	
Figure 5.11 The phylogenetic analysis of full-length nucleotide sequences of	97
the VP6 gene of selected human and porcine rotavirus strains	
Figure 5.12 The phylogenetic analysis of full-length nucleotide sequences of	99
the NSP4 gene of selected human and porcine rotavirus strains	
Figure 5.13 The phylogenetic analysis of full-length nucleotide sequences of	101
the NSP5 gene of selected human and porcine rotavirus strains	



q

LIST OF ABBREVIATIONS

bp	Basepair
cDNA	Complementary deoxyribonucleic acid
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
dsRNA	Double-stranded ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
g	Gram
GS	Genome segment
hr	Hour
Kbp	Kilobasepair
kDa	Kilodalton
min	Minute
ml	Milliliter
mM	Millimolar
mRNA	Messenger RNA
MW	Molecular weight
ng Copyright	Nanogram
nm All ri	Nanometer
NSPs	Nonstructural proteins
nt	Nucleotide
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
RNase	Ribonuclease

rpm	Revolution per minute
RT	Reverse transcription
RT-PCR	Reverse transcription-polymerase chain reaction
RVA	Group A rotavirus
RVB	Group B rotavirus
RVC	Group C rotavirus
RVD	Group D rotavirus
RVE	Group E rotavirus
RVF	Group F rotavirus
RVG	Group G rotavirus
RVH	Group H rotavirus
RVs	Rotaviruses
sec	Seconds
ssRNA	Single-stranded ribonucleic acid
TAE	Tris-acetate EDTA
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
VPs	Viral proteins
	MAI UNIVER

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF SYMBOLS

%	Percent, Percentage
°C	Degree Celsius, Temperature scale
μl	Microliter, volume unit
μg	Microgram, weight unit
μM	Micromolar, molecular mass



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

GLOSSARY

BLAST The Basic Local Alignment Search Tool: The program for comparison of nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. In addition, this program can be used to infer functional and evolutionary relationships between sequences as well as help identifying the members of gene families.

ELISA

MEGA

Enzyme-linked immunosorbent assay: A biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample, such as to detect immune responses in the body against infectious microbes

Molecular Evolutionary Genetics Analysis: An integrated tool for conducting sequence alignment, inferring phylogenetic trees, estimating divergence times, mining online databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses.

NCBI

The National Center for Biotechnology Information: The international resource for the scientific research community was established in 1988 by an act of the United States Congress providing access to public databases and software tools for analyzing biological data, as well as performing research in computational biology.

reserved

RCWG Rotavirus Classification Working Group: The working group established to develop a standardized nomenclature system for rotavirus strains, which is similar to that already established for influenza viruses, proposed by Matthijnssens, et al (2011) [Uniformity of rotavirus strain nomenclature proposed by the rotavirus classification Working Group (RCWG). Arch Virol 156(8), 1397-413.]

WHO

The World Health Organization: the directing and coordinating authority for health within the United Nations system-providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries, and monitoring and assessing health trends.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

ANG MAI