EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF DIARRHEAL VIRUSES IN ADULTS

BOONPA SUANTAI

MASTER OF SCIENCE
IN MICROBIOLOGY

THE GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
DECEMBER 2011

EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF DIARRHEAL VIRUSES IN ADULTS

BOONPA SUANTAI

A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN
PARTIAL FULUILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE
IN MICROBIOLOGY

THE GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
DECEMBER 2011

EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF DIARRHEAL VIRUSES IN ADULTS

BOONPA SUANTAI

THIS THESIS HAS BEEN APPROVED
TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
IN MICROBIOLOGY

EXAMINING COMMITTEE

Leera Kittigul ..... CHAIRPERSON
Assoc. Prof. Dr. Leera Kittigul

Niwat Maneekarn ..... MEMBER
Prof. Dr. Niwat Maneekarn

Pattara Khamri ..... MEMBER
Dr. Pattara Khamrin

THESIS ADVISORY COMMITTEE

Niwat Maneekarn ..... ADVISOR
Prof. Dr. Niwat Maneekarn

Pattara Khamri ..... CO-ADVISOR
Dr. Pattara Khamrin

20 December 2011

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Professor Dr. Niwat Maneekarn, my thesis advisor, for his professional guidance and excellent support throughout my study. In addition, I would also like to express my great appreciation to Dr. Pattara Khamrin, my co-advisor, for her excellent guidance during my Master Thesis experiments. Additionally, I would like to give special thanks for all staffs in the Department of Microbiology, Faculty of Medicine, Chiang Mai University who were always provide me with valuable advices and assistances.

Besides my advisors, I would like to thank the examining committee, Associate Professor Dr. Leera Kittigul for her valuable points of suggestions and comments to make my thesis manuscript in a better shape.

I would also like to express my appreciation to Miss Natthawan Chaimongkol for her kind compassion, great teaching the molecular techniques and phylogenetic analysis used in my study.

Great appreciations are also given to all nursing staffs at Maharaj Nakorn Chiang Mai Hospital and Mr. Wises Sonthisan for their kindness in helping me during the specimen collection.

Many thanks go to Miss Kattareeya Kumthip, Miss Pattranuch Chusri, Miss Wilaiporn Saikhreang, my laboratory colleagues and friends in the Department of Microbiology for their encouragement, sharing of love, and good friendships throughout my study.

Moreover, my appreciation go to Dr. Aksarakorn Kummasook and Miss Chansom Pantip for giving a good suggestion and precious guidance to me.

I would also like to express my appreciation to all of my teachers who have been teaching and supporting me in every aspects during my study.

Last but not least, I would like to give my deep gratitude to my parents and sister for their love, attention, understanding, and supporting me spiritually throughout my life.

Boonpa Suantai

Thesis Title	Epidemiology and Molecular Characterization of Diarrheal Viruses in Adults	
Author	Miss Boonpa Suantai	
Degree	Master of Science (Microbiology)	
Thesis Advisory Committee	Prof. Dr. Niwat Maneekarn	Advisor
	Dr. Pattara Khamrin	Co-advisor

ABSTRACT

Viral gastroenteritis is a common public health problem that causes morbidity and mortality worldwide, particularly in developing countries. The purpose of this study was to investigate the epidemiology and molecular characterization of the viruses detected in fecal samples collected from adult patients with diarrhea. A total of 332 fecal specimens collected between January to December, 2008 were screened for the presence of group A and C rotaviruses, sapovirus, Aichi virus, human parechovirus, noroviruses GI and GII, enterovirus, adenovirus and astrovirus by reverse transcription (RT) and multiplex-PCR methods. The genogroups and genotypes of these viruses were further identified by multiplex-PCR using genotype-specific primers and by nucleotide sequencing. Out of 332 fecal specimens tested, the overall detection rate of diarrheal viruses was 4.2%. Adenovirus and enterovirus were the most predominant viruses detected with the prevalent rate of 1.2%, followed by Aichi virus at 0.9%, and norovirus GII at 0.6%. In addition, mixed infection of norovirus GII and human parechovirus was also detected in one fecal specimen

(0.3%). However, rotaviruses of both groups A and C, astrovirus, norovirus GI and sapovirus were not detected in this study. Based on nucleotide sequence and phylogenetic analyses, the results indicated that all of 3 norovirus GII detected in the present study belonged to GII/4 genotype. Additionally, 3 strains of NoV GII/4 can be subdivided into 2 distinct variant groups, 2006a and 2006b variants. For adenoviruses, 3 genotypes were found to be circulated in this area, including AdV24, AdV25, and AdV40. For enterovirus, 4 genotypes of enterovirus were identified, including echovirus 30, enterovirus 99, poliovirus genotype 3 (PV3), and coxsackievirus A20 (CV-A20). Interestingly, Aichi viruses of both genotypes A and B were also detected in this surveillance. In conclusion, this study provided the molecular epidemiological data of wide variety of diarrheal viruses circulating in adults with diarrhea in Chiang Mai, Thailand.

EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF DIARRHEAL VIRUSES IN ADULTS

BOONPA SUANTAI

A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN

PARTIAL FULUILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

IN MICROBIOLOGY

THE GRADUATE SCHOOL

CHIANG MAI UNIVERSITY

DECEMBER 2011

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Professor Dr. Niwat Maneekarn, my thesis advisor, for his professional guidance and excellent support throughout my study. In addition, I would also like to express my great appreciation to Dr. Pattara Khamrin, my co-advisor, for her excellent guidance during my Master Thesis experiments. Additionally, I would like to give special thanks for all staffs in the Department of Microbiology, Faculty of Medicine, Chiang Mai University who were always provide me with valuable advices and assistances.

Besides my advisors, I would like to thank the examining committee, Associate Professor Dr. Leera Kittigul for her valuable points of suggestions and comments to make my thesis manuscript in a better shape.

I would also like to express my appreciation to Miss Natthawan Chaimongkol for her kind compassion, great teaching the molecular techniques and phylogenetic analysis used in my study.

Great appreciations are also given to all nursing staffs at Maharaj Nakorn Chiang Mai Hospital and Mr. Wises Sonthisan for their kindness in helping me during the specimen collection.

Many thanks go to Miss Kattareeya Kumthip, Miss Pattranuch Chusri, Miss Wilaiporn Saikhreang, my laboratory colleagues and friends in the Department of Microbiology for their encouragement, sharing of love, and good friendships throughout my study.

Moreover, my appreciation go to Dr. Aksarakorn Kummasook and Miss Chansom Pantip for giving a good suggestion and precious guidance to me.

I would also like to express my appreciation to all of my teachers who have been teaching and supporting me in every aspects during my study.

Last but not least, I would like to give my deep gratitude to my parents and sister for their love, attention, understanding, and supporting me spiritually throughout my life.

Boonpa Suantai

Thesis Title	Epidemiology and Molecular Characterization of Diarrheal Viruses in Adults	
Author	Miss Boonpa Suantai	
Degree	Master of Science (Microbiology)	
Thesis Advisory Committee	Prof. Dr. Niwat Maneekarn	Advisor
	Dr. Pattara Khamrin	Co-advisor

ABSTRACT

Viral gastroenteritis is a common public health problem that causes morbidity and mortality worldwide, particularly in developing countries. The purpose of this study was to investigate the epidemiology and molecular characterization of the viruses detected in fecal samples collected from adult patients with diarrhea. A total of 332 fecal specimens collected between January to December, 2008 were screened for the presence of group A and C rotaviruses, sapovirus, Aichi virus, human parechovirus, noroviruses GI and GII, enterovirus, adenovirus and astrovirus by reverse transcription (RT) and multiplex-PCR methods. The genogroups and genotypes of these viruses were further identified by multiplex-PCR using genotype-specific primers and by nucleotide sequencing. Out of 332 fecal specimens tested, the overall detection rate of diarrheal viruses was 4.2%. Adenovirus and enterovirus were the most predominant viruses detected with the prevalent rate of 1.2%, followed by Aichi virus at 0.9%, and norovirus GII at 0.6%. In addition, mixed infection of norovirus GII and human parechovirus was also detected in one fecal specimen

(0.3%). However, rotaviruses of both groups A and C, astrovirus, norovirus GI and sapovirus were not detected in this study. Based on nucleotide sequence and phylogenetic analyses, the results indicated that all of 3 norovirus GII detected in the present study belonged to GII/4 genotype. Additionally, 3 strains of NoV GII/4 can be subdivided into 2 distinct variant groups, 2006a and 2006b variants. For adenoviruses, 3 genotypes were found to be circulated in this area, including AdV24, AdV25, and AdV40. For enterovirus, 4 genotypes of enterovirus were identified, including echovirus 30, enterovirus 99, poliovirus genotype 3 (PV3), and coxsackievirus A20 (CV-A20). Interestingly, Aichi viruses of both genotypes A and B were also detected in this surveillance. In conclusion, this study provided the molecular epidemiological data of wide variety of diarrheal viruses circulating in adults with diarrhea in Chiang Mai, Thailand.

ชื่อเรื่องวิทยานิพนธ์	วิทยาการระบาดและการตรวจหาคุณลักษณะเฉพาะในระดับโมเลกุลของเชื้อไวรัสก่อโรคอุจจาระร่วงในผู้ใหญ่
ผู้เขียน	นางสาวบุญพา สวนใต้
ปริญญา	วิทยาศาสตรมหาบัณฑิต (จุลชีววิทยา)
อาจารย์ที่ปรึกษาวิทยานิพนธ์	ศ. ดร. นิวัตน์ มณีกาญจน์ อาจารย์ที่ปรึกษาหลัก อ. ดร. ภัทรา คำรินทร์ อาจารย์ที่ปรึกษาร่วม

บทคัดย่อ

โรคกระเพาะอาหารและลำไส้อักเสบหรือโรคอุจจาระร่วงที่เกิดจากการติดเชื้อไวรัสยังเป็นปัญหาที่สำคัญทางด้านสาธารณสุขทำให้เกิดการเจ็บป่วยและการตายที่พบได้บ่อยทั่วโลกโดยเฉพาะอย่างยิ่งในประเทศที่กำลังพัฒนา วัตถุประสงค์ของงานวิจัยนี้เป็นการศึกษาระบาดวิทยาและการตรวจหาคุณลักษณะเฉพาะในระดับโมเลกุลของเชื้อไวรัสก่อโรคอุจจาระร่วงในผู้ใหญ่ โดยนำตัวอย่างอุจจาระจากผู้ใหญ่ที่มีอาการอุจจาระร่วง จำนวนทั้งหมด 332 ตัวอย่าง ที่เก็บในช่วงเดือนมกราคมถึงเดือนธันวาคม พ.ศ. 2551 มาตรวจคัดกรองหาเชื้อไวรัสโรตา กรุ๊ป A และ C ไวรัสซาโป ไวรัสไอชิ ไวรัสพารีโค ไวรัสโนโร GI และ GII ไวรัสเอ็นเทอโร ไวรัสอะดิโน และไวรัสแอสโทร โดยวิธี revese transcription (RT) และ multiplex-PCR หลังจากนั้นนำไวรัสที่ตรวจพบไปจำแนก genogroups และ genotypes ต่างๆ โดยวิธี multiplex-PCR โดยใช้ primer ที่จำเพาะและโดยการทำ nucleotide sequencing เพื่อถอดรหัสพันธุกรรมของเชื้อ ผลการตรวจตัวอย่างจำนวน 332 ตัวอย่าง พบเชื้อไวรัสที่เกี่ยวข้องกับโรคอุจจาระร่วงในผู้ใหญ่ร้อยละ 4.2 โดยพบเชื้อไวรัสอะดิโนและไวรัสเอ็นเทอโร สูงสุดโดยแต่ละเชื้อพบร้อยละ 1.2 รองลงมาคือ ไวรัสไอชิ ร้อยละ 0.9 ไวรัสโนโร GII ร้อยละ 0.6 นอกจากนี้ยังพบการติดเชื้อร่วมระหว่างไวรัสโนโร GII กับไวรัสพารีโคในหนึ่งตัวอย่างตรวจคิดเป็นร้อยละ 0.3 อย่างไรก็ตาม การศึกษาในครั้งนี้ตรวจไม่พบไวรัสโรตา กรุ๊ป A และ C

ไวรัสแอสโทร ไวรัสโนโร GI และไวรัสซาโปเลย ผลจากการตรวจวิเคราะห์ลำดับนิวคลีโอไทด์และการวิเคราะห์วิวัฒนาการของตัวเชื้อโดยการวิเคราะห์ทาง phylogenetic พบว่าเชื้อไวรัสโนโร GII ทั้งหมด 3 ตัวอย่าง ที่พบในการศึกษาในครั้งนี้จัดอยู่ใน GII/4 genotype ซึ่ง 3 สายพันธุ์ของเชื้อไวรัสโนโรที่พบสามารถจำแนกย่อยออกเป็น 2 variants คือ variants 2006a และ 2006b สำหรับเชื้อไวรัสอะดิโนที่พบในการศึกษานี้พบว่ามีอยู่ทั้งหมด 3 สายพันธุ์ คือ AdV24, AdV25, AdV40 และเชื้อไวรัสเอ็นเทอโรพบ 4 สายพันธุ์ คือ echovirus 30, enterovirus 99, poliovirus 3, และ coxsackievirus A20 นอกจากนี้ยังพบว่าเชื้อไวรัสไอชิที่แยกได้จากการศึกษาในครั้งนี้มีทั้งชนิดที่เป็น genotype A และ B โดยสรุป การศึกษาในครั้งนี้ทำให้ได้ข้อมูลทางด้านระบาดวิทยาของเชื้อไวรัสหลากหลายชนิดที่ก่อโรคอุจจาระร่วงในผู้ใหญ่ในจังหวัดเชียงใหม่ของประเทศไทย

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

%	Percent
°C	Degree Celsion
µg	Microgram
µl	Microliter
AdV	Adenovirus
AGE	Acute gastroenteritis
AiV	Aichi virus
AstV	Astrovirus
AUS	Australia
BGD	Bangladesh
BRZ	Brazil
bp	Basepair
CDC	Center for Disease control and Prevention
CHN	China
cDNA	Complementary deoxyribonucleic acid
DEU	Germany
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
dsRNA	Double-stranded Ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay

EM	Electron microscopy
G	Genogroup
g	Gram
HuCVs	Human caliciviruses
HPeV	Human parechovirus
FRA	France
IEM	Immune electron microscopy
IND	Indonesia
JPN	Japan
kp	Kilobasepair
kDa	Kilodaton
KOR	Korea
M	Molar
mAbs	Monoclonal antibody
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
mRNA	Messenger ribonucleic aacid
MW	Molecular weight
NEP	Nepal
nm	Nanometer
ng	Nanogram
NoV	Norovirus

NoV GI	Norovirus genogroup I
NoV GII	Norovirus genogroup II
NSPs	Nonstructural proteins
NZL	New Zealand
ORFs	Open reading frames
PAGE	Polyacrylamine gel electrophosis
PBS	Phosphate buffer saline
PCR	polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolutions per minute
RT	Reverse transcriptase
RT-PCR	Revese-transciption polymerase chain reaction
rVLP	Recombination virus-like particle
RV	Rotavirus
SaV	Sapovirus
sec	Second
SG	Subgroup
SIG	Singapore
ssRNA	Single-stranded ribonucleic acid
TBE	Tris-borate EDTA
THA	Thailand
TUN	Tunisia

UK	United Kingdom
US	United State
VIE	Vietnam
VPs	Viral proteins