MOLECULAR EPIDEMIOLOGICAL STUDY ON NOROVIRUS INFECTION IN TWO DISTINCT HOSPITALS IN NORTHEASTERN THAILAND, 2013-2015

Ratigorn Guntapong[1,*], Kriangsak Ruchusatsawat[1,*], Boonnipa Suwannakan[2], Nucharat Panthasri[2], Worawit Kittiwongsunthorn[2], Vichai Chaichitwanitkul[3], Krissanapong Chumpon[4], Ratana Tacharoenmuang[1], Phakapun Singchai[1], Sompong Upachai[1], Michittra Boonchan[5], Naokazu Takeda[5,6], Somchai Sangkitporn[1] and Kazushi Motomura[5,6]

[1]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi; [2]Udon Thani Regional Medical Sciences Center; [3]Phen Hospital, Udon Thani; [4]Srivilai Hospital, Bueng Kan; [5]Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI), Nonthaburi, Thailand; [6]Research Institute of Microbial Diseases, Osaka University, Suita, Japan

Abstract. Nationwide epidemics due to norovirus (NoV) infections occur periodically in the winter season in the northern hemisphere. NoV outbreaks also have been reported in Thailand. In this study, 564 stool specimens were collected from patients with acute gastroenteritis in northeastern Thailand from October 2013 to May 2015. Partial genome sequences of the N-terminal Shell region of NoVs in the specimens were amplified revealing the majority of NoV cases were detected in samples from December 2013 to February 2014, and from March to April, 2015. The average humidity from December to April is the lowest every year in Thailand, suggesting a possible relationship between the occurrence of norovirus infection and low humidity. Six of 17 GI NoVs were grouped in GI.4 genotype, while 20/30 GII NoVs belonged to GII.4 cluster, of which 19 were closely related to Sydney_2012, a novel GII.4 variant spreading globally since early 2012, while 5/30 GII strains were grouped in GII.17 cluster, which was identified as a new emerging epidemic strain in 2014. The distribution of genogroups and genotypes in NoV in northeastern Thailand was consistent with those of other countries. Economic globalization has increased movements of people among countries so that NoV could easily have been carried into Thailand from other countries.

Keywords: acute gastroenteritis, norovirus, northeastern Thailand

INTRODUCTION

Norovirus (NoV) is a major etiological agent of both outbreaks and sporadic cases of acute gastroenteritis worldwide (Hutson *et al*, 2004; Estes *et al*, 2006). The virus causes diarrhea in patients of all ages with severe outcomes in infants, children, the elderly, and individuals

Correspondence: Kazushi Motomura, Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI), Nonthaburi 11000, Thailand.
Tel: +66 (0) 2965 9748; Fax: +66 (0) 2965 9749
E-mail: kmotomura@biken.osaka-u.ac.jp
[*]These authors contributed equally to the work.

with chronic diseases (Hutson *et al*, 2004; Estes *et al*, 2006). NoV is relatively stable in water containing chlorine (Keswick *et al*, 1985) and is prevalent in the natural environment (Hutson *et al*, 2004; Estes *et al*, 2006). Viral transmission occurs through ingestion of contaminated food and water (Hutson *et al*, 2004; Estes *et al*, 2006). NoV also spreads by direct person-to-person contact and by exposure to contaminated airborne vomitus droplets in a semi-closed community (Hutson *et al*, 2004; Estes *et al*, 2006). NoV commonly causes asymptomatic infection (Garcia *et al*, 2006; Monica *et al*, 2007; Ozawa *et al*, 2007) where the viral load of the carriers is similar to that of symptomatic individuals (Ozawa *et al*, 2007). These characteristics allow NoV to spread rapidly and extensively in subjects going about their daily activities and thereby raising major public health concerns in many countries.

NoV is a non-enveloped virus belonging to the family *Caliciviridae*. NoV has a single-stranded, positive-sense, polyadenylated RNA genome of about 7.5 kilobases (Xi *et al*, 1990). The RNA genome encodes three open reading frames (ORFs), namely, ORF1, ORF2, and ORF3. As is common in RNA viruses, NoV in nature is genetically and antigenically highly diverse (Katayama *et al*, 2002; Kageyama *et al*, 2004; Hansman *et al*, 2006). Although the International Committee on Taxonomy of Viruses has yet to reach a universal classification scheme, NoV is tentatively divided into five genogroups (GI to GV) and >25 genotypes based on the similarity to ORF2 capsid sequence. Among these, genogroup II genotype 4 (GII.4) is particularly important to public health, because this genotype has been the leading cause of NoV-associated acute gastroenteritis in humans since the middle 1990s in Asia, Australia, Europe, and North America (Kroneman *et al*, 2006; Phan *et al*, 2006; Bull *et al*, 2007; Ho *et al*, 2007; Motomura *et al*, 2008; Motomura *et al*, 2010).

As seen in many countries, nationwide epidemics of NoV infections periodically occur in the winter season in the northern hemisphere (Hutson *et al*, 2004; Estes *et al*, 2006). Previous studies in Thailand reported NoVs exist in various natural environments (Inoue *et al*, 2016) and several NoV outbreaks have occurred (Guntapong *et al*, 2004; Hansman *et al*, 2004; Phumpholsup *et al*, 2015), with a recent appearance of GII.17, a minor NoV genotype (Phumpholsup *et al*, 2015; Inoue *et al*, 2016; Boonchan *et al*, 2017).

The purpose of this study was to investigate whether norovirus was the cause of gastroenteritis outbreaks in Thailand, and if so, which strains were present. In order to obtain the genetic variations of the NoV strains circulating in Thailand, we determined 47 partial genome sequences of NoV variants from stool samples gathered from acute gastroenteritis patients at two sites in northeastern Thailand during October 2013 to May 2015.

MATERIALS AND METHODS

Stool specimens

A total of 564 stool specimens were collected from individuals of various ages with acute gastroenteritis admitted to Phen Hospital (487 specimens) and Srivilai Hospital (77 specimens), two different regional public hospitals in northeastern Thailand between October 2013 and May 2015. All stool specimens were stored at -80°C until used.

Questionnaire information was de-identified and re-coded so that no information could be linked to any individual participant. The protocol of this study

was reviewed and approved by the Ethics Committee of the Institute for the Development of Human Research Protections (IHRP), Thailand (Permit no.0032).

Viral RNA extraction, amplification and determination of nucleotide sequence

A 10% (w/v) fecal suspension in phosphate-buffered saline pH 7.2 (PBS) was centrifuged at 10,000*g* for 10 minutes and RNA extracted from the 10% (w/v) fecal suspension using QIAamp Viral RNA kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and stored at -80°C until used. RT-PCR was performed using QIAGEN One-Step PCR (QIAGEN) together with primer, G2SKR (anti-sense) (5′-CCRCCNGCATRHCCRTTRTACAT-3′) (H: Not G, R; A or G, N; any nucleotides). In brief, the 50-µl reaction mixture contained 5 µl of viral RNA, 2.0 µl of each primer (20 µM), 10.0 µl of 5X reaction buffer, 2.0 µl of dNTPs (10 mM), and 2.0 µl of enzyme mixture. The solution was incubated at 50°C for 30 minutes followed by 15 minutes at 95°C. Thermocycling was conducted in GeneAmp PCR System 9700 (Thermofisher, Waltham, MA) as follows: 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 1 minute. Nested PCR was performed using Ex-Taq system (TaKaRa Bio, Shiga, Japan). For amplification of a fragment of NoV GI the outer primer pair was COG-1F (sense) (5′-CGYTGGATGCGNTTYCATGA-3′) and G1SKR (anti-sense) (5′-CCAACCCARCCATTRTACA-3′), and inner primer pair, G1SKF (sense) (5′-CTGCCCGAATTYGTAAATGA-3′) and G1SKR (Y; C or T). For amplification of a fragment of NoV GII the outer primer pair was COG-2F (sense) (5′-CARGARBCNATGTTYAGRTGGATGAG-3′) and G2SKR (anti-sense) (5′-CCRCCNGCATRHCCRTTRTACAT-3′) and the inner primer pair, G2SKF (sense) (5′-CNTGGGAGGGCGATCGCAA-3′) and G2SKR (anti-sense) (B; Not A) (Kojima *et al*, 2002; Kageyama *et al*, 2003). A 5-µl volume of the RT-PCR product was added to 45 µl of the reaction mixture containing 2.0 µl of each primer (20 µM), 5.0 µl of 10X reaction buffer, 1.0 µl of dNTPs (10 mM), and 1.0 µl of Ex Taq enzyme (5 U/ml). Thermocycling was performed in GeneAmp PCR System 9700 (Thermofisher) for 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. Amplicons were purified using QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and directly sequenced using an automated ABI 3100 Analyzer (Thermofisher). The DDBJ accession numbers of the sequences are LC312716 - LC312762).

Quantitative (q)PCR

QPCR was carried out in a 50-µl volume reaction mixture containing of 5 µl cDNA, 25 µl of TaqMan Universal PCR Master Mix (Thermofisher) containing dUTP and uracyl N-glycosylase (UNG), 400 nM primer COG1F (5′-CGYTGGATGCGNTTYCATGA-3′) and primer COG1R (5′-CTTAGACGCCATCATCATTYAC-3′), and 5 pmol of RING1-TPA [5′-(FAM) AGATYGCGATCYCCTGTCCA(TAMRA)-3′] and RING1-TPB [5′-(FAM)AGATCG CGGTCTCCTGTCCA(TAMRA)-3′] fluorogenic probes for NoV GI detection. For NoV GII detection, 400 nM primer COG2F (5′-CARGARBCNATGTTYAGRTGGATGAG-3′) (B; not A) and COG2R (5′-TCGACGCCATCTTCATTCACA-3′), and 5 pmol of RING2-TP [5-(FAM)TGGGAGGGCGATCGCAATCT(TAMRA)-3′] fluorogenic probe were used. Thermocycling was performed in an ABI 7500 Real-Time PCR Systems (Thermofisher) as follows: 50°C for 2 minutes (to activate UNG); 95°C for 10 minutes; 45 cycles of

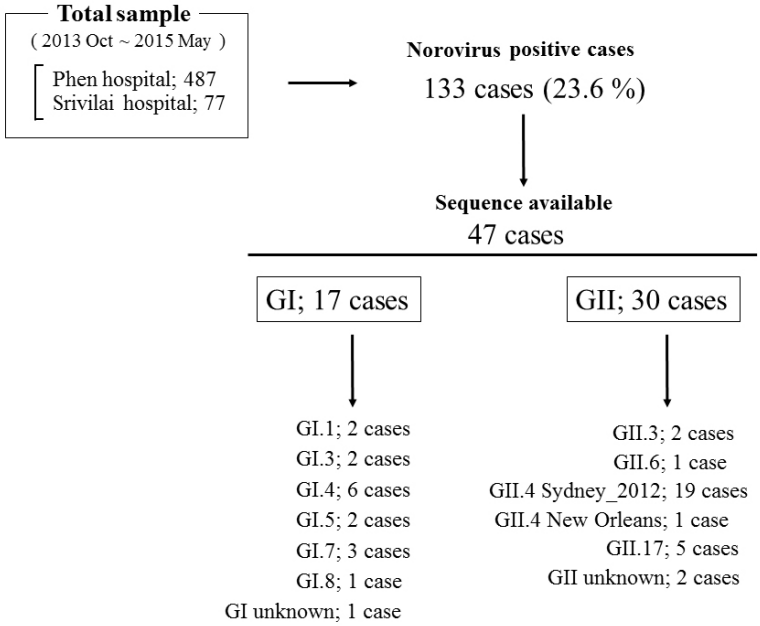

Fig 1–Determination and classification of norovirus genotypes from patients with acute gastroenteritis admitted to two hospitals in northeastern Thailand, 2013 - 2015.

95°C for 15 seconds and 56°C for 1 minute (Kageyama *et al*, 2003; Kageyama *et al*, 2004). Amplification data were collected and analyzed using a Sequence Detector software version 1.6 (Thermofisher). NoV GI- and GII-specific standard curves were generated by a 10-fold serial dilution (10^1 to 10^7 copies) of purified NoV GI and GII DNA plasmids. The cut-off value was 15 copies.

Phylogenetic analysis.

Neighbor-joining (Saitou and Nei 1987), maximum-likelihood and UPGMA trees were generated with 100 bootstrap replicates from the matrix numbers using MEGA, version 5.0 (Kumar *et al*, 1994). Partial genome sequences of the global and Japanese epidemic GI or GII subtypes were included.

RESULTS

NoV-positive samples from 133 stool specimens collected from October 2013 to May 2015 from hospitalized patients in northeastern Thailand included 71 GI-positive, 81 GII-positive and 19 both GI- and GII-positive samples (Fig 1). Fifty-seven (43%) cases occurred between December 2013 and February 2014, and 43 (32%) between January 2015 and April 2015 (Fig 2). Fifty-nine (44%) patients were <5 years old and 27 (20%) were in the 50-79 age group (Fig 3).

The average temperature in the dry season from December 2013 to February 2014 was low, and the average humidity was consistently low (Fig 2). On the other hand, the temperature between January and April, 2015, especially March and April, was high (~30°C), but the humidity remained low.

Only 47 partial genome sequences of NoV GI and GII strains were determined as the remaining samples contained insufficient amplicon material. All 47 partial genomes had no indels when compared to sequences of reference strains. The evolutionary relationships of the sequences were examined by phylogenetic analysis. Sequences of well-recognized reference strains from the global GI and GII epidemics during the past ~20 years were included as well. A representative GI neighbor-joining tree shows that the genome sequences from the 2013-2015 samples in Thailand were mainly divided into

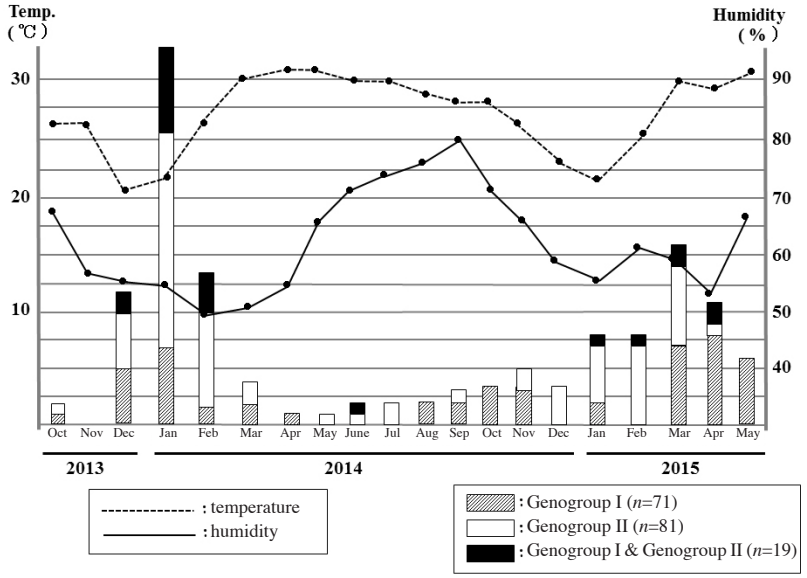

Fig 2–Seasonality of NoV prevalence in northeastern Thailand. The number of NoV cases is shown above each bar. Dots and block lines indicate the average temperature and average humidity in Udon Thani Province, respectively based on the Weather History Website (https://www.wunderground.com).

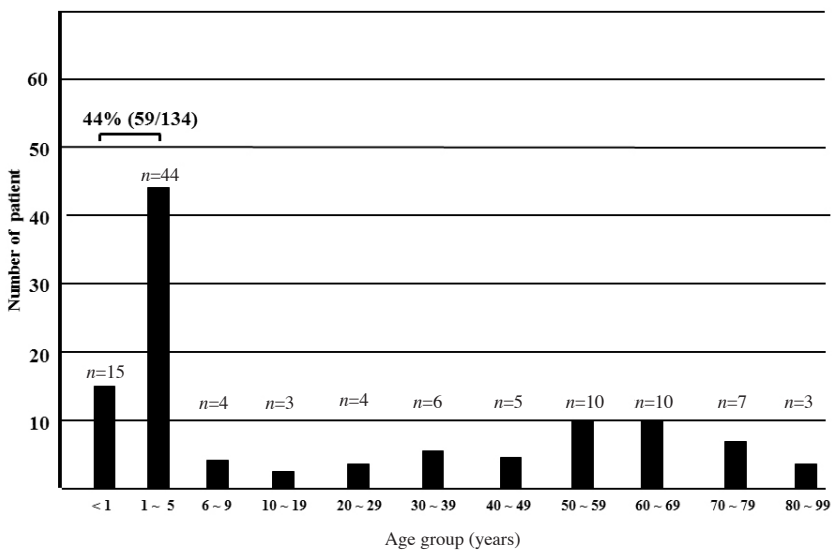

Fig 3–Distribution of age group of NoV infection in northeastern Thailand. Patients with NoV-positive stool samples are classed into 11 different age groups. The number of NoV cases for each age group is shown.

five genetic groups. Six of 17 (35%) GI sequences formed a monophyletic group within the GI.4 cluster, and 5 (29%) formed a monophyletic group within the GI.7 cluster (Fig 4A). A representative GII neighbor-joining tree shows that the GII sequences were mainly classified into two major GII genotypes, namely, GII.4 (20/30; 67%) and GII.17 (5; 17%) group (Fig 4B). Among the GII.4 group, 19 (63%) sequences formed a monophyletic group within GII.4 Sydney_2012, a worldwide pandemic strain of 2012 - 2013 (Eden *et al*, 2013). One particular sequence from northeastern Thailand in 2013 appeared in GII.4 New Orleans_2009, which was prevalent in Japan in 2009. The five sequences clustere3d in GII.17 were identified previously in Thailand in 2014 (Phumpholsup *et al*, 2015) and also were reported in the outbreaks in China and Japan between 2014 and 2015 (de Graaf *et al*, 2015; Han *et al*,

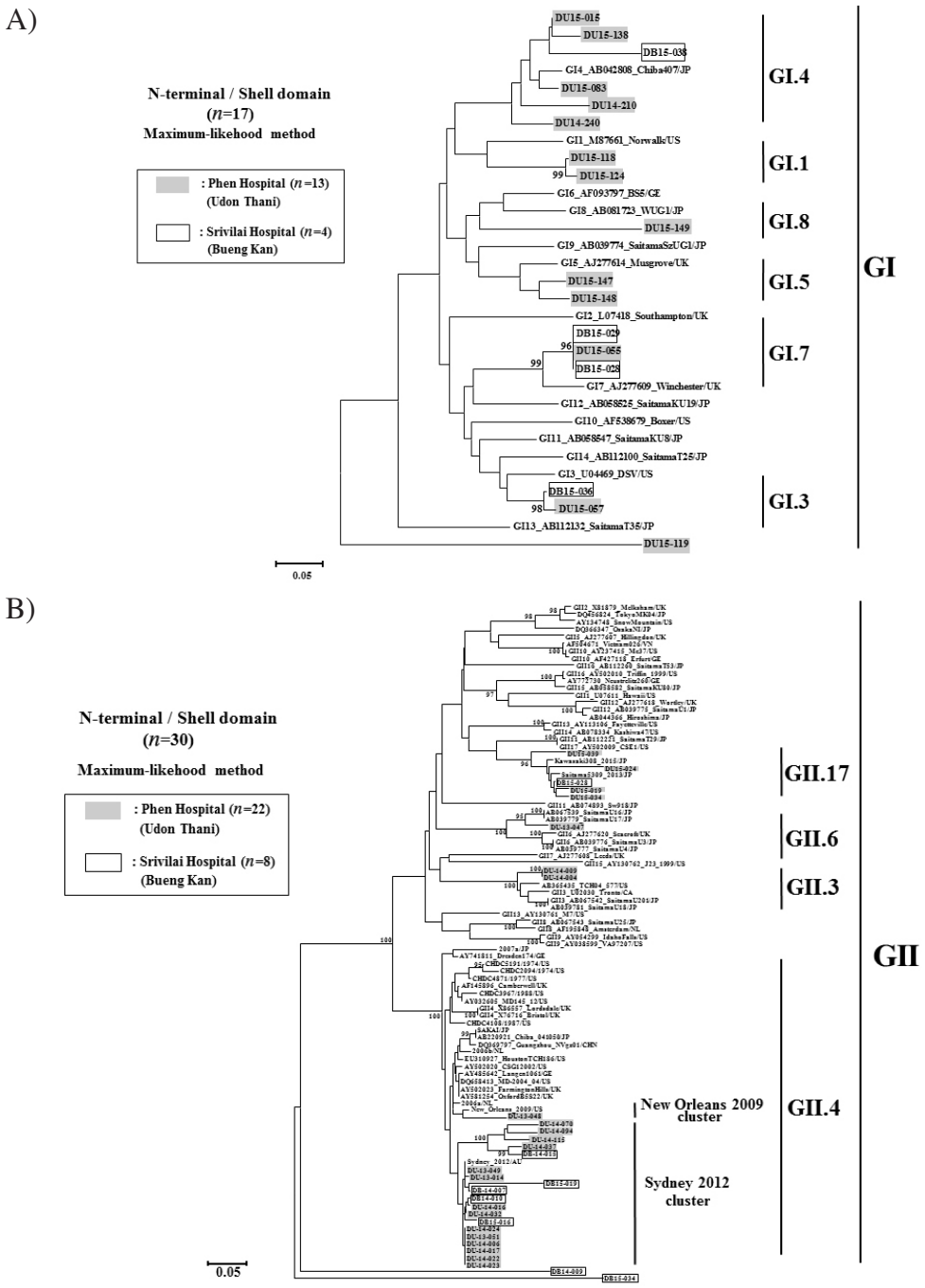

Fig 4–Phylogenetic relationship of among NoV strains. Neighbor-joining trees were constructed based on 47 NoV N-terminal Shell domain partial nucleotide sequences (300 bases) derived from (**A)** GI and **(B)** GII. Bootstrap values >0.95 are indicated at the nodes of the tree. The gray box indicates 2013-2015 NoV GI and GII sequences from Phen Hospital, Udon Thani Province and white and gray box indicates 2013-2015 NoV GI and GII sequences, respectively from Srivilai Hospital, Bueng Kan Province, northeastern Thailand.

2015, Lu *et al*, 2015; Matsushima *et al*, 2015). The monophyletic relationships of the GI and GII sequences were reproducible when the tree was constructed with maximum likelihood and UPMGA algorithms (data not shown).

DISCUSSION

In this study, 24% NoV-positive cases were identified by qPCR among patients with acute gastroenteritis admitted to two hospitals in northeastern Thailand between October 2013 and May 2015, a prevalence consistent with that of other Asian countries (Tang *et al*, 2013, Cho *et al*, 2014; Rahman *et al*, 2016). In addition, 47 partial genome sequences of NoV GI and GII strains were obtained.

The majority of the NoV-positive cases were detected between December and January in 2013-2014, and between March and April in 2015. The average temperature and humidity of December to March in Udon Thani was the lowest in that year. The temperature of March-April in 2014-2015 was nearly 30°C, but the humidity was <60%, suggesting that humidity was a possible important factor for NoV prevalence. Norovirus adhesion is mediated by interaction with hydrophobic residues normally exposed on the capsid surface at pH 3-8, under physiological ionic strength and low temperature (Samandoulgou *et al*, 2015); therefore, NoV particles might be physically strong and highly stable. However, the relationship between humidity and viral stability is unknown. Our results suggests that NoV virion may retain strong virion structure and high infectivity in cool and dry environment.

More than 40% of the NoV-positive cases were detected among children less than 5 years old. One possible reason is that children of <5 years old have not established adequate gut-immunity against viruses.

NoV GII.4 is a predominant genotype that has causes outbreaks in Thailand over the past two decades (Guntapong *et al*, 2004; Hansman *et al*, 2004). Our phylogenetic studies identified the majority of monophyletic GII.4 subtypes as belonging to GII.4 2012_Sydney that is predominated in both Phen and Srivilai hospitals. The distribution of the major genotypes and subtypes is consistent with that in many countries, including EU, Japan and USA, where this strain caused outbreaks during 2012-2013 (Eden *et al*, 2013; Zhang *et al*, 2015; Mans *et al*, 2016). Our study reveals that this strain was already present in Thailand in October 2013. Similar replacement of resident GII.4 by new GII.4 variant strains has periodically occurred in 2012-2013 global epidemic (Eden *et al*, 2013; Zhang *et al*, 2015; Mans *et al* , 2016). Enhancement in the physical stability of virions, viral infectivity or replication capability in cells may explain the periodical outgrowth of a new GII.4 variant strain as described previously (Motomura *et al*, 2008; Motomura *et al*, 2010). Thus, such changes of GII.4 strains may explain their outgrowth over other NoV genotypes and genogroups. Interestingly, five GII.17 strains detected in this study were the same GII.17 that caused outbreaks in China and Japan between December 2014 and March 2015 (de Graaf *et al*, 2015; Han *et al*, 2015; Lu *et al*, 2015; Matsushima *et al*, 2015). The sequences of GII.17 in the study is closely related to those of GII.17 reported previously (Phumpholsup *et al*, 2015), suggesting that GII.17 already existed and occurred in outbreaks in Thailand in 2014. The GII.17 genotype is

an emerging genotype with the potential of global spread (de Graaf *et al*, 2015). Our results indicate that the distribution of NoV genotypes and subtypes in Thailand was consistent with those of other countries, suggesting that NoV might have been brought into Thailand from other countries. Certainly globalization of recent years has increased the movement of individuals among countries.

In summary, we report for the first time NoV genome information from patients with acute gastroenteritis in northeastern Thailand over a period of 20 months. Acute gastroenteritis is an important public health problem in the Southeast Asian countries including Thailand. Our findings provide further evidence for dynamic changes in the periodical outgrowth of new NoV variants in the human population. The availability of the genome sequence information of new epidemic variants should help in the development of diagnostic assays and studies of the molecular biology of NoV in relation to the virus natural history.

ACKNOWLEDGEMENTS

This research is partially supported by the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) of the Ministry of Education, Culture, Sport, Science and Technology, Japan and the Japan Agency for Medical Research and Development (AMED). This study also was supported by the Department of Medical Sciences, Ministry of Public Health, Thailand.

CONFLICTS OF INTEREST

The authors declare no competing interests of either financial or non-financial nature regarding the work described in the present manuscript and its publication.

REFERENCES

Boonchan M, Motomura K, Inoue K, *et al*. Distribution of norovirus genotypes and subtypes in river water by ultra-deep sequencing-based analysis. *Lett Appl Microbiol* 2017; 65: 98-104.

Bull A, Tanaka M, White P. Norovirus recombination. *J Gen Virol* 2007; 88: 3347-59.

Cho G, Lee S, Kim J, *et al*. Molecular epidemiology of norovirus GII.4 variants in children under 5 years with sporadic acute gastroenteritis in South Korea during 2006-2013. *J Clin Virol* 2014; 61: 340-4.

de Graaf M, van Beek J, Vennema H, *et al*. Emergence of a novel GII.17 norovirus - End of the GII.4 era? *Euro Surveill* 2015; 20. pii:21178.

Eden S, Tanaka M, Boni M, Rawlinson D, White P. Recombination within the pandemic norovirus GII.4 lineage. *J Virol* 2013; 87: 6270-82.

Estes M, Prasad V, Atmar R. Noroviruses everywhere: has something changed? *Curr Opin Infect Dis* 2006; 19: 467-74.

Garcia C, DuPont H, Long K, Santos J. Ko G. Asymptomatic norovirus infection in Mexican children. *J Clin Microbiol* 2006; 44: 2997-3000.

Guntapong R., Hansman G, Oka T, *et al*. Norovirus and sapovirus infections in Thailand. *Jpn J Infect Dis* 2004; 57: 276-8.

Han J, Ji L, Shen Y, Wu X, Xu D, Chen L. Emergence and predominance of norovirus GII.17 in Huzhou, China, 2014-2015. *Virol J* 2015; 12: 139.

Hansman G, Katayama K, Maneekarn N, *et al*. Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. *J Clin Microbiol* 2004; 42: 1305-7.

Hansman G, Natori K, Shirato-Horikoshi H,

et al. Genetic and antigenic diversity among noroviruses. *J Gen Virol* 2006; 87: 909-19.

Ho C, Cheng P, Lau A, Wong A, Lim W. Atypical norovirus epidemic in Hong Kong during summer of 2006 caused by a new genogroup II/4 variant. *J Clin Microbiol* 2007; 45: 2205-11.

Hutson M, Atmar R. Estes M. Norovirus disease: changing epidemiology and host susceptibility factors. *Trends Microbiol* 2004; 12: 279-87.

Inoue K, Motomura K. Boonchan M, *et al*. Molecular detection and characterization of noroviruses in river water in Thailand. *Lett Appl Microbiol* 2016; 62: 243-9.

Kageyama T, Kojima S, Shinohara M, *et al*. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol* 2003; 41: 1548-57.

Kageyama T, Shinohara M, Uchida K, *et al*. Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to Norovirus in Japan. *J Clin Microbiol* 2004; 42: 2988-95.

Katayama K, Shirato-Horikoshi H, Kojima S, *et al*. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. *Virology* 2002; 299: 225-39.

Keswick H, Satterwhite T, Johnson P, *et al*. Inactivation of Norwalk virus in drinking water by chlorine. *Appl Environ Microbiol* 1985; 50: 261-4.

Kojima S, Kageyama T, Fukushi S, *et al*. Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J Virol Methods* 2002; 100: 107-14.

Kroneman A, Vennema H, Harris J, *et al*. Increase in norovirus activity reported in Europe. *Euro Surveill* 2006; 11: E061214 061211.

Kumar S, Tamura K, Nei M. MEGA: Molecular Evolutionary Genetics Analysis software for microcomputers. *Comput Appl Biosci* 1994; 10: 189-91.

Lu J, Sun L, Fang L, *et al*. Gastroenteritis outbreaks caused by norovirus GII.17, Guangdong Province, China, 2014-2015. *Emerg Infect Dis* 2015; 21: 1240-2.

Mans J, Murray T, Nadan S, Netshikweta R, Page N, Taylor M. Norovirus diversity in children with gastroenteritis in South Africa from 2009 to 2013: GII.4 variants and recombinant strains predominate. *Epidemiol Infect* 2016; 144: 907-16.

Matsushima Y, Ishikawa M, Shimizu T, *et al*. Genetic analyses of GII.17 norovirus strains in diarrheal disease outbreaks from December 2014 to March 2015 in Japan reveal a novel polymerase sequence and amino acid substitutions in the capsid region. *Euro Surveill* 2015; 20. pii: 21173.

Monica B, Ramani S, Banerjee I, *et al*. Human caliciviruses in symptomatic and asymptomatic infections in children in Vellore, South India. *J Med Virol* 2007; 79: 544-51.

Motomura K, Oka T, Yokoyama M, *et al*. Identification of monomorphic and divergent haplotypes in the 2006-2007 norovirus GII/4 epidemic population by genome-wide tracing of evolutionary history. *J Virol* 2008; 82: 11247-62.

Motomura K, Yokoyama M, Ode H, *et al*. Divergent evolution of Norovirus GII/4 by genome recombination over 2006-2009 in Japan. *J Virol* 2010; 84: 8085-97.

Ozawa K, Oka T, Takeda N, Hansman G. Norovirus infections in symptomatic and asymptomatic food handlers in Japan. *J Clin Microbiol* 2007; 45: 3996-4005.

Phan T, Kuroiwa G, Kaneshi K, *et al*. Changing distribution of norovirus genotypes and genetic analysis of recombinant GIIb among infants and children with diarrhea in Japan. *J Med Virol* 2006; 78: 971-8.

Phumpholsup T, Theamboonlers A, Wanlapakorn N, *et al*. Norovirus outbreak at a daycare center in Bangkok, 2014. *Southeast Asian J Trop Med Public Health* 2015; 46: 616-23.

Rahman M, Rahman R, Nahar S, *et al*. Norovirus diarrhea in Bangladesh, 2010-14: Prevalence, clinical features and genotypes. *J Med Virol* 2016; 88: 1742-50.

Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987; 4: 406-25.

Samandoulgou I, Hammami R. Morales R, Fliss I, Jean J. Stability of secondary and tertiary structures of virus-like particles representing noroviruses: effects of pH, ionic strength, and temperature and implications for adhesion to surfaces. *Appl Environ Microbiol* 2015; 81: 7680-6.

Tang M, Chen B, Chen S, Chou Y, Yu C. Epidemiological and molecular analysis of human norovirus infections in Taiwan during 2011 and 2012. *BMC Infect Dis* 2013; 13: 338.

Xi J, Graham N, Wang K, Estes M. Norwalk virus genome cloning and characterization. *Science* 1990; 250: 1580-3.

Zhang J, Shen Z, Zhu Z, *et al*. Genotype distribution of norovirus around the emergence of Sydney_2012 and the antigenic drift of contemporary GII.4 epidemic strains. *J Clin Virol* 2015; 72: 95-101.