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ORIGINAL ARTICLE

Norovirus and rotavirus infections in children less than five years of age hospitalized with acute gastroenteritis in Indonesia

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Abstract

Rotaviruses and noroviruses are the most important viral causes of acute gastroenteritis in children. While previous studies of acute gastroenteritis in Indonesia mainly focused on rotavirus, here, we investigated the burden and epidemiology of norovirus and rotavirus disease. Children less than five years of age hospitalized with acute gastroenteritis were enrolled in this study from January to December 2015 at three participating hospitals. Rotavirus was detected by enzyme immunoassay (EIA), followed by genotyping by reverse transcription PCR (RT-PCR). Norovirus genogroups were determined by TaqMan-based quantitative RT-PCR. Among 406 enrolled children, 75 (18.47%), 223 (54.93%) and 29 (7.14%) cases were positive for norovirus, rotavirus and both viruses (mixed infections), respectively. Most cases clinically presented with fever, diarrhea, vomiting and some degree of dehydration. The majority (n = 69/75 [92%]) of the noroviruses identified belonged to genogroup II, and several genotypes were identified by sequencing a subset of samples. Among 35 samples tested for rotavirus genotype, the most prevalent genotype was G3P[8] (n = 30/35 [85.6%]). Our study suggests that the burden of norovirus diseases in Indonesian children should not be underestimated. It also shows the emergence of rotavirus genotype G3P[8] in Indonesia.

Introduction

Acute gastroenteritis (acute diarrhea) is one of the most important global health issues, especially in children less than five years of age, and it is clinically characterized by acute symptoms of fever, abdominal pain, vomiting and diarrhea [1]. The mortality is primarily due to severe complications, including dehydration [2]. Control measures have resulted in significant progress towards decreasing the diarrheal burden of disease. The global diarrheal mortality across all ages has markedly declined, from an estimated 2.6 million annually in 1990 to approximately 1.3 million in 2013 [3].

Rotaviruses and noroviruses are the most common viral agents responsible for acute gastroenteritis in children less than five years of age [4]. Rotaviruses are segmented, double stranded RNA (dsRNA) viruses. Based on two outer-layer structural proteins, VP7 and VP4, they are classified into G- and P-genotypes, respectively [5]. Our previous national surveillance studies found a high incidence of rotavirus disease

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and identified G1P[8], G1P[6] and G2P[4] as the most common genotypes circulating in Indonesia [6–8]. Reassortment between different strains contributes to the genetic diversity of rotaviruses by creating novel combinations of G- and P-genotypes [9]. Thus, continuous strain monitoring is of great importance to identify novel genotypes.

Noroviruses are positive-sense single-strand RNA viruses that belong to the family *Caliciviridae* [10]. Globally, the prevalence is about 18% in children aged less than five years and mixed ages with acute gastroenteritis [11]. Members of norovirus genogroups I (GI), II (GII) and IV (GIV) have been found to infect humans, with a total of more than 30 characterized genotypes within those genogroups [10]. A single genotype, GII.4, is the most prevalent globally and is responsible for most norovirus-associated gastroenteritis outbreaks [12]. Importantly, noroviruses have emerged as the leading cause of acute gastroenteritis, especially in countries that have introduced universal rotavirus vaccination [13, 14].

Despite their importance as a cause of severe gastroenteritis, limited data are available on the burden and epidemiology of norovirus diseases in children less than five years of age in Indonesia. The norovirus genotypes responsible for acute gastroenteritis cases in hospitalized children in Indonesia is also unknown [15]. Therefore, the objective of this study was to investigate the prevalence, seasonality, clinical characteristics, and genotype distribution of norovirus and rotavirus infections in children less than five years of age hospitalized with acute gastroenteritis in Indonesia.

Materials and methods

Sample and clinical data collection

This study used stool samples collected from children less than five years of age who were hospitalized with acute gastroenteritis at 1) Mataram General Hospital, Nusa Tenggara Barat. 2) Dr. Sardjito General Hospital, Yogyakarta, and 3) Wates General Hospital (Kulon Progo District), Yogyakarta, during the Indonesian Rotavirus Surveillance Study conducted between January and December 2015. Stool samples were collected within the first 48 hours after admission according to the World Health Organization (WHO) protocol [16]. Stool specimens were stored at 4-8 °C before they were transported to the Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia. The specimens were then aliquoted into several tubes and stored at -20 °C. The patients' clinical presentation, such as fever, vomiting, dehydration and diarrhea, was obtained from the medical histories stored at Pediatric Research Office, Dr. Sardjito General Hospital Yogyakarta.

Severe dehydration was indicated by two or more of the following signs: lethargy (unconsciousness), inability to drink sufficiently, sunken eyes, and very slow recovery of pinched skin (> 2 seconds). Moderate dehydration was indicated by two or more of the following signs: restlessness, irritability, sunken eyes, eager drinking, thirst, and slow recovery of pinched skin [17].

Rotavirus detection and genotyping

All stool samples were tested for the presence of group A rotavirus by enzyme immunoassay (EIA) using an IDEIA^{TM} Rotavirus kit (DakoCytomation) according to the manufacturer's instructions. RNA was extracted from stool samples using a QIAamp RNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions, and rotavirus genotyping was performed as described previously [18]. Negative and positive controls were included in each experiment.

Norovirus genogroup determination

Norovirus RNA was extracted from stool samples using a QIAamp RNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. Determination of norovirus GI and GII was performed by TaqMan-based quantitative real-time polymerase chain reaction (qRT-PCR) on an ABI 7000 system (Applied Biosystems) as described previously [19]. Primers and probes to identify norovirus genogroup are listed in Supplementary Table 1.

The qRT-PCR reaction was conducted in a final volume of 25 µl containing 1 µl (10 µM) of forward primer, 1 µl (10 µM) of reverse primer, 0.3 µl (10 µM) of probe, 12.5 µl of 2X RT-PCR buffer, 1 µl of 25X RT-PCR enzyme mix, 4.2 µl of nuclease-free water, and 5 µl of RNA template. Reverse transcription for GI norovirus was performed at 61 °C for 3 minutes, followed by initial denaturation at 95 °C for 5 minutes and 45 cycles of 95 °C for 5 seconds; 57 °C for 45 seconds and 37 °C for 60 seconds. Reverse transcription for GII norovirus was performed at 61 °C for 3 minutes, followed by initial denaturation at 95 °C for 5 minutes and 45 cycles of 95 °C for 5 seconds, 60 °C for 45 seconds, and 37 °C for 60 seconds. Negative and positive controls were included in each experiment.

Norovirus sequencing

Norovirus RNA was extracted using a QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The primer sets MON432/G1SKR and MON431/G2SKR were used to amplify a 543- to 557-base-pair (bp) region of the ORF1–2 junction of genogroup I and genogroup II strains, respectively [20, 21]. The PCR reaction

Table 1 Characteristics of children less than five years old hospitalized with acute gastroenteritis [n = 406]

	Norovirus-positive[a] (n = 75)	Rotavirus-positive[b] (n = 223)	Mixed Infections[c] (n = 29)
Detection rate (%)	18.47	54.93	7.14
Age, month [median (IQR)]	15 (8-23)	14 (9-24)	21 (13-30)
Sex:			
Male [n (%)]	50 (66.7%)	146 (65.5%)	21 (72.4%)
Female [n (%)]	25 (33.3%)	77 (34.5%)	8 (27.6%)
Cases detected in each location:			
Dr. Sardjito Hospital Yogyakarta (n = 39)	12 (16.0%)	15 (6.73%)	4 (13.8%)
Wates Hospital Yogyakarta (n = 89)	17 (22.7%)	62 (27.8%)	10 (34.5%)
Mataram Hospital, Nusa Tenggara Barat (n = 278)	46 (61.33%)	146 (65.47%)	15 (51.7%)

[a]Norovirus-positive patients as determined by qRT-PCR

[b]Rotavirus-positive patients as determined by EIA

[c]Patients whose clinical samples were positive for both rotavirus and norovirus

was conducted in a final volume of 25 µl containing 0.5 µl (50 µM) of forward primer, 0.5 µl of (50 µM) reverse primer, 11 µl of nuclease-free water, 5 µl of 5X RT-PCR Buffer, 1 µl of dNTP mix (10 µM), 1 µl of RT-PCR enzyme (QIAGEN), 1 µl of RNase inhibitor (20 U), and 5 µl of RNA. The following cycling conditions were used: reverse transcription at 42 °C for 30 minutes, followed by an initial denaturation at 95 °C for 15 minutes and 40 cycles of 95 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. The PCR products were sent to 1st BASE (Malaysia). They were separated in 2% gels, and amplicons were then excised, purified, and sequenced using an ABI 3730xl DNA Sequencer (Applied Biosystems) using the same primer pairs as used in the qRT-PCR.

The sequences obtained in this study were deposited in the GenBank database under the accession numbers MK408504-MK408528.

Phylogenetic analysis

Electropherograms were visually analysed, and contiguous DNA sequences were created utilizing the software Geneious (version 9.1.7). The genogroup and genotype of each sample was determined using the Norovirus Typing Tool (Version 2.0) (https://www.rivm.nl/mpf/typingtool/norovirus/) [22]. Global norovirus strains were obtained from the Vipr database (https://www.viprbrc.org). Multiple nucleotide and amino acid alignments were constructed using the MUSCLE algorithm in MEGA X [23]. Nucleotide and amino acid distance matrixes were calculated using the p-distance algorithm in MEGA X.

The optimal evolutionary model was selected based upon the Akaike information criterion (corrected) (AICc) ranking implemented in MEGA X. Maximum-likelihood phylogenetic trees using the Kimura 2-parameter nucleotide substitution model were constructed using MEGA X [23]. The robustness of branches was assessed by bootstrap analysis using 1000 pseudoreplicate runs, and nodes with values greater than 50% were considered to be strongly supported in the phylogenetic analysis.

Statistical analysis

Data were computed with Stata 13 SE. Characteristics of norovirus, rotavirus and mixed infections are presented in frequencies and percentages. The chi-square test was used to measure odds ratios and *p*-values for relating pathogen detection to clinical manifestations of acute gastroenteritis of children less than five years of age in Indonesia.

Ethical approval

The research protocol was approved by the Ethical Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. Informed consent was provided by the parents or guardians of each child before the children were enrolled in the Indonesian Rotavirus Surveillance Study 2015.

Results

Study population

From January to December 2015, a total of 406 stool samples were collected from children less than five years of age hospitalized due to acute diarrhea at three participating hospitals. The samples were predominantly collected from Mataram General Hospital (68.5%). The age of the patients

ranged from 0 to 59 months (median, 15 months). There were 261 (64.29%) male and 145 (35.71%) female patients; the sex ratio (male/female) was 1.8.

Virus detection rates and clinical characteristics

Of the 406 stool samples collected, 75 (18.47%) and 223 (54.93%) were positive for norovirus (as determined by qRT-PCR) and rotavirus (as determined by EIA), respectively. Mixed infections of rotavirus and norovirus were identified in 29 (7.14%) patients. Norovirus, rotavirus, and mixed infections were most commonly identified in children with acute diarrhea at Mataram General Hospital and were more frequently identified in male patients, which accounted for 66.7%, 65.5%, and 72.4% of cases, respectively (Table 1).

Fever, vomiting, diarrhea and dehydration were clinical features commonly observed in norovirus and rotavirus infections. Fever was more likely present in patients with acute diarrhea due to norovirus (OR = 6.78; $P = 0.009$) and rotavirus (OR = 4.07; $P = 0.044$) infections. Vomiting was only significantly associated with rotavirus infection (OR = 22.32; $P = < 0.001$). Diarrhea as well as dehydration were not associated with norovirus, rotavirus or mixed infections (Table 2).

Seasonality and age distribution

Norovirus and rotavirus were identified in children less than five years of age with acute diarrhea throughout the year, following the year-round incidence of acute diarrhea. Norovirus seasonal distribution peaked in January-February and July-August. Meanwhile, rotavirus seasonal distribution was more sustained, concentrated in early May to the end of September, with a slight decrease in July and another peak identified in January (Fig. 1). Although norovirus and rotavirus were identified in all age groups, rotavirus infection was clustered in children 7-24 months of age, while norovirus clustered in children 7-36 months of age (Figure 2).

Molecular epidemiology

Genogrouping of 75 norovirus-positive stool samples showed that six (8%) and 69 (92%) contained GI and GII norovirus, respectively. Sequencing of a subset of 25 samples revealed multiple genotypes circulating in Indonesia during the study period. The most prevalent genotype was the GII.Pe-GII.4 Sydney 2012 (14/25 [56%]) variant, followed by GIP3-GI.3 (3/25 [12%]), GII.Pg-GII.1 (2/25 [8%]), and GII.P7-GII.6 (2/25 [8%]). Single detections (4%) of GII.P15-GII.15, GII.P16-GII.2, GII.P17-GII.17 and GII.P21-GII.21 were also identified (Table 3).

We performed PCR on 35 selected samples out of the 223 that were rotavirus positive by EIA to determine the rotavirus genotype. The genotypes G3P[8], G2P[6] and G9P[8] were identified in 30 (85.7%), 1 (2.9%) and 1 (2.9%), respectively. Three rotavirus samples could not be genotyped (8.5%) (Table 3).

Phylogenetic analysis of norovirus strains

Phylogenetic analysis was performed on an approximately 280-bp region of the 3' end of the RdRp gene and an approximately 310-bp region of the 5' end of the capsid gene. The Indonesian GII.Pe-GII.4 Sydney-2012 strains from this study shared 97.9-100% nucleotide (nt) and 100% amino acid (aa) sequence identity across the RdRp region and 97.5-100% nt and 96.0-100% aa sequence identity across the capsid region. These strains shared 96.5-97.9% nt and 100% aa sequence identity across

Table 2 Clinical features of norovirus and rotavirus infections among children less than five years old hospitalized with acute gastroenteritis [n = 406]

	Norovirus-positive (n = 75)	Rotavirus-positive (n = 223)	Mixed Infections (n = 29)
Clinical features			
Fever [n (%)]	45/75 (60.0%)	170/223 (76.2%)	22/29 (75.(%)
Diarrhea [n (%)]	74/75 (98.7%)	222/223 (99.6%)	29/29 (100%)
Vomiting [n (%)]	66/75 (88.0%)	209/223 (93.7%)	27/29 (93.1%)
No dehydration [n (%)]	14/75 (18.7%)	48/223 (21.5%)	5/29 (17.2%)
Some dehydration [n (%)]	59/75 (78.7%)	168/223 (75.3%)	24/29 (82.8%)
Severe dehydration [n (%)]	2/75 (2.7%)	7/223 (3.1%)	0/29 (0%)
Odds ratio of clinical features			
Fever [OR (*P*)]	6.78 (0.009)*	4.07 (0.044)*	0.21 (0.645)
Diarrhea [OR (*P*)]	0.44 (0.506)	0.57 (0.451)	0.23 (0.630)
Vomiting [OR (*P*)]	0.19 (0.665)	22.32 (< 0.001)*	1.18 (0.277)
Dehydration [OR (*P*)]	1.55 (0.462)	1.48 (0.48)	2.03 (0.362)

*Statistically significant

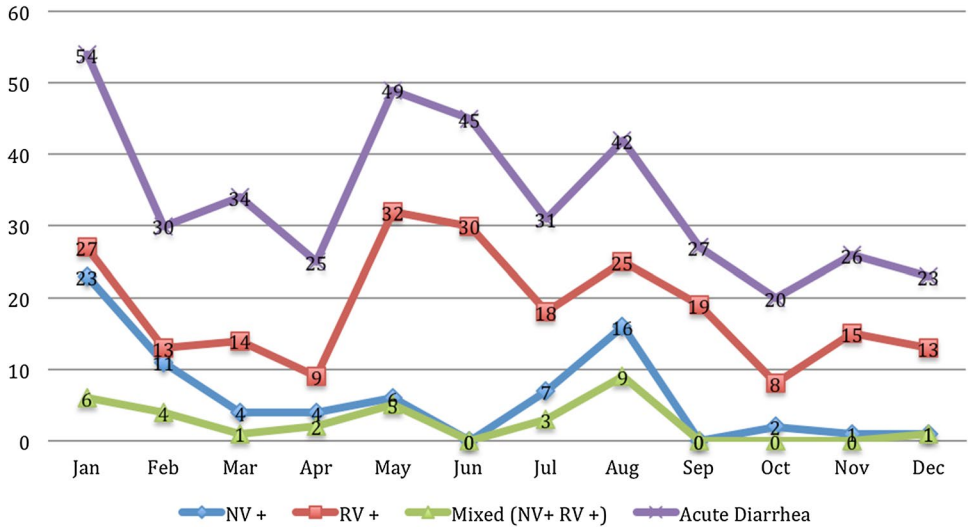


Fig. 1 Seasonal distribution of norovirus and rotavirus infections during January – December 2015 [n = 406]. NV + were norovirus-positive patients as determined by qRT-PCR; RV + were rotavirus positive patients as determined by EIA; and NV + RV + were norovirus- and rotavirus-positive patients (mixed infections)

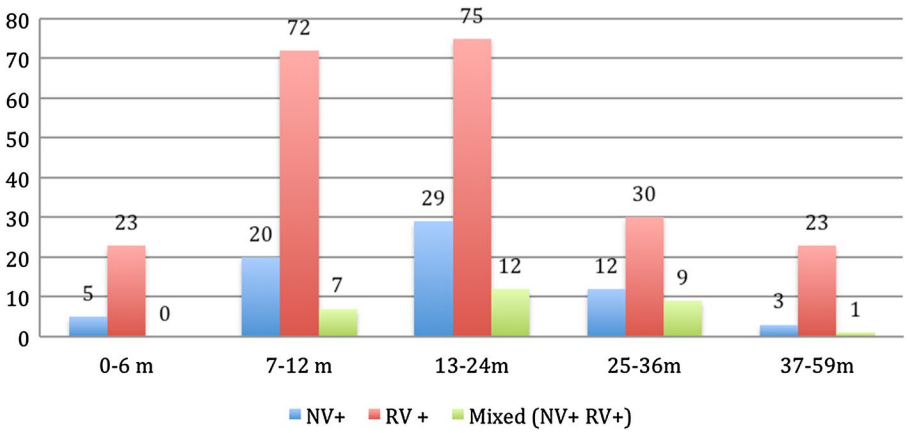


Fig. 2 Age distribution of hospitalized children with norovirus (NV +) and rotavirus (RV +) infections [n = 406]

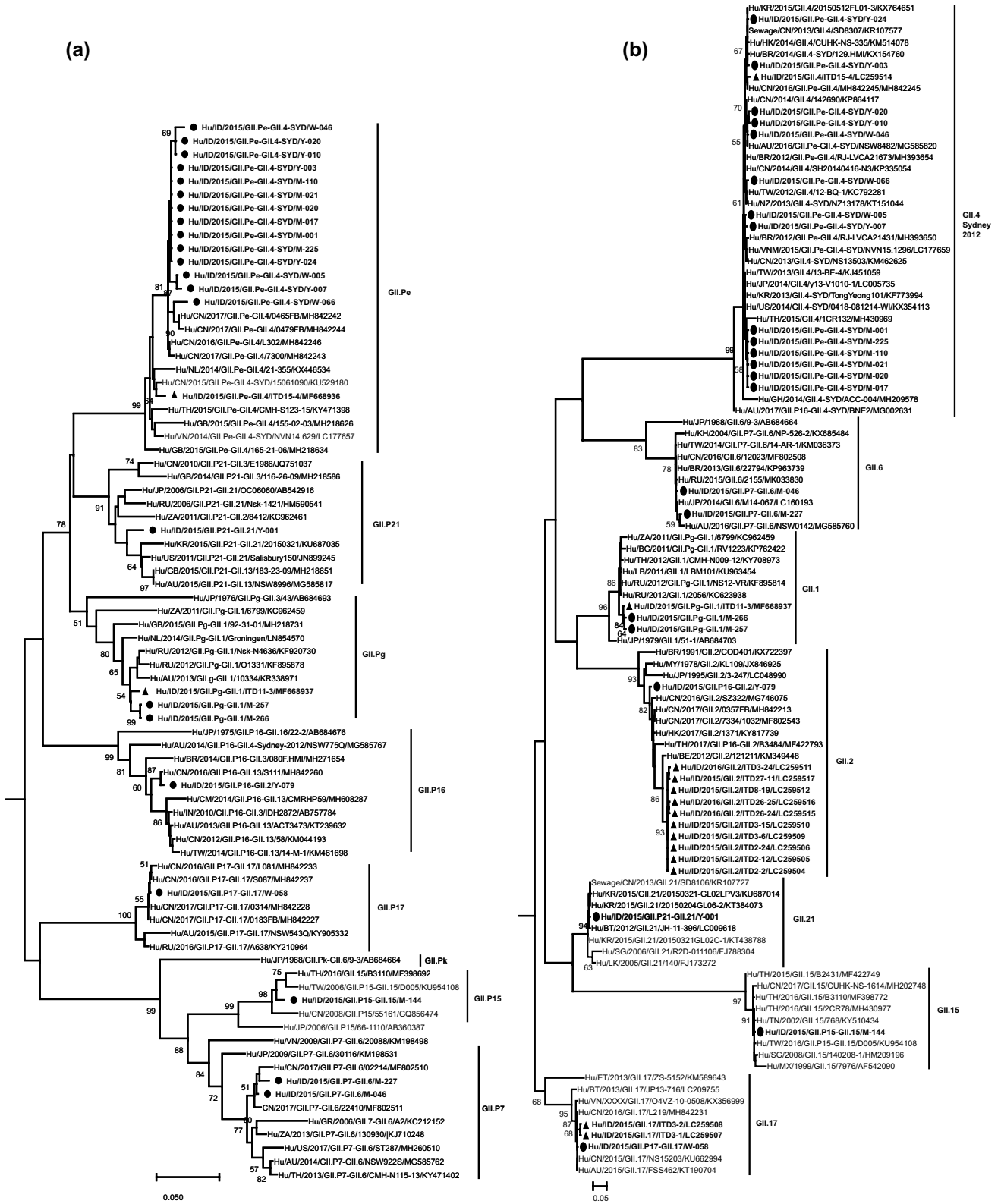
Table 3 Norovirus and rotavirus genotypes identified in this study

Viral Agent	n (%)
Norovirus genogroup (n = 75)	
Norovirus 1 (GI)	6 (8)
Norovirus 2 (GII)	69 (92)
Norovirus genotype (n = 25)	
GII.Pe-GII.4 Sydney 2012	14 (56)
GIP3-GI.3	3 (12)
GII.Pg-GII.1	2 (8)
GII.P7-GII.6	2 (8)
GII.P15-GII.15	1 (4)
GII.P16-GII.2	1 (4)
GII.P17-GII.17	1 (4)
GII.P21-GII.21	1 (4)
Rotavirus (n = 35)	
G2P[6]	1 (2.9)
G3P[8]	30 (85.7)
G9P[8]	1 (2.9)
Untypeable	3 (8.5)

the RdRp region and 97.0-98.1% nt and 98.9-100% aa sequence identity across the capsid region with a previously sequenced virus from an asymptomatic adult from Indonesia in 2015 [24]. The strains from this study formed multiple subclusters in the RdRp and capsid phylogenetic analysis, which may suggest multiple introductions into Indonesia, and these strains clustered with global contemporary strains, reflecting the widespread circulation of this pandemic variant (Fig. 3a and b).

A single GII.P17-GII.17 strain was identified in this study and was also previously identified in asymptomatic adults in Indonesia [24]. The capsid region of these strains shared 98.5% nt and 98.9% aa sequence identity. In phylogenetic trees based on the RdRp and capsid regions, these Indonesian strains clustered with contemporary strains from China (Fig. 3a and b).

The two GII.Pg-GII.1 strains from this study shared 100% nt and aa sequence identity in the RdRp region and 99.3% nt and 100% aa sequence identity in the capsid region. These strains were most closely related to a previously



characterised strain from Indonesia isolated from an asymptomatic adult in 2015 [24] (sharing 98.6% nt and 100%% aa sequence identity in the RdRp region and 99.3% nt and 100% sequence identity in the capsid region), and these strains clustered with sporadic strains from Russia detected in 2012 (Fig. 3a and b).

◂**Fig. 3** Maximum-likelihood phylogenetic trees of **(a)** 288 bp of the RdRp region (3'-ORF1) of norovirus genogroup II samples and **(b)** 306 bp of the capsid region (5'-ORF2). Genetic distances were calculated using the Kimura 2-parameter model with the gamma substitution model employed in MEGA X. Bootstrap values greater than 50% are shown. Strains sequenced in this study are indicated by a filled circle symbol, and previously sequenced Indonesian strains are indicated by a filled triangle symbol. The scale bar shows genetic distance expressed as nucleotide substitutions per site. For both trees, the strain GIII/Bo/GB/1994/Dumfries/AY126474, which is not shown, was used as an outgroup

Two GII.P7-GII.6 strains were characterised in this study. These strains shared 89.5% nt and 100% aa sequence identity in the RdRp region and 98.3% nt and 100% aa sequence identity in the capsid region. These strains clustered with geographically diverse strains in the phylogenetic analysis based on the RdRp and capsid regions (Fig. 3a and b).

A single GII.P16-GII.2 strain was characterised in this study. In the RdRp and capsid phylogenetic trees, the strain clustered with contemporary strains, in particular with strains from China (Fig. 3a and b). GII.2 strains were previously detected in asymptomatic adults in Indonesia [24]. These strains shared 92.9-93.7 nt and 88.7-100% aa sequence identity in the capsid region, suggesting that distinct variants may be circulating in the adult and paediatric populations in Indonesia.

A single GII.P21-GII.21 strain was detected, which clustered with contemporary strains from South Korea (Fig. 3a and b). A single GII.P15-GII.15 strain was also identified, which clustered with strains from Thailand and Taiwan.

Globally, GI norovirus strains are detected less frequently than GII strains, which was also observed in this study. The Indonesian GIP3-GI.3 strains shared 97.8-99.3% nt and 100% aa sequence identity across the RdRp region and 99.0-100% nt and 99.0-100% aa sequence identity across the capsid region. In the phylogenetic tree based on the RdRp, the Indonesian GIP3-GI.3 strains clustered with strains from the UK and Japan circulating in 2014 and 2015 (Fig. 4a), and in the phylogenetic tree based on the capsid region, they clustered with strains from Japan, Thailand and Nicaragua circulating in 2014, 2015 and 2010, respectively (Fig. 4b).

Discussion

Currently, there is a lack of nationwide studies to assess the burden of norovirus diseases in Indonesia, despite the importance of these viruses as the main agents of acute gastroenteritis outbreaks globally [15]. Most of the previous studies were conducted more than a decade ago in only two regions (Jakarta and Surabaya) for limited surveillance periods [15]. Those studies also did not determine the genogroups and genotypes of norovirus circulating in Indonesia [25–28]. Consequently, there is a lack of information regarding the burden of disease, epidemiology, and diversity of norovirus strains in Indonesia, as is the case in other developing countries [29].

Our study showed that the prevalence of norovirus in children less than five years of age hospitalized with acute gastroenteritis in Indonesia was 18.47%, similar to findings of worldwide studies [11]. Since the previous nationwide surveillance of acute gastroenteritis was mainly focused on rotaviruses [7], we therefore suggest that the burden of norovirus diseases in Indonesia should not be underestimated. In 2015, the Ministry of Health reported 21 diarrhea outbreaks in Indonesia, with more than 1,200 patients affected and 30 deaths, resulting in a case fatality rate (CFR) of 2.47% [30]. Unfortunately, the etiological causes of these outbreaks were not further investigated. It is possible that a proportion of these reported outbreaks were caused by noroviruses, given that it is the most common cause of diarrhea in all age groups. In fact, norovirus has been reported in diarrhea outbreaks in neighboring countries of Indonesia, including Singapore [31, 32] and Thailand [33, 34].

Based on our findings, norovirus and rotavirus infections commonly occurred at between 7 and 24 months of age, with a median age of 15 and 14 months, respectively. Maternal antibodies could be a protective factor for children early in their life during the breastfeeding period [35]. Administration of rotavirus vaccines in the first 6 months of life should therefore reduce the prevalence of rotavirus diarrhea at later ages. The overall clinical characteristics of norovirus and rotavirus infections are similar and therefore cannot be easily distinguished clinically. With regard to seasonality, both viruses were detected throughout the year, with the exception of June and September, when noroviruses were not detected. We found a peak of rotavirus-positive cases in the cool, dry season in May and June, consistent with our previous studies [6, 7]. A limitation of the current study is that this interpreted seasonality is only based on data from a single year. In temperate countries of the northern and southern hemispheres, peaks of norovirus and rotavirus infections have been observed during winter season [36, 37]. Environmental factors, such as temperature and humidity, as well as population factors, such as travel and food consumption, all contribute to norovirus and rotavirus transmission in humans [6, 38].

We found that GII norovirus was significantly more prevalent (92%) than GI norovirus (8%). To our knowledge, this is the first study that identified norovirus genogroups in hospitalized children less than five years of age with acute gastroenteritis in Indonesia and only the second study to perform sequencing of norovirus strains. It is noteworthy that the important genotypes currently circulating in other regions of Asia and globally were also identified in our study. Globally, GII.4 norovirus is the most prevalent

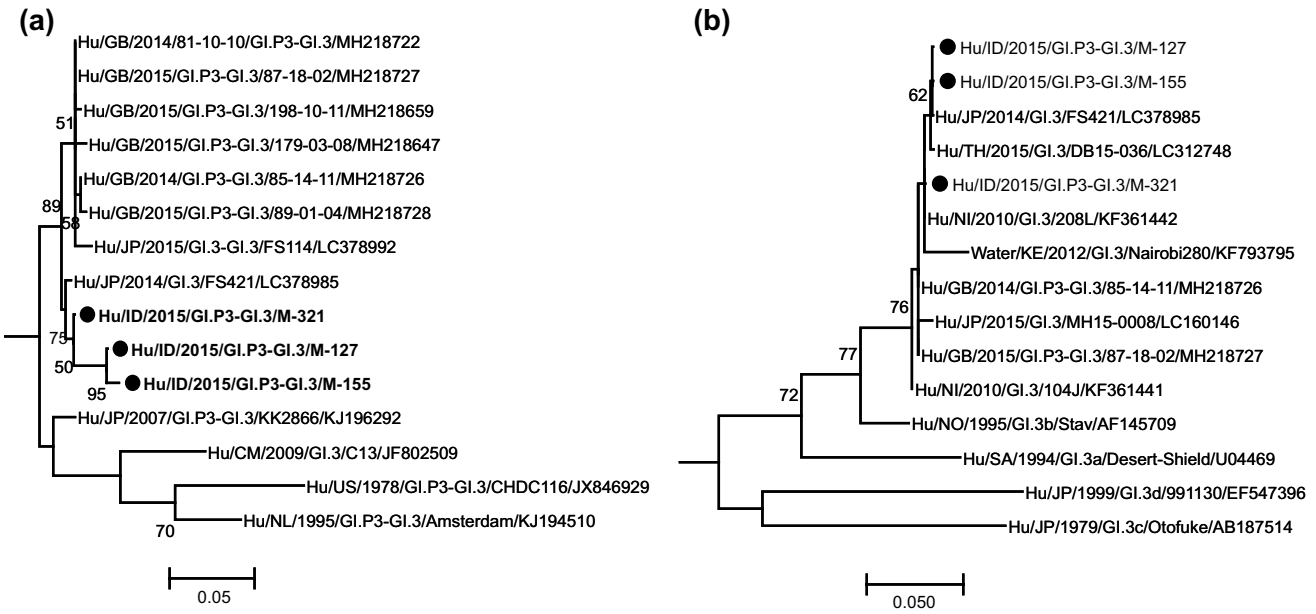


Fig. 4 Maximum-likelihood phylogenetic trees of **(a)** 282 bp of the RdRp region (3'-ORF1) of norovirus genogroup I samples and **(b)** 297 bp of the capsid region (5'-ORF2). Genetic distances were calculated using the Kimura 2-parameter model with the gamma substitution model employed in MEGA X. Bootstrap values less than 50% are shown. Strains sequenced in this study are indicated by a filled circle symbol, and previously sequenced Indonesian strains are indicated by a filled triangle symbol. The scale bar shows genetic distance expressed as nucleotide substitutions per site. For both trees, the strain GI/Hu/DE/1997-8/GI.P6-GI.6/BS5/AF0937975, which is not shown, was used as an outgroup

genotype causing clinical diseases [10]. Interestingly, in a recent study in Surabaya, Indonesia, norovirus shedding was detected in asymptomatic healthy adults [24]. Norovirus genotyping revealed that most of the viruses were of genotype GII.2. Norovirus strains associated with outbreaks have also been detected, including GII.4 Sydney-2012 and GII.17 [24], as also found in our study. The frequent detection of GII.Pe-GII.4 Sydney-2012 strains in this study reflects the global dominance of this pandemic variant between 2012 and 2014 [39]. It has also been detected in gastroenteritis in the United States [40], China [41], and Taiwan [42]. In Southeast Asia, the GII.Pe-GII.4 Sydney-2012 variant was replaced by the GII.17 Kawasaki variant [43–45], which was associated with outbreaks in Japan and China [10, 46]. Our study revealed the circulation not only of these globally dominant genotypes but also of rarer genotypes that have more localised circulation, including GII.Pg-GII.1 and GII.P7-GII.6. GII.Pg-GII.1 strains have been identified in outbreaks in Europe [47, 48], and several GII.P7-GII.6 strains have been identified both in sporadic cases and as the cause of large outbreaks in Europe and Asia [49]. A single GII.P16-GII.2 strain was characterised in this study, and this recombinant emerged in Asia in 2016 [50–52]. A single GII.P21-GII.21 was also detected; the GII.P21 RdRp is more commonly detected in combination with the GII.3 capsid in children. A single GII.P15-GII.15 strain was also identified, and this genotype is rarely detected globally. Based on all these findings, the diversity of circulating strains, even in a single year, should not be underestimated and should be the main focus of our future studies.

In this study, we did not investigate the source and route of transmission of the infecting norovirus strain. Norovirus genotypes differ in their mode of transmission. GI norovirus is frequently associated with waterborne transmission. In contrast, GII.4 genotype is more often associated with person-to-person transmission than non-GII.4 genotypes [10]. It has been hypothesized previously that immunocompromised individuals serve as reservoirs of emerging norovirus strains, which are then responsible for large outbreaks [53]. This hypothesis was challenged by recent evidence suggesting that these individuals were unlikely to contribute at the epidemic level [54]. It is noteworthy that multiple norovirus infections have been observed in asymptomatic healthy subjects with a relatively high viral load [24], raising the question of whether healthy asymptomatic individuals could potentially serve as reservoirs to increase the likelihood of sporadic cases and outbreaks. In nosocomial settings, however, norovirus transmission is more likely to occur from symptomatic patients [55, 56]. Therefore, these studies suggest that symptomatic individuals are also more likely to contribute to norovirus transmission in community settings. A better understanding of transmission routes and effective prevention strategies are urgently required to reduce the burden of norovirus disease in Indonesia.

Our study found a high incidence of rotavirus infection in Indonesia, similar to the findings of our previous national surveillance conducted in 2006, 2009 and 2010 [7, 8]. The prevalence of rotavirus infection (54.93%) was indeed higher than that of norovirus (18.74%). These results were not surprising, given the fact that rotavirus vaccines are not yet included in the National Immunization Program of Indonesia, although parents can obtain the vaccines through private health facilities. However, the use of rotavirus vaccines has been limited because of limited knowledge of their importance and availability as well as financial constraints on our general population [57]. Importantly, in several countries where rotavirus vaccines have been universally introduced, norovirus infection become more prevalent than rotavirus infection as the cause of acute gastroenteritis in children [13, 14, 58, 59].

Rotavirus genotyping demonstrated that G3P[8] was the most common genotype. This finding was unexpected, since G3P[8] has rarely been detected in previous surveillance studies conducted in several regions of Indonesia [7, 8, 18, 60, 61]. Worldwide surveillance data from 1996 to 2007 showed that the G3P[8] genotype circulates to a lesser extent than the G1P[8], G9P[8] and G2P[4] genotypes in humans [62]. However, its prevalence has increased in the last decade in many countries, and it has replaced the previously dominant G1P[8] genotype in some regions [63–68]. Whether these changes were associated with mass vaccination or were simply the result of the natural evolution of rotaviruses remains to be determined. Interestingly, the G3 genotype has the broadest host range within human rotavirus group A. It has been detected in many species, including humans, pigs, monkeys, horses, cats, dogs and rabbits [69]. Therefore, further sequence analysis is essential to determine the origin and evolution of G3P[8] strains in Indonesia. These results also highlight the importance of continuous strain monitoring to detect alterations in the circulating genotypes.

In conclusion, our study describes the considerably high burden of norovirus and rotavirus gastroenteritis in Indonesian children less than five years of age. Strengthening collaborations between the government, public health experts, virologists and research laboratories is critical for assessing the true burden of norovirus diseases in Indonesia and formulating appropriate prevention and control strategies. In addition, norovirus genotyping should be continuously performed in our future studies to provide databases of potentially emerging strains and to support the development of effective vaccines.

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Author contributions HN and CMD contributed to the study concept and design, acquisition of data, analysis and interpretation of data and drafting the manuscript; YM, NSM, AI and ATA contributed to acquisition of data and critical revision of the manuscript; MPP, YS and QP contributed to the scientific discussion and critical revision of the manuscript; MSH contributed to study concept and design, obtained funding, study supervision and critical revision of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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