



Research paper

Occurrence of norovirus infection in an asymptomatic population in Indonesia



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ABSTRACT

Norovirus (NoV) is a major cause of nonbacterial acute gastroenteritis worldwide in all age groups, and asymptomatic individuals may contribute to NoV transmission as a reservoir. Nonetheless, little information is available regarding asymptomatic NoV infection in Indonesia. We performed an epidemiological analysis of NoV infection among asymptomatic healthy volunteers in the city of Surabaya, Indonesia (population ~2.75 million). A total of 512 stool samples from 18 individuals (age range 20–42 years) collected from July 2015 to June 2016 were examined. The detection of NoV and the genotype classification were carried out by a reverse transcription-polymerase chain reaction (RT-PCR) direct sequencing method. NoV was detected in 14 of the 512 stool samples (2.7%), with 7 individuals (38.9%) having at least 1 positive stool sample. All 14 of the NoV strains detected belonged to genogroup GII. The phylogenetic analysis indicated that 10 strains (71.4%) were grouped with GII.2, 2 (14.3%) were GII.17, 1 was GII.4 Sydney 2012, and 1 was GII.1. The circulation of GII.Pg/GII.1 and GII.Pe/GII.4 Sydney 2012 recombinant variants was detected among an asymptomatic population in Surabaya, Indonesia. Of the 7 positive individuals, 2 were repeatedly infected with the same strain and heterogenous strains. Taken together, our results suggest that the excretion of NoV from healthy individuals is one of the sources of NoV outbreak.

1. Introduction

Norovirus (NoV) is considered a leading cause of both sporadic cases and outbreaks of nonbacterial gastroenteritis in all age groups (Bon et al., 2005; Khamrin et al., 2016; Zheng et al., 2010), and is responsible for 1.45 million deaths worldwide every year (Ahmed et al., 2014). Moreover, diarrhea, the most common symptom of gastroenteritis, remains a major cause of morbidity and mortality in all age groups in Southeast Asia. Diarrhea is the third leading cause of overall morbidity and the leading cause of infant mortality in Indonesia (Agtini et al., 2005). However, whether NoV is a major cause of diarrhea in Indonesia or, more generally, Southeast Asia, remains uncertain due to the dearth of epidemiological data.

NoV is a positive-sense, single-stranded, non-enveloped RNA virus that belongs to the family *Caliciviridae* (Jiang et al., 1993). The NoVs

are classified into 7 genogroups (GI to GVII) based on the complete capsid protein VP1 sequences. The GI and GII genogroups are further classified into 9 and 22 genotypes, respectively (Vinjé, 2015).

NoV is highly contagious and can be transmitted in various modes including contact with contagious individuals, contaminated environments, and the consumption of contaminated foods and even ice (Parashar et al., 1998). The personal hygiene practices of infected food handlers are considered to be the most important contributing factor in the spread of foodborne diseases (Koopmans and Duizer, 2004).

Several studies have shown that asymptomatic NoV infections are relatively common within different populations (García et al., 2006). Data from Western countries and Japan have demonstrated that NoVs are responsible for 5%–30% of NoV asymptomatic infection in adults (Centers for Disease Control and Prevention, 2011; Ozawa et al., 2007), and that asymptomatic persons can spread the virus although their viral

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Table 1
Demographic and virologic characteristics of NoV positive individuals.

ID	Number of infection	Sex	Age	Virus titer (copies/g)	Genotype	Nucleotide similarity	Interval between Nov detections
ITD2	ITD2-2	1	F	23	<10 ¹	GII.2	} 100% } 72 days 81 days
	ITD2-12	2			10 ³	GII.2	
	ITD2-24	3			<10 ¹	GII.2	
ITD3	ITD3-1*	1	F	26	<10 ¹	GII.17	} 100% } 15 days
	ITD3-2*	1			<10 ¹	GII.17	
	ITD3-6	2			10 ¹	GII.2	} 100% } 29 days 69 days
	ITD3-15	3			10 ²	GII.2	
	ITD3-26	4			<10 ¹	GII.2	
ITD8	ITD8-19	1	F	23	<10 ¹	GII.2	} 99.6% } 138 days
ITD11	ITD11-3	1	F	23	10 ⁵	GII.1	
ITD15	ITD15-4	1	M	25	<10 ¹	GII.4 Sydney 2012	
ITD26	ITD26-24**	1	F	24	<10 ¹	GII.2	} 100% } 6 days
	ITD26-25**	1			<10 ¹	GII.2	
ITD27	ITD27-11	1	F	27	<10 ¹	GII.2	

NoV: norovirus; *, **: one infection.

		Year 2015												Year 2016												Total																												
Month		July			Aug			Sept			Oct			Nov			Dec			Jan			Feb			Mar			Apr			May			Jun																			
ID/Week		IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I					
ITD2	1	2	3	4	5	6	-	7	8	9	10	11	12	13	14	15	16	17	18	-	19	20	21	22	23	24	-	-	25	26	27	28	29	30	31	32	33	34	35	36	-	37	38	39	40	-	41	42	43	-	44			
ITD3	-	1	2	3	4	5	-	6	7	8	9	-	10	11	12	13	14	-	15	-	16	17	18	-	19	-	-	20	21	-	-	22	-	-	23	24	25	-	-	-	-	-	-	26	27	-	28	29	30	31	-	32		
ITD4	-	1	2	3	4	5	6	-	7	8	9	-	10	11	12	13	14	15	16	-	17	18	19	-	20	-	-	21	22	23	24	25	-	26	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
ITD5	-	-	1	2	3	4	5	-	6	7	8	9	10	11	12	13	14	-	15	-	16	-	-	17	18	19	20	21	22	23	24	25	-	26	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
ITD6	-	1	2	3	-	4	5	6	7	8	-	-	9	10	11	12	13	14	15	16	-	17	-	18	19	20	21	22	-	-	23	24	25	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
ITD8	-	1	2	3	4	5	-	6	7	8	9	10	11	12	13	14	15	16	17	-	18	19	-	-	-	-	-	-	20	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
ITD11	-	-	1	2	3	4	-	5	6	7	8	-	9	-	10	11	-	-	-	-	12	13	14	-	15	-	16	17	18	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ITD12	1	-	2	3	4	5	-	6	7	8	9	10	-	11	12	13	14	15	-	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	-	35	36	-	-	-	-	-	-	-	-	-	37	38	39	
ITD15	-	1	2	3	4	5	6	-	7	8	9	-	10	11	12	13	-	-	-	14	15	-	16	17	18	19	20	21	22	23	24	-	25	26	-	27	28	29	30	31	32	-	33	34	35	36	37	38	39	40	-	41		
ITD17	-	1	2	3	4	5	6	-	7	8	9	10	11	12	13	14	15	-	16	17	18	19	20	21	22	-	-	-	23	24	25	26	27	28	29	-	30	-	31	32	-	-	-	-	-	-	-	-	-	-	-			
ITD18	-	1	-	2	3	4	5	6	7	8	9	-	10	11	12	13	-	-	-	14	-	15	-	16	17	-	18	-	19	-	20	-	21	22	-	23	24	25	-	-	-	-	-	-	-	-	-	-	-	26	27	-		
ITD19	1	-	-	-	2	3	-	-	4	-	5	-	6	7	8	9	10	11	12	13	14	-	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	-	30	31	32	33	-	-	-	-	-	-	34	-	35	36		
ITD21	1	-	2	3	-	4	5	6	7	8	9	10	11	12	13	-	-	-	-	14	-	-	-	15	16	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ITD24	1	-	2	3	4	5	6	-	7	8	9	10	11	12	13	14	15	-	16	-	17	-	18	19	20	21	22	-	23	24	25	26	27	-	28	29	30	31	-	32	-	-	-	-	-	-	-	-	33	-	34	35	-	36
ITD26	-	1	2	3	4	5	6	-	7	8	9	-	10	11	12	13	14	15	-	16	-	-	17	18	19	20	-	21	22	23	24	25	26	27	28	29	30	31	-	-	-	-	-	-	-	-	-	-	-	32	33	34	-	35
ITD27	-	1	2	3	4	5	-	6	7	8	9	10	-	11	12	13	14	15	16	17	-	-	18	-	-	-	-	-	19	-	20	21	22	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
ITD28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3	-	4	-	5	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
ITD29	-	-	-	-	-	-	-	1	2	-	3	4	5	6	7	8	-	9	10	-	11	12	13	14	15	16	-	-	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
*		15			68				65				75					47				62				51			40						41							18				24			6	512				
**		2			3				1				2					2				1				1			1						0								1			0		0	14					
*** (%)		13.3			4.4				1.5				2.7					4.3				1.6				2.0			2.5						0.0							5.6			0.0		0.0	2.7						

Fig. 1. The prevalence of NoV asymptomatic infection from July 2015 to June 2016 in asymptomatic Indonesian adults. The numbers in each column are the serial numbers of the collected samples. NoV-positive samples are shown in bold font within a square. *The total number of samples collected per month. **The number of samples positive for NoV per month. ***NoV positivity per month.

titer is usually lower than those of symptomatic persons. Moreover, NoV infections sometimes result in subclinical symptoms, which makes clinical diagnoses difficult (Ayukekbong et al., 2015). Asymptomatic NoV infection seems to contribute more to the spread of NoV infection in developing countries than in developed countries, because the number of people living in a household in developing countries is usually higher and the environmental sanitation level in these regions is often not optimal.

Little is known about NoV infection or its symptomatic manifestations in Indonesia (population > 257 million), and little epidemiological data is available in regard to asymptomatic cases in this country (D.S. Subekti et al., 2002a; D. Subekti et al., 2002b), whereas there are many epidemiological reports from Western countries and East Asia.

We conducted the present study to determine the prevalence of asymptomatic NoV excretion in an adult population living in Surabaya, Indonesia as well as the genetic diversity of NoVs circulating in this

community, the absence or presence of recombination events, and any other relevant characteristics of NoV infection.

2. Materials and methods

2.1. Study population

This study took place at a medical research institution in Surabaya, the second largest city in Indonesia with a population of about 3 million inhabitants. The staff members in the institution are mostly technicians and office personnel. Sources of drinking water are mainly commercially purchased bottled water or boiled water, both of which are recommended by the Indonesian government for hygienic reasons. Although western toilets have recently been introduced in the urban areas, traditional toilets without cover are still commonly used. Participants were invited to enroll voluntarily in this study, and were

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