

Identification of rotavirus infection during diarrhoea outbreaks among children under five years of age in Lampung, Indonesia

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This study aimed to estimate the proportion of rotavirus infection during a diarrhoea outbreak among children under five years of age and to investigate the possible risk factors causing rotavirus diarrhoea. A cross-sectional study was conducted during the September-December 2016 and June-July 2018 periods. Stool samples and socio-demographic characteristics were collected from inpatients and outpatients aged less than five years with diarrhoea. Identification of rotavirus by an immunochromatography test and polymerase chain reaction was carried out with the immunochromatography dipstick method, Eiken Rota stick and Taq PCR Master Mix kit. The proportion of rotavirus infection was 74.3%. The proportion of rotavirus infection was higher in male as compared with female patients. The highest infection rate was identified in children under 2 years of age. There was no statistically significant difference in breastfeeding history and socio-economic status between the rotavirus-positive and -negative groups. In conclusion, the proportion of rotavirus infection was high among children aged less than five years with acute diarrhoea in Lampung, Indonesia.

Key words: ROTAVIRUS; DIARRHEA; INDONESIA

INTRODUCTION

Diarrheal disease is one of the leading causes of death in children under five years of age (1). Most children under five years of age have had the experience of being infected by rotavirus. Rotavirus is the main cause of severe acute diarrhoea among children in both developed and developing countries. Rotavirus was estimated to have caused 528,000 deaths in 2000 all over the world (2). Laboratory testing is the only way to confirm the diagnosis of rotavirus (3). Several rapid methods such as the immunochromatographic test and the latex agglutination test have been evaluated and compared to other methods such as enzyme assays and polymerase chain reaction (PCR) and shown a wide range of sensitivity and specificity (4).

Rotavirus is the primary infection responsible for diarrhoeal disease in children in Indonesia (5). In previous studies that

have reported different percentages of rotavirus infection among Indonesian children under five years of age, rotavirus accounted for 40% of diarrhoea in hospitalized children in Surabaya (6), 67% in Jakarta (5), and 67% in Papua (7). Moreover, rotavirus vaccine (RVV) has been commercially available in Indonesia since 2011 and has been embedded

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into the Indonesia Pediatric Association Recommended Immunization Schedule for Children and Adolescents (0-18 years of age) (8). However, it has not been included in the Indonesia National Immunization Program (NIP). Furthermore, Lampung Province is a region in Indonesia where a diarrhoea outbreak occurred in 2014 (9). In 2015, this province had no diarrhoeal rotavirus case data (10).

In Indonesia, the lack of data on rotavirus cases has led to poor prioritization of rotavirus vaccines as a prevention strategy. Thus, to investigate the proportion of rotavirus infection and help health providers manage diarrhoeal rotavirus in 2016 and 2018 in Lampung, Indonesia, this study determined the proportion of diarrhoea caused by rotavirus infection among children under five years of age.

PATIENTS AND METHODS

A cross-sectional study was conducted in Pasar Ambon Primary Health Care, Panjang Primary Health Care, Tjokrodipo General Hospital, and Abdoel Moeloek General Hospital. Seventy-four stool samples were collected randomly from hospitalized children and children in the community who showed diarrhoeal symptom from September to December 2016 and from June to July 2018. Stool samples were sent to the Institute of Tropical Disease, Universitas Airlangga, Surabaya for further analysis. Three inclusion criteria were employed, as follows: children aged 1-60 months with a history of diarrhoea defined as more than 3 stools *per* day of liquid consistency, and showing diarrhoea less than 3 days prior to hospitalisation. Information on patient age and gender, breastfeeding history, rotavirus immunization status, and socio-demography of parents/guardians was obtained through face-to-face interviews with parents/guardians of the patients.

This study was approved by the Ethics Committee of Teaching Hospital of Airlangga University. Informed consent was obtained from parents or guardians of eligible children before their inclusion in the study.

Rotavirus antigen in stool samples was detected by the immunochromatography (ICT) technique using the immunochromatography dipstick method Eiken Rota stick (Eiken Chemical Co., Tokyo, Japan). All faecal samples were tested with polymerase chain reaction (PCR) assays for rotavirus gene identification as the reference. Samples were defined as positive for rotavirus if the respective viral nucleic acid was detected by PCR. Extraction of viral RNA from 10% faecal suspensions in phosphate-buffered saline was done by QIAmp® Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instruction. The extracted RNA was used in cDNA synthesis using Superscript® III Reverse Transcriptase (Invitrogen, USA). Amplification DNA for VP7 gene was

carried out with Taq PCR Master Mix kit (Qiagen, Hilden, Germany) using a method described before (11). The nucleotide length was 881 bp. Statistical analysis was performed using SPSS statistics 16.0 software.

RESULTS

Seventy-four stool samples were collected from inpatient and outpatient children under five years of age with acute diarrhoea. Forty-one (55.4%) and 55 (74.3%) out of 74 stools were positively infected by rotavirus by ICT and PCR, respectively (Table 1).

TABLE 1. Comparison of results obtained by ICT and PCR

Characteristic	PCR		Total
	Positive	Negative	
ICT	Positive	41 (55.4%)	41 (55.4%)
	Negative	14 (18.9%)	33 (44.6%)
Total	55 (74.3%)	19 (25.7%)	74 (100%)

ICT = immunochromatography; PCR = polymerase chain reaction

Not all patients had received rotavirus vaccination.

Higher rates of rotavirus infections were recorded in male (56.4%) than in female (43.6%) patients, and the highest rate of infection was detected in children aged 7-23 months (60%). Children with working parents had the highest proportion of rotavirus infection (58.2%), while parents/guardians with secondary education had children with the highest proportion of rotavirus infection (47.3%). There was no statistically significant difference between rotavirus-positive and -negative groups according to patient gender, age, breastfeeding pattern or socio-economic status of parents/guardians.

DISCUSSION

This was the first study on rotavirus in Lampung, Indonesia. Rotavirus was identified in as much as 74.5% of the total of 74 children with acute diarrhoea. Rotavirus remains the primary cause of acute diarrhoea in children and previous studies in Indonesia showed that the high proportion of rotavirus infection was the cause of acute diarrhoea in children (5).

This study found that male patients had a higher rate of rotavirus infection (68.4%) than female patients (31.6%). Similar values were obtained in some studies conducted in Indonesia (5, 6, 12). This result is similar to the findings of studies conducted in Nigeria, indicating that males were twice as susceptible as females (13). A study in Vietnam showed that this virus predominantly affected male patients (14).

TABLE 2. Rotavirus infection (PCR positive) distribution according to patient sex, age, breastfeeding and socio-economic characteristics of parents/guardians

Characteristic	Number of patients enrolled (N=74)		p-value
	Rotavirus-negative	Rotavirus-positive	
Sex:			
Male	13 (68.4%)	31 (56.4%)	0.356*
Female	6 (31.6%)	24 (43.6%)	
Total	19 (100%)	55 (100%)	
Age:			
0-6 months	6 (31.6%)	13 (23.6%)	0.826**
7-23 months	9 (47.4%)	33 (60.0%)	
24-59 months	4 (21.1%)	9 (16.4%)	
Total	19 (100%)	55 (100%)	
Educational background of parents/guardians:			
Primary education	2 (10.5%)	12 (21.8%)	0.56**
Secondary education	11 (57.9%)	26 (47.3%)	
Tertiary education	6 (31.6%)	17 (30.9%)	
Total	19 (100%)	55 (100%)	
Occupation of parents/guardians:			
Working parents/guardian	11 (57.9%)	32 (58.2%)	0.289*
Housewife	8 (42.1%)	23 (41.8%)	
Total	19 (100%)	55 (100%)	
Breastfeeding:			
Yes	9 (47.4%)	22 (40%)	0.6*
No	10 (52.6%)	33 (60%)	
Total	19 (100%)	55 (100%)	

*calculated by χ^2 ($p < 0.05$); **calculated by Spearman correlation ($p < 0.05$)

However, there was no statistically significant difference ($p > 0.05$) (Table 2).

Most cases (62.2%) of rotavirus disease occurred in children aged <2 years (Table 2). Age distribution of children with rotavirus infection in our study was consistent with studies conducted in Europe, which show that the life span of infection is in the first two years of life (15), and other studies in some provinces in Indonesia (5, 12). This can be explained by the fact that at the age <6 months, maternal antibodies have a protective effect, while at the age >23 months, natural immunity may have developed due to recurrent rotavirus infections. The life span of infection is in the first two years of life (16). The results of a cohort study on 200 Mexican infants followed until the age of 2 years show that during this period, only 4% experienced a second rotavirus infection (17). There were three (9.09%) children aged 36-59 months positive for rotavirus. After 3 years of age, children rarely suffer severe rotavirus diarrhoea. It is presumed to be

the influence of active immunity from repeated infections throughout life (18). There was no statistically significant difference in age group distribution between rotavirus-positive and rotavirus-negative groups.

The educational background of the parents/guardians of these children was not statistically significantly correlated with the status of rotavirus infection ($p > 0.05$) (Table 2). Parents with secondary education had children with the highest proportion of rotavirus infection. This result is comparable to a previous study in Kenya (19). However, these occupational characteristics were not statistically significant ($p > 0.05$). Children whose parents worked had the highest prevalence of rotavirus infection. A previous study in Nigeria has shown similar result (13). These results indicate that the observed factors may be due to chance and not a certainty, and this implies that rotavirus infects children regardless of the parent's or guardian's occupation and education.

Breastfeeding did not show any significant difference between the case group and rotavirus infection. There may be no direct correlation between rotavirus diarrhoea and breastfeeding (20). Breastfeeding did not protect children from rotavirus diarrhoea (21). Studies conducted in Bandung, Indonesia, showed that exclusive breastfeeding was not associated with severe dehydration in rotavirus diarrhoea (22).

In this study, two different tests were used to detect rotavirus, i.e. ICT and PCR (Table 1). Sensitivity and specificity value of ICT for rotavirus was 74.5% and 100%, respectively. A study in Vietnam compared the ICT method with RT-PCR in 104 samples to detect group A rotavirus. The results indicated sensitivity of 87.8% and specificity of 93.3% (23). In another study in Ghana, the ICT sensitivity and specificity for rotavirus was 75% and 95%, respectively (24). Molecular techniques are more sensitive than ICT (25). Negative ICT results can be related to the low concentration of viral load that cannot be detected by the kit, especially VP6 protein in rotavirus antigen (23). The ICT based testing tool to detect specific RNA viruses required 2×10^5 - 5×10^7 PFU/mL (26).

Some limitations for evaluation should be considered. The patients enrolled may not have represented all children having acquired rotavirus during the outbreak. Children with very mild disease may have been less likely to seek medical care and be captured during surveillance. Our results did not differentiate between children who were exclusively breastfed and those who were partially breastfed.

In Indonesia, rotavirus vaccine is only available in paediatric private practices. Although Indonesia had a trial rotavirus vaccine in Yogyakarta, Indonesia in 2013 and achieved good results in decreasing rotavirus cases, other regions do not have regulation for this program yet.

CONCLUSION

Overall, the results of this study highlight the proportion of high acute diarrhoea caused by rotavirus in Lampung, Indonesia. Recent qualitative research has shown low awareness of rotavirus disease among policymakers, healthcare practitioners, and the community (27). Finally, increasing public awareness of rotavirus infection and suggestion of considering vaccine use are important in prevention strategies for reducing the number of cases.

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SAŽETAK

Identifikacija infekcije rotavirusom tijekom epidemije proljeva kod djece mlađe od pet godina u Lampungu, Indonezija

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Cilj studije bio je procijeniti razmjere infekcije rotavirusom tijekom epidemije proljeva kod djece mlađe od pet godina te istražiti moguće čimbenike rizika koji uzrokuju proljev izazvan rotavirusom. Ovo poprečno istraživanje provedeno je od rujna do prosinca 2016. i od lipnja do srpnja 2018. godine. Uzorci stolice i sociodemografski podaci prikupljeni su za bolničke i izvan-bolničke bolesnike s proljevom mlađe od pet godina. Identifikacija rotavirusa imunokromatografskom metodom i lančanom reakcijom polimeraze provedena je pomoću Eiken Rota i Taq PCR Master Mix. Stopa infekcije rotavirusom bila je 74,3%. Stopa infekcije rotavirusom bila je viša kod muških nego kod ženskih bolesnika, a najviša je utvrđena kod djece mlađe od 2 godine. Nije bilo statistički značajne razlike u povijesti dojenja i socioekonomskom statusu između skupine pozitivne na rotavirus i skupine negativne na rotavirus. U zaključku, stopa infekcije rotavirusom bila je visoka među djecom mlađom od pet godina s akutnim proljevom u Lampungu, Indonezija.

Ključne riječi: ROTAVIRUS; PROLJEV; INDONESIA