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Molecular Characteristics of Rotavirus Isolated from a Diarrhea Outbreak in October 2008 in Bintuni Bay, Papua, Indonesia

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ABSTRACT

BACKGROUND: Viral diarrhea continues to be a health problem in Indonesia that often causes outbreaks; in particular, acute viral diarrhea in young children. Rotavirus is the leading cause of severe diarrhea in children under two years of age. This study aimed to determine the genotypes of rotavirus in Bintuni Bay, Papua.

METHODS: Stool specimens from 15 patients were collected and analyzed for rotavirus using an enzyme immunosorbent assay (EIA) and reverse transcriptase-polymerase chain reaction (RT-PCR). Subsequently, we sequenced the genetic material of rotavirus positive samples by RT-PCR and analyzed the results using Mega-4 software.

RESULTS: Two rotavirus serotypes were identified from the diarrhea outbreak in Bintuni, Papua in October 2008: serotype G1 with G1P[6] (50%) and G1P[8] (16.7%) strains, and serotype G2 with G2P[4] (23.3%) strain. Phylogenetic tree analyses of VP7 protein showed that rotavirus-infected diarrhea in Bintuni Bay, Papua at that time was dominated by the G1 serotype (83%).

CONCLUSION: The laboratory results showed that G1 serotype rotavirus was a cause of the outbreak of diarrhea in October 2008 in Bintuni, Papua.

KEYWORDS: diarrhea, Papua, rotavirus, serotype

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Introduction

Diarrhea is caused by infection, malabsorption, allergies, toxicity, and other factors. Diarrhea often results in outbreaks, as evidenced by the high numbers of patients and deaths, especially from acute diarrhea resulting from infections and food poisoning. These outbreaks occur frequently in areas with poor sanitation, insufficient and unclean water supplies, and poor nutritional status.[1] Diarrhea continues to be a health problem in Indonesia. The data from a rotavirus surveillance conducted in six hospitals in six cities (Jakarta, Palembang, Bandung, Denpasar, Mataram, and Yogyakarta) in Indonesia from January until December 2006 demonstrated that approximately 60% of children hospitalized with diarrhea were rotavirus-positive. Although the mortality rate has dropped significantly, morbidity remains high.[2]

Until the 1970s, bacterial infection was considered to be responsible for the majority of diarrheal disease in Indonesia.[3] However, a study conducted from 2005 to 2006 at a referral hospital in Yogyakarta showed that the prevalence of bacterial diarrhea was only 5%, suggesting a very large percentage of non-bacterial pathogenic causes of diarrhea, such as viruses, parasites, and fungi.[3] One of the most common pathogens causing gastroenteritis is rotavirus.[4]

Rotavirus infection is recognized as the leading cause of severe diarrhea in children aged less than two years, both in the industrialized and developing world.[5] The data from Kapikian et al showed that approximately 40–60% of children in developed countries and 20–40% of children in developing countries are exposed to rotavirus infection. It is estimated that in developing countries, there are approximately 18 million cases

of moderate to severe diarrhea with high mortality occurring in infants and children under five years of age.[6]

The first report of rotavirus diarrhea among Indonesian children, published in January 1978 from a study in Yogyakarta, reported an incidence of 12%. Between 1978 and 1979, acute diarrhea cases reported as rotavirus rose to 38%.[7] The low prevalence reported from these studies may have been due to the use of electron microscopy as the diagnostic tool. Further examination by enzyme immunosorbent assay (EIA) discovered that 42% of acute diarrhea cases were associated with rotavirus infection.[8] A recent study reported that the overall prevalence of rotavirus diarrhea among children aged <5 years with acute diarrhea in Indonesia was 45.5%.[3]

Rotavirus belongs to the Reoviridae family and is a non-enveloped virus that consists of 11 segments of double-stranded RNA, encoding 6 structural (VP1, VP2, VP3, VP4, VP6, VP7) and 6 nonstructural proteins (NSP1–NSP6). The virus particle consists of a triple-shelled capsid comprised of outer, intermediate, and inner layers. The outer capsid is formed by VP4 and VP7 proteins, the intermediate layer is formed by VP6, and the inner layer by VP2, which enfolds two other proteins, VP1 and VP3. As a result of the segmented nature of the rotavirus genome, genetic reassortment occurs at a high frequency during mixed infection.[9,10]

Rotaviruses carry three important antigenic specificities: group, subgroup, and serotype. According to this group specificity, rotavirus can be classified into seven groups, namely A, B, C, D, E, F, and G, predominantly by VP6. The groups of rotaviruses most often infecting humans and animals are A, B, and C, where human rotavirus (HRV)-associated infections are predominantly caused by group A, and less commonly by groups B or C. Group A is a common cause of diarrhea in children, whereas groups B and C mainly affect adults.[11] VP7 serotype-specific rotavirus is called G (glycoprotein), whereas VP4 is named P (protein sensitive to protease enzymes). There are currently 15 known G serotypes and 10 of those can infect humans. Strains G1, G2, G3, G4, and G9 are the most widely distributed globally. There are 22 known P serotypes, 10 of which can infect humans. Of those 10 P serotypes, P2, P4, and P8 are dominant.[12]

Identification of rotavirus infection requires laboratory tests such as the detection of antigen using EIA and latex agglutination (LA) tests, both of which are commercially available and widely used in hospitals and clinical laboratories. These tests are easy to use and have high specificity, although they cannot definitively distinguish between strains of the virus that cause gastroenteritis and the sensitivity for detecting antigen is low. Between EIA and LA, the sensitivity and specificity of EIA is more than 98%.[13] Various molecular techniques have been explored to develop a highly sensitive and rapid examination to detect the causative agent of viral gastroenteritis. RT-PCR has been enhanced to detect rotavirus A and the sensitivity is 48% higher than EIA or electron microscopy.[13]

In a diarrheal disease outbreak that occurred in Bintuni from January through October 2008, 242 toddlers were reported to have been admitted to hospitals with massive diarrhea accompanied by vomiting, fever and dehydration. The number of cases slightly increased between September and October 2008 in the Weriagar district with 60 cases and 11 deaths, mostly from those who lived in Taroy village.[14] The specimens could not be collected from January through September 2008 because the outbreak cases, which occurred in a very remote area, were underreported. The specimens could be collected in October 2008, which was during the latter part of the outbreak. Although many diarrheal outbreaks occurred in Bintuni Bay, Papua, the causes of these outbreaks are still unknown. Therefore, this study aimed to observe the infectious agent associated with the diarrheal disease outbreaks in Bintuni in October 2008.

Methods

This study was conducted at the Virology Laboratory, Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia. The research received IRB approval from the National Insitute of Health Research and Development. Only 15 stool specimens were collected from a diarrhea outbreak from September to October 2008 in five villages, Bintuni Bay District, Papua. Among these 15 cases, there were seven males and eight females. The age distribution was 6 months through 41 months. Initially, we performed tests for bacterial detection from the stool samples. The test results were negative for bacteria, and so samples were further examined for rotavirus infection using EIA followed by viral RNA detection by reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing.

We used a rapid EIA kit (Standard Diagnostic Bioline Rotavirus Kit: No. Cat. 14FK10-02-1), according to the manufacturer's instructions, to screen for antigen against group A rotavirus. We then performed RT-PCR according to the methods previously described.[15] Extraction of viral RNA from stool specimens was performed using QIAamp Viral RNA Mini Kit 250 (Qiagen: Cat. No. 52.906) and amplified with Superscript III One Step RT-PCR kit with Platinum Taq (Invitrogen: Cat No. 12574-026). Each sample was pre-incubated in the PTC-100 Thermal Cycler BioRad at 50°C for 30 minutes. The first round consists of VP7 amplification for 39 cycles of 1 minute at 94°C, 2 minutes at 46°C, and 3 minutes at 68°C, followed by a final incubation at 68°C for 10 minutes. The VP7 forward primer used was GGCTTTAAAAGAGAGAATTTCCGTTTGG, and the reverse primer used was GGTCACATCATACAACTCTAATCT.[15,16] The amplicons were analyzed on 2% agarose gel with an expected product of 1062 bp.

Sequencing was performed on capillary Genetic Analyzer 16 (AB-3130xl, Japan), followed by Blast into GenBank

Table 1. Sequence result of HRV group A in Bintuni Bay, Papua.

NO	SPECIMEN	SEROTYPE
1	RV1 G2P[4]	G2
2	RV2 G2P[4]	G2
3	RV3 G1P[6]	G1
4	RV5 G1P[8]	G1
5	RV6 G1P[6]	G1
6	RV8 G2P[4]	G2
7	RV10 G1P[6]	G1
8	RV11 G1P[6]	G1
9	RV12 G1P[6]	G1
10	RV13 G1P[8]	G1
11	RV14 G1P[6]	G1
12	RV15 G1P[6]	G1

access based on July 21, 2009 records and analyzed using Mega 5-software.

Results

EIA and molecular testing by RT-PCR to determine G and P serotypes were performed on 15 specimens. The EIA results showed that 10 of 15 specimens (67%) tested positive for rotavirus. However, RT-PCR analyses found that 12 of 15 specimens (80%) tested positive for group A rotavirus with a majority of strains belonging to the G1 serotype (83%) and the remainder belonging to the G2 serotype. The G1 serotype consisted of 50% G1P[6] strain, 16.7% G1P[8], and 8.3% G2P[4]. In addition, three serotypes of P were also found (Table 1).

Discussion

In this study, we examined the serotype-specific rotavirus infection in a diarrheal disease outbreak in Bintuni, Papua. Fewer positive results were obtained by EIA compared to RT-PCR. This demonstrates that RT-PCR was more useful than EIA in detecting specific rotavirus strains. The results, which are in accordance with those obtained by O'Neill,[13] show that the use of RT-PCR can improve rotavirus A detection, with 48% higher sensitivity than that of EIA or electron microscopy.

The G1P[6] strains in this study fall into one of the rare serotypes in the distribution of strains in the world, with a global incidence of only 0.8% (Table 1).[17] Rotavirus strains circulating throughout the world are constantly changing. The presence of a rare strain in this study did not rule out the possibility of existence of a few minority strains in Eastern Indonesia. This indicates that the tendency of the G1P[6] strain to be present in Bintuni Bay, Papua is approximately 40% higher than elsewhere in the world. A recent study in 2006 and 2007 from several hospitals in six provinces in Indonesia reported that the majority of strains were serotype G1 followed by serotype G2.[2,18] The results from these studies indicate that the majority of serotypes that circulate in Indonesia from different provinces are similar. The G serotypes isolated from an outbreak in Bintuni Bay, Papua in 2008, were two out of four of the G serotypes that have been reported globally to be of importance in the epidemiology of rotavirus diarrhea in humans.[19]

Based on the phylogenetic tree mapping strains of rotaviruses (Fig. 1), it appears that there are four groups of smaller base adjacent arrangement with similarity to each

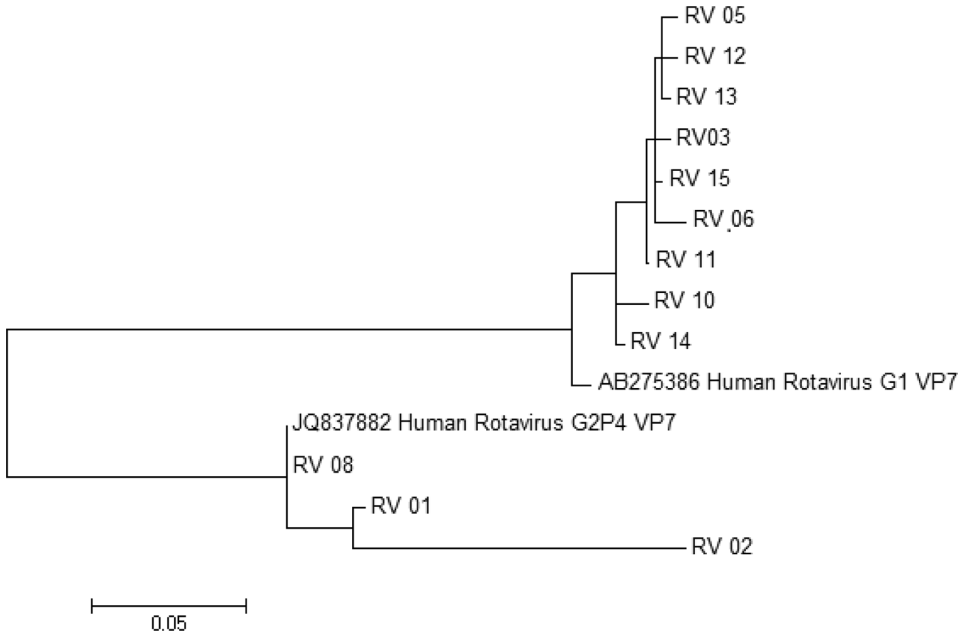

Figure 1. Phylogenetic tree stool specimens from diarrhea patients in Bintuni Papua. The phylogeny was constructed using the Mega 5 version AA Neighbor-Joining bootstrap analysis (1000 replicates).

other. In this study, we conducted PCR using VP7 primers because the VP7 is a glycoprotein that is more abundant and constitutes the major portion of the outer surface of the virion. For diagnosis, it is important to detect the rotavirus by its glycoprotein.

In conclusion, based on the results of the PCR assay and EIA and confirmed by sequencing, the majority of the 15 specimens that had been collected from diarrheal outbreaks in Bintuni Bay District in October 2008 were positive for rotavirus. From phylogenetic tree analysis, we found that the strains of rotavirus circulating in Bintuni, Papua belonged to two serotypes of G, namely G1 and G2, with the G1 frequency four times greater than the G2 frequency (Fig. 1). Although this study identified rotavirus A in 15 patients, we do not intend to draw the conclusion that rotavirus A is the only cause of this outbreak in Bintuni Bay, Papua. Testing on more specimens need to be conducted to represent the number of patients affected by this outbreak.

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Author Contributions

Conceived and designed the experiments including data analyses: EP. Wrote the first draft of the manuscript: EP. Contributed to the writing of the manuscript: VS, RHP. Agree with manuscript results and conclusions: EP, VS and RHP. Jointly developed the structure and arguments for the paper: EP, VS. Made critical revisions and approved final version: EP, VS, RHP. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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