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Short Communication

G2P[4] rotavirus outbreak in Belu, East Nusa Tenggara Province, Indonesia, 2018

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ABSTRACT

Rotavirus is a major cause of acute gastroenteritis (AGE) in children worldwide. However, rotavirus outbreak has rarely been reported in Indonesia. This study aims to identify the causative agent for AGE outbreak among children in Belu, East Nusa Tenggara, Indonesia in 2018. All the samples were negative for bacteria (*Salmonella, V. cholera*) and Norovirus. Ten out of 11 stool samples were rotavirus-positive by immunochromatography testing. Reverse-transcription polymerase chain reaction (RT-PCR) and phylogenetic analyses revealed that rotavirus G2P[4] was the possible causative agent for the AGE outbreak, although sample size was limited. These findings suggest that the AGE outbreak was caused by rotavirus G2P[4], highlighting the importance of rotavirus surveillance.

Group A rotavirus (RVA) remains an important cause of childhood gastroenteritis in Indonesia [1]. Despite the approval of rotavirus vaccines in 2013, Indonesia has not included RVA vaccines in its national immunization program yet [2]. Like other developing countries, children whose parents are well educated and wealthy may have more chances to get vaccinated with RVA in Indonesia. In this low vaccination coverage, an effective nationwide surveillance is necessary to monitor and evaluate the RVA infections. In collaboration with the Center for Environmental Health and Disease Control (CEHDC) in Surabaya, we examined the cause of acute gastroenteritis (AGE) outbreak which is one of the most significant health issues among children in Indonesia.

On September 1st, 2018, CEHDC was notified of an outbreak of AGE from unknown pathogen, affecting 631 individuals at all of the 17 primary health centers (PHCs) in Belu, the easternmost region of East Nusa Tenggara province, Indonesia, which borders Timor-Leste. The outbreak first started at one of the PHC located in a mountainous region of Belu. Out of 631 individuals, 435 were infants and young children under 5 years of age, and the rest was children over 5 years of age and adults (age unknown). There was no difference in the sex ratio. In order to control the outbreak, patients were prohibited from traveling temporarily. However, new diarrhea cases were found at other PHCs in distant regions. Public health officials conducted tracing around the area of every patient's house and found more diarrhea cases which had not been reported to any other health facilities. All the children received oral rehydration solution and zinc as supportive treatments. There were 4 death cases (7-month-old girl, 10-month-old girl, 9-month-old boy and 2-year-old girl) due to severe dehydration.

We were able to collect rectal swab samples from 11 children (age range, 11–38 months) with diarrhea, fever, and vomiting at five representative PHCs on September 4th, 2018. These five PHCs represented various geographic areas of Belu which comprise mountainous, urban, and coastal areas. No antibiotic had been administered to all of the 11 children. All the samples were negative for bacteria (*Salmonella, V. cholera*) and Norovirus. RVA was detected from 10 samples (90.9%) by immunochromatography testing (Eiken Chemical Co., Tokyo). To examine the sample, 10% (w/v) stool suspension of each sample in distilled water was prepared by centrifuging at 21,130 × *g* for 10 min. Viral RNA was extracted from 140 μl of the supernatant using a QIAamp Viral RNA mini kit (Qiagen, Valencia, CA). All the 10 samples were determined

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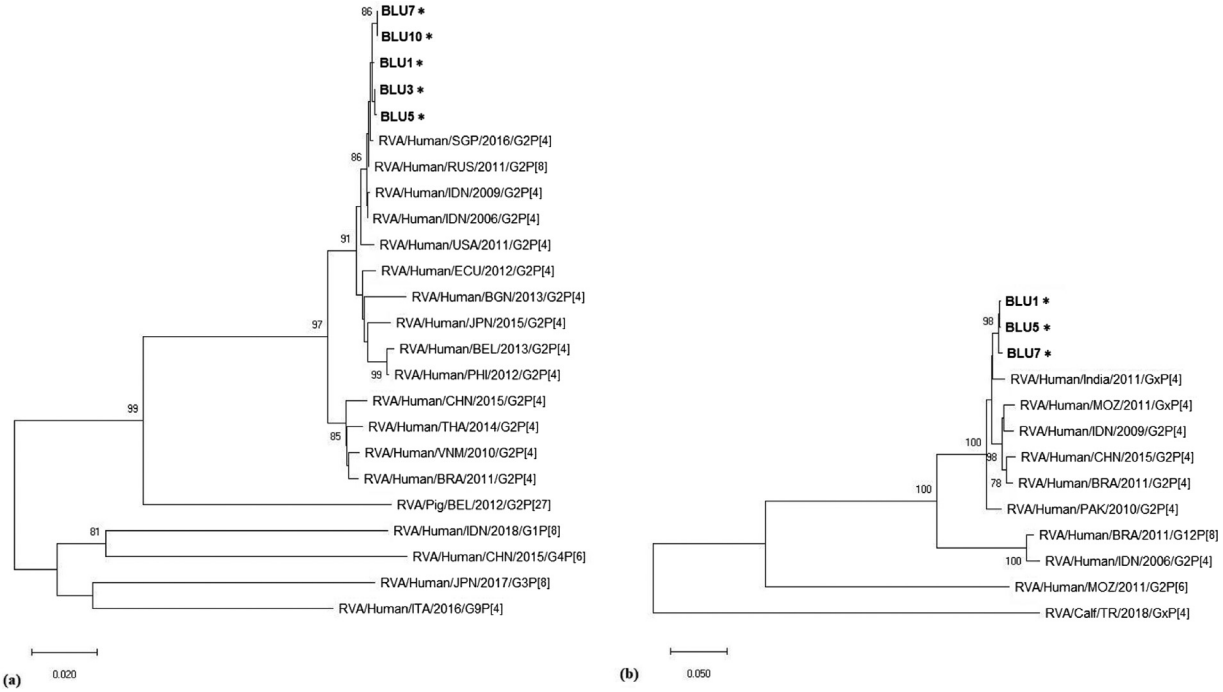

Fig. 1. Phylogenetic analysis of RVA (a) VP7 (G genotype) and (b) VP4 (P genotype) gene. The tree was constructed using the Neighbor-joining Maximum Composite Likelihood (MCL) method. Bootstrap values (>70) are shown at the branch nodes. Strains in the present study are shown in bold font with an asterisk.

to be positive by RVA immunochromatography testing and were subjected to genotyping in the VP7 (G typing) and VP4 genes (P typing) by multiplex Reverse Transcription-PCR (RT-PCR) [3]. RVA was detected only in 5 samples probably because of low amounts of RVA RNAs extracted from the rectal swab samples. Another possibility is that RNA-negative results were obtained due to the presence of inhibitors in the extracted samples. To determine RVA sequences, gene analysis was performed for all of the five positive samples by RT-PCR. The sequences were determined directly from the PCR products with the BigDye terminator v3.1 cycle sequencing kit and Applied Biosystems 3500XL Genetic Analyzer (Applied Biosystems, Waltham, MA). All the five samples were genotyped as G2 in the VP7 gene. In the VP4 gene, three samples were genotyped as P[4], and two samples were non-typeable, because PCR products were not amplified by RT-PCR using the 2nd PCR primer set. Further phylogenetic analyses using MEGA X revealed high sequence identity among RVA isolates in both VP7 (98.7–99.2%) and VP4 genes (98.5–99.4%) in this study (Fig. 1). The nucleotide sequences of the eight strains were deposited in the GenBank database under accession numbers LC500699-LC500706. None of the subjects had received rotavirus vaccinations. No vaccine coverage data in Belu was available just as other parts of Indonesia [4].

All the detected RVAs were determined as genotype G2P[4] and showed genetically close relationship among the isolates in this study, suggesting that an outbreak emerged among children in Belu in 2018 and that G2P[4] RVA is a possible causative agent. However, the number of samples analyzed and the number of RNA-positive samples in this study were too small. The small sample size of the present study was the limitation with regard to the ability to generalize the information obtained from the present study. Some countries have reported that G2P[4] became predominant, in an inverse proportion to the decrease in G3P[8]/G1P[8] after vaccine introduction [5–7]. Although numerous RVA outbreaks have been reported globally, there are only two RVA outbreaks reported in Indonesia to date [8,9]. Both of the outbreaks were reported in the last decade and the predominance of G1 RVA was highlighted as the causative etiology. Together with the current study, these results conform with aforementioned findings on the rise of G2P[4] genotype circulating in the post-vaccine introduction era. The incidence of RVA outbreak in Indonesia may be underrepresented because rotavirus surveillance has not been well established in Indonesia. It is important to elucidate whether the emergence of G2P[4] in this study was due to the effect of vaccination or due to natural variation. Rotavirus surveillance would monitor changes in the prevalence, genotype and pathogenicity. Consequently, informed public health interventions and national policies on rotavirus infections could be achieved, including the continuous promotion and development of vaccines. Two currently available rotavirus vaccines, Rotarix® (GSK Biologicals, Belgium) and RotaTeq® (Merck & Co., Inc., West Point, PA, USA), have been proven efficacious for prevention of severe AGE caused by various RVA strains thus far [10].

In this investigation, we demonstrated an outbreak of G2P[4] RVA among young children in Belu, East Nusa Tenggara, Indonesia in 2018. Surveillance must be continually and effectively implemented to monitor and evaluate the emergence and circulation of rotaviruses in Indonesia.

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Competing interests

None declared.

Ethical approval

The study protocol was reviewed and approved by the ethics committees of Airlangga University in Indonesia and of Kobe University in Japan. Informed consent from parents or guardians of all the children was obtained by medical examination according to the ethical principles of the Declaration of Helsinki.

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