A LARGE OUTBREAK OF PROBABLE ROTAVIRUS IN NUSA TENGGARA TIMUR, INDONESIA

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Abstract. An outbreak of acute diarrheal disease was reported in Kupang, Nusa Tenggara, Indonesia, in August 2002. An investigative team carried out a retrospective historical review of records, and a case-control study involving data and specimen collections. Etiologic determination involving stool specimens was based on an enzyme-linked immunosorbent assay, with a reverse transcriptase–polymerase chain reaction performed for serotyping purposes. Two thousand six hundred probable cases were identified from hospital records during the outbreak months of June, July, August, and September 2002. Previous enteric outbreaks were recognized from the same months in the preceding years and all annual outbreak episodes following a period of prolonged, low rainfall. In contrast to previous outbreaks discerned from trend analysis, the overwhelming burden of disease fell upon the pediatric population versus the young and old in previous outbreak instances. Rotavirus was found to be the causative etiology, with serotype 1 predominating.

INTRODUCTION

Rotavirus has been long been recognized as a leading cause of acute diarrheal disease in young children throughout both the developing and developed world.1-3 The debilitating nature of rotavirus is reflected in that an estimated one-third of all diarrheal pediatric hospital admissions globally can be attributed to this viral agent. The actual number of rotavirus infections with associated diarrhea is probably much greater because severity appears diminished, resulting from immunologic tolerance evolving from repeat exposures.4

Rotavirus infection is generally characterized by vomiting, fever, and watery diarrhea, with incubation ranging from 24 to 72 hours. Severe, debilitating diarrheal disease and associated fatalities are frequently attributed to this enteric viral pathogen, particularly in developing countries where young children are often already immunologically compromised, e.g., poor nutritional status. Person-to-person transmission is principally via fecal-oral contamination and exposure. While seasonality implicating the colder months has been recognized in more temperate climates, occurrence in tropical regions is year round.3-6

This investigation, prompted by anecdotal diarrheal outbreak reports from Kupang, West Timor, Indonesia was designed to provide recognition of 1) evidence of epidemic occurrence via trend analysis, 2) associated etiology, 3) affected population and community, 4) community impact, and 5) determinants contribution to transmission.

RECOGNITION, AREA, MATERIALS, AND METHODS

Outbreak recognition. In mid August 2002, local health officials in Kupang reported to the World Health Organization (WHO) an outbreak of diarrhea from an unknown pathogen affecting mainly children and infants less than five years of age. More than 2,000 cases had been reported during the previous month, with 12 reported deaths. The United States Naval Medical Research Unit No. 2 (U.S. NAMRU-2) in Jakarta, Indonesia, as a WHO Collaborating Center for Emerging and Re-Emerging Diseases, confirmed this report with the Indonesian Ministry of Health through its Surveillance Directorate, the Center for Communicable Diseases and Prevention and Environmental Health (P2M-PLP) in Jakarta. An investigation team consisting of personnel from the P2M-PLP, the Indonesian National Institutes of Health and Research Development (Litbangkes), and U.S. NAMRU-2 was dispatched to Kupang from August 21–29, 2002 to support local district health officials in investigating the outbreak.

Outbreak area. The district city of Kupang, located on the western coast of Timor, is the provincial capital of Indonesia’s East Nusa Tenggara Province. With an estimated population of 522,944, it is the largest urban concentration in the province (Figure 1). In general, most inhabitants in Kupang get their water from piped municipal supplies, sourced from a nearby mountain range. Geographically, the city is located at 10.16°S, 123.66°E at an elevation at or near sea level (0–10 meters). Rainfall varies between 500 and 2,000 mm a year. Mean annual rainfall is approximately 1,000 mm, but the area has an 8–9-month dry season. The wet season lasts from December to March and the dry season from March to November. The climate is tropical and sub-humid, influenced for much of the year by dry winds from the Australian landmass some 700 km to the southeast.

Outbreak investigation. An investigation team designed a case-control study of new acute diarrhea cases in one hospital (Yohanes Hospital) and four of six community health centers within Kupang city. A case of diarrhea was defined as an episode with three or more watery stools over a period of 24 hours, with or without other symptoms during the outbreak period. All available patients in the clinics were admitted into the study. Patients from the same hospital or community health centers during the outbreak period reporting no diarrhea but age and sex matched with case were selected as controls. The cross-sectional study was also conducted in two nearby villages (Tenau and Tode Kisar) with no report of diarrhea cases during the outbreak period as control villages.

Specimen collection. The investigation team collected 27 stool samples via rectal swab from consenting patients presenting with acute diarrhea at Yohanes Hospital and four of six community health centers in Kupang city (Alak, Kupang Kota, Pasir Panjang, and Sikumana) during the phase of investigation. Twenty-three patients, age and sex matched and reporting no diarrhea, were also selected as control subjects. From the control village, rectal swabs from 38 healthy infants...
and children without reported acute diarrhea were collected for comparative study purposes. All rectal swabs collected from subject cases (27) and controls (23) were stored in Cary-Blair medium (BBL, Cockeysville, MD) and 1.5 mL of phosphate-buffered saline (PBS), while specimens from the 38 control village participants were kept in Cary-Blair transport medium only. The swabs in Cary-Blair medium were transported to Jakarta in an icebox, while specimens in PBS were stored in a liquid nitrogen tank (dry shipper).

All subjects completed questionnaires, which gathered demographic information such as age, home address, water source, recent diet, sick contacts, and health history. Since subjects were infants and young children, parents or guardians provided the information. Investigative study enrollment was predicated on informed, voluntary consent. This outbreak response activity satisfied human use requirements as determined by the Institutional Review Board of U.S. NAMRU-2, Jakarta.

Laboratory analysis. Laboratory diagnostics for detecting rotavirus infection was performed at the U.S. NAMRU-2 laboratory in Jakarta included an enzyme-linked immunosorbent assay (ELISA) and a polymerase chain reaction (PCR). In addition, bacteriologic cultures for detecting enteric pathogens other than rotavirus, such as Salmonella sp., Campylobacter sp., Vibrio cholera sp., and Shigella sp., were also performed. Bacteriologic culture was done from the rectal swabs stored in the Cary-Blair medium transport. The EIA and PCR were performed on the specimens kept in PBS.

Enzyme-linked immunosorbent assay. The Premier Rotaclove Enzyme Immunoassay, a commercial kit from Meridian Diagnostic, Inc. (Cincinnati, OH), was used according to the manufacturer's direction for all case and control specimens collected from the outbreak-affected area. This assay detects rotavirus antigen in human fecal specimens.

Reverse transcriptase–polymerase chain reaction (RT-PCR). Viral RNA was extracted from rectal swabs in PBS suspension using the QIAmp Viral RNA Mini Kit (Qiagen Inc, Valencia, CA) following the manufacturer's protocols and supplied reagents. The procedure for PCR typing was performed according to the methods developed in previous studies.7,8 The amplification was carried out in two stages. Amplification of the DNA fragment of the full-length VP7 gene in the first stage was followed by a second amplification of the DNA fragment using serotype-specific primers and the copy of the full-length VP7 gene as a template. Serotype-specific primers were set in variable regions, so that PCR products of different sizes are amplified depending on different serotypes. The first amplification used C1 and C2 primers, followed by a second amplification of DNA fragments that used S1, S2, S3, S4, S8, S9, and C1 primers as previously described by Taniguchi and others.7 The PCR products were separated by electrophoresis on 2% agarose gels and visualized with ultraviolet light after staining with ethidium bromide.

Supporting data. Historical data on monthly diarrheal cases throughout Kupang city from January 1997 to December 2002 were obtained from the local health authority office (Dinas Kesehatan Kupang). Community demographic information, the most recent census data (2002), and a map of Kupang city were also provided. Monthly rainfall data records

FIGURE 1. Map of the community health center (Puskesmas) in Kupang, Indonesia.
FIGURE 2. Trend analysis of diarrheal cases from six community health centers by age categories in Kupang, Indonesia, 1997–2002.


Data for February, March, May 1997 & December 2001 not available

Data of June 1997 & December 2001 not available

Data of August 1997 & February & December 2001 not available

Data of December 2001 not available

Data of January 1997, December 1998 & December 2001 not available

* Outbreak period

< 5 year

≥ 5 years old
collected during the period from January 1997 through September 2002 was provided by the Kupang Meteorology and Geophysics Office.

RESULTS

Retrospective historical review. Analysis of trend of routine data depicting clinically recognized cases of acute diarrheal disease clearly reflect the dramatic increase in episodes during the purported outbreak period (June 14 to September 14, 2002), which was suggestive of epidemic occurrence. The reach of the outbreak extended throughout Kupang, as shown in Figures 2 and 3. Notable was the proportional increase in outbreak cases from the Alak, Pasir Panjang, Bakunase, and Sikumana Community Health Centers, representing the four principal sub-districts of Kupang. Earlier instances of epidemic diarrheal transmission are apparent from 2001, 1999, 1998, and 1997. Comparative analysis, controlling for potential seasonality, showed little variance in the total number of diarrheal cases during the outbreak months of June, July, August, and September 2002, and proceeding years: 2600, namely, 2,469 in 2001, 2,189 in 1998, and 2,521 in 1997. Similarly, the monthly mean ± SD number of diarrheal cases for the four outbreak months in 2002 approximated that in the same period in 2001, 1998, and 1997: 640 ± 751.4 (ranging from 130 in June to 1,750 in July), weighted against 617.25 ± 152.11 (ranging from 413 in June to 776 in September), 630.25 ± 376.6 (ranging from 300 in June to 795 in September), and 630.25 ± 334.98 (ranging from 394 in June to 1,127 in July), respectively. The years 2000 and 1999 were omitted from analysis, controlling for seasonality (the months of June, July, and August), in the absence of local reporting for this period.

Figure 2 and 3 clearly reflect concordance in age-related trend between populations < 5 years old and ≥ 5 years old in all instances of peak diarrheal occurrence, except for the June through August 2002 outbreak period. Moreover, this trend is highlighted in five of the six community health centers (Figure 2). Whereas earlier, diarrheal peak episodes affected all age groups, most of the patients affected by the 2002 outbreak were less than five years of age.

Outbreak epidemiology. Weekly versus daily case occurrence, which is reflected in the epidemic curve (Figure 4), showed a slow incremental increase in outbreak cases beginning week 25 (June 14–20, 2002), then a dramatic increase during week 31 (July 25–31, 2002), nearly two-fold. The outbreak peaked during week 32 (August 1–7, 2002), with the first notable decrease in cases during week 35 (August 22–28, 2002). There was no evidence of continued diarrheal outbreak transmission beyond week 37 (September 5–11, 2002).

The overall attack rate (AR) in Kupang was 1.1% (2,600 of 236,630 inhabitants), with a case fatality rate of 0.46% (12 of 2,600). In none of the four sub-districts comprising the city did the AR reach 2%. Controlling for age clearly shows the preponderance of outbreak-associated risk in the population < 1 year of age: AR = 27.9% (ranging from 19.4% in the Maulafa sub-district to 42.8% in the Alak sub-district), AR = 4.6% (ranging from 4% in the Maulafa sub-district to 6.9% in the Alak sub-district), and AR = 0.39% (ranging from 0.27% in the Kelapa Lima sub-district to 0.57% in the Alak sub-district), in the age groups < 1, 1–5, and > 5 years old, respectively (Table 1).

Case-control comparative data. The results in Table 2 attest to age-based criteria in the selection of cases versus controls, showing no significant differences. There was a negli-
gible difference between cases and controls as to piped or well water sources: 48% versus 52% (piped water) and 48% versus 39% (well water), respectively. Similarly, there was no notable difference in boiling of drinking water between cases and controls: 96% versus 100%, respectively.

**Laboratory findings.** Laboratory evidence indicative of recent rotavirus infection was recognized in 48% (13 of 27) of case stool specimens, compared with 13% (3 of 23) of control stool specimens ($P < 0.01$). The mean ± SD age of cases testing positive for rotavirus was less ($P > 0.05$) than for those testing negative: 11 ± 8.8 (range = 3–32) months versus 15 ± 11.6 (range = 3–40) months, respectively. In the control population, the mean ± SD age of ELISA-positive cases (30 ± 25.7 months, range = 14–60) was higher than for negative cases (19 ± 13.1 months, range = 4–39) ($P > 0.05$). The male to female ratio was 1:1.2 among ELISA-positive cases and 1:81 among the negative cases. None of the case specimens examined were culture positive for *Shigella dysenteriae*, *S. sonnei*, or *V. cholerae*. One of 23 control subjects was culture positive for *S. sonnei*. Among 38 people from the control village, 2 were positive for *S. dysenteriae*.

**Serotyping.** All 13 case specimens recognized as positive for rotavirus by ELISA were typed as serotype 1 (Group A) using RT-PCR technology. Additionally, 3 of 23 patient controls examined with evidence of rotavirus by ELISA also typed positive for serotype 1.

**Climatic influences.** Rainfall reported in (mm) historically appears low just prior to (and sometimes during) epidemic diarrheal peak episodes (Figure 3). In the months leading up to and during the June, July, August, and September outbreak period, negligible rainfall was recorded: 48.9 mm in April and none in May, June, July, and August 2002. Cumulative mean ± SD rainfall measures for 2001, 1998, and 1997 were $46 ± 59.7$ mm (range $= 5.4–114$) in April, $4.2 ± 5.3$ mm (range $= 0–10$) in May, 17.7 ± 27.5 mm (range $= 1.2–49.4$) in June, 15.9 ± 14.5 mm (range $= 0–28$) in July, and 0 in August.

**DISCUSSION**

The almost routine temporal nature of diarrheal case occurrence suggests a seasonal trend, no less than a yearly outbreak phenomenon. Such an occurrence predisposes a similar etiology associated with annual dry seasons. However, there are two distinguishing features recognized from the 2002 outbreak. First, the break in trend, which shows the population <5 years old to mirror that of the population ≥5 years old in the years proceeding 2002, is consistent with rotavirus, which is nominally specific to pediatric groups. The concordance

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overall attack rate from six community health centers</th>
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<tbody>
<tr>
<td>Age distribution (years)</td>
<td>Total population</td>
</tr>
<tr>
<td>&lt;1</td>
<td>3,984</td>
</tr>
<tr>
<td>1–5</td>
<td>21,656</td>
</tr>
<tr>
<td>&gt;5</td>
<td>210,990</td>
</tr>
<tr>
<td>Total</td>
<td>236,630</td>
</tr>
</tbody>
</table>

FIGURE 4. Epidemic curve of the diarrheal outbreak in Kupang, Indonesia, week 1 through week 51. Weekly data were obtained from six community health centers.
exhibited in age-specific trends during previous outbreaks becomes discordant during the June–August 2002 outbreak period. This break in trend indicates prior outbreaks occurring with seasonal regularity that are likely attributed to pathogens other than rotavirus. Indeed, *V. cholerae* 01 Ogawa strain was identified as the causative etiology during every diarrheal outbreak episode from 1993 to 1999 across the Indonesian archipelago.9 Second, the actual number of suspect outbreak cases in 2002 far exceeded the norm observed in early outbreak/season episodes.

Importantly, retrospective trend findings are replicated in almost all the community health centers, except for the Kupang Kota Community Health Center. This ensures that recognized outbreak occurrence is not merely a function of a reporting phenomenon, which is suggestive of a collection bias. This finding also elucidates an outbreak that was not localized to one area within the Kupang municipality.

The small number of case and control specimens scrutinized does not provide necessary assurance in attributing definitive outbreak causation. Delays in responding to suggestive outbreak warnings limited access to acute phase cases and corresponding controls. The generalization of etiologic findings from a small number of specimens to the excess bolus of outbreak cases is speculative.

That serotype 1 (Group A) was predominate in the case specimens from patients with debilitating diarrhea is consistent with the literature. This finding lends credence to a virulence component associated with infecting serotype.4 However, studies carried out in Indonesia and Australia have shown serotype 1 to occur far less frequently than serotypes 2, 3, and 4.

There is an apparent seasonal influence on outbreak occurrence that is reflective of rainy and dry climatic conditions. In every instance, transmission occurred following periods of low rainfall. In the case of the Kupang outbreak, subnormal rainfall led to severe water shortages, forcing water to be distributed to communities in water-carrying tanker trucks. The viability of the virus to persist in this environment, particularly in water contaminated by human excreta, makes such a scenario plausible.10 It is possible that such an occurrence contributed in small or large measure to the outbreak. Unfortunately, there was no opportunity to identify the source of trucked water or to carry out sample collections for laboratory analysis.

The absence of recognized outbreaks involving rotavirus in Indonesia may reflect the relatively few immunologically (naive) susceptible individuals in the populations. Rotavirus has clearly been shown as the principal associated etiology in sporadic (laboratory diagnosed) pediatric diarrheal disease in numerous hospital-based studies carried out throughout the country.12,13

<table>
<thead>
<tr>
<th>Status</th>
<th>Mean ± SD age, months (range)</th>
<th>Male:female ratio</th>
<th>Source of water</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n = 27)</td>
<td>13.2 ± 10.8 (3–40)</td>
<td>1:0.8</td>
<td>Piped water</td>
<td>0.17</td>
</tr>
<tr>
<td>Controls (n = 23)</td>
<td>20.1 ± 14.9 (4–60)</td>
<td>1:0.6</td>
<td>Well</td>
<td>0.03</td>
</tr>
<tr>
<td>Control village (n = 38)</td>
<td>16.8 ± 12.8 (1–44)</td>
<td>1:1.7</td>
<td>Boiled water</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Recognition of the outbreak potential of rotavirus, as expressed through reported findings, should establish at least a level of suspicion in considering cause. These findings provide evidence of possible rotavirus in the context of outbreak occurrence in Indonesia. Public Health Authorities, appreciative of the outbreak potential of rotavirus, should be prepared, very much as they would with cholera, to make oral rehydration solution available to the community, and encourage a high level of sanitary practice, including consumption of commercially bottled or boiled water, and possible warnings regarding day care preschool practices.

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