TRANSMISSION OF EPIDEMIC DENGUE HEMORRHAGIC FEVER IN EASTERNMOST INDONESIA

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Abstract. In April 2001, a second suspected outbreak of dengue hemorrhagic fever in the easternmost region of Indonesia was investigated in Merauke, a town located in the southeastern corner of Papua, by the Indonesian Ministry of Health and the U.S. Naval Medical Research Unit No. 2. Principal case criteria of hemorrhagic disease provided for a study enrollment of 15 clinically acute and 37 convalescing subjects. Additionally, 32 comparable age/sex controls were selected from neighboring households. Laboratory diagnosis involved three testing methodologies: virus isolation by cell culture, a reverse transcriptase–polymerase chain reaction (RT-PCR) assay, and serologic assays. Antibody (IgM) to dengue virus was detected in 27% of the acute clinical cases, 30% of the convalescing cases, and only 3% of the matched controls. Dengue 3 was the only viral serotype detected from acute serum samples by the RT-PCR. The mean ± SD age of the acute and convalescing cases was 7.8 ± 5.4 years. Overall hospital records accounted for 172 suspected outbreak cases, all urban residents of Merauke with no recent travel history outside the area. The estimated outbreak-associated case fatality rate among all suspected dengue cases was 1.2%. A seven-year retrospective review of hospital records in Merauke showed negligible disease reporting involving hemorrhagic disease prior to the outbreak.

INTRODUCTION

Epidemic dengue hemorrhagic fever (DHF) is well documented in Indonesia, and was first recognized in 1968 on the island of Java.1,2 A 1960 serologic survey of dengue (DEN) virus infection in the Jayapura-Sentani area of northeastern Papua (formerly called Irian Jaya) showed evidence of past exposures to DEN-1 and DEN-2 viruses among adults, but not in the population less than 18 years of age.3 This suggested that dengue viruses had been absent from this region for the past 10–15 years. However, survey and outbreak investigations in neighboring Papua New Guinea showed that DEN-1 and DEN-2 viruses were circulating there in the 1970s.4 The first case of DHF in Papua was reported in 1979 (Provincial Center for Communicable Disease Control, Papua, 1979–1994, unpublished data).

The first instance of epidemic DHF transmission in Papua was reported in 1993–1994.4 Evidence of a second DHF outbreak in Papua, as presented in this report, again highlights the epidemic potential of these viruses in an area with no known historical experience with DHF (Figure 1).

Dengue virus serotype 3 (DEN-3) has been recognized as the predominant serotype in many recent epidemic occurrences of DHF in Indonesia.5 This was also evident during the 1993–1994 outbreak reported from Papua, although DEN-1 and DEN-2 viruses were also detected.

The objective of this investigation was to confirm the outbreak occurrence of a hemorrhagic disease in Merauke, Papua and subsequently determine its etiology. The confirmation and determination of suspected dengue virus serotype(s) involved was considered necessary in an area with no previous instances of epidemic DHF. Moreover, this outbreak provided an opportunity to profile the epidemiology of epidemic DHF from easternmost Indonesia.

BACKGROUND

Area. The district town of Merauke, with an estimated population of 78,000, is located in the southeast corner of Papua, which shares a common border with Papua New Guinea. Situated at 8°50′S, 140°40′E, at an elevation of near sea level (0–8 meters), urban Merauke is divided by the Maro River system and buttressed by extensive swampy marshlands (Figure 1).

Chronology. In April 2001, national newspaper accounts described a major diarrheal outbreak in the town of Merauke. Immediate follow-up on anecdotal reports proved somewhat contradictory, and suggested that the outbreak was associated with hemorrhagic disease manifestations. An investigation team was assembled and dispatched from April 16 to April 26, 2001 by the Indonesian Ministry of Health, with institutional representation from the National Institutes of Health Research and Development (LITBANGKES), the Centers for Communicable Diseases (P2M-PLP), and the United States Naval Medical Research Unit No. 2 (U.S. NAMRU-2) to support local district health services response.

MATERIALS AND METHODS

Specimen collection. To carry out a retrospective case-control investigation, acute and convalescing hospital-recognized DHF patients from RSUD Merauke (the regional public hospital) were enrolled for outbreak investigative purposes. The case definition for DHF in the hospital was based upon the criteria of the World Health Organization.6 Patients who presented with 1) fever, 2) hemorrhagic manifestation including a positive tourniquet test result, 3) thrombocytopenia, and 4) hemoconcentration were diagnosed as having
DHF. This included 15 acute, clinically recognized cases and 37 convalescing subjects at home, who were identified from hospital records. Additionally, 32 age- and sex-comparable controls, matched with recovering patients, were selected from neighboring households. A 5–7-mL venous blood sample was obtained from each volunteer case and control subject using a Vacutainer® tube (Becton Dickinson, Franklin Lakes, NJ) (one tube with no additive). Additionally, a thick and thin blood smear was made for examination for possible malaria parasitemia. An interview questionnaire was administered to each participant. Investigative study enrollment was predicated on informed, voluntary consent. This outbreak response activity satisfied human use requirements as determined by the Internal Review Board of U.S. NAMRU-2, Jakarta. Laboratory evaluation, carried out at U.S. NAMRU-2 in Jakarta, involved three testing methodologies: 1) virus isolation by cell culture, 2) reverse transcriptase–polymerase chain reaction (RT-PCR) for detection of DEN serotype-specific RNA, and 3) serologic assays for IgG and IgM antibodies to dengue virus.

Laboratory tests. Diagnostic testing using all three methodologies was applied only to the sera from the 15 patients with acute disease. Convalescing and control patient sera were examined using an enzyme-linked immunosorbent assay (ELISA).

Enzyme-linked immunosorbent assay. Antibody to dengue virus was detected using the dengue fever virus IgM capture ELISA kit (MRL, Cypress, CA).

Reverse transcriptase–polymerase chain reaction. The QIAamp viral RNA mini-kit (Qiagen GmbH, Hilden, Germany) was used to extract viral RNA from sera. Target viral RNA was amplified by a semi-nested RT-PCR using dengue-specific oligonucleotide primers. In the first part of the procedure, viral RNA was reverse transcribed into cDNA with continued amplification in a single tube using the RT-PCR Access System (Promega, Madison, WI). Commercially obtained PCR reagents (Perkin Elmer, Norwalk, CT) were used during the semi-nested amplification step. Final amplicons were separated by electrophoresis on a 2% agarose gel and stained with ethidium bromide. Serotype identification was determined by the basepair size of the final product.

Virus isolation. Serum samples were assayed for the presence of live virus by the method of Singh and Paul with some minor modifications. Patient’s serum was diluted 1:10 in phosphate-buffered saline (PBS) and inoculated onto confluent monolayers of C6/36 Aedes albopictus cells (Cell Repository Line 1660; American Type Culture Collection, Manassas, VA). Following incubation for one hour at 30°C, cell monolayers were washed in PBS and a fresh culture medium was added. Cell cultures were then maintained at 30°C for 14 days. All cultures were monitored daily for the appearance of cytopathic effects (CPEs). Cultures demonstrating CPEs were harvested, and the cells were then applied to immunofluorescence slides and stained with a series of dengue virus serotype-specific monoclonal antibodies (from an IgG mouse hybridoma cell line) for virus identification.
Microscopy for malaria identification. Venous blood was used for blood slide preparation and malaria parasite examination. Thick and thin blood films were prepared on the same slide, stained with Giemsa, and examined under a 100× oil-immersion lens.

Supporting data. Historical data were obtained from RSUD Merauke. These included demographic and clinical information from the suspected (presumptive diagnosis) 172 DHF inpatients by day of admission from November 2000 to July 2001. Additionally, monthly aggregate inpatient data were tallied from 1995 to 2001. Monthly rainfall and temperature data recordings were obtained for the period of January 1998 through July 2001 from the Merauke Meteorology and Geophysics Office. Community demographic information, the most recent census data, and a map of the Merauke subdistrict were kindly provided by the Merauke Health Authority Office.

RESULTS

Hospital case detection. Clinical epidemiology. One hundred seventy-two suspected DHF patients were identified from hospital records on the basis of DHF selection criteria in conjunction with outbreak occurrence. Detailed clinical data also were obtained from 172 recognized cases and are reported herein. A case fatality rate of 1.2% was calculated on the basis of two outbreak-attributed deaths from the 172 suspected cases.

Epidemic curve. The temporal window of epidemic DHF transmission (cumulative period of 34 weeks) began during week 47 (November) in 2000 and ended in week 28 (July) in 2001. Analytical interpretations were extracted from clinical observations of 172 suspected cases for this 34-week period. The epidemic curve presented in Figure 2 indicates a gradual increase in cases, peaking during week 14. Intermittent increases in case detection (at intervals approximating five weeks) during weeks 1, 7, and 14 were followed by notable decreases. A historical review of comparable monthly hospital records over a six-year period, from January 1995 to November 2000, revealed no previous instances of clinical hemorrhagic disease, either suggestive of epidemic or sporadic DHF (Table 1).

Demographic information. The mean ± SD age of the 172 cases was 7.4 ± 4.6 years of age (range = 10 months to 24 years). Ninety-three percent of suspected cases were less than 15 years of age: 25% were < 5 years old, 52% were 5–9 years old, and 16% were 10–14 years old. The male:female ratio of suspected DHF cases was nearly equal (1:1.1) among child cases (< 15 years old) and 1:0.6 among adults (≥ 15 years old).

Geographic case distribution. The outbreak cases were distributed by residence in six of eight kelurahan (arbitrary divides that separate cities, villages, and rural areas into political areas) and in only one of 50 villages present in the non-urban Merauke subdistrict (Figure 3). The attack rates, based on extrapolation when matching case findings with census data, among the six urban kelurahan ranged from 162 suspected cases/100,000 persons in Kelapa Lima to 826 suspected cases/100,000 persons in Samkai. In the rural village of Tanah Miring, a much lower attack rate of 78 suspected cases/100,000 persons was estimated (Figure 4).
Laboratory findings. Laboratory results. All blood smears microscopically screened for malaria parasites were negative. Serologic examination of 84 specimens found significant titers of IgM antibody to dengue virus present in 27% of 15 sera from patients with acute disease, 30% of 37 convalescent patient sera; and only 3% of 32 control sera (Table 2). Sera from seven patients with suspected acute diseases were positive by either the dengue IgM ELISA (two specimens), the RT-PCR (three specimens), or by both testing methods (two specimens). Dengue-3 was the only dengue virus serotype detected from five serum samples using the RT-PCR. Viral isolates were not recovered in the C6/36 cell culture.

Demography. The mean ± SD age was 10.2 ± 10.3 years for patients with acute disease who tested positive for IgM antibody to dengue virus and 6.2 ± 2.1 years for convalescing subjects. In the three control subjects positive for IgM antibody to dengue virus, the mean ± SD age was 5 ± 1.1 years (Table 3).

Climatic influences. Figure 5 indicates that the outbreak coincided with a period of subnormal rainfall in the months leading up to it (cumulative rainfall = 94.5 mm from June to September 2000), and was followed by relatively heavy rainfall (226.9 mm) in October 2000, the month immediately preceding the outbreak. A comparative review of mean cumulative rainfall for June to September 1998 and 1999 showed equal to moderately higher rainfall than for the same period leading up to the outbreak in 2000. Total rainfall during outbreak months of November 2000 to July 2001 (160 mm) was notably lower than that of the comparable months in 1998–2000 (229 mm). Similarly, with a decrease in average accu-
mulative rainfall, there was a notable increase in ambient temperatures during the actual outbreak months. Mean ambient temperatures from June through September 2000 (25.3°C, range = 24.5–26.2°C) increased significantly in October (28.4°C), the month preceding the outbreak, and decreased only slightly during the months of actual outbreak transmission (27.1°C, range = 25.6–28°C).

DISCUSSION

Serologic evidence presented in this report is clearly indicative of a DHF outbreak in Merauke in 2001. Historical hospital records from 1995 to October 2000, suggested a recent introduction of dengue virus into the affected area of southeastern Papua. Additionally, the absence of any historical and/or anecdotal reporting reflect possible first-time epidemic dengue virus transmission in Merauke.

As reported from the outbreak investigations in Jayapura in 1993, and in Palembang, South Sumatra in 1998, the largest proportion of recognized dengue cases were found in the population less than 15 years of age.\(^4,5\) The near equal representation of male and female cases in the segment of the population less than 15 years old contrasted significantly with

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<th>Age (years)</th>
<th>Acute cases(†)</th>
<th>Convalescing cases(‡)</th>
<th>Controls§</th>
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<tbody>
<tr>
<td>1–9</td>
<td>8/27 (30%)</td>
<td>4/14 (29%)</td>
<td>1/17 (6%)</td>
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<tr>
<td>10–19</td>
<td>2/9 (22%)</td>
<td>1/1 (100%)</td>
<td>0/14 (0%)</td>
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<td>20–29</td>
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<td>30–39</td>
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<tr>
<td>40</td>
<td>1/1 (100%)</td>
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Mean ± SD age (range) 10.2 ± 10.3 (4–40) 6.2 ± 2.1 (4–9) 5 ± 1.1 (5–39)

\(*\) ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; ND = not done.

† From a case-control study conducted in the hospital.
‡ From a case-control study conducted in the community.
§ Seven controls from a hospital case-control study; 25 controls from a community case-control study.
the overwhelming disease burden among adult males compared with adult females. This demographic outbreak feature may reflect an additional, as yet unknown, occupational risk associated with adult exposures. Unfortunately, the relatively few (12) adult outbreak cases preclude any meaningful interpretation of this observation.

Dengue-3 was the only dengue virus serotype detected by the RT-PCR in samples collected. All cell culture results were negative for recovery of viral isolates. The possible explanation for this is because the specimen collection for isolation attempts were made, in some instances, well into the convalescing (post-acute phase) period or more than five days of illness. The implication as to the predominance of DEN-3 virus in association with DHF was documented in other instances of epidemic DHF occurrences in Indonesia, including the Palembang and Jayapura outbreak.4,5 The predominance of DEN-3 virus in this and other outbreak findings throughout Indonesia may represent a sampling bias because most specimen collections have targeted inpatient populations who present more severe clinical hemorrhagic manifestations. While there may indeed be a virulence component associated with an infecting viral serotype, it is also recognized that other serotypes may be more prevalent among less severe cases.5

There was no apparent spatial case clustering when outbreak episodes were plotted on a map by kelurahan. However, with the analytical advantage of having census data, we observed that attack rates were clearly highest in Samkai, along the Arafura coast, and neighboring Mandala. Moreover, RSUD Merauke was located in Maro kelurahan, where the attack rate was relatively low at 293 suspected cases/100,000 persons, suggesting that geographical proximity did not affect hospital use.

It is always intriguing to speculate what factors influence or generate outbreaks: interrelated environmental, ecologic, biologic, and demographic factors all play a role. However, probably none is as important as the mosquito vector itself. Unfortunately, many dengue outbreak investigations are often hampered by a lack of supporting entomologic data, and very little is understood about the intrinsic and extrinsic factors that influence vector bionomics and behavior during times of epidemics. In this regard, the Merauke investigation was limited to examining the climatic data that may have predisposed the dengue permissive area to a DHF outbreak.

In comparing previous years of rainfall data from Merauke, we suspect that the precipitation patterns in the months before and during the 2001 outbreak probably had only a minor influence on increasing transmission or dramatically altering the ecology and behavior of the mosquito vector in favor of
an increased risk of disease. Furthermore, the influence of the above average ambient temperatures on enhanced virus transmission before and during the outbreak is also not clear. However, there is compelling evidence to suggest that increased temperatures may have helped precipitate this Merauke epidemic. Higher ambient temperatures have been shown to enhance dengue virus replication and shorten the extrinsic incubation period (EIP) in the vector.\textsuperscript{10} Furthermore, a relationship between increased frequency of vector feeding during hot, dry and rainy periods and DHF epidemics has been noted.\textsuperscript{11} These observations imply that temperature-induced variations in vector efficiency for \textit{Aedes aegypti} (i.e., greater feeding frequency, shortening of the EIP) are among the important determinants of periodic variation in the incidence of DHF.

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