

HEMORRHAGIC FEVER IN CAMBODIA IS CAUSED BY DENGUE VIRUSES: EVIDENCE FOR TRANSMISSION OF ALL FOUR SEROTYPES

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Abstract. Hemorrhagic fever (HF) has been widespread in Cambodia and thought to be due to dengue virus although laboratory confirmation has been lacking. Between 1980 and 1995, 49,420 cases of HF and 3,032 deaths were reported. Cases increased during this period; large epidemics of HF occurred every two to three years. In 1995 there were 10,208 cases of HF with 424 deaths. Over a two day period in August 1995, 40 consecutive cases were investigated at the National Pediatric Hospital in Phnom Penh, Cambodia. All 40 cases were confirmed as dengue by virus identification and/or serology. Mean age was 6.5 years. Of 39 patients with complete medical records, the diagnoses were: dengue fever (n=3), dengue hemorrhagic fever (DHF) grade 2 (n=21), DHF grade 3 (n=10), and DHF grade 4 (n=5). The serologic response was secondary in 95%. Dengue virus was identified in 13 of 40 cases. All four dengue serotypes were identified. The high frequency of secondary infections, the low mean age of admission, and identification of all four dengue serotypes support the national statistics to show that DHF is highly endemic in Cambodia.

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a mosquito-borne disease endemic to Southeast Asia that is believed to be widely prevalent in Cambodia. The principle vector mosquito, *Aedes aegypti*, lives in and near households with a predilection to breed in water collected for domestic use (Kittayapong and Strickman, 1993). Dengue infection may manifest as undifferentiated fever (viral syndrome), dengue fever (DF), or DHF. DF is a self-limited illness which usually presents with high fever, headache, retro-orbital pain, muscle pain, and joint pain. A positive tourniquet test or mild hemorrhage are common. Although DHF is indistinguishable from DF early in the course of illness, around the time of

defervescence DHF becomes distinguishable via plasma leakage resulting in hemoconcentration, pleural effusion, and ascites leading to hypovolemic shock in approximately half of cases (dengue shock syndrome=DSS). DHF is also defined by thrombocytopenia and hemorrhagic diatheses are more severe than in DF, frequently resulting in petechiae, epistaxis, hematemesis, melena, or menorrhagia (Innis, 1995). As there is no specific therapy or vaccine available, treatment is supportive by careful administration of fluids and/or colloid which is effective and life saving (Nimmannitya, 1993).

Since 1980, the Kingdom of Cambodia has experienced seasonal epidemics of hemorrhagic fever (HF) (1983, 1985, 1987, 1990, 1992; see Table 1, Fig 1) with the highest incidence during the rainy season from April to October (peak rates from June to August) (Sopha, 1993). Children aged 6 months to 15 years have been most affected, with peak age-specific attack rates among 4-6 years olds (Dr Oum

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Table 1

Reported hemorrhagic fever cases from all 21 provinces in Cambodia, 1980-1995.

Year	Cases	Deaths	CFR (%) ^a	Provinces Reporting ^b
1980	347	20	5.8	19%
1981	498	46	9.2	33%
1982	692	58	8.4	43%
1983	3,545	256	7.2	43%
1984	647	80	12.4	48%
1985	5,980	505	8.4	62%
1986	2,129	170	8.0	67%
1987	3,716	409	11.0	62%
1988	426	45	10.6	67%
1989	2,237	119	5.3	76%
1990	7,247	341	4.7	62%
1991	1,528	136	8.9	67%
1992	4,809	172	3.6	81%
1993	3,913	169	4.3	100%
1994	1,498	82	5.5	100%
1995	10,208	424	4.2	100%
Totals	49,420	3,032	6.1	

^aCase fatality rate

^bPercentage of provinces submitting data for at least one month in the year

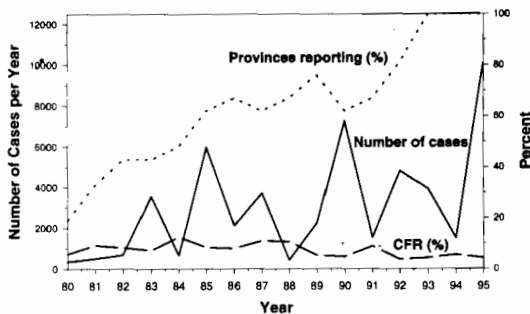


Fig 1 – Hemorrhagic fever cases, case fatality rate (CFR), and percentage of provinces submitting reports for at least one month of the year by year from 1980 through 1995.

Sophal, unpublished data). The cases have been assumed to be DHF, but laboratory confirmation has been lacking. We suspect that the bulk of these cases were likely due to dengue virus infection. To determine the cause of HF among children in Cambodia, consecutive HF cases were investigated in August 1995 at the National Pediatric Hospital in

Phnom Penh. We now report clinical and laboratory data from that series including some of the first dengue virus isolates from Cambodia. Moreover, we present Ministry of Health statistics for HF in Cambodia in 1995 and summarize total cases by year since 1980 when HF surveillance was started.

MATERIALS AND METHODS

National pediatric case series

From 16-18 August 1995, clinical and laboratory data were collected from 40 consecutive patients admitted to the HF ward of the National Pediatric Hospital (NPH), one of two referral pediatric hospitals in Cambodia, located in Phnom Penh. Patient history and clinical signs and symptoms at the time of presentation were collected from patient charts. A clinical diagnosis following the WHO guidelines for dengue diagnosis was provided at the time of discharge (Anonymous, 1986).

Paired sera were collected one day apart, transported on dry ice to the Armed Forces Research Institute of Medical Sciences in Bangkok, and tested for IgG and IgM antibody against dengue and Japanese encephalitis by an antibody capture enzyme-linked immunoassay (EIA) (Innis *et al*, 1989). Virus isolation was attempted by inoculating *Toxorhynchites splendens* mosquitos with acute serum specimens (Rosen and Gubler, 1974; Rosen and Shroyer, 1985). Identification of virus genome was attempted by reverse transcription polymerase chain reaction (PCR) (Lancioti *et al*, 1992).

National statistics

National data were compiled from reports from the Provincial Health Departments to the National Dengue Control Committee, organized by the Department of Health Information Systems, Ministry of Health.

Statistics

All statistical procedures were performed using SPSS for Windows (SPSS, Inc, Chicago, IL). Correlations were sought using the Spearman correlation coefficient. Independent means were compared using the Mann-Whitney test.

RESULTS

National pediatric hospital case series

Twenty-three girls (58%) and 17 boys (42%) with a mean age of 6.5 years old (range 2 to 15 years) were evaluated. Complete clinical information was available for 39 of the 40 patients (one clinical record missing). The majority resided in Phnom Penh (22 cases) or the adjacent province, Kandal (9 cases). There were two cases each from nearby provinces of Takeo and Kampong Speu and one each from more distant provinces of Kompot, Kompong Cham, Kompong Thom and Preyveng. The mean duration of hospitalization for all cases was 4 days (range 3-12 days).

Clinical findings for 39 of the 40 cases are summarized in Table 2. At discharge three patients were classified presumptively as DF and 36 as

DHF (15 of those as DSS). All made complete recoveries. Thirty-eight of 39 patients were febrile at presentation. Twenty-eight of 29 had a positive tourniquet test. Bleeding occurred in 30 patients: petechial hemorrhage in 16/39 (41%), melena in 6/39 (15%), and hematemesis in 5/39 (13%). Shock consistent with DHF grade III occurred in 10 of 39 while shock without measurable blood pressure consistent with DHF grade IV occurred in 5 of 39. Clinical laboratory values were available for 19 of the DHF cases revealing a mean increase in hematocrit during plasma leakage of 25% (95% confidence interval 18%, 31%) and a mean minimum platelet count of 46,000/mm³ (95% confidence interval 31,000 to 61,000).

Serology and virus isolation data are summarized in Table 2. Blood was drawn, on average, four days following the onset of illness and again 24 hours later. All 40 patients had evidence of acute dengue virus infection. EIA confirmed that 37 patients experienced acute dengue infections. Two of 37 patients experienced primary flavivirus infections (one DHF grade 2 case and the case for which the clinical records are missing). Among the three patients with non-diagnostic serology, a diagnosis of dengue infection was confirmed by virus isolation and/or PCR. Dengue viremia, detected by virus isolation or PCR in 13/40 (32%), was associated with a higher body temperature at the time of venipuncture (mean body temperature 37.7°C for patient with viremia versus 37.1°C for those without a virus isolated, $p=0.03$).

National data

From January to December 1995, 10,208 hospitalizations and 424 deaths due to HF were reported to the Ministry of Health, Cambodia. The number of cases increased dramatically in March and April (Fig 2). Most cases were reported from five of 21 provinces in Cambodia: Battambang (25%); Phnom Penh (21%); Kandal (17%); Banteay Meanchey (10%); and Kg Cham (5%). There were fewer reported cases from other provinces and no reports for most months from four remote provinces. The mean case fatality rate (CFR) for 1995 was 4.2%. The CFR was particularly high in Battambang (6.1%) and Banteay Meanchey (8.2%) during this period. The CFR for Phnom Penh was 1.4%, the lowest among any reporting province.

Over the past 15 years the number of HF cases has increased with periodic epidemics ($r=0.57$,

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Table 2

Discharge diagnosis and clinical findings among 40 patients with dengue fever and dengue hemorrhagic fever (DHF).

Clinical diagnosis (n)	Percent	Mean change in hematocrit	Mean minimum platelet count mm ³	Total isolates (n if >1)	By method	
					Culture	PCR
Unknown (1)	2.5%	-	-	D1	D1	D1
Dengue fever (3)	7.5%	4.3%	23,200	D2,D3	D2	D2,D3
DHF grade 1(0)	0.0%	-	-	-	-	-
DHF grade 2 (21)	52.5%	22.4%	60,150	D1,D2(3), D3(2),D4	D1,D2 (3),D4	D2,D3 (2),D4
DHF grade 3 (10)	25.0%	23.3%	43,100	D3(2)	none	D3(2)
DHF grade 4 (5)	12.5%	27.1%	36,600	D4	D4	D4
Totals or mean values	100%	22.2%	50,362	13/40	8/40	10/40

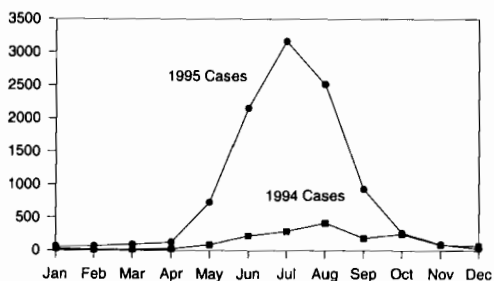


Fig 2 - Hemorrhagic fever cases in Cambodia by month for 1994 and 1995.

p=0.02) as has the percentage of provinces submitting monthly reports to the Ministry of Health (Fig 1). During this time there was a modest decrease in the CFR ($r=-0.57$, $p=0.02$) and the CFR was inversely correlated with the number of cases ($r=-0.58$, $p=0.02$).

DISCUSSION

In a series of typical cases of HF among children in Cambodia, all of 40 children with a presumptive

diagnoses of dengue based on World Health Organization criteria had their diagnoses confirmed in the laboratory. This limited sample confirms the clinical acumen of pediatricians at one teaching hospital and supports the inferences that case reports of HF in children throughout Cambodia are likely reports of dengue infection. As children with febrile illnesses on non-HF wards were not evaluated, no estimate of the frequency of under-reporting is possible.

Although more widely spaced specimen collection is desirable to diagnose dengue based on antibody response, this study demonstrates that testing paired sera as close as 24 hours apart using EIA is sensitive to diagnose dengue. The dengue virus isolation rate was 8 of 40 (20%), which is consistent with the isolation rate at the Bangkok Children's Hospital where children also present relatively late in the illness (Ananda Nisalak, personal communication). PCR identified 5 additional cases of dengue type 3 to bring the total to 32.5% of cases with a virus identified.

Several factors from this small cases series support the implications of the national statistics that dengue illness is widespread in Cambodia. The low mean age of admission (6 years) and the antibody response (95% secondary) suggest hyper-endemic

dengue virus transmission for an extended period. Identification of all four dengue virus types over a two day period in a single hospital also suggests favorable circumstances for virus transmission.

On a national level periodic epidemics of DHF have occurred in Cambodia from 1980 (data unavailable prior to 1980) until present. In 1995, 10,208 reported cases and 424 deaths succeeded a lower number of cases in 1994. The pattern of epidemics every few years is similar to that of Thailand (Ungchusak and Kunasol, 1988). Using a population estimate of 10 million persons for Cambodia, the data suggests an attack rate of 102 per 100,000 for 1995. However, reporting is admittedly incomplete and age specific attack rates were much higher among children. The overall trend has been an increase in total cases since 1980; much of this may be explained by improved reporting. Since 1992 all provinces have made reports of DHF in at least one month of the year. In 1995 only five of 21 provinces failed to provide reports for all twelve months.

Over the past 15 years the CFR has decreased. This may reflect an improvement in care provided, a decrease in the lethality of the disease, or an increase in recognition with resultant reporting of less severely ill cases. CFRs dropped when there were more reported cases suggesting that during the larger outbreaks, the index of suspicion for DHF was increased resulting in more less-serious cases being reported to the Ministry. In 1995, CFRs remained higher in the outlying provinces than in the capital province. Clinical studies carried out to identify factors associated with a high CFR showed that late referral and inappropriate treatment prior to hospitalization were risk factors (Hong Rathavuth, unpublished data).

There is room for improvement in the current CFR of 4.2%. In Cambodia the WHO criteria for diagnosis and treatment of DHF (Anonymous, 1986), has been simplified and translated into the Khmer language. It is now used by senior pediatricians to train pediatricians and physicians working in provincial and district level hospitals who deal with dengue patients. This must be encouraged and further promoted. One or more pediatric referral hospitals should be developed to emphasize dengue teaching and research to include the ability of perform simple serologic testing (eg dengue/Japanese encephalitis EIA) to identify epidemics early in the year and to assist physicians to

refine their diagnostic and treatment skills. Increased public health measures to reduce vector breeding sites and to increase awareness among parents to prompt earlier consultation with a health care provider should also help to decrease mortality from dengue in Cambodia.

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