Title : Epidemiology of Dengue Hemorrhagic Fever

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Objective:

To determine the factors which influence the occurrence and spread of mosquito-borne hemorrhagic fever in Southeast Asia.

Description:

Data on hospitalized hemorrhagic fever cases in Bangkok-Thonburi hospitals were collected by monthly visits to each hospital by public health nurses in the same manner as had been used since 1962. Reports on hemorrhagic fever cases occurring outside of Bangkok were requested from hospitals. Additional data were available from Department of Public Health records of reports from provincial health officers.

Reports of large or unusual outbreaks were investigated by a team consisting of at least one physician and two public health nurses. Attempts were made to collect clinical and epidemiologic information and obtain specimens for virologic studies.

Epidemiologic data on hemorrhagic fever in Saigon are being collected through the cooperation of the US Army Medical Research Team, Vietnam, and the Public Health Division of United States Agency for International Development, Saigon. USAID public health nurses collected data on hospitalized hemorrhagic fever cases by visiting hospitals weekly and reviewing clinical records.

An epidemic of hemorrhagic fever on the island of Koh Samui in the gulf of Thailand off the coast of Surat Thani province was investigated in detail by a team made up of personnel from the Departments of Epidemiology, Entomology and Virology. The purpose of the studies was to determine the epidemiologic patterns of dengue hemorrhagic fever in a relatively isolated population in which the disease had recently appeared, and to determine whether serologic response to infection and the epidemiologic patterns are consistent with the "second infection" hypothesis of the dengue shock syndrome. In addition, studies were carried out to determine the relative prevalence of Aedes aegypti and Aedes albopictus on the island and to obtain information concerning the role of Aedes albopictus as a dengue vector.

Progress:

Hemorrhagic Fever in Bangkok. During 1966 hemorrhagic fever continued as a major cause of morbidity and a significant cause of mortality in Thai Children. A total of 3,663 cases were admitted to hospitals in Bangkok and Thonburi with 139 fatalities. The case fatality rate in 1966 was 3.8% essentially the same as the rate of 3.9% seen in 1965.

The distribution of cases by month (Figure 1) shows the peak incidence in June through August which was similiar to the seasonal pattern seen from 1962 to 1964. There were a relatively large number of cases during the dry months from December 1965 to April 1966 and a sharp decrease in the dry season beginning November 1966.

It has become apparent from discussions with pediatricians at Children's Hospital and Chulalongkorn Hospital that admitting policies have been changed since 1964. At these two hospitals, which care for approximately half of all hospitalized cases, prior to 1965 most cases diagnosed as hemorrhagic fever were admitted. During 1965 and 1966 most mild cases were treated as outpatients and only patients with shock, bleeding, or signs of impending severe illness were admitted. Therefore the gross figures for 1965 and 1966 are not directly comparable to previous years and tend to underestimate in 1965 and 1966 since they are based on a more severely ill group of patients.

Age distribution remains essential unchanged from previous years with over 50% of cases occurring in the 3-6 year age group. Age specific rates given in Table 1 are based on the 1960 census.
Hemorrhagic Fever in Udorn Thani. In June-July 1966 hemorrhagic fever was reported for the first time in and near the city of Udorn Thani in Northeast Thailand. Clinical descriptions of cases were typical of hemorrhagic fever and serologic studies on 9 cases confirmed the dengue etiology.
Hemorrhagic Fever in Saigon. During 1966, 457 cases of hemorrhagic fever were admitted to 3 Saigon hospitals (Nhi Doug, Grall, and seventh Day Adventist). Of the 457 cases, 214 had clinical evidence of shock and there were 67 deaths reported. Distribution of cases by month, shown in Figure 2, reveals the majority of cases occurred during the southwest monsoon season (May to September) with a sharp peak in June and July. The seasonal variation is very similiar to that seen in Bangkok.

Age distribution (Table 2) is also very similiar to that seen in Bangkok except the relative number of cases under one year is lower.

The mortality rate in Saigon of 14.9% is considerable greater than the rate in Bangkok in 1966 (3.9%) and comparable to the mortality rate of 12.8% in Bangkok in 1958.

In addition to the Saigon-Cholon area, hemorrhagic fever was reported in the following provinces of South Vietnam: Gia Dinh, Bien Hoa, Long An, Long Khanh, Binh Duong, Binh Long, Hua Nghia, and Phoc Tuy.

Hemorrhagic Fever on Koh Samui:

Koh Samui is the main island in a small archipelago located in the gulf of Thailand, about 9° North of the equator. (Figure 3). The nearest mainland port is 5 hours away by boat.

Prior to December 1965, the island's two physicians had not seen hemorrhagic fever among the populace. Both men were familiar with the disease, and readily recognized its appearance in December 1965, when 20 cases, with 1 death, occurred in Ang Tong. On being informed of this outbreak, a team was sent to carry out a preliminary serological and entomological survey on the island. The diagnosis of dengue hemorrhagic fever was serologically confirmed, a high prevalence of dengue antibodies was found in a sample of children, and dengue—1 virus was isolated from one patient. Aedes aegypti were present in large numbers.

It appeared likely that a major epidemic of hemorrhagic fever might occur during the next monsoon season and preparations were made to study it should it occur. The anticipated epidemic began in July 1966 and lasted into October. A team from SMRL was present on the island throughout this time. The epidemiologic studies are described below.

Materials and Methods.

Description of the area. Koh Samui is about 12 miles long and 10 miles wide, with an area of almost 90 sq. mile. The population is approximately 25,000. The remoteness of the island, while rendering

study difficult from the point of view of access, was an advantage in that it limited the mobility of the population, particularly the age group in which we were most interested. Most of the population lives on the coastal plain of the island, which ranges in width from 2 to 4 miles. The center of the island is mountainous, the highest peak reaching over 1800 feet. Except in the mountainous center, the dominant form of vegetation is the coconut palm (Cocos nucifera); copra production is the economic mainstay of the island. Piles of coconut shells and husks are found in and around every village and scattered over the entire coastal plain.

The climate of Koh Samui is dominated by the tropical monsoon. Mean daily temperatures vary from 26° to 29°C throughout the year. Mean humidity ranges from 76 to 88%. During the dry season, from January through April. monthly rainfall averages 13-68 mm. The southwest monsoon (May-September) brings 135-170 mm of rain per month, and the Northeast monsoon season (October through December) has up to 300 mm of rain per month.

Clinical and Virologic Methods. All patients were seen by one of us (S.N.) who was the only practicing physician on the island at the time. A case record was kept of each patient seen in the course of the study. This record included name, age, sex, place of residence, date of onset, initial signs and symptoms and a careful description of the clinical course. Blood was obtained from each patient at the time of initial interview; this was usually within 3 days of onset of illness. A convalescent blood specimen was usually obtained 10-14 days after the acute specimen. Following clot separation and centrifugation, sera were stored on dry ice for shipment to Bangkok. Serum pairs were identified only by code number; serologicol and virological studies were carried out by personnel having no access to clinical records.

Each serum was acetone extracted and tested for hemagglutination-inhibiting (HI) antibody against dengue-1, 2, 3 and 4, Japanese encephalitis, and chikungunya antigens. Virus isolation from acute sera was done by plaque method in LLC-$MK_2$ cell culture and plaque forming agents were identified by plaque reduction neutralization test using reference monkey antiserum to prototype strains.

Based upon completed clinical records, patients were categorized by syndrome without reference to serologic or virologic data. Clinical categories were determined using the diagnostic criteria and nomenclature suggested by the World Health Organization. Patients with fever alone were classified as undifferentiated fever (UF). Patients with febrile disease exhibiting a positive tourniquet test or a few scattered petechiae were considered as having dengue fever (DF) syndrome. Patients were classified as hemorrhagic fever (HF) cases if in addition to fever and positive tourniquet test they exhibited one or more of the following: extensive petechiae, purpura, ecchymoses, epistaxis, hematemesis, hematuria or melena. Patients with shock (S), defined as pulse pressure less than 20 mm Hg., or evidence of a fall in systolic pressure below 90 mm Hg., were originally considered as a separate subcategory of the HF group, without reference to the presence or absence of hemorrhagic manifestations, for reasons discussed below.

The serologic response of each patient was independently classified by the following criteria: serum pairs which showed no detectable HI antibody, or no rise in titer from acute to convalescent were considered negative. A response which consisted of absence of antibody in the acute serum and a four-fold or greater rise in titer in the convalescent serum was designated a primary response. Demonstrable antibody in the acute specimen obtained within 3 days of onset of illness, plus either isolation of virus from the same specimen or a four-fold rise in titer to 1:640 or greater was taken as evidence of a secondary type antibody response. Specimens which showed antibody in an acute specimen obtained 4 or more days after onset plus either virus recovery or with a four-fold rise in HI titer were considered to be positive, but undifferentiable into primary or secondary response. Sera were rejected as incomplete if one serum of a pair was missing or if volumes were not sufficient to complete serologic examination.

At the completion of the study comparisons were made of clinical and serological classification of all patients.

Results.

A total of 148 individuals with 150 illnesses were included originally. From these, 139 complete adequate serum pairs were available for serologic and/or virologic study. The remaining 11 illnesses, for which complete serum pairs were not available, were excluded from further consideration. Table 3 presents the distribution of the 139, by admission clinical diagnosis and serologic response. Note that two individuals are represented twice, each having two episodes of undifferentiated fever (UF) at least one month apart. Since in both cases dengue was not implicated in the first episode of UF it was considered that they remained at risk; in one case the second UF was not dengue related, but in the other instance dengue virus was isolated from serum collected during the second UF episode. Therefore, they are treated as four separate cases.

Ninety illnesses were shown to be associated with dengue, in 21 cases dengue virus was isolated from the acute serum specimen. The additional 69 cases were associated with dengue on serologic grounds alone. Since no other agents were incriminated in more than 2 cases, we felf justified in considering this a dengue epidemic.

Figure 3 presents the geographic distribution of the 90 cases of dengue-associated disease, with the earliest date of onset recorded for each affected village. Figure 4 is the distribution of cases by week of onset. The disease first appeared on 10 July on the north coast of the island, later being reported from scattered villages over virtually the entire island. The last known case occurred on 5 October. The 3 week first "peak" in the epidemic curve (Fig. 4) coincides with the epidemic in and around the village of Mae Nam. A survey of the 87 households in this village indicated there were 224 children under age 15 living in Mae Nam. Thirty two become ill with dengue-associated disease during the epidemic; of these, 7 had dengue hemorrhagic fever with shock syndrome and 14 had hemorrhagic fever without shock. Thus, in a period of less than 2 months 9% of the children of Mae Nam had HF. An additional 5% were ill with DF or UF for a total dengue attack rate of 14.3%. Three other severe HF cases, with 1 fatality (presumably due to shock) had occurred in Mae Nam at the onset of the epidemic, before the arrival of the investigating team; these are not included in the data presented.

Considering the population age 15 and under at risk, the estimated dengue attack rate for the island as a whole was approximately 0.75% and in no village except Mae Nam was it over 2%.

The second "peak" in the epidemic corresponds to the simultaneous appearance of the disease in several scattered locations south of Ang Thong, primarily in Lipanoi and Talingngarm. No consistent pattern in the appearance of the disease in the scattered villages is apparent. The dates of onset do not seem to correlate with distance by road from the area on the north coast where the epidemic was centered. Table 5 and Figure 5 present the age and sex distribution of the 90 cases of dengue. The youngest child affected was one year of age, the eldest, fifteen. The median of the distribution is 6.6 years, the mode 7 years. Median and mode did not vary with sex, and since only 9 individuals exhibited primary antibody response, comparison by serologic classification is not revealing. When cases of DF and UF are combined, and their age distribution compared to that for HF and shock cases (Figure 6) the median (6.4 years) and mode (6 years) are lower than those for the HF age distribution (median 7.3 years, mode 7 years). The female preponderance seen in Table 5 (52:38) is more apparent if HF cases alone are considered (20:11). However, these ratios are not significantly different from the ratio of 1:1 which holds for the island population under age 15.

Dengue viruses were isolated from the acute serum of 21 patients, an isolation rate of 23%. Of these, 15 have been identified as to serotype. Three serologic types were represented among the 15 strains. The lack of relationship of virus type to clinical syndrome is shown in Table 6a. Dengue-2 and dengue-3 were both associated with shock cases as well as mild illness. The single dengue-1 strain came from a case of dengue fever syndrome.

Geographic and chronologic distribution of the dengue strains isolated from human cases are listed in table 6b. Dengue-2 and dengue-3 were widespread over the island during the period of the epidemic.

## Discussion

The observations made from clinical and serologic studies during this epidemic indicate the difficulty of diagnosing disease due to dengue virus solely on clinical grounds. Undifferentiated fevers, dengue fever syndrome, and hemorrhagic fever were sometimes due to causes other than dengue virus infection. Considerable caution, therefore, is necessary in interpreting epidemiologic data from "hemorrhagic fever" epidemics in which the diagnosis is based solely on clinical grounds. Meaningful patterns can be obscured by the presence of other agents producing similiar syndromes. Of interest is the fact that no cases of chikungunya infection were seen in contrast to Bangkok epidemics where as high as 10% of HF cases (all mild illnesses) were due to chikungunya.

In this series 64 cases of UF were seen; only 35 (55%) were due to dengue. Of 36 cases of "dengue fever" syndrome, only 24 (68%) were shown to be dengue-caused. "Hemorrhagic fever syndrome" (i.e. without shock) was no more specific. Only eighteen (69%) of 26 cases were dengue-associated. The thirteen cases which exhibited shock, on the other hand, were all dengue-associated. The number is small, so that generalization with a high degree of confidence is not possible, but the data support the suggestion made elsewhere that the shock syndrome seen in connection with hemorrhagic fever epidemics is specifically associated with dengue. Dengue shock syndrome, or dengue hemorrhagic fever with shock, is the only segment of this clinical spectrum that can be differentiated by clinical examination with reasonable precision.

It is probable that all cases of hemorrhagic fever which occurred on the island during the epidemic are represented here. As has been noted, only one medical clinic was in operation during the epidemic, and all the cases included were treated at this central location. In addition, periodic visits were made to outlying areas by the attending physician as well as by members of the SMRL team, and no additional cases were found or reported. The island population is fairly closely knit; they were familiar with hemorrhagic fever from outbreaks which had occurred elsewhere in Thailand, and were generally sympathetic to the work of the investigating team so it is unlikely that any cases were concealed from or missed by the investigators. However, in general, no concerted effort was made to include all undifferentiated febrile illness, so this category (UF) represents an unknown but probably small proportion of febrile illness which occurred on the island during the time of the study.

Previous studies in Bangkok indicated that "cases tended to occur multiply in households". In an area when the "primary attack rate" was 3.6%, it was noted that of 271 families with at least one case of HF., 35 (13.2%) had two or more cases. This however does not indicate that these two rates bear a relationship analogous to primary/secondary attack rates, and cannot be used as evidence that siblings of cases are at higher risk than children in general. In the Mae Nam study where denominator data were available, it has been pointed out that the overall attack rate was 14.3%. Thirty two cases occurred in 22 households, having a child population of 82. If the first case in a household is considered the index case, then 60 siblings were at risk. Of these 10 had clinical illness; the attack rate among siblings of index cases was thus 16.7%, not strikingly different for that of the population as a whole. Thus the "clustering" in families appears to be a chance phenomenon entirely.

The HF cases in this epidemic appear to be generally older than has been reported elsewhere in Thailand or in Vietnam, and similiar to the ages reported in Malaysian epidemics.

The fact that all dengue shock cases did not exhibit hemorrhagic manifestations has been alluded to above. In this series, 9 cases with shock had hemorrhagic signs consistent with a diagnosis of hemorrhagic fever syndrome had shock been absent. However four cases, including the only fatality, had only fever and a positive tourniquet test or a few scattered petechiae as sole concomitant evidence of infection. If the "dengue shock syndrome" was an extreme manifestation of the same mechanism which produced the dengue hemorrhagic fever syndrome (i.e. the extreme end of the dengue infection spectrum) then all shock cases would be expected to exhibit severe hemorrhagic signs. This does not appear to be the cases; but neither

do the data suggest that shock is entirely independent of other symptoms; rather that the dengue shock syndrome is produced by a mechanism related to, but not necessarily identical with that resulting in hemorrhagic diathesis.

The data were examined to compare other characteristics of the group of dengue shock syndrome with those of cases of hemorrhagic fever syndrome without shock. Shock cases occurred throughout the epidemic and no evidence of geographic clustering was seen. Age distribution data (Figure 6) indicate that the average (median) age of HF cases may be higher than that of shock cases. Dengue viruses type 2 and 3 were isolated from shock cases, providing further evidence that the antigenic type of the infecting virus is not the sole determinant of the infecting virus is not the sole determinant of the production of the shock syndrome.

The hemorrhagic fever syndrome has been observed to result from an initial dengue infection; on Koh Samui one individual with a primary type antibody response to dengue had a clinically typical example of the syndrome. In contrast none of the thirteen shock cases were accompanied by primary type antibody response. Nine cases had secondary type antibody response and four were serologically positive for dengue, but response could not be classified with complete assurance as either primary or secondary, due to delay in obtaining acute specimens. In these four, initial serum specimens had high titers which probably indicate secondary-type serologic response.

The observations made thusfor support the hypothesis that dengue shock syndrome is produced by a specific immunologic phenomenon elicited by a second, heterotypic, dengue infection occurring a certain critical period of time following an initial dengue infection. The initial, or primary, dengue infection may produce a mild form of hemorrhagic fever but either undifferentiated fever or dengue fever syndrome are more commonly produced. Shock does not occur. Following recovery, patients have immunity to heterologous dengue infection for a variable period of time (of the order of 6 months). During this time "sensitization" may be occurring in certain individuals, depending upon host factors. Following the immune period, in "sensitized" individuals, there occurs a period where a second, heterotypic, dengue infection results in development of shock syndrome and/or hemorrhagic fever syndrome, the period of susceptibility for the latter probably extending beyond that for the former syndrome. Thus a critical "configuration" of circumstances, age at initial infection, interval between infections, and possibly also dengue type and dose, are necessary to produce the dengue shock syndrome.

The finding on Koh Samui of three distinct dengue serotypes (dengue-1, 2 and 3) present during the epidemic is consistent with the previous findings that in areas where dengue hemorrhagic fever is prevalent (e.g. Bangkok, Manila, Singapore and Saigon) three or four dengue serotypes have been found.

Until it is possible to directly demonstrate the pathogenic mechanism (s) of shock syndrome in man or suitable animal models, it is necessary to continue careful and detailed studies of epidemiologic patterns manifested by this imperfectly understood disease.

Entomologic Studies on Koh Samui. The initial SMRL survey on Koh Samui in February 1966 established that both Aedes aegypti and Aedes albopictus were present on the island. Aedes aegypti was subsequently found to be widely distributed throughout the island, both in villages and in isolated farmhouses, and during both the rainy season (May—December) and dry season (January—April) (Table 7a).

During November a survey of larval habitats of Aedes aegypti and Aedes albopictus was carried out in the vicinity of homes of September cases of THF in tambons Mae Nam and Taling Ngam on the island of Koh Samui. The frequent occurrence of albopictus larvae in artificial containers (e.g., water jars, gasoline drums, plant containers, etc.) in close proximity to houses (inside a house on one occasion) and sharing of the same habitat with aegypti larvae was observed (Table 7b). During this same period adults of both species were frequently collected resting and biting inside houses, however aegypti adults were collected biting outdoors on only one occasion (Table 7c).

During the course of the 1966 HF epidemic both A. aegypti and A. albopictus adults were collected from the vicinity of the dwellings of recent cases of the disease for virus isolation attempts. Nine strains of dengue virus were isolated from a total of 122 A. aegypti collected from houses in Ang Thong and Mae Nam in July. Four additional isolations were obtained from a total of 1110 A. albopictus collected during September. The occurrence of these two vector species together in the presence of this dengue outbreak is strikingly similar to the situation in Saigon and Singapore and quite dissimilar to that of Bangkok (and probably Manila) where only aegypti appears to be involved in dengue transmission.

In February 1967 collections of A. aegypti and A. albopictus were made from several areas on Koh Samui, from Koh Pangan (10 kilometers north of Koh Samui) and from Koh Paluai (25 kilometers west of Koh Samui) for purposes of insecticide susceptibility studies. All houses visited on these smaller, more isolated islands were infested with A. aegypti (22/22 on Paluai and 25/25 on Pangan), but there was no evidence that A. albopictus was present although the ecological conditions there apparently were similar to those on Koh Samui. The apparent absence of albopictus on these outer islands may have been due to a decline in its population caused by the dry season, for albopictus was found with difficulty in areas of Koh Samui where during the previous November it had been abundant. Susceptibility tests of larvae of A. aegypti from Ang Thong and Taling Ngam on Koh Samui and from Koh Paluai and Koh Pangan indicated that this species has become resistant to DDT and dieldrin in those areas (details of these tests given under the report on mosquito studies). Results of tests of the insecticide susceptibility of A. albopictus from Koh Samui are not yet available.

## SUMMARY

Dengue hemorrhagic fever continues as a major public health problem in the pediatric age group in Bangkok and many other areas in Thailand. In 1966 a further spread of this disease was documented in the city of Udorn Thani in northeast Thailand.

Epidemic dengue hemorrhagic fever occurred in Saigon in 1966 with a peak incidence during the monsoon season in June and July. The age distribution in Saigon is similar to that seen in Bangkok. However, the reported mortality rate is considerably higher.

Epidemiologic studies were carried out during an epidemic of dengue hemorrhagic fever which occurred on the island of Koh Samui between 10 July and 5 October 1966.