INTRODUCTION

Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are caused by infection with dengue viruses, transmitted to man through mosquito bites, and are ranked among the arboviral (arthropod-borne) diseases. After feeding on a viremic person, an Aedes aegypti female can transmit the virus after an extrinsic incubation period of 7-12 days. Host changes when the blood meal is interrupted contribute to increasing the potential for transmitting infected vectors (Gubler, 1988). After infection with one of the four dengue serotypes, the host immune response produces neutralizing antibodies (IgM, IgG), predominantly against that serotype (primary dengue infection). The first attack by one virus serotype gives temporary protection against the other three serotypes because of cross-reactions among the four dengue serotypes. Therefore, a person living in an endemic area can have a secondary, or sequential infections, only after three months, when the non-specific antibody titer due to the previous infection decreases (Nimmanitya, 1991). Most infected hosts are asymptomatic or present a mild, non-specific febrile illness. The factors determining whether a person develops mild or severe illness are not well understood and disease expression can be influenced by a number of factors, including virus and vector strain, immune status or the genetics of the infected hosts (Guzman et al, 1984; Henchal et al, 1986).

The increasing rate of dengue virus transmission appears as one of the major factors involved in the emergence and persistence of a dengue outbreak. Most of the case reports have been limited to severe infection (DHF, DSS). Therefore, a high level of persistent transmission can be hidden and the recognition of early epidemic manifestations delayed, dramatically reducing the
efficiency of control measures.

In order to contribute to early active surveillance in an endemic area, a serosurvey was performed in Ratchaburi Province, Thailand, from July 2000 to June 2001, to evaluate the rate of infection in a normal population. For reason of practicality, schoolchildren, the most sensitive population with the lowest immunity, were targeted for the seroconversion survey in the present study.

MATERIALS AND METHODS

Study site

The study was performed in Ratchaburi Province, 100 km west of Bangkok (Fig 1), where the prevalence of dengue virus transmission, through reported DHF cases, has been permanent for over 14 years, according to the Annual Report of the Ministry of Public Health (MOPH, Annual Epidemiological Reports, 1988-2001). During the 1997-1999 epidemic, the DHF morbidity rate reached 379 per 100,000 inhabitants (Fig 2).

The age distribution for the Ratchaburi population, per age class, was calculated using the same age classes as the MOPH (Fig 3).

The study area is a flat agricultural zone; the climate is tropical with average temperatures ranging from 25°C to 35°C and relative humidity from 65 to 90%. In 2000, annual rainfall was 1,037.9 mm. The serological survey was done in Hin Gong subdistrict (63.75 km²; 13°35’N, 99°44’E) which is comprised of 11 villages, with 6,136 inhabitants and 1,424 households. The four local surveyed primary schools were located in different villages and among them, only school in Nong Taluang village was selected for longitudinal survey.

Study design

The seroepidemiological survey was targeted on the 341 schoolchildren of the 4 schools in the study area. The first serosurvey, in July 2000, was conducted to collect baseline data, and the children were monitored again for evidence of dengue virus infection in June 2001 (48th week).

Schoolchildren in the village of Nong Taluang consisted of 28 kindergarten children (3-4 years old) and 62 primary schoolchildren (5-12 years old).
proved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (TM-IRB 030/2001).

Specimens were tested on Vero cell lysate antigen, by an enzyme linked immunosorbent assay (ELISA) for dengue IgG antibody and by capture ELISA for IgM antibody screening, as described elsewhere (Kuno et al., 1987; Ansari et al., 1993). For quality control, a positive control and three negative controls were always included in each plate, and individual specimens collected several times were simultaneously tested. The cut-off value for positive designation was defined when the OD value was higher than the average of three negative controls plus three standard deviations. Significant serological changes were interpreted as follows: IgG increase by four-fold; IgM increase by two-fold.

Retrospective data
Epidemiological records were obtained from Ratchaburi Provincial Hospital, Ratchaburi Provincial Health Office and the MOPH, Thailand.

RESULTS
We were able to test 283 specimens among 341 schoolchildren (83%) included in the survey, and of them, 71% (200/283) were found positive for dengue IgG antibody at baseline, in July 2000 (Table 1).

From these 283 schoolchildren, 205 were subsequently monitored in June 2001 and 192 were interpretable. Among the IgG negative group (68 children), 7 (10%) exhibited a seroconversion to IgG positive or IgM positive due to a past (less than 12 months) or recent infection (less than 6 months), respectively. Among the IgG positive group (124 children), 11 (8%) presented an IgM seroconversion (Table 1).

The immunity rate showed an average of 1.0% yearly increase, from 5 to 12 years of age. At 12 years group of age, the immunity of the study population reached 74% (Fig 4) and the estimated absolute (from birth) average yearly increase was 6%.

The immunological status (57.8% seropositivity for dengue virus infection) of 90 schoolchildren from Nong Taluang did not differ significantly from the other schools (p>0.05). Also, the 5-12 year old group showed a significantly (p<0.01) higher rate of immunity (69.3%) than the younger years old). A total of 90 schoolchildren were followed up for dengue reacting antibodies at the 8th, 24th and 48th weeks after the primary serosurvey.

Hospitalized cases who originated from Hin Gong subdistrict and blood samples of clinically diagnosed DF/DHF cases were collected during the acute (admission date) and convalescent (discharge date) phases to establish immune status.

Laboratory analysis
All blood specimens were collected by venipuncture (3-5 ml). Clotted blood was centrifuged at 1,500 rpm for 10 minutes at 25°C and sera were aliquoted and kept frozen at -80°C until used. Blood specimens were taken only from primary school students (5-12 years old) on behalf of whom parents or guardians had provided written informed consent. The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (TM-IRB 030/2001).

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### Table 1

<table>
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<th>2000</th>
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<td></td>
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<td>IgG+, M+</td>
<td>IgG-, M-</td>
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<tr>
<td>Total</td>
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*a Number of children tested for dengue reacting antibody; - = negative; + = positive.

### Table 2

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- = negative    + = positive

### Table 3

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<td>IgG+, M+</td>
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</tbody>
</table>

- = negative    + = positive

The group (3-4 years old; 32.1%). During the study period, the average (3.4%) rate of immunity increased among these 2 groups (Tables 2 and 3).

Eighteen hospitalized cases from Hin Gong subdistrict were admitted to Ratchaburi Provincial Hospital during the study period; 11 were clinically diagnosed as DF, and 7 presented DHF syndrome, according to WHO criteria (WHO, 1980).

### DISCUSSION
Two-thirds of schoolchildren in the 5 year-old age group had experienced dengue virus infection, and this survey revealed an average 1% annual increase in immunity. The children in the 5-8 year old age group were the most susceptible...
for dengue virus infection, compared with the 9-12 year group, with statistical significance (p<0.01). The result is consistent with the highest number of hospital-admitted cases of ages 5-8 years (50.4%) in Ratchaburi Provincial Hospital, in the year 2000, where 685 cases were hospitalized dengue cases (Fig 5), which is also similar to other previous findings in Thailand (Chareonsook et al, 1999; Strickman et al, 2000). It can be explained from retrospective studies, decreasing former DHF incidence can affect exposure and protective immunity and increase the risk of infection in consequent generations.

Changes in seroprevalence among experienced (8.0%) and naïve (10.3%) schoolchildren were not significantly different (p>0.05). The rate of dengue infection in the endemic study area was 8.8% among schoolchildren. However, current estimates suggest that 2.5 billion people in tropical and subtropical countries throughout the world are infected with the dengue virus, 2-4% of dengue cases occur annually, and only 1% present as severe DHF (Vaughn et al, 1997). Therefore, estimation of the dengue situation should not ignore silent transmission, as described by Chen et al (1996) where 2.8% of schoolchildren, 8-15 years of age, were IgM-positive, despite the fact that no epidemic had been reported in the previous 10 years.

In the main town of Ratchaburi Province, an explosive outbreak occurred in the dry season, February 2001 (43 DHF cases), whereas the first DHF case in Hin Gong subdistrict was not identified until January 2001. However, we also observed an increase in seroprevalence among schoolchildren in December 2000, before the dry season epidemic. Such observation appears of importance and will fuel further studies.

Establishment of serologic surveillance can be an effective measurement, either in a low level of inapparent infection, or in an intermittent epidemic area. Since the susceptible population living in an endemic area is at risk of sequential infection, we can see not only seronegative, but also seropositive, at baseline data change in serological profile and/or symptom appearance.

ACKNOWLEDGEMENTS

This survey was supported by the Thailand Research Fund (TRF) for Royal Golden Jubilee, Thailand, Institut de Recherche pour le Développement (IRD-Research Unit 034) and AVENTIS, France. We are indebted to Dr Bernadette Murgue and Dr Hervé Zeller from the Centre de Référence de Virus, Pasteur Institut (France) for kindly providing dengue antigen and Mr Somnuek Palabodeewat from CVD, Mahidol University, for his help in developing the ELISA technique at the RCEVD. We are also grateful to the personnel from Ratchaburi Provincial Health Office, Ratchaburi Provincial Hospital for providing data, and Hin Gong Health Station and local schools for their kind cooperation.

REFERENCES


