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Assessment of the factors associated with flavivirus seroprevalence in a population in Southern Vietnam

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SUMMARY

Dengue and Japanese encephalitis flaviviruses cause severe disease and are hyperendemic in southern Vietnam. This study assesses associations between sociodemographic factors and flavivirus seroprevalence in this region. Sera were collected from 308 community and hospital-based subjects between April 1996 and August 1997 and tested with an indirect ELISA. The factors associated with seroprevalence were assessed using multivariate logistic regression. In this first report of adjusted prevalence odds ratios (POR) for flavivirus infection in Vietnam, seropositivity was associated with increasing age in children (multiple regression coefficients for a child compared to an adult $= -4{\cdot}975$ and for age in children $= 0{\cdot}354$) and residence in the city compared to surrounding rural districts. The association with age indicates that subjects were most likely to have acquired infection in early childhood. This is key to the design of Vietnamese health education and immunization programmes.

INTRODUCTION

Dengue, Japanese Encephalitis (JE) and hepatitis C viruses are the only documented flaviviruses circulating in southern Vietnam [1–6]. Major dengue epidemics have occurred in this region annually since 1975 [4]. These have been associated with the severe forms of disease, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), with an incidence from 31 to 381 DHF/DSS cases per 100 000 persons in the population per year [4, 7]. The case fatality rate of reported DHF and DSS cases from 1956 to 1995 ranged between 0·3 and 1·9% [8]. The dengue vector in Vietnam is the urban mosquito, *Aedes aegypti* [9].

Japanese encephalitis virus is transmitted in a cycle involving swine, wading birds, humans and possibly other vertebrates by the mosquito vector, *Culex tritaeniorhynchus* [3, 10]. This vector is prolific in rural areas since the larvae breed in flooded rice fields [11]. Between 1973 and 1995, the annual number of JE cases in Vietnam reported to the World Health Organization (WHO) and to the Ministry of Health ranged from 832 to 4935 [3, 12]. The mean annual case fatality rate was 15% (95% confidence intervals (95% CI) = 8%, 23%) [3, 12]. The majority of

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dengue and JE virus epidemiological data from Vietnam comprises the number of clinical disease cases reported to research laboratories and to the WHO. This is likely to underestimate the number of cases in the community as a whole, data which are critical for planning intervention strategies.

Risk factor studies in this country and in neighbouring Thailand have focused on severe disease [6, 13, 14]. These ignore asymptomatic and milder infections which also contribute to virus transmission. The incidence of such infections is most accurately measured by conducting a cohort study.

Where this approach is not possible, serological screening of a population for IgG antibodies to dengue and JE viruses yields estimates of past and present infection experience in the population. This is due to the fact that these antibodies remain elevated for several decades after infection [15, 16]. Serological screening of a community-based sample from Mexico permitted multivariate analysis of the associations between environmental and meteorological factors and dengue seroprevalence [17]. Associations were identified with the presence of *Ae. aegypti* larvae (adjusted prevalence odds ratio (POR) = 1·9, 95% CI = 1·4, 2·5) and uncovered water containers on the premises of study subjects (adjusted POR = 1·9, 95% CI = 1·4, 2·7).

The objective of the present study was to assess the associations between demographic and socio-economic factors and flavivirus seroprevalence in a population sample from Dong Thap province in southern Vietnam.

METHODS

Study population

All subjects were participants of a matched case-control study designed to investigate the epidemiology of typhoid (conducted by C. L. and paper submitted to the Transactions of the Royal Society of Tropical Medicine and Hygiene). Sampling was carried out from April 1996 to August 1997 at Cao Lanh hospital and surrounding district in Dong Thap province, southern Vietnam (Fig. 1). This province is covered with rice fields and canals to the Mekong River and rice farming is the major occupation. All study subjects lived in concrete (31%) or bamboo (69%) houses. Only families in urban areas had running water. Based on observation however, water storage for domestic use is a common practice in the area. At the time of sampling, the population size of Cao Lanh city was 134 022 and that of Cao Lanh district was 175 101.

The study population consisted of three groups: culture-confirmed hospitalized cases of typhoid fever (group A), hospital controls (group B) and community controls (group C). Inclusion criteria included residence in Cao Lanh city or district for more than three months. Hospital and community controls were matched to members of Group A by gender and 5-year age group. They were excluded if they had a recent history of, or clinically suspected typhoid fever. Group B subjects were identified from the admission register of each ward in Cao Lanh hospital at the time of admission of the Group A subject. Group C subjects were selected by visiting the house on the left side of the case's home (or the following house if a match could not be found) within one month of interviewing the Group A subject.

Demographic and socio-economic data for each subject were collected with a standardized questionnaire, designed and pre-tested to identify risk factors for typhoid fever. Interviews were carried out by three doctors. Where possible, the same doctor interviewed the typhoid case and their matched controls. The percentage of persons approached to participate in the study who did so was 100%.

Laboratory methods

The serological assay employed was a commercial anti-dengue IgG antibody indirect enzyme-linked immunosorbent assay (ELISA) (PanBio, Brisbane, Australia). Positive, negative and cut-off calibrator sera were provided with the assay, and these were included in each microtitre plate. Sera from Vietnamese individuals taken either during, or 3 months after virologically confirmed dengue infection were also used as positive controls. The assay was performed according to the manufacturer's instructions outlined by McBride et al. [18]. All test plates fulfilled the criteria required for their validity to be confirmed. However, it was not possible to differentiate between anti-dengue and anti-JE virus IgG antibodies due to their cross-reactivity.

Statistical analysis

The associations between age, gender, place of residence (Cao Lanh city or surrounding district), highest educational level achieved by the subject (or mother if

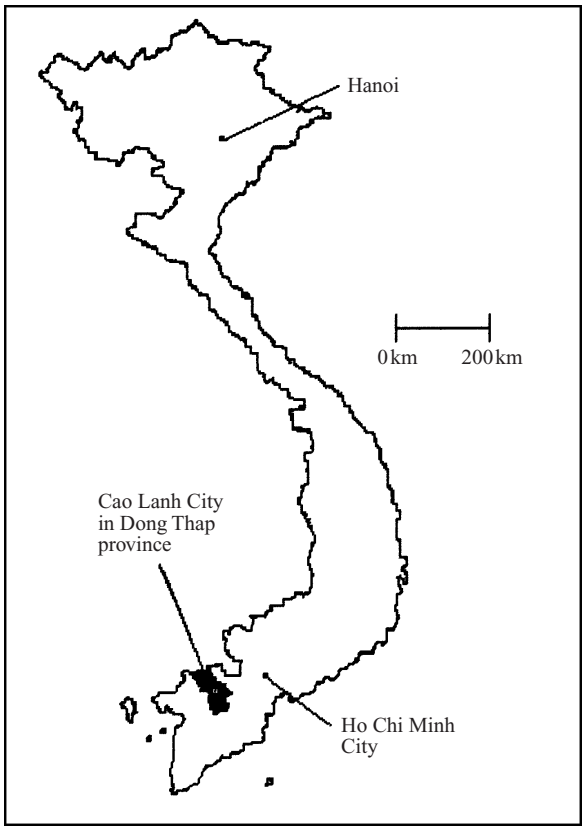

Fig. 1. Map of Vietnam.

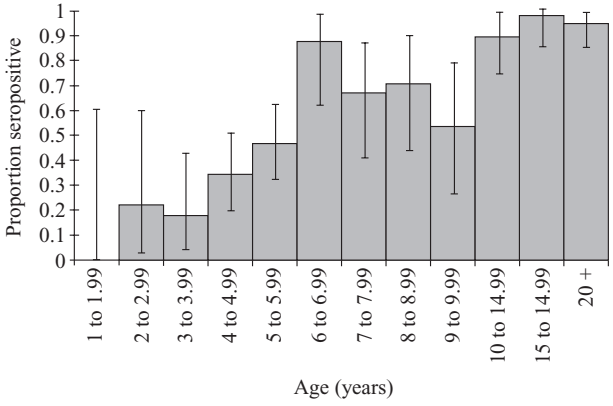

Fig. 2. Flavivirus seroprevalence among study subjects stratified by age. Legend: bars represent exact binomial 95% confidence intervals.

the subject was younger than 16 years old), whether water storage containers at the household were covered, possession of a television in the household, rearing pigs and flavivirus seroprevalence were assessed. Study group (A, B or C) was considered as a potential effect modifier. Subjects were excluded from the analysis if any of these data were missing. Bivariate analysis of the factors involved the calculation of crude PORs and their 95% CIs. Multivariate analysis was performed using logistic regression techniques.

Variables were selected *a priori* according to their biological importance and then on the basis of their strength in the bivariate analysis. Bivariate analyses demonstrated that the prevalence of infection increased with age until the age of 15 years and then reached a plateau (Fig. 2). Therefore, an index variable was created for children who were defined as 15 years or younger. The variable was coded one in children and zero in adults (who were defined as 16 years or older). To model the increase in prevalence with age in children, an interaction between age and the index variable was included. This measured the difference between adults and children (index variable) and the effect of age in children (interaction term). Variable selection for the final model was based on the likelihood ratio test. The goodness-of-fit of this model was assessed according to Hosmer and Lemeshow [19]. All analyses were performed using STATA for Windows 95® [20].

Ethics

The study was approved by the Scientific and Ethical Committee of the Centre for Tropical Diseases, Ho Chi Minh City, Vietnam. Informed consent to participate in the study was obtained from each subject (or from their parents or guardians if the subject was 15 years or younger).

RESULTS

Less than 1% of the screened study population (3 out of 311 subjects) were excluded from the analysis. This included the single infant in the study population who may have possessed maternally derived antibodies to dengue. The remaining two subjects were excluded because of the absence of demographic or socio-economic data. The age range of the final study population was 1 to 59 years, and the male to female ratio was 1·05:1.

Crude seropositivity was 63·2% (95% CI = 54·4, 72·0%), 63·4% (95% CI = 54·5, 72·3%) and 73·2% (95% CI = 63·6, 82·8%) in groups A, B, and C, respectively. The distribution of the socio-demographic factors among the study population is presented in Table 1. Due to similarities in crude seroprevalence, age distribution and other potential risk factors in Groups A and B, these groups were pooled and the original grouping was replaced with two strata

Table 1. *Distribution of socio-demographic factors among the study subjects*

Variable	Number observed	Number seropositive (%)	Number seronegative (%)
N	308	203 (65·9)	105 (34·1)
Age in years*			
Gender			
Males	158	97 (61·4)	61 (38·6)
Females	150	106 (70·7)	44 (29·3)
District			
Cao Lanh City	172	126 (73·3)	46 (26·7)
Surrounding district	136	77 (56·6)	59 (43·4)
Study group			
Group A	114	72 (63·2)	42 (36·8)
Group B	112	71 (63·4)	41 (36·6)
Group C	82	60 (73·2)	22 (26·8)
Highest educational level achieved†			
No schooling	25	18 (72·0)	7 (28·0)
Primary school	178	116 (65·2)	62 (34·8)
High school/College	105	69 (65·7)	36 (34·3)
Whether water storage containers are covered			
Yes	297	195 (65·7)	102 (34·3)
No	11	8 (72·7)	3 (27·3)
Possession of a TV in household			
Yes	138	88 (63·8)	50 (36·2)
No	170	115 (67·6)	55 (32·4)
Pig rearing			
Yes	101	63 (62·4)	38 (37·6)
No	205	139 (67·8)	66 (32·2)

* The average age of seropositives was 5·6 years (standard deviation = 4·2 years) whereas that of seronegatives was 14·3 years (standard deviation = 10·2 years).
† Highest educational level achieved by the subject if 16 years or older, or of mother of subject, if the subject was 15 years or younger.

('hospital sample' and 'community sample'). Bivariate analysis revealed that the associations between the highest educational level reached, possession of a television, pig rearing, covering water storage containers and flavivirus seroprevalence were negligible (Table 1).

Adjusted prevalence odds ratios from multivariate analysis indicated that seroprevalence among children increased substantially with age. Lower seroprevalence was associated with residence in Cao Lanh district compared to that in Cao Lanh city (POR = 0·5, 95% CI = 0·28, 0·89). An interaction between gender and place of sampling occurred such that males sampled from the community had higher seroprevalence compared to those sampled from Cao Lanh hospital (POR = 3·58, 95% CI = 1·45, 8·87). However, this interaction was not observed among the female subjects (POR = 1·07, 95% CI = 0·41, 2·8). Nor was seroprevalence notably different when comparing females to males in the hospital sample (POR = 1·68, 95% CI = 0·85, 3·33) and when comparing those in the community sample (POR = 0·5, 95% CI = 0·16, 1·55). The factors which exhibited weak associations with seroprevalence in the bivariate analysis (Table 1) had negligible associations in the multivariate analysis. The crude PORs and those derived from the final logistic regression model are listed in Table 2. Estimated regression coefficients from the model and their 95% CIs are listed in Table 3.

Table 2. *Crude prevalence odds ratios (and 95% confidence intervals) and those from the final logistic regression model*

Variable	Crude POR (95% CI)	Adjusted POR (95% CI)
Children compared to adults*	0·06 (0·02, 0·17)	NA
Age in children	1·43 (1·26, 1·61)	NA
Age of children compared to being an adult:		
1 year	0·01 (0·003, 0·03)	0·01 (0·003, 0·03)
3 years	0·02 (0·007, 0·06)	0·02 (0·006, 0·06)
5 years	0·04 (0·01, 0·12)	0·04 (0·01, 0·12)
7 years	0·08 (0·03, 0·24)	0·08 (0·03, 0·24)
9 years	0·17 (0·06, 0·52)	0·17 (0·05, 0·52)
11 years	0·35 (0·1, 1·17)	0·34 (0·10, 1·18)
13 years	0·70 (0·18, 2·74)	0·69 (0·17, 2·76)
15 years	1·43 (0·31, 6·56)	1·40 (0·29, 6·64)
Cao Lanh district compared to city	0·48 (0·30, 0·77)	0·50 (0·28, 0·89)
Females compared to males in:		
Hospitalized group	2·20 (1·27, 3·82)	1·68 (0·85, 3·33)
Community group	0·52 (0·20, 1·37)	0·5 (0·16, 1·55)
Community group compared to hospitalized group in:		
Males	3·15 (1·44, 6·85)	3·58 (1·45, 8·87)
Females	0·74 (0·33, 1·64)	1·07 (0·41, 2·8)

* 'Children' defined as subjects 15 years or younger; 'adults' defined as 16 years or older.
NA, not applicable.

Table 3. *Multiple regression coefficients and their 95% confidence intervals from the final logistic regression model*

Variable	Coefficient estimate	95% CI
Constant	2·900	1·772, 4·028
Child or adult*	−4·975	−6·255, −3·695
Child or adult × age	0·354	0·231, 0·477
District	−0·700	−1·283, −0·116
Place where sampled	1·275	0·368, 2·182
Gender	0·520	−0·163, 1·204
Place where sampled × gender	−1·207	−2·525, 0·111

* 'Child' defined as 15 years or younger; 'adult' defined as 16 years or older.

DISCUSSION

To our knowledge, this is the first study in Vietnam to assess the adjusted strength of associations between socio-demographic factors and flavivirus seroprevalence. Dengue and JE virus infection were assumed to be the most likely causes of seropositivity in this population. Antibodies to hepatitis C virus infection could not be detected by the dengue IgG indirect ELISA [21]. Immunization of subjects against JE infection was of little concern because this was not carried out in the study region prior to, or during the study period.

Sixty-six percent (95% CI = 60·6, 71·2%) of the total study population and 54·9% (95% CI = 48·4, 61·4%) of 1–15 year olds were seropositive. The few documented dengue seroepidemiological surveys performed in the region prior to the current study employed the haemagglutination inhibition (HI) test for serological screening. This test is similar to the indirect dengue IgG ELISA since it detects antibodies specific to flaviviruses rather than to dengue alone [22]. The sensitivity of the dengue IgG indirect ELISA in comparison with the HI test was calculated as 99·17% (95% CI = 98·07, 99·73%) and the specificity as 96·18% (95% CI = 93·78, 97·84%), permitting a fair comparison between the current and previous serological studies [18]. A survey conducted in 1986 among healthy 1- to 4-year-olds from Long Xuyen and Can Tho towns yielded seroprevalence estimates of

19·6% (95% CI = 14·6%, 24·6%) and 13·7% (95% CI = 8·3, 19·1%), respectively [4]. These towns are less than 50 km from Cao Lanh city. Our estimate of flavivirus seroprevalence in the same age group was 26·8% (95% CI = 17·7, 35·9%). This higher seroprevalence may reflect in part, an increase in flavivirus transmission between the two study periods. The only JE virus seroprevalence estimate from southern Vietnam in the literature was 72·3% (95% CI = 70·5, 74·1%) and was measured in 1979 in children and adults [4]. However, neither the age distribution nor the serological assay used were reported.

Multivariate analysis of the data from the current study demonstrated that increasing age in children was strongly associated with flavivirus seroprevalence. A similar association (adjusted for other factors) was detected from a serological survey of populations from dengue-endemic regions of Peru [23]. Although other explanatory and potential confounding variables were not considered, an increase in flavivirus seroprevalence with age was also demonstrated in children from Ho Chi Minh City screened in 1964 [24]. Fifty-two percent of subjects aged 1–2 years old were seropositive (95% CI = 41·9, 62·5%) but this increased to 74·6% of subjects aged 6–9 years old (95% CI = 69·9, 79·4%). This and the findings from the current study suggest that flavivirus infection in this region occurs at an early age. If this is the case, dengue and JE virus control efforts should be targeted at young children.

A strong association between place of residence and flavivirus seroprevalence was also identified from the multivariate analysis, the seroprevalence being lower in Cao Lanh district compared to that in Cao Lanh City. Dengue virus transmission has been reported to be higher in urban than in rural areas due to the generation of a higher density of *Ae. aegypti* oviposition sites in the former [25]. Thanh and Giao [7] implicated overcrowding as a contributory factor to the dengue epidemics in Ho Chi Minh City. This may also apply to other cities in southern Vietnam. In consideration of migration of subjects prior to sampling, the most common migratory pattern in Vietnam is from rural to urban environments. Consequently, migration would most likely have reduced the strength of the observed association between place of residence and seroprevalence.

Water storage containers are recognized oviposition sites for *Ae. aegypti* in Vietnam [26]. However, covering these containers had a negligible effect on the flavivirus seroprevalence in the study population. A likely explanation is that only a small number of subjects (11 out of 308) responded negatively when asked whether they cover their water containers. Consequently, the statistical power for analysis of this factor was low.

Possession of a television and education of the subject were included in the analysis because television, radio and newspapers have been used for dengue health education in southern Vietnam for a period of years. Education was also shown to be an important factor contributing to the success of a dengue health education study in Singapore [27]. Rearing pigs was considered because pigs amplify JE virus and transmit it to man via *Culex* species mosquitoes [28, 29]. Despite the potential roles of these factors in flavivirus transmission or its prevention, their associations with flavivirus seroprevalence were negligible. These associations may have been affected by the time of exposure of the subjects to the factors relative to their infection with a flavivirus. It is also possible that health education would have benefited only study subjects who were immunologically naïve when the campaign started, or those born after this time.

The association between gender and seroprevalence within the community sample was also extremely weak. This agrees with the findings from the dengue seroprevalence study conducted in Ho Chi Minh City in 1964 [24] and those from studies in other dengue-endemic areas including Tahiti [30] and China [31].

However, the higher seroprevalence observed in males from the community than in hospitalized males requires further investigation. Reduced immunocompetence of hospitalized males as a result of HIV infection was unlikely to be responsible, since HIV prevalence in Dong Thap province was only 0–0·4% at the time of sampling [32, 33].

The findings from this study provide a basic assessment of the prevalence of flavivirus infection in a population from southern Vietnam. This is an essential step in formulating hypotheses relating to risk factors for infection. The strength of these risk factors must be measured to identify the elements that are modifiable and which should therefore, be targeted when devising control strategies. None of the factors identified for their association with flavivirus seroprevalence (age, gender and place of residence) were modifiable. Nevertheless, the hypothesis that flavivirus infection occurs early in childhood is pertinent to the design of health education campaigns, JE immunization programmes and dengue immunization pro-

grammes in the future. The former control strategies are currently employed in southern Vietnam and future research should be aimed at evaluating their cost-effectiveness.

The advantages of this seroprevalence study were its low cost and practicability. It was limited by the inability of the assay to differentiate between dengue and JE viruses. Differentiation would be possible if using prospective cohort or nested case-control study designs. The risk factors for infection with each virus could then be identified and hence, effective control strategies could be devised.

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