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Spatial distribution and risk factors of dengue and Japanese encephalitis virus infection in urban settings: The case of Vientiane, Lao PDR.

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SUMMARY

Objectives: To evaluate the prevalence of flavivirus infection in Vientiane city (Lao PDR), to describe the spatial distribution of infection within this city, and to explore the link between flavivirus seroprevalence and urbanization levels of residential neighbourhoods.

Methods: A seroprevalence survey was carried out in 2006 including 1,990 adults (≥ 35 years) and 1,568 children (≥ 6 months and < 6 years) randomly selected.

Results: The prevalence of individuals with previous flavivirus infection (i.e. negative for both DEN and JE IgM but positive for DEN IgG) was 57.7%, with a significantly ($p < 0.001$) higher prevalence among adults (84.6%; 95%CI=82.4-86.8) than children (9.4%; 95%CI= 7.2-11.6). The prevalence of individuals with recent flavivirus infection (i.e. positive for DEN and/or JE IgM) was 6.5% and also significantly ($p < 0.001$) higher among adults (10.0%; 95%CI= 8.3-11.7) than children (2.5%; 95%CI= 1.5-3.5). In terms of spatial distribution, IgG prevalence was significantly ($p < 0.001$) higher among individuals living in the central city (60.1%; 95%CI= 56.2-64.1) as compared to those living in the periphery (44.3%; 95%CI=41.5-47.2). In contrast, seroprevalence of recent flavivirus infections was significantly ($p < 0.001$) higher among individuals living in the periphery (8.8%; 95%CI= 6.9-10.7) than in the central city (4.0%; 95%CI= 2.9-5.2). This association was also statistically consistent ($p < 0.01$) in multivariate logistic regression after controlling for individual risk factors indicating that level of urbanization of residential neighbourhoods influences the risk of flavivirus infection.

Conclusions: This study underscores the variable spatial distribution of flavivirus infection even within a small city of less than 300,000 habitants such as Vientiane.

KEYWORDS: Vientiane, spatial distribution, urbanization, residential neighbourhood, serosurvey, Dengue and Japanese encephalitis

INTRODUCTION

Dengue virus (DENV) and Japanese encephalitis virus (JEV) are classified within the Flaviviridae family, genus *Flavivirus*, and are mosquito-borne viruses that cause significant morbidity and mortality worldwide. Dengue fever (DF) is globally one of the most rapidly spreading vector-borne diseases (Rigau-Perez *et al.* 1998; Kyle & Harris 2008). The incidence and severity of DF have been expanding particularly rapidly within south-east Asia, where the number of reported DF cases and deaths increased from 2006 to 2007 by 18% and 15%, respectively (WHO/SEARO 2007), presumably due to global population growth and the associated uncontrolled urbanization (Gubler 1998). Although dengue is an important public health problem in the Mekong basin countries, not much is known on the recent epidemiological profile of the disease (van Panhuis *et al.* 2005). The most important mosquito vector of DENV, *Aedes aegypti*, commonly breeds within household water containers in urban areas, such as jars, drums or tanks. From larvae surveys conducted in Vientiane by the Centre of Malariology, Parasitology and Entomology (CMPE), *Aedes aegypti* appear to be a dominant species in central Vientiane, whereas *Aedes albopictus* is found more commonly on the periphery (B. Sidavong, Centre of Malariology, Parasitology and Entomology in Lao PDR, personal communication). Prevalence of DF is thus impacted by rapid and unplanned urban development, and is also commonly associated with poor water storage and unsatisfactory sanitary conditions (WHO 2007).

JEV circulates throughout Asia and is the leading cause of viral encephalitis, with estimated 35-40,000 cases and 10,000 deaths annually (Halstead & Deen 2002). The predominant vectors of JEV in Asia are rice field breeding *Culex* species (e.g.: *C. tritaeniorhynchus*, *C. vishnui*, *C. gelidus*) and the disease is more frequently associated with irrigated agriculture, pig farming, and rural rather than urban environments. From larvae collection of CMPE, major vector species for Japanese encephalitis appear to be present in Vientiane city.

In the Lao People's Democratic Republic (PDR; Laos), dengue is endemic in most areas of the country. Large epidemics (with more than 7,000 annual cases reported in hospitals) occurred in 1987, 1995, 1996, 1998 and 2003 (WHO DengueNet 2008). Current surveillance for dengue in Laos is limited to hospitalized cases and only a very small fraction is laboratory-confirmed. The system likely suffers from both over-reporting due to clinical mis-diagnosis and under-reporting due to poor access to health facilities and inadequate data collection. Previous epidemiological studies of dengue transmission in Laos have been restricted to small-scale serosurveys (less than 300 individuals) either from hospital patients in Vientiane's Mahosot Hospital in 1990 (Bounlu *et al.* 1992; Fukunaga *et al.* 1993) or from patients in Attapeu province in 2001 (Peyerl-Hoffmann *et al.* 2004). These studies showed evidence for high levels of endemic transmission with 88% and 79% of individuals, respectively, testing positive for dengue IgG.

Here we report the first large-scale serosurvey for flaviviruses conducted in Vientiane city, capital of Laos. Vientiane city is the most populated urban area in the country, and represents 5% of the national population with a density of 3255 people/km². The objectives of the study were to evaluate flavivirus endemicity in Vientiane, to describe the spatial distribution of infection, and to explore the link between flavivirus seroprevalence within the human population and level of urbanization of residential communities.

MATERIAL AND METHODS

Study site and target population

The present study was carried out within the administrative boundaries of Vientiane metropolitan area. Vientiane was estimated at 277,000 inhabitants in 2005. The urban area is delineated into 148 administrative units called «villages» ('ban' in lao). These urban neighbourhoods constituted the primary sampling unit for the randomized cross-sectional seroprevalence survey. Neighbourhoods were classified by level of urbanization using thirteen different human and environmental indicators derived from a 1999 aerial photographic coverage (Rossi *et al.* 2003) and from the 1995 census made by the Lao National Statistical Center (<http://www.nsc.gov.la/>). Three strata were identified: 1) the central zone; 2) the first urbanized belt; and 3) the second urbanized belt (Vallée *in press*). Within each stratum, nine neighbourhoods were selected (i.e. a total of 27 neighbourhoods) as representative of the variability of the global urban population (Vallée *et al.* 2007). In each neighbourhood, households were selected randomly from a list produced with the help of the neighbourhood authorities. The probability of household selection was proportional to the number of eligible people in the household. Within each selected

household, a maximum of one adult (≥ 35 years) and one child (≥ 6 months and < 6 years) were randomly selected and interviewed during February and March 2006. To measure the association of residence with seroprevalence rates, study participants were limited to adults who claimed continued residence in a single neighbourhood of the study area for a minimum of five years, and children who had lived permanently in the same neighbourhood since birth.

Sample size calculations were based on the following formula: $n = t^2 * r * (1-r) / m^2$ (n = number of people to interview per stratum; t = confidence level (95%) then $t = 1.96$; m = precision; r = estimated prevalence). A total sample size of 2000 adults and 2000 children was chosen for budgetary reasons, yielding an estimated precision of 1.3% for a prevalence of 10% at the city scale. The same number of individuals (74 adults and 74 children) was surveyed in each of 27 neighbourhoods, allowing comparisons at the same level of precision (Vallée et al. 2007).

Ethical issues

Informed written consent forms were signed by all participants prior to survey administration and sample collection. Parents or legal guardians were asked to sign on behalf of children. Ethical approval for the study was granted by the Lao National Ethics Committee for Health Research in Lao PDR (no 046) as well as the Oxford University Tropical Research Ethics Committee (no 003-06).

Household and individual survey

Standardized questionnaires were administered to assess demographic and socioeconomic information from the study population and specific data on urban lifestyle variables, and morbidity within the last three months. Questionnaires were first validated in two stages by independent back-translation to compare the source with the translated text in Lao language, and secondly by pretesting on 35 Lao Vientiane residents.

A household deprivation index was synthesized from household data (e.g. house building materials, access to running water, types of cooking energy, possession of motorbike, car, refrigerator, washing machine and computer).

Human serological testing

The seroprevalence survey took place in February and March 2006, during the dry season (rains in Laos generally last from May to October). Blood samples of approximately 25 μ l were collected by fingerprick and absorbed on filter papers (Whatman, ProteinsaverTM) for subsequent analysis as previously described (Parker & Cubitt 1999). Samples were stored at room temperature for one month until receipt at the laboratory in Bangkok, where they were placed at -80°C for storage until processing.

Laboratory analyses

Filter paper blood samples were cut and calibrated using a 0.5 cm diameter punch. For each sample, two discs were eluted in 800 μ l of PBS buffer (5% Nonfat Dried Milk/Phosphate-buffered Saline). After vortexing (1 min), samples were incubated for one hour at room temperature, vortexed again, and then centrifuged for 10 min at 10,000 rpm at 4°C. Supernatants were recovered corresponding to 1:100 dilution of original serum samples. DEN and JE IgM MAC-ELISA, and DEN indirect IgG ELISA were performed as previously described (Kuno *et al.* 1991) using sucrose-acetone extracted viral antigens and commercially available mouse anti-virus monoclonal antibodies (Center for Vaccine Development, MU, Thailand) (Tuntaparsat *et al.* 2003). For IgM detection, samples were considered positive with a DOD value > 0.5 . For IgG, samples were considered positive for a DOD value > 2 . Serum samples positive for DEN IgM and/or JE IgM were scored as 'recent flavivirus infection'.

A more precise interpretation was also made: samples were scored (1) as 'recent dengue infection' if they were positive for DEN IgM and negative for JE IgM, (2) as 'recent JE infection' if positive for JE IgM and negative for DEN IgM and (3) as 'recent undetermined flavivirus infection' if they were positive for both DEN and JE IgM. Serum samples negative for both DEN and JE IgM but positive for DEN IgG were scored as 'previous flavivirus infection'.

Spatial and statistical analysis

Proportions and their 95% confidence intervals (CI) were calculated taking into account the two-stage sample design. Differences in seropositivity among adults and children and among Vientiane urban areas were calculated by Pearson chi-squared test. A *p* value < 0.05 was considered statistically significant. The associated factors with recent and previous flavivirus infection were assessed using multivariate logistic regression for adults and children separately. Odds ratios (OR) and their 95% CI were calculated. Multivariate logistic regressions were performed using Intercooled® Stata 8.0 (Stata Corporation, College Station, Tx, USA) that fits a random-effect logit model at the neighbourhood scale. Maps were generated using Geographic Information System (GIS) ArcGis® 8.3 software (ESRI, USA).

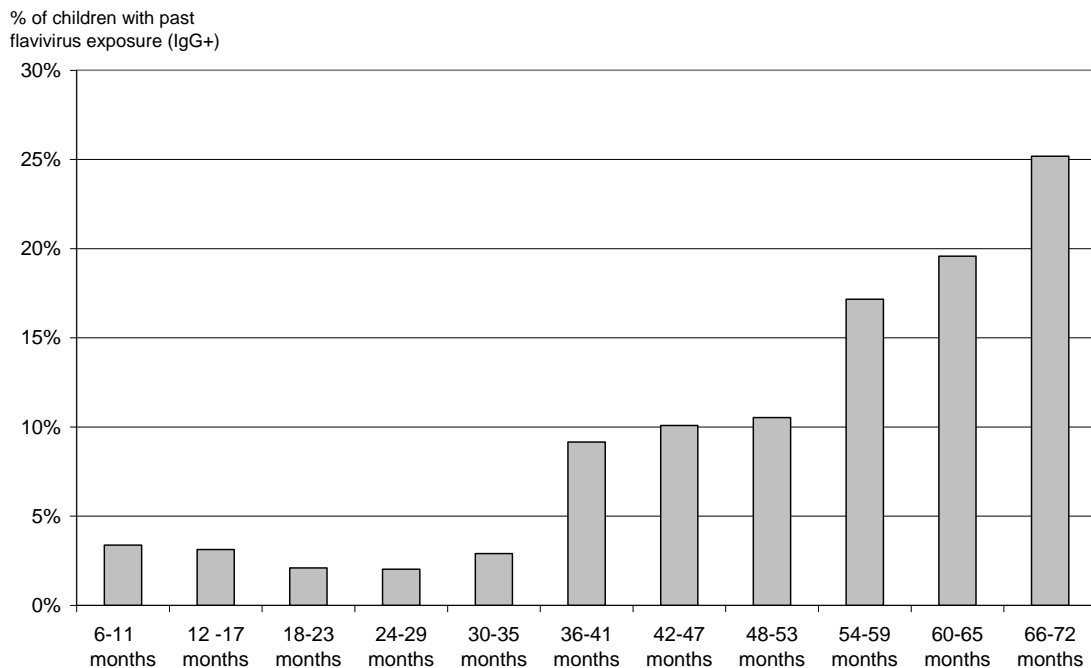
RESULTS

A total of 3692 individuals were interviewed during February and March 2006 and 3634 blood samples were taken. 76 samples were omitted from analysis due to inadequate specimen sampling and/or registration. Data from 3558 participants (1990 adults and 1568 children) were included in the study. Among the survey participants, males and females were equally represented in all defined age classes.

Flavivirus seroprevalence

The overall prevalence of previous flavivirus infections (i.e. positive for DEN IgG but negative for both DEN and JE IgM) among survey participants was 50.2% (95% CI= 48.4-52) with significantly (*p*<0.001) higher prevalence among adults (84.6%; 95% CI= 82.4-86.8) than children (9.4%; 95% CI= 7.2-11.6) (Table 1). Seropositivity for flavivirus reacting IgG antibody was found in children as young as 6 months and showed significant increase with age, reaching 25% by 6 years of age (Figure 1). Seroprevalence did not appear to be related to sex within any of the age groups.

Figure 1. Percentage of flavivirus antibody reacting sera (IgG tested by ELISA) detected among children under six years old (by 6 month age classes), Vientiane city, 2006.



The total number of recent flavivirus infections (i.e. IgM positive) was 232 cases (6.5%), including a majority of DEN infections (126 cases, 3.5%) and a lesser number of JE infections (78 cases, 2.1%). The prevalence of recent flavivirus infection was significantly higher (*p*<0.001) among adults (10.0%; 95% CI= 8.3-11.7) than children (2.5%; 95% CI= 1.5-3.5) (Table 1).

Table 1. Flavivirus antibody reacting sera in a representative population of Vientiane city, 2006.

	Adults n = 1990		Children n = 1568		p
	No. (%)	95% CI	No. (%)	95% CI	
Previous flavivirus infection*	1686 (84.6)	82.4-86.8	147 (9.4)	7.2-11.6	<0.001
Recent flavivirus infection**	195 (10.0)	8.3-11.7	37 (2.5)	1.5-3.5	<0.001
Positive Dengue IgM only	99 (5.1)	3.8-6.4	27 (1.9)	1.1-2.7	<0.001
Positive for Japanese encephalitis IgM only	70 (3.6)	2.7-4.4	8 (0.5)	0.1-0.8	<0.001
Positive for both Dengue and JE IgM	26 (1.0)	0.6-1.4	2 (0.1)	0-0.3	0.002

* negative for both DEN and JE IgM but positive for DEN IgG

** positive for DEN and/or JE IgM

Note: Proportions and their 95% confidence intervals (CI) were performed taking into account the two-stage of sample design

Clinical manifestation

Adults testing IgM positive did not report increased health problems in the retrospective morbidity survey as compared to IgM negative individuals. Among the 195 adults testing positive for IgM, 86 (44%), 96 (49%) and 20 (10%) reported dizziness, headache, or severe fatigue, respectively, within the last three months, which was not significantly different from levels reported by IgM negative adults. Taking into consideration only the 94 IgM-positive adults who declared health problems in the two weeks prior to the survey, 8 (8.5%) reported that they sought health care, but none were diagnosed as suffering from dengue or Japanese encephalitis virus infection.

Spatial distribution of seroprevalence

High levels of previous flavivirus infections were detected throughout the studied area indicating a high exposure to flavivirus (>40%) in all sampled neighbourhoods (Figure 2). However, the prevalence of previous flavivirus infections was significantly ($p < 0.001$) higher in the central zone (60.1%; 95% CI=56.2-64.1) as compared to the first (51.1 %; 95% CI=48.7-53.5) and second (44.3%; 95% CI=41.5-47.2) urbanized belts (Table 2). In contrast, seroprevalence of recent flavivirus infections was significantly ($p < 0.001$) higher among people living in the periphery than in the central zone: 4.0% IgM positive in central zone (95% CI= 2.9-5.2) as compared to 5.4% (95% CI=3.7-7.1) and 8.8% (95% CI=6.9-10.7) for first and second urbanized belts, respectively (Table 2).

Table 2. Percentage of Flavivirus antibody reacting sera of the study population (Adults and Children) according to the level of urbanization of their living place within Vientiane city, 2006.

	Central zone n =1031		First urbanized belt n=1203		Second urbanized belt n=1324		p
	No. (%)	95% CI	No. (%)	95% CI	No. (%)	95% CI	
Previous flavivirus infection*	626 (60.1)	56.2-64.1	621 (51.1)	48.7-53.5	586 (44.3)	41.5-47.2	<0.001
Recent flavivirus infection**	43 (4.0)	2.9-5.2	72 (5.4)	3.7-7.1	117 (8.8)	6.9-10.7	<0.001

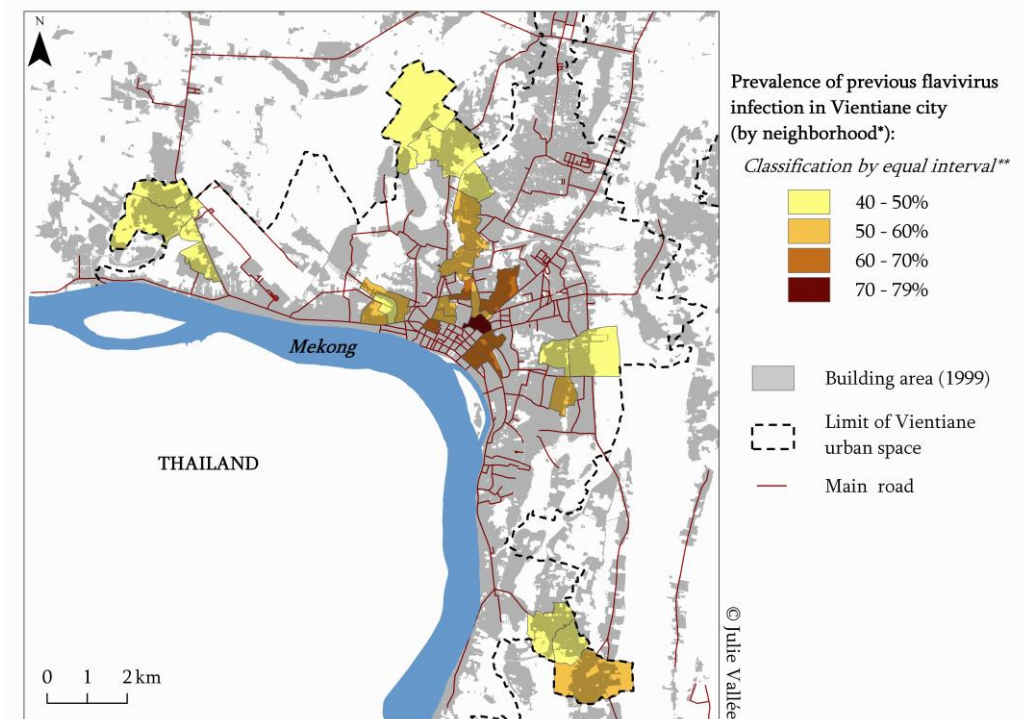
* negative for both DEN and JE IgM but positive for DEN IgG

** positive for DEN and/or JE IgM

Note: Proportions and their 95% confidence intervals (CI) were performed taking into account the two-stage of sample design

Recent flavivirus infections were detected more frequently in a few neighbourhoods of the western and northern outskirts of Vientiane with a minor difference in spatial distribution of recent dengue and JEV infection: the three neighbourhoods in the northern outskirts of Vientiane had a prevalence of JE infection higher than city overall prevalence but a prevalence of recent dengue infection lower than city overall prevalence (Figure 3).

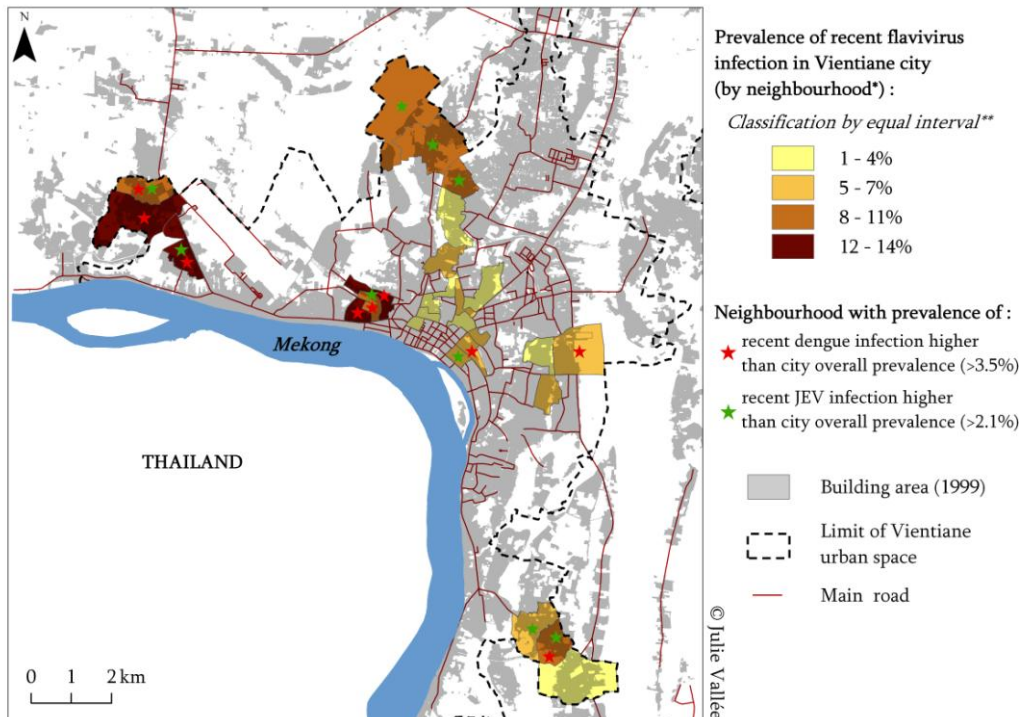
Figure 2. Spatial distribution of previous flavivirus infections (adults and children IgG positive) in Vientiane city in 2006.



* The « neighbourhood » is the primary administrative unit in Laos and constituted the primary sampling unit of the seroprevalence survey within Vientiane

** Equal Interval Classification method divides a set of attribute values into groups that contain an equal range of values
 Note: Cartographic files from "Atlas Infographique de Vientiane" (Rossi *et al.* 2003)

Figure 3. Spatial distribution of recent flavivirus infections (adults and children IgM positive) in Vientiane city in 2006.



* The « neighbourhood » is the primary administrative unit in Laos and constituted the primary sampling unit of the seroprevalence survey within Vientiane

** Equal Interval Classification method divides a set of attribute values into groups that contain an equal range of values

Note: Cartographic files from "Atlas Infographique de Vientiane" (Rossi *et al.* 2003)

Individual and environmental risk factors

In multivariate analysis, no significant associations between seroprevalence and sex, education level or household deprivation were found (Table 3). However, adults with a longer residence in Vientiane were at significant greater risk for past flavivirus infection (OR = 1.3; 95%CI= 1.0-1.7) and a lower risk for recent infection (OR = 0.7; 95%CI= 0.5-1.0) than those with a shorter residence (<2/3rds lifetime spend in Vientiane). After accounting for individual and household risk factors, urbanization level remained significantly linked to risk of transmission: adults living in neighbourhoods in the central zone were at greater risk for past flavivirus infection (OR = 2.2; 95%CI= 1.6-3.0) and at lower risk for recent infection (OR = 0.3; 95% CI= 0.2-0.6) than those living in neighbourhoods from the second urbanized belt (Table 3).

Table 3. Analysis of individual, household and environmental factors regarding flavivirus infection in adults, Vientiane city, 2006

Risk Factor	Odd Ratio [IC 95 %] / p	
	Previous flavivirus infection*	Recent flavivirus infection**
Male (Versus Female)	1.0 [0.7-1.2] / >0.10	0.9 [0.6-1.3] / >0.10
55 - 64 years old (Vs. 35 - 54 yrs)	1.2 [0.9-1.7] / >0.10	0.8 [0.5-1.2] / >0.10
65 years old up (Vs. 35 - 54 yrs)	1.1 [0.7-1.6] / >0.10	0.9 [0.5-1.4] / >0.10
Primary school (Vs. No education)	0.8 [0.5-1.3] / >0.10	1.1 [0.6-1.9] / >0.10
Secondary school up (Vs. No education)	0.9 [0.6-1.6] / >0.10	1.1 [0.6-2.0] / >0.10
Household with high income (Vs. middle and low)	1.2 [0.8-1.7] / >0.10	0.9 [0.7-1.2] / >0.10
> 2/3rds lifetime spend in Vientiane (Vs.< 2/3rds)	1.3 [1.0-1.7] / 0.03	0.7 [0.5-1.0] / 0.03
1st Urbanized Belt (Vs. 2 nd Belt)	1.8 [1.3-2.4] / <0.01	0.6 [0.4-1.0] / <0.01
Central Zone (Vs. 2 nd Belt)	2.2 [1.6-3.0] / <0.01	0.3 [0.2-0.6] / <0.01

* negative for both DEN and JE IgM but positive for DEN IgG

** positive for DEN and/or JE IgM

DISCUSSION

Intensity of flavivirus transmission in Vientiane

This study revealed the classic linear association between age and increasing IgG antibody prevalence as expected for areas of endemic flavivirus transmission. Seroprevalence among children (6 months - 6 years) was 9.4% and reached 25% by 6 years of age, confirming relatively high levels of endemic transmission within Vientiane. However, the Vientiane seroprevalence rate is lower than rates found in two similar serosurveys conducted in southeast Asia, such as a study in southern Vietnam (Binh Thuan Province) showing 53% dengue IgG prevalence among children of 7 years (Thai *et al.* 2005), or a study from Thailand (Mueang district, Ratchaburi Province) showing 69% dengue IgG prevalence among children (5-12 years) (Tuntaparsat *et al.* 2003).

The IgG seroprevalence of Vientiane adults aged > 35 years (84.6%) was similar to past prevalence estimates of 86.4% (78/94) in Attapeu province in 2001 (Peyerl-Hoffmann *et al.* 2004). Interestingly, our estimates of IgG seroprevalence among children (9.4%) are lower than those reported by Bounlu *et al.* 1992 and Fukunaga *et al.* 1993. However, given the differences in study design, inferences regarding changes in flavivirus transmission intensity based on these studies alone are not warranted. Our 2006 estimates of seroprevalence were based on randomly sampled healthy individuals within the 3 strata sampling design, whereas the 1990 estimates were based on patients presenting to health centers.

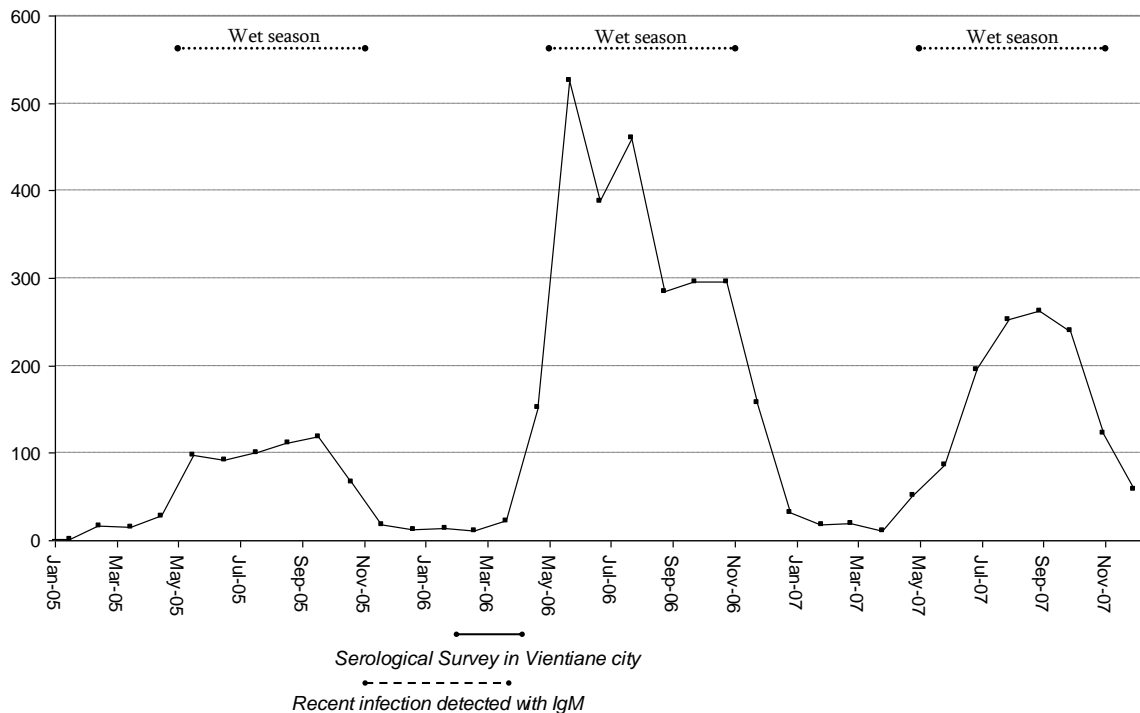
Flaviviruses other than dengue and JEV viruses (such as yellow fever virus and West Nile virus) may be responsible for the IgG positivity. Discriminating the previous infecting virus in IgG-positive sera is complicated by serological cross-reactivity across the flavivirus group.

Distinguishing between past infections with dengue or JEV would typically require plaque reduction neutralization tests, which was not feasible within the limitations of this study. However, our analysis of IgM results does suggest that transmission of dengue virus was more frequent than transmission of Japanese encephalitis virus in Vientiane in early 2006, since DEN infections (ie anti-dengue IgM positive) were more frequent (126 cases, 3.5%) than JE infections (78 cases, 2.1%). This result confirms and extends previous findings of Bounlu *et al.* 1992 and Fukunaga *et al.* 1993. In addition, our results suggest possible differences in the spatial distribution of recent dengue and JEV infections within Vientiane city.

Reported dengue cases in Vientiane hospitals

The number of dengue cases reported monthly by Vientiane hospitals in 2005 and 2006 may be used to put results from our seroprevalence survey into their temporal context. The survey was conducted during the dry season of 2006, when approximately 20 clinical cases (without systematic laboratory confirmation) were registered per month in hospitals of Vientiane Municipality. In contrast, during the previous wet season from May to October 2005, the average number of reported clinical cases was 100 cases/month (Figure 4).

Figure 4. Dengue case monthly report among Vientiane Municipality hospitals between 2005 and 2007



Source: Epidemiology Section, National Center for Laboratory and Epidemiology, Ministry of Health

Taking into account that IgM antibodies persist two or three months after infection (Gubler, 2008) and that the wet season ends in October, it is of interest to note that 6.5% of survey participants remained IgM positive at the time of sampling (February and March 2006). This suggests moderate levels of sustained flavivirus transmission throughout the dry season (Figure 4).

The majority of recent flavivirus infections detected in this survey may have been subclinical, as evidenced by the lack of individual recall of symptoms, and the relatively few individuals (8/94) who sought health care. The ratio of symptomatic to asymptomatic cases of dengue within endemic regions is difficult to measure and varies considerably according to studies. Burke *et al.* performed a prospective cohort study of school students living in Bangkok, Thailand and found a vast majority (87%) of either asymptomatic or minimally symptomatic cases (Burke *et al.* 1988). In a more recent study among schoolchildren in Thailand (Kamphaeng Phet Province) Endy *et al.* concluded that inapparent and symptomatic dengue infection occurred with nearly equal incidence (Endy *et al.* 2002).

Urban residence and flavivirus transmission

Due to the absence of similar seroprevalence surveys in other parts of Laos, it is not possible to contextualize our results on a national level. However, we observed that long-time inhabitants of Vientiane were more at risk for past infection than those living for a short time in the city, regardless of age and location of residence within the city. We also observed that people living for a short time in the city were more at risk for recent infection than long-time inhabitants of Vientiane. The prevalence of IgM positive cases was indeed higher among people living for a short time in Vientiane: 11.1% of adults living in Vientiane for less than two third of life time were IgM-positive, whereas 8.2% of those living in Vientiane for more than two third of life time were IgM-positive. This relationship held true even after controlling for individual, household and environmental risk factors. The reasons for this relationship are unclear. One possibility is that newcomers to Vientiane have settled in places particularly favourable for peridomestic mosquitoes breeding, such as areas with poor waste disposal and relatively poor sanitary conditions. An additional complementary hypothesis is that immunity of recent newcomers in Vientiane is lower than immunity within long-term residents, as was suggested by the high prevalence of IgG positive cases amongst long-time inhabitants of Vientiane.

Spatial distribution of recent and past flavivirus infections within Vientiane

Our study reveals an interesting contrast between the spatial distribution of past versus recent flavivirus infections; IgG-positive individuals were found more frequently in the city centre (Figure 2), whereas IgM-positive individuals were found more frequently in the periphery (Figure 3). This spatial differentiation cannot be explained by length of residence within the city or by other individual risk factors, because the relationship between urbanization and flavivirus infection remained significant in the multivariate logistic regression even after controlling for these variables.

The outward expansion of Vientiane city may explain why adults living in neighbourhoods within the central zone were at greater risk for past flavivirus exposure and at lower risk for recent infection than those living in neighbourhoods from second urbanized belt. We hypothesize that flavivirus transmission rates may have been higher within the city center of Vientiane during previous decades, but that in recent years the intensity of transmission has been relatively higher in the neighbourhoods of the periphery. In the past, the 'central zone' was characterized by poor water storage and unsatisfactory sanitary conditions that were favourable for mosquito breeding. The central zone has since undergone remarkable transformation. Currently, major environmental transformations are occurring in the periphery: what we call the 'second urbanized belt' has now become favourable to mosquito breeding. This view is supported by the 1994 study of Sisouk *et al.* that reported an outbreak of DF/DHF for the first time in a rural neighbourhood (in Hatxaifong district) of Vientiane municipality (Sisouk *et al.* 1995). Historically, DF has been reported as occurring predominantly among urban populations. However, contemporary literature shows that dengue transmission occurs also in rural settings in both Asia and Latin America (Guha-Sapir & Schimmer 2005). Today, Thailand has an incidence rate that is higher in rural than urban areas (Chareonsook *et al.* 1999).

One important assumption of our study is that flavivirus infections were most likely to occur near the home residence, or within the individual's residential neighbourhood. We are aware that inhabitants could have become infected in other locales outside their residential neighbourhood (i.e. place of work, leisure, travel etc.) or at one of their former places of residence. Unfortunately, our study did not permit controlling for spatial variability on such a fine scale. Our findings, however, clearly demonstrate a non-random variation in seroprevalence between residential neighbourhoods. Furthermore, level of urbanization and length of residence were the two most significant factors in predicting individual risk of flavivirus infection. We conclude there is a significant association between flavivirus seroprevalence and urbanization within Vientiane city, and suggest the need for additional studies to further explore the spatial distribution and public health significance of flavivirus transmission in urban and rural settings of Lao PDR.

Authorship statement: JV performed the sample design, organized the seroprevalence survey and conducted spatial and statistical analysis; ADP and JPG organized serological data analysis; PO and CS kindly provided dengue surveillance data from Vientiane; JV, ADP, JEB and JPG drafted the manuscript. All authors read and approved the final manuscript.

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