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นิพนธ์ต้นฉบับ

Serological survey of dengue infections among individuals in Rayong, Thailand

(การสำรวจความชุกของภูมิคุ้มกันต่อไวรัสเด็งกี่ของประชากรในจังหวัดระยอง ประเทศไทย)

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Abstract

Background: Dengue diseases have been a major public health problem in Thailand over the past 50 years. Clinical dengue has traditionally affected children with rare cases among adults. Even though the incident number of cases does not seem to have decreased, a shift towards older age groups has been observed over the past years. The reasons for this shift have not been elucidated.

Methods: The age-stratified serological study conducted among school aged children living in the district of Mueang district in Rayong, Thailand. Schools and classrooms were sampled probabilistically from all schools serving the district. Samples were analyzed using single dilution neutralization testing. We estimated and compared the age-specific seroprevalence for the two serosurveys in 1980 and 2010. To explore the factors associated with seropositivity in 2010, we fit a mixed-effects model with a random intercept for school.

Results: A total of 1,811 children, from 90 classes in 25 schools participated in the study. The overall response rate for the study was 53%. Overall, 69% of the sampled children showed evidence of prior exposure to Dengue virus and 46% of the samples showed immunological evidence of exposure to Japanese encephalitis. The age-specific seroprevalence to dengue virus in the 2010 serosurvey as compared to the 1980 serosurvey while according to the survey conducted in 1980, 96% of the population had been exposed to dengue by age 11 years, the 2010 survey suggests that only 74% of children aged 11 years a have been exposed. By age 18 years, 16% of the population remains susceptible to dengue virus. As expected, age was the factor most strongly associated with seropositivity in all analyses (OR 1.16, 95%CI 1.10, 1.23). Attending one of the schools in the single industrial subdistrict was also associated with a significantly greater risk of seropositivity (OR 3.17, 95%CI 1.51, 6.67). Although there was a trend towards a negative association

between owning a car/motorbike and seropositivity (OR 0.48, 95%CI 0.22, 1.02).

Discussion: Our results, from two studies conducted in the same location, 30 years apart, and show that underlying this shift there has been a significant increase in the age at exposure to dengue virus. Multiple factors might be responsible for the changes in age at exposure. A decrease in the hazard of infection may indicate decreased contact with the mosquito, as a result of decreasing vector populations or to improved housing.

Keywords: dengue, seroprevalence, school, Thailand

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Introduction

Infection by dengue virus continues to cause significant morbidity in Thailand; the number of cases reported during 2010 was 48,514, and of these 25,661 were cases of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). However, even though the number of cases has remained high, there has been a significant shift in the age distribution of dengue fever (DF) and DHF/DSS cases over the last 30 years. [1-3] While in the 1980's the mean age of clinical cases was 8-10 years of age, the mean age of cases reported during more recent years has increased to 18 years. Similar shifts have been described in other Southeast Asian countries. [4, 5]

For most infectious diseases, an increase in the mean age of cases can be interpreted as a decrease in the hazard of infection (transmission intensity) leading to exposure later in life. This is the case of many childhood diseases such as measles and rubella, in which the age distribution of cases can be expected to be representative of the age distribution of infections. [6] However, for diseases where the probability of clinical disease after infection depends on age, inference from age distribution of cases can be misleading. In such instances, age at exposure can only be determined through age stratified serological surveys.

Dengue virus consists of four closely related, but genetically distinct serotypes. [7] Infection by one serotype confers lifelong immunity against infection by that same serotype but seems to enhance subsequent heterologous infections. [7, 8] The spectrum of disease ranges from asymptomatic and mild febrile illness (DF) in the majority of infections, to severe hemorrhagic fever (DHF) in a varying proportion of cases. [9,10] [11] Even though secondary infection is considered to be the main risk factor for severe disease, the role of other factors such as age, sequence of infecting serotypes and viral factors is not clear. [9] The association between age, risk of infection and clinical disease has been particularly elusive; in many hyperendemic settings severe dengue occurs almost exclusively in children but this age range coincides with that of secondary infections. In addition, some studies have suggested that primary infection might be more severe when it occurs among adults.

Understanding whether the epidemiologic shift that is being observed in Thailand and much of Southeast Asia reflects an increase in the age at exposure has implications for clinical practice and public health. The shift could certainly reflect a decrease in the hazard of infection, but changes in the surveillance patterns and/or in the virus itself could potentially have generated similar phenomena. Insight on the nature of the shift and of the factors that might be driving it is fundamental in order to adequately plan future control interventions.

An age-stratified, school based serological survey was conducted in Mueang district, Rayong, Thailand, in 1980 as part of an ongoing cohort study.[12] Blood samples were collected from 1,009 children aged 0-11 years and tested for dengue neutralizing antibodies. We conducted a new serosurvey, in the same location, in December 2010. To make the results comparable, our study was designed to resemble the original serosurvey.

Methods

Study design

School based age-stratified serological survey conducted in Mueang district, Rayong province, Thailand. Serum samples were obtained from children aged 4-18 years in eligible schools and classes and tested for neutralizing antibodies against dengue virus.

Study setting

Rayong province, located in southeast Thailand, is hyperendemic for dengue and has historically reported one of the highest number of cases in Thailand. In 2010, the incidence of dengue was 202.9/100,000 persons and of these, 111.7/100,000 persons were cases of DHF Mueang district is the provincial capital, with a total area of 514.5 km^2 and a population of 243,502 (2010). [13] There are 68 schools in the district.

The Thai school system consists of 14 basic years (2 pre-primary, 6 primary levels and 6 secondary levels). It is estimated that over 95% of children attend to primary grade 6 or higher.

Participants

We obtained a two-stage probabilistic sample of schools and classes. After selecting schools (primary sampling unit), we obtained a sample of classes (secondary sampling unit), stratified by grades. Figure 1 shows the location of the participating schools.

All children enrolled in the selected classes and aged 4-18 years were invited to participate. Exclusion criteria included medical conditions that precluded blood sample collection and inability to give assent Signed consent was obtained from parents during information meetings and by sending information packages home prior to sample collection.

Study procedures

Participants were asked to provide a 3 mL of peripheral venous blood sample and to complete a brief questionnaire that asked for basic demographic (e.g. age, sex) and socioeconomic (e.g. household characteristics) information. All study procedures were completed during a single visit.

Blood samples were collected in anticoagulant-free Vacutainer tubes and transported to Rayong hospital where they were centrifuged within 4 hours of collection. Samples were then transported to AFRIMS, Bangkok, and stored at -20°C until serological testing. Prior exposure to dengue virus was measured using the single dilution neutralization test (SDNT).

Single dilution neutralization testing

A detailed description of the SDNT assay used is available in the study.[14] Briefly, serum samples were considered positive for dengue neutralizing antibodies if they neutralized > 50% of plaques at a single 1:10 dilution. This dilution/neutralization level has been shown to be optimal to differentiate people who have been exposed to dengue from those who are immunologically naïve, but is suboptimal for classifying homotypic vs heterotypic immunity.

Statistical analyses

Characteristics of participants were compared using tests of equality of proportions and t-tests. To compare age to exposure in 1980 and 2010, we estimated and compared the age-specific seroprevalence for the two serosurveys. To explore the factors associated with seropositivity we fit a mixed-effects model with a random intercept for school. All statistical analyses were performed in STATA IC/10.1.

Ethical review

The protocol for this study was reviewed and approved by the institutional review boards of the Institute for the Development of Human Research Protection (IHRP), Thailand on 17th March 2010. All of the samples were collected in December 2010.

<u>Results</u>

<u>Characteristics of the sample</u>

A total of 1,811 children, from 90 classes in 25 schools participated in the study. Three additional selected schools refused to participate. Enrolled schools were located in 9/15 of the subdistricts within Mueang district representing urban, rural and industrial areas. Figure 2 shows the locations of the enrolled schools.

The overall response rate for the study was 53%. Participation rates varied between schools and grades and were lowest among children enrolled in the two grades of pre-primary education (26% and 24% respectively). Due to the low response rate, children in these two grades were not included in statistical analyses.

Table 1 summarizes the characteristics of participants. The majority of children (n=1,016, 56%) were enrolled in urban schools. The proportion of females enrolled was higher in rural and industrial schools (57% and 57%, respectively) than in urban schools (51%). Reported access to electricity was universal (>99% of the enrolled children reported having electricity at home), but only 82% of children reported tap water at home. The reported access to tap water was significantly lower in children enrolled from rural and industrial settings (72% and 75%, respectively) than in children enrolled in urban schools (88%). Children enrolled in the single industrial subdistrict reported having lived in that particular location 2.8 years less than children from urban subdistricts, and this was significant even after adjusting for age.

<u>Prior exposure to dengue virus</u>

Overall, 69% of the sampled children showed evidence of prior exposure to dengue virus. And 46% of the samples showed immunological evidence of exposure to Japanese encephalitis, but this result has to be interpreted with caution due to known cross-reactivity with other flaviviruses. Figure 3 shows the age-specific seroprevalence to dengue virus in the 2010 serosurvey as compared to the 1980 serosurvey. While according to the survey conducted in 1980, 96% of the population had been exposed to dengue by age 11 years, the 2010 survey suggests that only 74% (95%CI 61-87%) of children aged 11 years a have been exposed. By age 18 years, 16% (95%CI 0-32%) of the population remains susceptible to dengue virus.

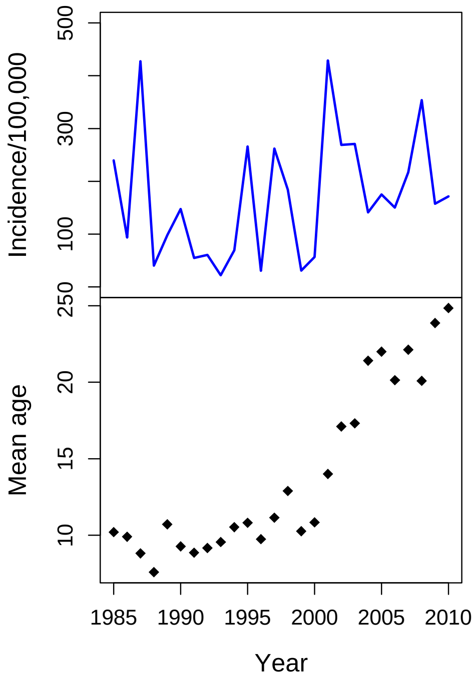

Figure 1: Dengue incidence and mean age of dengue cases in Rayong, 1985-2010

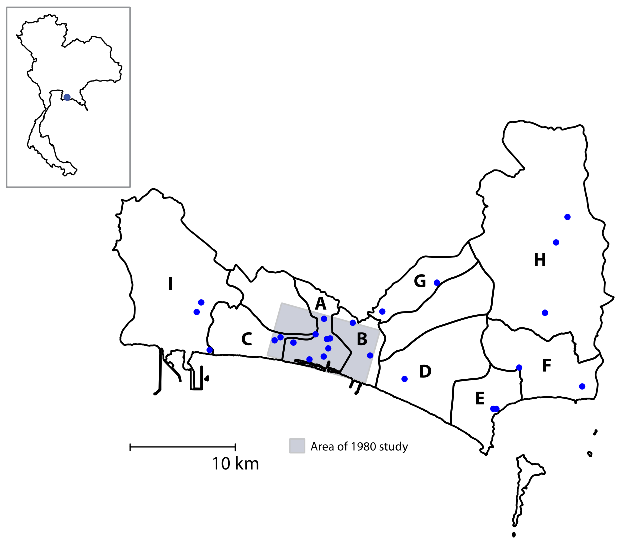

Figure 2: Map of Rayong showing location of schools, areas, and area of original study

<u>Factors associated with seropositivity</u>

As expected, age was the factor most strongly associated with seropositivity in all analyses (OR 1.16, 95%CI 1.10, 1.23). Attending one of the schools in the single industrial subdistrict was also associated with a significantly greater risk of seropositivity (OR 3.17, 95%CI 1.51, 6.67). Although there was a trend towards a negative association between owning a car/motorbike and seropositivity (OR 0.48, 95%CI 0.22, 1.02), none of the socio-economic characteristics measured were significantly associated at the 5% significance level. There was significant heterogeneity in seropositivity between schools. After adjusting for age and other individual level covariates, differences between schools accounted for 10% (95%CI 5%, 9%) of the variance in seropositivity to dengue. Including school-level covariates, in particular whether the school was urban, rural or industrial, accounted for part of this variance but differences between schools still accounted for 5% (95%CI 2%, 10%) of variance in the outcome. The fit of the model did not improve by including additional levels of clustering, such as the specific class or the subdistrict of the school.

Table 1: Characteristics of study participants

Characteristics	Urban *(A, B, C, F)*	Rural *(B, D, E, G, H)*	Industrial *(I)*	All
No. Children	1016	564	228	1808
No. Schools	16	9	3	25
No. Subdistricts	4	5	1	9
Mean age (se)	13.1 (.11)	11.7 (.15)	10.8 (.23)*	12.3 (.09)
Female% (n)	51 (522)	57 (324)**	57 (129)	54 (975)
Years in location (se)	9.5 (.2)	8.1 (.2)*	6.7 (.3)*	8.7 (.2)
Household Characteristics				
Median no. of members (range)	4 (1-11)	4 (1-10)	4 (2-10)	4 (1-11)
Electricity% (n)	100 (1016)	99 (560)**	100 (228)	99.8 (1804)
Tap Water% (n)	88 (896)	72 (409)**	75 (171)**	82 (1476)
Auto/motorbike% (n)	97 (989)	96 (543)	96 (218)	97 (1750)

3 children excluded from analysis because of insufficient/damaged sample

** p<0.05 as compared to Urban (reference) in t-test*

*** p<0.05 as compared to Urban (reference) in test of equality of proportions*

Table 2: Factors associated seropositivity in school-age students, Mueang district, Rayong, Thailand, 2010

Factors	Univariate model		Adjusted (individual-level model)		Adjusted Model (Full-model)	
	Hazard Ratio	95% CI	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Individual-level predictors						
Age in years	1.16	1.09-1.23	1.17	1.10-1.25	1.16	1.10-1.23
Female	1.27	1.01-1.59	1.21	0.96-1.51	1.19	0.95-1.49
Number of household members	1.09	0.99-1.15	1.07	0.99-1.16	1.07	0.99-1.16
Auto/motorbike	0.48	0.23-1.01	0.48	0.22-1.02	0.48	0.22-1.02
Electricity	1.13	0.15-8.74	2.33	0.29-18.60	2.19	0.28-17.4
Tap Water	0.81	0.60-1.10	0.84	0.62-1.14	0.84	0.62-1.14
Years in location	1	0.97-1.02	0.98	0.96-1.01	0.98	0.96-1.01
School-level predictors						
Private School	0.83	0.29-2.46			0.87	0.41-1.83
Location of school						
Urban	*Ref*	-			*Ref*	
Rural	0.82	0.47-1.43			0.79	0.50-1.27
Industrial	3.83	1.61-9.12			3.17	1.51-6.67

Discussion

Thailand and several Southeast Asian countries have experienced a dramatic shift in the age distribution of dengue cases over the last 30 years. While traditionally severe dengue affected young children, during recent years cases have become increasingly common among adolescents and young adults [3,4], in spite of a roughly constant incidence. Our results, from two age-stratified serological studies conducted in the same location, 30 years apart, and show that underlying this shift there has been a significant increase in the age at exposure to dengue virus. Multiple factors might be responsible for the changes in age at exposure. A decrease in the hazard of infection may indicate decreased contact with *Aedes aegypti*, the principal vector involved in dengue transmission, as a result of decreasing vector populations or to improved housing. [15] Vector control programs started in Thailand in the 1960's and efforts were reinforced in the 1990's, particularly against *Ae. aegypti*. It has also been proposed that demographic changes such as the decrease in birth rate and the increase in life expectancy might be driving the decrease in hazard of infection[3].

Even thought the 2010 study was designed to resemble the serological study conducted in 1980, there are several differences between the two studies that must be taken into account.

First of all, the 1980 seroprevalence study were limited schools within the municipal area of Rayong and adjacent suburban villages. The total area of the 1980 study region was 73 km^2 while the area covered in the 2010 serosurvey included the full district, with an area of approximately 514 km^2 (Figure 1) [12,16]. Limiting our analysis to the schools within the same area as the original study does not change the results, but does obscure the heterogeneity observed in schools.

Moreover, Rayong district has changed considerably over the past 30 years, becoming an industrial and touristic center in central/eastern Thailand and attracting migrants from other districts and provinces. The registered population of Rayong city has increased from 38,000 in 1980 to 58,000 in 2010, and might be much larger if the population that has recently migrated is taken into account. [17, 18] Similarly, the density of the population has increased from 2,194/km^2 to 3,439/km^2 in the city.

The serologic test used for the 1980/81 study was the single dilution neutralization assay (SDNT) at a 1:30 dilution (70% reduction) along with full PRNT (multiple sequential dilutions) on a subset of samples. [12] The decision to use a single 1:10 dilution (50% reduction) for the current study was based on it's better performance when classifying exposed vs. unexposed individuals. A single 1:10 dilution has shown a very high sensitivity and specificity in classifying individuals as exposed/unexposed, at the expense of a very poor performance in classifying immunity as monotypic or heterotypic. Higher dilutions perform better at differentiating the type of immune response but tend to misclassify some exposed people as unexposed.

The fact that this study does not provide information about the age at primary vs. secondary exposure is a limitation. Secondary exposure is regarded as the principal risk factor for developing severe clinical manifestations and therefore, knowing whether children are monotypically or multitypically immune would inform about age-groups that are at risk of severe disease. [9] Multiple dilution neutralization assays are considered the gold-standard, both in terms of classifying people as exposed/unexposed and monotypic/heterotypic, but there is no universal agreement of the optimal cut-point when analyzing data from serological surveys. [19]

Acknowledgments

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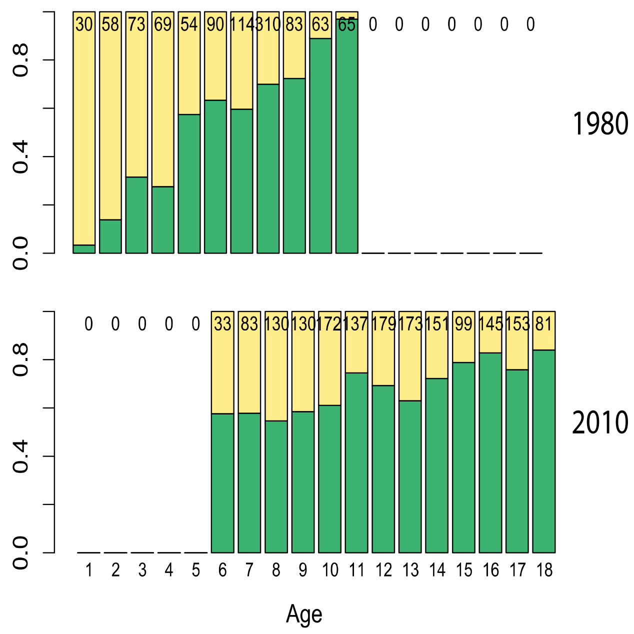

Figure 3: Age specific seroprevalnce according to the 1980 and 2010 serosurveys

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แนะนำการอ้างอิงสำหรับบทความนี้

โรม บัวทอง, โสภณ เอี่ยมศิริถาวร. การสำรวจความชุกของภูมิคุ้มกันต่อไวรัสเด็งกี่ของประชากรในจังหวัดระยอง ประเทศไทย. รายงานการเฝ้าระวังทางระบาดวิทยาประจำสัปดาห์. 2561; 49: 449-56.

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การสำรวจความชุกของภูมิคุ้มกันต่อไวรัสเด็งกี่ของประชากรในจังหวัดระยอง ประเทศไทย

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ความเป็นมา: โรคติดเชื้อไวรัสเด็งกี่เป็นปัญหาสาธารณสุขของประเทศไทยมานานกว่า 50 ปี โดยประชากรเด็กเป็นโรคติดเชื้อเด็งกี่มากกว่าประชากรผู้ใหญ่ แม้ว่าอุบัติการณ์ของโรคติดเชื้อเด็งกี่ในประเทศไทยยังคงสูงลอยมาต่อเนื่องและไม่มีแนวโน้มที่จะลดลง แต่กลับพบว่าอายุเฉลี่ยของผู้ติดเชื้อมีแนวโน้มสูงขึ้นตลอดทุกปี และยังไม่มีคำตอบที่แน่ชัด

วิธีการศึกษา: การสำรวจความชุกของการติดเชื้อไวรัสเด็งกี่ในน้ำเหลืองครั้งนี้ เป็นการสำรวจเด็กนักเรียนในพื้นที่อำเภอเมืองของจังหวัดระยอง โดยทำการเจาะเลือดเด็กนักเรียนตั้งแต่ชั้นอนุบาล จนถึงชั้นมัธยมศึกษาปีที่ 6 และนำน้ำเหลืองมาตรวจโดยวิธี Single dilution neutralization testing (SDNT) ซึ่งสามารถแยกการติดเชื้อชนิดปฐมภูมิ และทุติยภูมิ นอกจากนี้ยังนำผลลัพธ์ที่ได้ไปเปรียบเทียบกับการศึกษาในอดีตที่ทำการวิจัยแบบเดียวกันในจังหวัดระยอง ในปี พ.ศ. 2523 รวมทั้งการศึกษาหาปัจจัยเสี่ยงของเด็กที่มีภูมิคุ้มกันของไวรัสเด็งกี่เปรียบเทียบกับเด็กที่ไม่มีภูมิของไวรัสเด็งกี่

ผลการศึกษา: มีเด็กทั้งหมด 1,811 คนที่เข้าร่วมโครงการและได้รับการเจาะเลือดจาก 90 ห้องเรียน ใน 25 โรงเรียน โดยมีผลการตอบรับเข้าร่วมโครงการร้อยละ 53 โดยเด็กนักเรียนมีภูมิคุ้มกันต่อไวรัสเด็งกี่อย่างน้อยหนึ่งชนิดร้อยละ 69 และมีภูมิคุ้มกันต่อไวรัสเจอีร้อยละ 46 เมื่อเปรียบผลความชุกของภูมิคุ้มกันในปี พ.ศ. 2523 และ ปี พ.ศ. 2553 พบว่าเด็กอายุ 11 ปี ในปี พ.ศ. 2523 มีภูมิคุ้มกันต่อไวรัสเด็งกี่ร้อยละ 96 และเด็กอายุ 11 ปี ในปี พ.ศ. 2553 มีภูมิคุ้มกันต่อไวรัสเด็งกี่ลดลงมากเพียงร้อยละ 74 (95% CI 61-87%) และเด็กอายุ 18 ปี ในปี พ.ศ. 2553 ร้อยละ 16 (95%CI 0-32%) ยังคงมีความเสี่ยงต่อการติดเชื้อไวรัสเด็งกี่ ส่วนปัจจัยที่ผลต่อการมีผลบวกต่อภูมิคุ้มกันของไวรัส ได้แก่ อายุที่เพิ่มขึ้น (OR 1.16, 95%CI 1.10, 1.23) การเรียนพื้นที่อุตสาหกรรมมีความเสี่ยงเพิ่มขึ้น (OR 3.17, 95%CI 1.51, 6.67) และเด็กที่ครอบครัวมีเศรษฐานะที่ดี เช่น การมีรถยนต์ เป็นปัจจัยป้องกันต่อการติดเชื้อเด็งกี่ (OR 0.48, 95%CI 0.22, 1.02)

อภิปรายผล: จากผลการศึกษาในครั้งนี้สรุปได้ว่ามีการเปลี่ยนแปลงและแตกต่างของการมีภูมิคุ้มกันต่อไวรัสเด็งกี่ ระหว่างปี พ.ศ. 2523 และ ปี พ.ศ. 2553 โดยพบว่าเด็กในปี พ.ศ. 2553 มีภูมิคุ้มกันของไวรัสเด็งกี่ลดลง หรือติดเชื้อไวรัสเด็งกี่ที่อายุเพิ่มขึ้น ทั้งนี้มีหลายปัจจัยที่ทำให้เกิดการเปลี่ยนแปลง ได้แก่ การที่ประชากรเด็กได้รับการป้องกันการติดเชื้อได้ดีขึ้น โดยมีการควบคุมยุงนำโรคที่ดี ทำให้การแพร่เชื้อของไวรัสเด็งกี่ลดลง

คำสำคัญ: ไวรัสเด็งกี่, ความชุก, ภูมิคุ้มกัน, นักเรียน, ประเทศไทย