Zika Virus Immunoglobulin G Seroprevalence among Young Adults Living with HIV or without HIV in Thailand from 1997 to 2017

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Abstract: Zika virus (ZIKV) epidemiological data in Thailand are limited. We assessed ZIKV IgG seroprevalence among young adults during 1997–2017 and determined factors associated with ZIKV IgG seropositivity. This retrospective laboratory study included randomly selected subjects aged 18–25 years participating in large clinical studies conducted in Thailand during 1997–2017. Stored plasma samples were analyzed for ZIKV IgG using an ELISA test (Anti-Zika Virus IgG, EUROIMMUN, Lübeck, Germany). Sociodemographic, clinical and laboratory data were used in univariable and multivariable analyses to identify factors associated with ZIKV IgG positivity. Of the 1648 subjects included, 1259 were pregnant women, 844 were living with HIV and 111 were living with a 25.2% prevalence. Factors independently associated with ZIKV IgG positivity included an age of 23–25 years as compared to 18–20 years, an HIV RNA load below 3.88 log_{10} copies/mL and birth in regions outside northern Thailand. Our study shows that a large proportion of the population in Thailand probably remains susceptible to ZIKV infection, which could be the ground for future outbreaks. Continued surveillance of ZIKV spread in Thailand is needed to inform public health policies.

Keywords: Zika virus; epidemiology; IgG; seroprevalence; Thailand; HIV; pregnant women

1. Introduction

Zika virus (ZIKV) is an enveloped, positive single-strand RNA virus belonging to the Flaviviridae family and Flavivirus genus. The RNA genome is composed of about 10,800 nucleotides encoding three structural proteins, the capsid, precursor membrane and envelope protein, and seven nonstructural (NS) proteins i.e., NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 [1]. ZIKV is mainly transmitted to humans through infected Aedes mosquito
bites. Since its discovery in 1947 in Uganda, ZIKV infection was not considered as a public health concern until the outbreaks in the Pacific region between 2007 and 2013 [2–4]. Indeed, an acute infection is usually asymptomatic or exhibits mild and self-limiting symptoms. When present (less than 20%), symptoms include a non-specific febrile syndrome with a maculopapular rash, arthralgia, or conjunctivitis [3,5].

In 2007, the first ZIKV outbreak outside Africa and Asia occurred in Yap Island (Federated States of Micronesia) [3,5]. It was followed in 2013 with an outbreak in French Polynesia, which was responsible for severe neurological complications in adults and malformation in neonates [2,6,7]. The virus subsequently spread to South and Central Americas in 2015 [8], especially in Brazil, where ZIKV infection was associated with neurological complications, including microcephaly in newborns or Guillain–Barré syndrome (GBS) in adults [2,9,10]. As a result of this extensive spread of ZIKV and its associated neurological complications, the World Health Organization designated ZIKV a “Public Health Emergency of International Concern” in February 2016 [11].

Initial serologic tests performed on stored samples suggest that ZIKV has circulated in Thailand since 1954 [12]. In 2013, the Ministry of Public Health of Thailand (Thai-MOPH) rapidly implemented in the local healthcare centers a system to report ZIKV infections following the report of a symptomatic ZIKV infection in a traveler upon returning to Canada after visiting Thailand in May 2013 [13] and the suspicion of a ZIKV outbreak in several areas. Shortly after, the Thai-MOPH conducted ZIKV investigations throughout the country. A retrospective analysis of neutralization antibody in stored plasma samples collected in 2012 from two patients with exanthematous fever identified that they had been infected with ZIKV [14]. However, no outbreaks and no severe complications have ever been reported.

We present herein the seroprevalence of immunoglobulin G (IgG) against ZIKV among young adults in Thailand over several time periods between 1997 and 2017 and factors associated with positive ZIKV IgG.

2. Materials and Methods

2.1. Study Population

This is a retrospective laboratory study of ZIKV IgG among subjects enrolled between 1997 and 2017 in large clinical studies conducted in Thailand on the prevention of perinatal transmission of HIV [15–19] or hepatitis B virus (HBV) [20] or in an HIV testing research program [21] (ClinicalTrials.gov Identifier: NCT00386230, NCT00398684, NCT00142337, NCT00409591, NCT01511237, NCT01745822, NCT02752152, respectively). Since cumulative exposure to mosquitoes increases over an individual’s lifetime, the risk of being ZIKV-IgG-positive may increase with age. For this reason, only subjects aged 18–25 years were included in this study. For this study, we used socio-demographic and clinical data, laboratory results, and stored blood samples that were collected during the course of those studies.

Of the 8347 subjects enrolled between December 1997 and December 2017 across the seven studies and with a stored sample, 3675 met the age range criterion. Only 97 women enrolled in the perinatal HIV prevention studies conducted during the period 2004–2007. These 97 pregnant women were not included in this Zika study since their number was too low to allow for an appropriate random age-based selection. We, thus, considered five time periods, based on the years in which those studies were conducted: 1997–2000 (742 subjects), 2001–2003 (833 subjects), 2008–2011 (158 subjects), 2012–2014 (238 subjects) and 2015–2017 (1704 subjects). We used a proportionate sampling approach to obtain the target number of subjects for each period, i.e., 400, 250, 150, 150, and 400, respectively (Figure 1). To homogenize the study population, the subjects at each time period were separated into four sub-groups according to the subjects’ age quartiles. We then applied an age-matched draw procedure to select the subjects from each time period.
Figure 1. Study population: subjects were enrolled between 1997 and 2017, over five time periods: 1997–2000, 2001–2003, 2008–2011, 2012–2014 and 2015–2017. The target numbers of subjects randomly selected for each period were 400, 250, 150, 150 and 400, respectively. An additional group of 400 HIV-uninfected pregnant women enrolled in the 1997–1999 period was included. The bottom row indicates the number of selected subjects with the available samples.

2.2. Laboratory Testing

Stored plasma samples collected before any antiretroviral treatment were tested for ZIKV IgG using an indirect ELISA test (Anti-Zika Virus IgG ELISA, EUROIMMUN, Lübeck, Germany; Product number: EI 2668-9601 G; 78.9% sensitivity and 99.8% specificity [22]) according to the manufacturer’s instructions. Each test run was validated with the kit positive and negative controls as internal controls. A test was considered ZIKV-IgG positive if the signal per cut-off ratio was >1.1 and ZIKV-IgG negative if the ratio was ≤1.1.

2.3. Statistical Considerations

The characteristics of the subjects are described using counts and percentages for categorical data and medians with interquartile ranges (IQR) for continuous data. The characteristics included age at enrollment, region of birth, occupation, education level, blood chemistry and hematology tests, HIV, hepatitis B and C virus and syphilis infection statuses and HIV-1 RNA load. The percentage of women with ZIKV IgG antibodies, along with the corresponding Clopper–Pearson 95% confidence interval (CI), are provided for each group. ZIKV IgG seroprevalence during the 1997–2000 period was compared between the HIV-infected and HIV-uninfected pregnant women. ZIKV IgG seroprevalence was analyzed at each of the five time periods and compared to the ZIKV IgG seroprevalence in 1997–2000 using a chi-square test.

Logistic regression models were used to identify whether time periods and other factors were associated with ZIKV IgG positivity. Continuous variables were transformed into categorical variables using common cut-off or median values. All factors with a p-value < 0.2 in the univariate analysis were considered for inclusion in the multivariate analysis, and the backward elimination procedure was applied to select only independent factors associated with ZIKV IgG positivity. All data analyses were performed using Stata™ version 14.1 software (Statacorp, College Station, TX, USA). Differences were considered statistically significant if the p-value was ≤0.05.
3. Results

3.1. Study Population Characteristics

Of the 1750 randomly selected subjects, 1648 had a sample available (386 in the period 1997–2000, 248 in the period 2001–2003, 102 in period the 2008–2011, 113 in period the 2012–2014, 399 in the period 2015–2017 and 400 in the HIV-uninfected pregnant women) (Figure 1). The median age was 22 years (IQR: 20–23 years). Of the 1648 subjects with samples available, 1464 (88.8%) were females, of whom 1295 were pregnant, with a median gestational age of 25 weeks at time of blood draw. Almost half of subjects were born in the northern region of Thailand (Table 1), and 956 (58.4%) completed secondary or higher education.

Eight hundred and forty-four subjects (51.2%) were positive for HIV antibodies. The median HIV-1 RNA load was $3.88 \log_{10}$ copies/mL (IQR: 3.21–4.46). The median CD4 T-cell count was 410 cells/mm$^3$ (IQR: 280–550), and 13.5% (110 of 814) had a CD4 T-cell count below 200 cells/mm$^3$. The hepatitis B surface antigen was positive in 111 of 1245 subjects (8.9%) and the HCV antibody was positive in 22 of 1246 (1.8%). Other socio-demographic data, laboratory test results and substance use information are described in Table 1 and Table S1.
Table 1. Characteristics of the study population.

| Characteristics          | Overall (n = 1648) | HIV-Uninfected Pregnant Women 1997–2000 | Period 1997–2000 | Period 2001–2003 | Period 2008–2011 | Period 2012–2015 | Period 2015–2017 |
|--------------------------|--------------------|----------------------------------------|------------------|------------------|------------------|------------------|------------------|
|                          | n/N or n           | Percentage or Median (IQR)              | n/N or n         | Percentage or Median (IQR) | n/N or n         | Percentage or Median (IQR) | n/N or n         | Percentage or Median (IQR) |
| **Sex**                  |                    |                                        |                  |                  |                  |                  |                  |                  |
| Female                   | 1464/1648          | 88.8                                   | 400/400          | 100.0            | 386/386          | 100.0            | 248/248          | 100.0            |
| Male                     | 174/1648           | 10.6                                   | 0/400            | 0.0              | 0/386            | 0.0              | 0/248            | 0.0              |
| Other                    | 10/1648            | 0.6                                    | 0/400            | 0.0              | 0/386            | 0.0              | 0/248            | 0.0              |
| **Age (years old)**      |                    |                                        |                  |                  |                  |                  |                  |                  |
| 1648                     | 22.0 (20.0, 23.0)  | 372                                    | 22.0 (20.0, 23.0) | 386              | 22.0 (20.1, 23.0) | 248              | 22.0 (20.0, 23.0) | 102              |
|                         |                    |                                        |                  |                  |                  |                  |                  |                  |
| **Pregnancy (denominator: females)** |        |                                        |                  |                  |                  |                  |                  |                  |
| 1295/1464                | 88.5               | 400/400                                | 100              | 386/386          | 100              | 248/248          | 100              | 113/113          |
| **Gestational age (weeks)** |                |                                        |                  |                  |                  |                  |                  |                  |
| 1262                     | 25.0 (16.7, 29.7)  | 347                                    | 15.9 (11.3, 22.9) | 386              | 21.4 (16.9, 25.3) | 248              | 29.7 (28.0, 33.0) | 102              |
|                         |                    |                                        |                  |                  |                  |                  |                  |                  |
| **Region of birth**      |                    |                                        |                  |                  |                  |                  |                  |                  |
| Central                  | 250/1522           | 16.4                                   | 19/354           | 5.4              | 46/339           | 13.6             | 60/244           | 24.6             |
| Northern                 | 757/1522           | 49.7                                   | 251/354          | 70.9             | 156/339          | 46.0             | 39/244           | 16               |
| Northeastern             | 173/1522           | 11.4                                   | 4/354            | 1.1              | 5/339            | 1.5              | 83/244           | 34               |
| Eastern                  | 273/1522           | 17.9                                   | 78/354           | 22               | 125/339          | 36.9             | 46/244           | 18.9             |
| Western                  | 25/1522            | 1.6                                    | 0/354            | 0.0              | 0/339            | 0.0              | 7/244            | 2.9              |
| Southern                 | 38/1522            | 2.5                                    | 2/354            | 0.6              | 7/339            | 2.1              | 9/244            | 3.7              |
| Foreign country          | 6/1522             | 0.4                                    | 0/354            | 0.0              | 0/339            | 0.0              | 0/244            | 0.0              |
| **Region of enrollment** |                    |                                        |                  |                  |                  |                  |                  |                  |
| Central                  | 250/1644           | 15.2                                   | 19/398           | 4.8              | 58/386           | 15.0             | 73/242           | 30.2             |
| Northern                 | 868/1644           | 52.8                                   | 256/398          | 64.3             | 168/386          | 43.5             | 37/242           | 15.3             |
| Northeastern             | 70/1644            | 4.3                                    | 0/398            | 0.0              | 0/386            | 0.0              | 39/242           | 16.1             |
| Eastern                  | 413/1644           | 25.1                                   | 121/398          | 30.4             | 152/386          | 39.4             | 81/242           | 33.5             |
| Western                  | 7/1644             | 0.4                                    | 0/398            | 0.0              | 0/386            | 0.0              | 0/242            | 0.0              |
| Southern                 | 36/1644            | 2.2                                    | 2/398            | 0.5              | 8/386            | 2.1              | 12/242           | 5.0              |
| **Education**            |                    |                                        |                  |                  |                  |                  |                  |                  |
| Higher than bachelor’s degree | 5/1638           | 0.3                                    | 0/396            | 0.0              | 0/383            | 0.0              | 0/248            | 0.0              |
| College/University       | 369/1638           | 22.5                                   | 21/396           | 5.3              | 25/383           | 6.5              | 25/248           | 10.1             |
| High school              | 188/1638           | 11.5                                   | 37/396           | 9.3              | 24/383           | 6.3              | 24/248           | 9.7              |
| Secondary school/Vocational certificate | 394/1638       | 24.5                                   | 92/396           | 23.2             | 95/383           | 24.8             | 67/248           | 27.0             |
| Primary school           | 482/1638           | 29.4                                   | 179/396          | 45.2             | 164/383          | 42.8             | 88/248           | 35.5             |
| Lower than primary school | 175/1638          | 10.7                                   | 55/396           | 13.9             | 75/383           | 19.6             | 36/248           | 14.5             |
| Others                   | 25/1638            | 1.5                                    | 12/396           | 3.0              | 0/383            | 0.0              | 8/248            | 3.2              |
Table 1. Cont.

| Characteristics | Overall (n = 1648) | HIV-Uninfected Pregnant Women 1997–2000 | Period 1997–2000 | Period 2001–2003 | Period 2008–2011 | Period 2012–2015 | Period 2015–2017 |
|-----------------|-------------------|------------------------------------------|------------------|------------------|------------------|------------------|------------------|
|                 | n/N or n          | Percentage or Median (IQR)               | n/N or n         | Percentage or Median (IQR) | n/N or n         | Percentage or Median (IQR) | n/N or n         |
| **Marital status** |                   |                                          |                  |                   |                  |                   |                  |
| Living with partner | 834/892         | 93.5                                     | n.a. 1           | 372/384          | 96.9             | 224/247          | 90.7             | 91/102           | 89.2             | 102/113          | 90.3             | 45/46            | 97.8             |
| Divorced/Not living with partner/Widowed/Single Others | 53/892      | 5.9                                      | n.a. 1           | 12/384           | 3.1              | 19/247           | 7.7              | 10/102           | 9.8              | 11/113           | 9.7              | 1/46             | 2.2              |
| Others | 5/892           | 0.6                                      | n.a. 1           | 0/384            | 0.0              | 4/247            | 1.6              | 1/102            | 1.0              | 0/113            | 0.0              | 0/46             | 0.0              |
| **Number of household members** |                   |                                          |                  |                   |                  |                   |                  |
| 1 (Living alone) | 101/611          | 16.5                                     | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 3/102            | 2.9              | 0/112            | 0.0              | 96/397           | 24.7             |
| 2 people | 136/611          | 22.3                                     | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 26/102           | 25.5             | 32/112           | 28.6             | 78/397           | 19.6             |
| 3 people | 96/611           | 15.7                                     | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 15/102           | 14.7             | 16/112           | 14.3             | 65/397           | 16.4             |
| 4 people | 110/611          | 18                                       | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 21/102           | 20.6             | 28/112           | 25               | 61/397           | 15.4             |
| More than 4 people | 168/611          | 27.5                                     | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 37/102           | 36.3             | 36/112           | 32.1             | 95/397           | 23.9             |
| **Multiple partner** |                |                                          |                  |                   |                  |                   |                  |
| Unemployed or Housewife | 487/1603       | 30.4                                     | 89/397           | 22.4             | 25/386           | 6.5              | 243/248          | 98.0             | 56/102           | 54.9             | 64/110           | 58.2             | 10/360           | 2.8              |
| Agriculturist/Fishery | 176/1603       | 11                                       | 106/397          | 26.7             | 63/386           | 16.3             | 2/248            | 0.8              | 3/102            | 2.9              | 2/110            | 1.8              | 0/360            | 0.0              |
| Commercial/Private business | 128/1603      | 8.0                                      | 34/397           | 8.6              | 42/386           | 10.9             | 1/248            | 0.4              | 22/102           | 21.6             | 21/110           | 19.1             | 8/360            | 2.2              |
| Self-employed Office man | 152/1603      | 9.5                                      | 27/397           | 6.8              | 124/386          | 32.1             | 0/248            | 0.0              | 1/102            | 1.0              | 0/110            | 0.0              | 0/360            | 0.0              |
| Labor/ Housekeeper | 292/1603       | 18.2                                     | 131/397          | 33               | 121/386          | 31.3             | 0/248            | 0.0              | 12/102           | 11.8             | 19/110           | 17.3             | 9/360            | 2.5              |
| Student Others | 303/1603       | 18.9                                     | 3/397            | 0.8              | 2/386            | 0.5              | 0/248            | 0.0              | 3/102            | 2.9              | 3/110            | 2.7              | 292/360          | 81.1             |
| Others | 65/1603         | 4.1                                      | 7/397            | 1.8              | 9/386            | 2.3              | 2/248            | 0.8              | 5/102            | 4.9              | 1/110            | 0.9              | 41/360           | 11.4             |
| **Risk behavior** |                |                                          |                  |                   |                  |                   |                  |
| Alcohol consumption | 290/404        | 71.8                                     | n.a. 1           | 24/24            | 100.0            | 16/16            | 100.0            | 7/7              | 100.0            | 9/9              | 100.0            | 234/348          | 67.2             |
| Smoking | 59/349          | 16.9                                     | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 59/349           | 16.9             |
| Drug use | 73/351          | 20.8                                     | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | n.a. 1           | 73/351           | 20.8             |
| Any of these | 301/407        | 74                                       | n.a. 1           | 24/24            | 100.0            | 16/16            | 100.0            | 7/7              | 100.0            | 9/9              | 100.0            | 245/351          | 69.8             |
Table 1. Cont.

| Characteristics | Overall (n = 1648) | HIV-Uninfected Pregnant Women 1997–2000 | Period 1997–2000 | Period 2001–2003 | Period 2008–2011 | Period 2012–2015 | Period 2015–2017 |
|-----------------|-------------------|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | n/N or n          | Percentage or Median (IQR)             | n/N or n        | Percentage or Median (IQR) | n/N or n        | Percentage or Median (IQR) | n/N or n        | Percentage or Median (IQR) | n/N or n        | Percentage or Median (IQR) |
| Anti-HIV positive | 844/1645          | 51.2                                   | 2/386           | 100.0           | 247/248         | 99.6            | 101/101         | 100.0           | 106/112         | 94.6            | 4/398           | 1.0            |
| HIV RNA load     | 838               | 3.88 (3.21, 4.46)                      | 2/386           | 3.92 (3.32, 4.40) | 246             | 4.0 (3.33, 4.70) | 102             | 3.57 (2.16, 4.19) | 99             | 3.81 (4.52, 3.04) |
| HIV RNA load     | 834               | 3.87 (3.21, 4.45)                      | 2/386           | 3.92 (3.32, 4.40) | 246             | 4.0 (3.33, 4.70) | 102             | 3.57 (2.16, 4.19) | 99             | 3.81 (4.52, 3.04) |
| HBsAg positive   | 111/1245          | 8.9                                    | n.a. 386        | 7.3             | 16/246          | 6.5             | 5/102           | 4.9             | 8/113           | 7.1             | 54/399          | 13.5           |
| Anti-HCV positive | 22/1246           | 1.8                                    | n.a. 384        | 3.4             | 3/248           | 1.2             | 5/105           | 4.9             | 0/113           | 0.0             | 1/399           | 0.3            |
| Syphilis positive | 3/353             | 0.8                                    | n.a. 129        | 3.97 (3.54, 4.40) | 32              | 32              | 102             | 20.5 (16.5, 31.5) | 34             | 20 (16, 24)     | 46             |
|                 |                   |                                        |                 |                 |                 |                 |                 |                 |                 |                 |                 | 19 (16, 22)     |
|                 |                   |                                        |                 |                 |                 |                 |                 |                 |                 |                 |                 | 17.5 (12, 20)  |
| Blood chemistry testing |               |                                        |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Fasting blood sugar (mg/dL) | 112          | 82 (73, 91)                            | n.a. 17         | 90 (85, 109)    | 7               | 88.8 (83, 94)   | 42              | 78.5 (71, 84)   | 43              | 77 (71, 84)    | 3               |
| Cholesterol (mg/dL) | 248              | 217 (180, 258.5)                       | n.a. 32         | 197 (160.5, 225) | 38              | 173.5 (152, 206) | 102             | 244 (217, 280) | 106             | 211.5 (182, 232) | 6               |
| AST (IU/L) | 184              | 32 (21, 46)                            | n.a. 32         | 30.5 (21, 46)   | 32              | 22 (18, 34.5)   | 40              | 20.5 (16, 31.5) | 34              | 20 (16, 24)     | 46             |
| ALT (IU/L) | 866              | 14 (10.0, 20.0)                        | n.a. 385        | 14 (10.0, 20.0) | 239             | 16 (11.15)      | 102             | 14 (10.0, 21)  | 111             | 12 (9, 15)      | 46             |
| Hemoglobin (g/dL) | 893              | 10.8 (11.6, 10)                        | n.a. 384        | 10.6 (9.9, 11.4) | 248             | 11 (10.1, 11.6) | 102             | 10.9 (10.2, 11.6) | 113             | 11 (10.2, 11.6) | 46             |
| Hematocrit (%) | 895              | 33.0 (35, 30.9)                        | n.a. 386        | 33.0 (30.9, 35.1) | 248             | 32.9 (30.35, 34.85) | 102         | 32.7 (31.0, 34.4) | 113             | 32.9 (30.35, 34.4) | 46             |
| RBC count (million cells/mL) | 516     | 4.0 (3.6, 4.4)                         | n.a. 129        | 3.97 (3.54, 4.40) | 127             | 4.33 (3.95, 4.72) | 102             | 3.71 (3.49, 3.95) | 112             | 3.94 (3.61, 4.26) | 46             |
| Platelet count (thousand/mm³) | 596   | 78.5 (180.0, 258.5)                     | n.a. 87         | 241 (197, 286)  | 271 (230, 318.5) | 102             | 271.5 (233, 328) | 102             | 253 (217, 296) | 254.5 (219, 283) | 46             |
| WBC count (cells/mm³) | 877    | 8880 (10,600, 6400)                     | n.a. 124        | 8800 (7800, 10,700) | 248             | 6525 (7400, 10,700) | 102         | 8790 (7650, 10,490) | 113             | 9000 (7700, 10,120) | 46             |
| Absolute CD4 T-cell (cells/mm³) | 814    | 410 (280, 550)                          | n.a. 358        | 378.5 (250, 540) | 248             | 405.5 (266.5, 541) | 102             | 518.5 (413, 541) | 102             | 394.5 (292, 516) | 4               |
| Absolute CD4 T-cell among pregnant women (cells/mm³) | 810 | 409.5 (280, 550)                        | n.a. 358        | 378.5 (250, 540) | 248             | 405.5 (266.5, 541) | 102             | 518.5 (413, 541) | 102             | 394.5 (292, 516) | n.a. 1         |

Note: 1 Not available; 2 Not applicable.
3.2. ZIKV IgG Seroprevalence in HIV-Infected versus HIV-Uninfected Pregnant Women during 1997–2000

During 1997–2000, 88 of 386 (22.8%, 95%CI: 18.7–27.3) HIV-infected pregnant women tested positive for ZIKV IgG antibody versus 103 of 400 (25.8%, 95%CI: 21.5–30.3) HIV-uninfected pregnant women ($p$-value = 0.335) (Figure 2).

![Figure 2. ZIKV IgG seroprevalence among pregnant women in Thailand according to HIV status during 1997–2000. The whisker error bars represent the 95% confidence intervals.](image)

3.3. The Evolution of ZIKV IgG Seroprevalence during 1997–2017

The evolution of ZIKV IgG seroprevalence among all subjects over the five time periods was initially analyzed. In the period 2001–2003, 68 of 248 (27.4%, 95%CI: 22.0–33.4) subjects were ZIKV-IgG-positive. In the period 2008–2011, 25 of 102 subjects (24.5%, 95%CI: 16.5–34.0) were ZIKV-IgG-positive. In the period 2012–2014, 30 of 113 subjects (26.5%, 95%CI: 18.7–35.7) were ZIKV-IgG-positive. In the period 2015–2017, 66 of 399 subjects tested positive (16.5%, 95%CI: 13.0–20.6) for the ZIKV IgG antibody. ZIKV IgG seroprevalence was significantly lower during the period 2015-2017 as compared to other periods, likely as a result of the population enrolled during that period. Indeed, a large proportion of subjects were young men and non-pregnant women enrolled only in one city of northern Thailand.

When we restricted the analysis to pregnant women, ZIKV IgG seroprevalence looks stable over all the time periods: 24.3% in 1997–2000, 27.4% in 2001–2003, 24.5% in 2008–2011, 26.5% in 2021–2014 and 26.1% in 2015–2017. No significant differences were observed between periods (Figure 3).
3.4. Factors Associated with ZIKV IgG Seropositivity among Pregnant Women

In the univariable analysis, older age, being born or residing outside northern Thailand and a lower HIV-1 RNA load were significantly associated with ZIKV IgG positivity Table 2). In the multivariable analysis, factors found to be independently associated with ZIKV IgG positivity were older age (23–25 years versus 18–20 years: adjusted odd ratio (aOR) = 1.65, 95%CI: 1.03-2.63), being born outside northern Thailand (aOR = 1.95, 95%CI: 1.32-2.88) and lower HIV RNA (≤3.88 versus >3.88 log_{10} copies/mL: aOR = 1.46, 95%CI: 1.05-2.04).

Table 2. Factors associated with ZIKV IgG positivity among pregnant women (N = 1295).

| Characteristics | n/N | % (Univariable) | Odds Ratio (95%CI) | p | Adjusted Odds Ratio (95%CI) | p |
|-----------------|-----|----------------|-------------------|---|-----------------------------|---|
| Period          |     |                |                   |   |                             |   |
| 1997–2000       | 88/386 | 22.8 | 1 | 1.28 (0.89–1.85) | 0.19 |
| 2001–2003       | 68/248  | 27.4 | 1.10 (0.66–1.83) | 0.72 |
| 2008–2011       | 25/102  | 24.5 | 1.22 (0.76–1.98) | 0.41 |
| 2012–2014       | 30/113  | 26.5 | 1.20 (0.59–2.41) | 0.62 |
| 2015–2017       | 12/46   | 26.1 | 1.20 (0.59–2.41) | 0.62 |
| Age             |     |                |                   |   |                             |   |
| 18–20 years     | 47/221  | 21.3 | 1 | 1.08 (0.69–1.69) | 0.74 | 1.06 (0.65–1.73) | 0.81 |
| >20–22 years    | 51/226  | 22.6 | 1.26 (0.81–1.97) | 0.30 | 1.36 (0.85–2.20) | 0.20 |
| >22–23 years    | 55/216  | 25.5 | 1.60 (1.04–2.45) | 0.03 | 1.65 (1.03–2.63) | 0.04 |
| >23–25 years    | 70/232  | 30.2 | 1 | 1.08 (0.69–1.69) | 0.74 |
| Gestational age (N = 1262) | | | | | |
| 1–13 weeks      | 11/46   | 23.9 | 1 | 1.14 (0.56–2.33) | 0.72 |
| >13–28 weeks    | 111/466 | 23.8 | 1.09 (0.49–2.02) | 0.99 |
| >28 weeks       | 101/383 | 26.4 | 1.14 (0.56–2.33) | 0.72 |
| Region of birth (N = 1177) | | | | | |
| North           | 44/254  | 17.3 | 1 | 2.00 (1.38–2.90) | <0.001 | 1.95 (1.32–2.88) | <0.001 |
| Other           | 168/569 | 29.5 | 1 | 2.00 (1.38–2.90) | <0.001 | 1.95 (1.32–2.88) | <0.001 |
| Region of enrollment (N = 1293) | | | | | |
| North           | 45/277  | 16.3 | 1 | 2.09 (1.45–3.00) | <0.001 | 1 | n.i. 2 |
| Other           | 178/618 | 28.8 | 1 | 2.09 (1.45–3.00) | <0.001 | 1 | n.i. 2 |

Figure 3. Evolution of ZIKV IgG seroprevalence among pregnant women in Thailand during 1997–2017. The whisker error bars represent the 95% confidence intervals.
Table 2. Cont.

| Characteristics                  | n/N   | %    | Univariate Analysis | Multivariate Analysis |
|----------------------------------|-------|------|---------------------|-----------------------|
|                                 |       |      | Odds Ratio (95%CI)  | p         | Adjusted Odds Ratio (95%CI) | p         |
| Education (N = 1288)            |       |      |                     |           |                             |           |
| Lower than secondary school     | 99/421| 23.5 | 1                   |           |                             |           |
| Secondary school/Vocational     | 76/283| 26.9 | 1.19 (0.84–1.69)    | 0.32      | 1.16 (0.81–1.68)            | 0.27      |
| Higher than secondary school    | 46/177| 26   | 1.14 (0.76–1.71)    | 0.52      |                             |           |
| Other                           | 2/11  | 18.2 | 0.72 (0.15–3.40)    | 0.68      |                             |           |
| Marital status (N = 892)        |       |      |                     |           |                             |           |
| Divorced/Not living with partner | 18/53 | 34   | 1.62 (0.90–2.92)   | 0.11      | 1.45 (0.77–2.75)            | n.s.³     |
| Widowed/Singer                  | 201/834| 24.1 | 2.10 (0.35–12.65)  | 0.42      | 1.98 (0.33–12.09)           | n.s.³     |
| Living with partner             | 2/5   | 40   | 1                   |           |                             |           |
| Other                           | 2/5   | 40   | 1                   |           |                             |           |
| HIV status (N = 1292)           |       |      |                     |           |                             |           |
| HIV negative                    | 118/452| 26.1 | 1.24 (0.67–2.30)   | 0.50      |                             |           |
| HIV positive                    | 207/840| 24.6 | 1                   |           |                             |           |
| HIV-1 RNA load (N = 834)        |       |      |                     |           |                             |           |
| ≤3.88 log 10 copies/mL         | 120/420| 28.6 | 1.55 (1.13–2.13)   | 0.01      | 1.46 (1.05–2.04)            | 0.03      |
| >3.88 log 10 copies/mL         | 85/414| 20.5 | 1                   |           |                             |           |

Note: ¹ Not applicable, ² not included due to collinearity with region of birth, ³ not significant.

4. Discussion

In the absence of systematic data collection on ZIKV infection over time in Thailand, public health measures to limit potential ZIKV outbreaks cannot be taken. This study assessed the ZIKV IgG seroprevalence in adults aged 18–25 years in Thailand from 1997 to 2017, which covers the period when outbreaks of ZIKV infection were reported. We found no association between HIV-infection status and ZIKV IgG positivity among pregnant women. ZIKV IgG seroprevalence in pregnant women was stable over this 20 year period, ranging from 24.3 to 27.4%. Older age, being born outside northern Thailand and a lower HIV-1 RNA load were found to be independently associated with ZIKV IgG positivity.

To the best of our knowledge, this is the first study assessing ZIKV IgG seroprevalence among young HIV-infected or HIV-uninfected pregnant women. We found similar ZIKV IgG positivity rates among these two groups, which may be due to the relatively preserved immunity in the HIV-infected women randomly selected in this study. Indeed, the median CD4+ T-cell count was 410 cells/mm³ (95% CI: 280–540), and none of the subjects living with HIV had severe clinical complications before enrolling in the original clinical studies. Our results also suggest that HIV infection may not have impaired the immune response to ZIKV.

Our study provides new indirect data on the circulation of ZIKV over the past two decades. ZIKV IgG seroprevalence was stable, ranging from 24.3 to 27.4% during 1997–2017. These results are consistent with the overall ZIKV IgG seroprevalence of 29% found in HIV- or HBV-infected pregnant women with a median age of 25.2 years in Thailand during 1997–2015 [23]. Our data of ZIKV IgG seroprevalence in the 1997–2000 period suggests that ZIKV was circulating in Thailand before 1997, which supports findings by Ponds et al. of ZIKV positive serology in Thailand since 1954 [12]. Another study using time-resolved phylogenetic tree analyses of ZIKV sequences obtained in Thailand also suggested a persistent circulation of ZIKV in Thailand since at least 2002, although this estimation was based on sequence data that were dated, at the earliest, in 2006 [24].

The risk of being ZIKV-IgG-positive was doubled in subjects who were born/living in regions outside northern Thailand as compared to those born/living in the northern
region. When the region of enrollment was considered instead of region of birth in the multivariable analysis model, the same factors were found to be independently associated with ZIKV IgG positivity. ZIKV spread depends on various factors: mosquito vectors, environments for mosquitoes breeding and host behaviors, including people’s lifestyle and socioeconomic status. A possible hypothesis could be the different distribution of the mosquito vector and the variation in the environmental conditions needed for optimal mosquito breeding [25]. The ZIKV IgG seroprevalence during the last period, 2015–2017, was lower in the non-pregnant population as compared to the prevalence in the pregnant population. This may be explained by the fact that most of subjects were enrolled in Chiang Mai and living in urban areas. In northern Thailand, lower temperatures and humidity conditions may be less favorable for the spread of mosquitoes [26]. The less favorable conditions for mosquito breeding in northern Thailand was shown in a survey study of the *Aedes* population using an Ovitrap to number the eggs laid by mosquitoes [27]. This study, conducted during 2012–2019 across 32 provinces of Thailand, showed the highest average eggs per trap and percent of *Aedes*-positive traps in the south, followed by the central, northeast and north regions, [27]. In addition, those living in cities may benefit from better mosquito control campaigns. This combination may contribute to an overall lower exposure to mosquitoes and, thus, to ZIKV.

Since the risk of exposure of an individual to mosquito bites increases with the age, the cumulative risk of infection is greater in older individuals. This is consistent with our finding that ZIKV IgG prevalence was higher among pregnant women aged 23–25 years compared to those aged 18–20 years (30.2% vs. 21.3%, p = 0.04). It is unclear why the prevalence of ZIKV IgG was higher among HIV-infected pregnant women with HIV RNA levels below the median. One hypothesis is that those individuals may have less inflammation and a better immunity. However, this warrants further confirmation.

Our study has some limitations. First, a high proportion of subjects were pregnant women infected with HIV or HBV [15–20], while the last period (2015–2017) samples were collected from a young population of male or female subjects seeking testing for HIV or other infections [21]. However, when we restricted the analysis to pregnant women only, the ZIKV IgG seroprevalence was stable. Second, as some clinical and socio-economic information was not available for analysis, further study is needed to confirm our findings and identify other potential confounding factors. Third, this study was conducted in endemic areas of the dengue virus. Thus, a cross-reactivity from the pre-existing anti-DENV IgG may have led to an overestimation of the ZIKV IgG seroprevalence. However, our ZIKV IgG seroprevalence results are consistent with the low exposure to ZIKV of healthy Thai people reported in 2017: 20% of 135 healthy subjects (95% CI: 14.0–28.2%) were positive for the ZIKV neutralizing antibody [28].

5. Conclusions

There was no evidence that the overall ZIKV IgG seroprevalence in populations aged 18–25 years in Thailand has evolved during 1997–2017, and it appeared to be stable at around 25%, suggesting that ZIKV has been circulating for more than 20 years. This study suggests that a large proportion of the population in Thailand probably remains susceptible to ZIKV infection, which could be the ground for future outbreaks affecting non-immune pregnant women with a potential for severe adverse pregnancy outcomes. Continued surveillance of the ZIKV spread in Thailand is needed to inform public health policies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v14020368/s1, Table S1: Characteristics of the study population.

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Informed Consent Statement: This non-interventional study included no additional procedures. Informed consent for storage and further use of samples was obtained from all participants.

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