# Long-term circulation of Zika virus in Thailand: an observational study

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## Summary

**Background** **Little is known about the historical and current risk of Zika virus infection in southeast Asia, where the mosquito vector is widespread and other arboviruses circulate endemically. Centralised Zika virus surveillance began in Thailand in January, 2016. We assessed the long-term circulation of Zika virus in Thailand.**

**Methods** **In this observational study, we analysed data from individuals with suspected Zika virus infection who presented at hospitals throughout the country and had biological samples (serum, plasma, or urine) tested for confirmation with PCR at the National Institute of Health laboratories in Bangkok. We analysed the spatial and age distribution of cases, and constructed time-resolved phylogenetic trees using genomes from Thailand and elsewhere to estimate when Zika virus was first introduced.**

**Findings** **Of the 3089 samples from 1717 symptomatic individuals tested between January, 2016, and December, 2017, 368 were confirmed to have Zika virus infection. Cases of Zika virus infection were reported throughout the year, and from 29 of the 76 Thai provinces. Individuals had 2·8 times (95% CI 2·3–3·6) the odds of testing positive for Zika virus infection if they came from the same district and were sick within the same year of a person with a confirmed infection relative to the odds of testing positive anywhere, consistent with focal transmission. The probability of cases being younger than 10 years was 0·99 times (0·72–1·30) the probability of being that age in the underlying population. This probability rose to 1·62 (1·33–1·92) among those aged 21–30 years and fell to 0·53 (0·40–0·66) for those older than 50 years. This age distribution is consistent with that observed in the Zika virus epidemic in Colombia. Phylogenetic reconstructions suggest persistent circulation within Thailand since at least 2002.**

**Interpretation** **Our evidence shows that Zika virus has circulated at a low but sustained level for at least 16 years, suggesting that Zika virus can adapt to persistent endemic transmission. Health systems need to adapt to cope with regular occurrences of the severe complications associated with infection.**

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## Introduction

Zika virus is a flavivirus spread by *Aedes* spp mosquitoes that was first discovered in Uganda in 1947, with the first reported presence in Asia in 1966 in Malaysia.[1,2] Although infection in humans usually causes either mild disease or no symptoms,[3] Zika virus infection can be associated with the development of Guillain Barré syndrome and infection during the early stages of pregnancy has been linked to congenital microcephaly syndrome.[4,5] Much of the interest in Zika virus has been focused on South America, where the virus spread widely following its introduction in 2013.[6] The situation in Asia is much less clear.

Zika virus is proposed to have been circulating silently in Asia for years, as has previously been shown with another arbovirus—chikungunya virus.[7,8] Poor understanding of the long-term transmission patterns of Zika virus is not surprising. Even when symptoms do occur, laboratory testing for Zika virus is rarely done and clinical misdiagnosis is common, especially given the potential for serological cross-reactivity with dengue virus, which has circulated endemically in much of southeast Asia for decades.[9] In support of sustained transmission are a handful of viral isolates obtained in the 1960s in Malaysia, from 2006 in Thailand, and from 2012 in the Philippines—all before the emergence of the Asian lineage into South America.[6,10–12] Seroprevalence studies in the 1950s also showed some population immunity to Zika virus; however, interpreting the results of serology is complicated because of flavivirus cross-reactivity.[3,13] By contrast, the viruses responsible for an outbreak in Singapore in 2016 showed little viral diversity, consistent with emergence; however, they were genetically closest to viruses isolated from other Asian countries than to South American strains, consistent with an introduction from the surrounding region.[14]

Whether or not there has been widespread long-term circulation of Zika virus in the region, resulting in high levels of population immunity, is still unknown. The epidemic versus endemic nature of Zika virus has consequences for tackling the pathogen and the risk of severe complications. Zika virus epidemics have been suggested to be likely to burn themselves out after just a

## Research in context

### Evidence before this study

To assess the evidence base of Zika virus in Thailand, we searched Google Scholar for all publications before September, 2018 with "Zika" and "Thailand" in the title. We found 25 documents, mostly consisting of individual case reports. Two articles showed that cases of Zika virus had been found in different places in Thailand; however, no previous studies considered the endemicity of the virus in the country and the likely level of underlying immunity. An equivalent search in PubMed yielded 12 results, but no additional documents.

### Added value of this study

Using data from the national surveillance system, we present a comprehensive assessment of the Zika virus situation in Thailand. We use information from symptomatic individuals who tested negative for Zika virus infection to control for underlying spatial differences in health-care seeking. We show that the age distribution of cases of Zika virus is largely consistent with that from a known emergent setting (Colombia) and different from dengue virus, where population immunity is high. We use time-resolved phylogenetic approaches to show that Zika virus has circulated in the country for at least 16 years.

### Implications of all the available evidence

Our findings are consistent with sustained transmission of Zika virus within a single country for many years. This suggests that other countries that have also experienced Zika virus outbreaks might also be affected by the virus in future years. Our findings support the need for the deployment of long-term sustainable surveillance and intervention strategies.

few years, with resulting population immunity leading to absence of the virus for decades.[15] In such a scenario, efforts to contain the virus and enhanced surveillance for Guillain Barré syndrome and microcephaly might not be an effective use of resources, given the lead time and logistical constraints in establishing such measures. By contrast, if Zika virus can transition to sustained endemic circulation, the development of long-term intervention strategies and the establishment of systematic surveillance for the severe complications associated with infection will be necessary.

Analysing the Zika virus situation in Thailand provides an opportunity to understand the long-term epidemic potential of Zika virus. *Aedes* spp mosquitoes are found throughout Thailand and all four serotypes of dengue virus, which is transmitted by the same mosquitoes, circulate endemically throughout the year.[9,16] Thailand has been identified by phylogenetic studies as a potential source of dengue virus in the region.[17] Following the renewed global interest in Zika virus, in 2016 the National Institute of Health in Thailand started the centralised testing of serum, plasma, and urine samples of individuals with symptoms consistent with Zika virus infection from throughout the country. We present the results of this surveillance. Additionally, we use the age distribution of cases to infer the immunity to Zika virus in the population and use phylogenetic analyses to infer the current diversity in circulating viruses.

## Methods

### Data collection

In January, 2016, the National Institute of Health in Thailand established the centralised testing of samples from individuals with suspected Zika virus infection who presented at hospitals throughout the country. The symptoms of suspected infection were presence of maculopapular rash and a fever, or just a rash in provinces where cases of Zika virus had already been identified. Acute serum, plasma, or urine samples from individuals with suspected Zika virus infection were sent to the National Institute of Health laboratories in Bangkok where they were tested for evidence of infection with PCR. The date of symptom onset, date of testing, district of the hospital, age of the individual, and results of the testing were recorded. Data were collected between January, 2016, and December, 2017.

This study is based on secondary analysis of data collected as part of Thai governmental surveillance activities during the global Zika virus emergency. No ethical approval was required because the data were anonymised.

### Phylogenetic analyses

To assess whether the observed cases of Zika virus in Thailand in 2016–17 are consistent with a reintroduction of Zika virus following the large outbreak in South America or from sustained transmission within Thailand, we downloaded all available full genome sequences from Thailand (eight) from GenBank. We also selected a random sample of other sequences from Asia (Cambodia [three], Singapore [three], Philippines [one], Indonesia [one], and Malaysia [two]), French Polynesia (five), Brazil (nine), and Colombia (five). We used these sequences to build time-resolved phylogenetic trees using BEAST software, version 2.2.1, with a general time reversible nucleotide substitution model using a discrete γ distribution (ie, GTR+G) and a random local clock, as has previously been identified as optimal for Zika virus phylogenetic analyses.[14,18]

To estimate the date that Zika virus first emerged in Thailand, we randomly selected 100 time-resolved trees from the posterior distribution. For each tree, we extracted the date for the most ancient (ie, furthest back in time) most recent common ancestor (MRCA) separating any two Thai viruses. We then calculated the mean date and 95% CIs across the 100 trees.

To assess the diversity in viruses within any location over time, we randomly selected 100 trees from the posterior distribution. For each tree, we extracted the evolutionary distance separating each pair of tips. For all pairs of viruses coming from the same country and isolated within a year of one another, we calculated the proportion that had an MRCA within different time limits going back in time, ranging from 1 month to 10 years in the past. We then calculated the mean proportion over the 100 trees.

The GenBank accession numbers for the sequences used in these analyses are presented in the appendix.

### Statistical analysis

We used the age distribution of cases to gain insight into population immunity. If Zika virus had circulated endemically for decades, cases would be concentrated in children because most adults would be immune from previous infection. By contrast, a novel emergence would result in the case distribution being similar to the age distribution of the population. This assumes that no large-scale differences by age exist in the probability of becoming infected, developing symptoms, or seeking care. We used data on the age distribution in Thailand and compared that with the age distribution in Zika virus cases.[19] We also compared the age distribution of dengue virus cases sent to the laboratory between 2011 and 2016. For each virus, we calculated the proportion of cases who were within each 10-year age group and divided this percentage by the proportion of the underlying population within that age group. Values of this relative proportion of one indicate that individuals in that age category had a similar risk of becoming a case as anyone from the population, consistent with no immunity in the population. Relative proportions greater than one in younger individuals and less than one in older individuals are consistent with immunity in the population shifting cases to younger individuals.[20] To assess uncertainty, we used a bootstrap approach. We repeatedly randomly resampled all cases with replacement and recalculated the relative proportion. 95% CIs were obtained from the 2·5th and 97·5th percentiles of the resultant distribution. To compare the age distribution to Zika virus in a known emergent setting, we calculated the same value for Zika virus cases from Colombia between 2015 and 2016.[21]

The probability that an individual with symptoms of Zika virus infection presented to health-care facilities, and the subsequent probability that health-care facilities sent the samples to be tested by the National Institute of Health, was likely to differ spatially and temporally. To assess whether Zika virus cases persisted in the same district for many months, allowing for spatial and temporal biases in observation, we used the spatial distribution of both the individuals who tested positive and those who tested negative. This test-negative approach assumes that the individuals who tested negative were similar in their health-care seeking to the individuals with Zika virus infection.[22] To increase the probability that test-negative individuals were true negatives, we only included individuals who provided biological samples within 7 days of symptom onset.

To characterise the spatial dependence among individuals with Zika virus infection, we calculated the odds of an individual with symptoms within 30 days of an index case who lived within the same district testing positive for the infection, relative to the odds of anyone testing positive within that timeframe. We repeated the analysis considering individuals with Zika virus infection who lived in nearby districts (defined as other districts

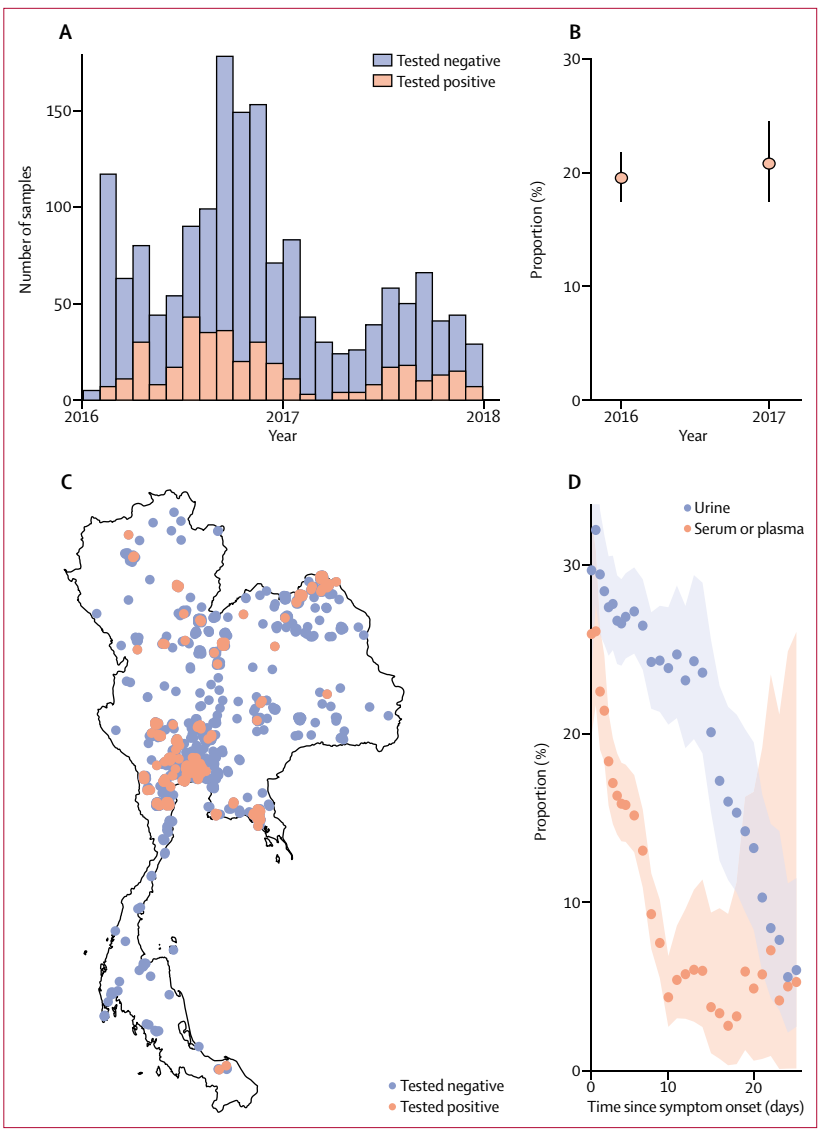


***Figure 1:*** **Location of suspected Zika cases and results of testing**
(A) Temporal distribution of the results of testing of symptomatic individuals from throughout Thailand from January, 2016, to December, 2017. (B) Proportion of symptomatic individuals who tested positive in each year. (C) Spatial distribution of samples. (D) Proportion of samples that tested positive as a function of time and type of sample. Each point represents the proportion positive from a 10-day time window. The shaded area represents 95% exact binomial CIs.

See Online for appendix

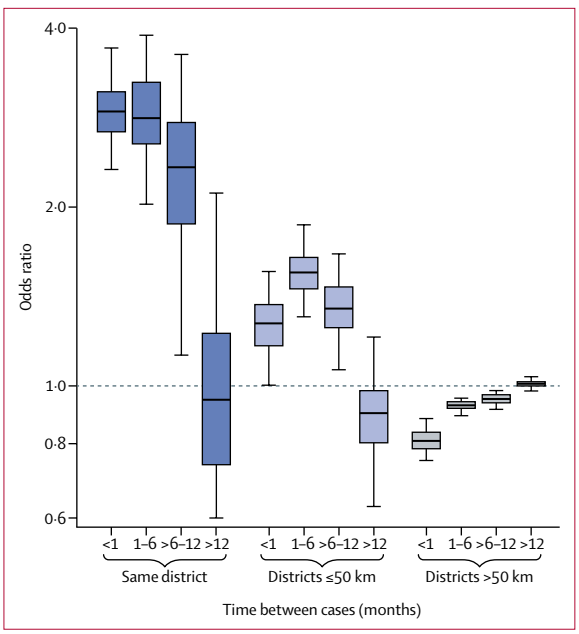


***Figure 2:*** **Spatial dependence between location of cases**
Odds of a symptomatic individual testing positive for Zika virus infection when they live at different spatial distances (within district [dark blue], neighbouring district [light blue; ≤50 km], and distal district [grey; >50 km]) of a confirmed case of Zika virus within different temporal windows relative to the odds of a symptomatic individual testing positive for Zika virus infection anywhere within that same temporal window. The box plots represent means with IQRs and 2·5% and 97·5% bootstrapped CIs.

within ≤50 km) and those who lived in distal districts (>50 km). We then considered time periods longer than 30 days (ie, 1–6 months, >6–12 months, and >12 months). This approach has previously been used to characterise the spatial and temporal dependence among individuals with dengue virus infection.[23] To assess uncertainty, we used a bootstrap approach where all the cases were resampled with replacement and the odds ratio recalculated. 95% CIs were obtained from the 2·5th and 97·5th percentiles of the resultant distribution.

To assess whether particular regions had a greater risk of Zika virus than others, we divided the country into six regions (north, northeast, central, south, west and east), and identified the region for each symptomatic individual. We then calculated the odds of testing positive among individuals within each region, relative to the odds of anyone testing positive. Values greater than one indicate a greater risk of observing a case in that region compared with the country as a whole, whereas values less than one indicates a reduced risk.

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

Between January, 2016, and December, 2017, 3089 samples were tested from 1717 symptomatic individuals for evidence of Zika virus infection. 368 (21%) of 1717 individuals tested positive for the infection (figure 1). There were 1247 serum or plasma samples (181 positive [15%]), 1831 urine samples (413 positive [23%]), and ten samples from other sources (saliva, cerebrospinal fluid, with none positive) received, with some patients providing multiple samples. For serum and plasma, the probability of testing positive was higher if samples were collected less than 7 days from symptom onset (150 [17%] of 879 samples) than if collected more than 7 days from symptom onset (21 [7%] of 283 samples; figure 1). For urine samples, 281 (28%) of 1015 samples were positive when collected less than 7 days from symptom onset, compared with 111 (17%) of 650 samples collected more than 7 days from symptom onset. Individuals with confirmed Zika virus infection were identified throughout the year, although with greater concentration in the second half of the year with 265 (72%) of 368 infections identified between July and December. The probability of symptomatic individuals testing positive was consistent across the 2 years (257 [20%] of 1258 in 2016 *vs* 111 [24%] of 459 in 2017). Although 224 (61%) of 368 individuals with confirmed Zika virus infection were female, once we accounted for the probability of being tested (1038 [62%] of 1666 symptomatic individuals sent for testing were female), we observed no difference in the odds ratio of testing positive by sex (OR 1·0, 95% CI 0·8–1·3).

Samples from individuals with symptoms consistent with Zika virus infection were sent from 60 of the 76 provinces in Thailand. 29 provinces (48%) and 77 districts, covering all regions in the country, had at least one case, consistent with a widespread distribution of the virus (figure 1). We observed a small increased risk of a symptomatic individual testing positive for Zika virus infection in the northeast (relative risk [RR] 1·5, 95% CI 1·2–1·8) and east (1·6, 1·2–2·2), and a reduced risk in the south (0·3, 0·03–0·9), relative to the country as a whole (appendix). Of the 223 symptomatic individuals tested, 53 confirmed Zika virus cases were from Bangkok, located within the central region, with the probability of individuals from the capital testing positive being the same as the country as a whole (1·1, 0·8–1·4).

Within a district we observed strong spatial dependence between cases. Symptomatic individuals had 2·8 times (95% CI 2·3–3·6) the odds of testing positive for Zika virus infection if they were from the same district and were sick within the same year of a confirmed Zika virus case relative to the odds of any symptomatic individual testing positive in that time interval (figure 2). These odds fell to 0·9 (0·2–1·8) at time intervals greater than a year. This spatial dependence extended to neighbouring districts too, but at a reduced scale (figure 2).

The mean age of individuals with Zika virus infection was 32 years (SD 17), slightly younger than the mean

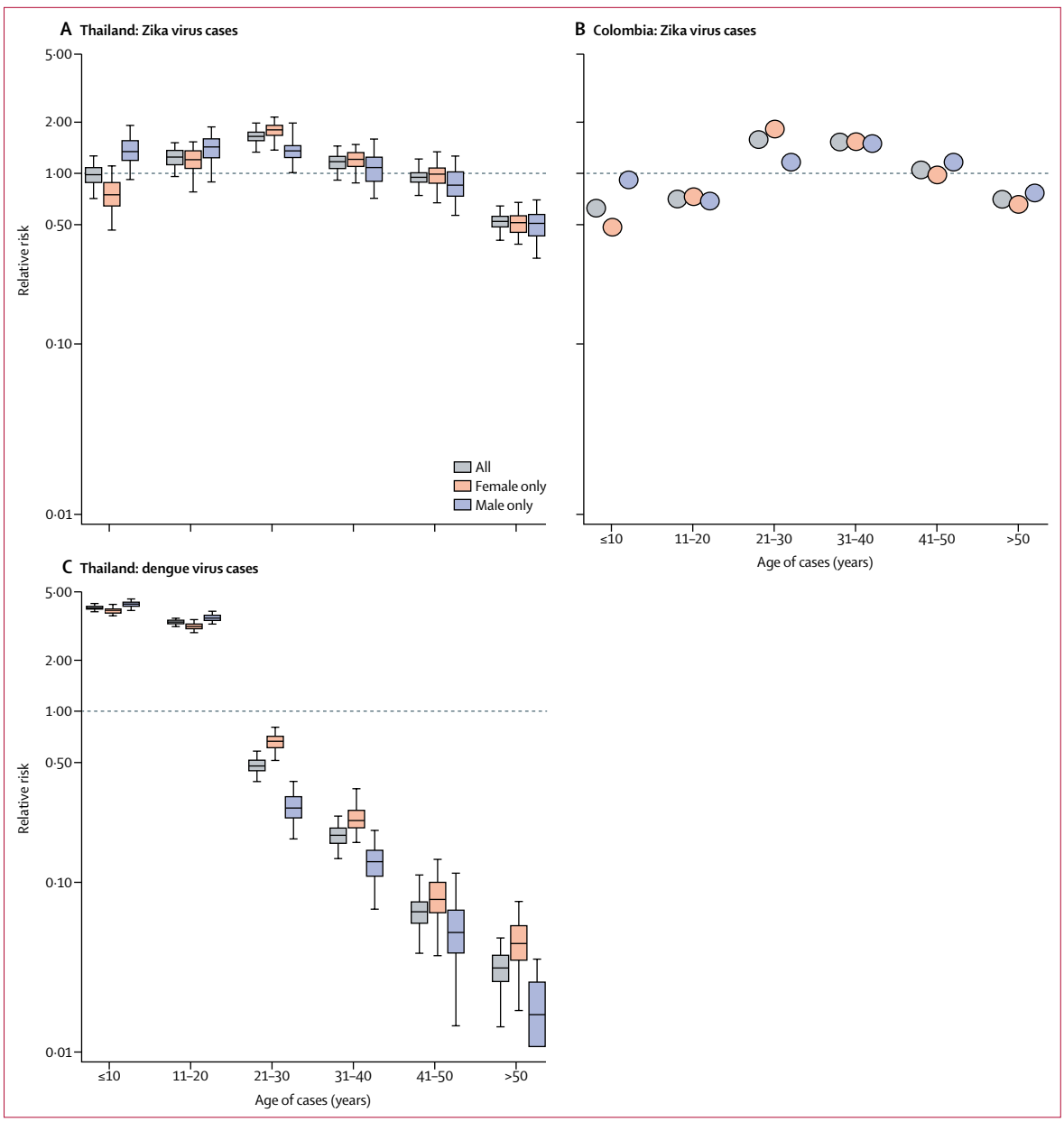


***Figure 3:*** **Age distribution of cases compared with underlying population**

Proportions of Zika virus cases in Thailand (A) and Colombia (B) that are within each age group relative to the proportion of the underlying population that are within that age group. (C) The proportion of dengue cases in Thailand that are within each age group relative to the proportion of the underlying population that are within that age group. The box plots represent means with interquartile ranges and 2·5% and 97·5% bootstrapped CIs.

age of the population (37 years). Among individuals with Zika virus infection, the probability of being younger than 10 years was 0·99 times (95% CI 0·72–1·30) the probability of being within that age range in the underlying population (figure 3). This probability rose to 1·62 times (1·33–1·92) among those aged 21–30 years and fell to 0·53 times (0·40–0·66) for individuals older than 50 years. As females of reproductive age might be more likely to seek care because of the risk of microcephaly, we repeated the analysis using males only, finding consistent results. These cases were also largely consistent with the age distribution of cases in Colombia, where the RR of being infected with Zika virus was lowest in individuals younger than 10 years (RR 0·63) and older than 50 years (RR 0·70), and highest in individuals aged 21–30 years (RR 1·58), with broadly similar patterns between females and males (figure 3). The mean age of a person with Zika virus infection in Colombia was 31 years, similar to the mean age in the population (30 years).[21] As a comparison,

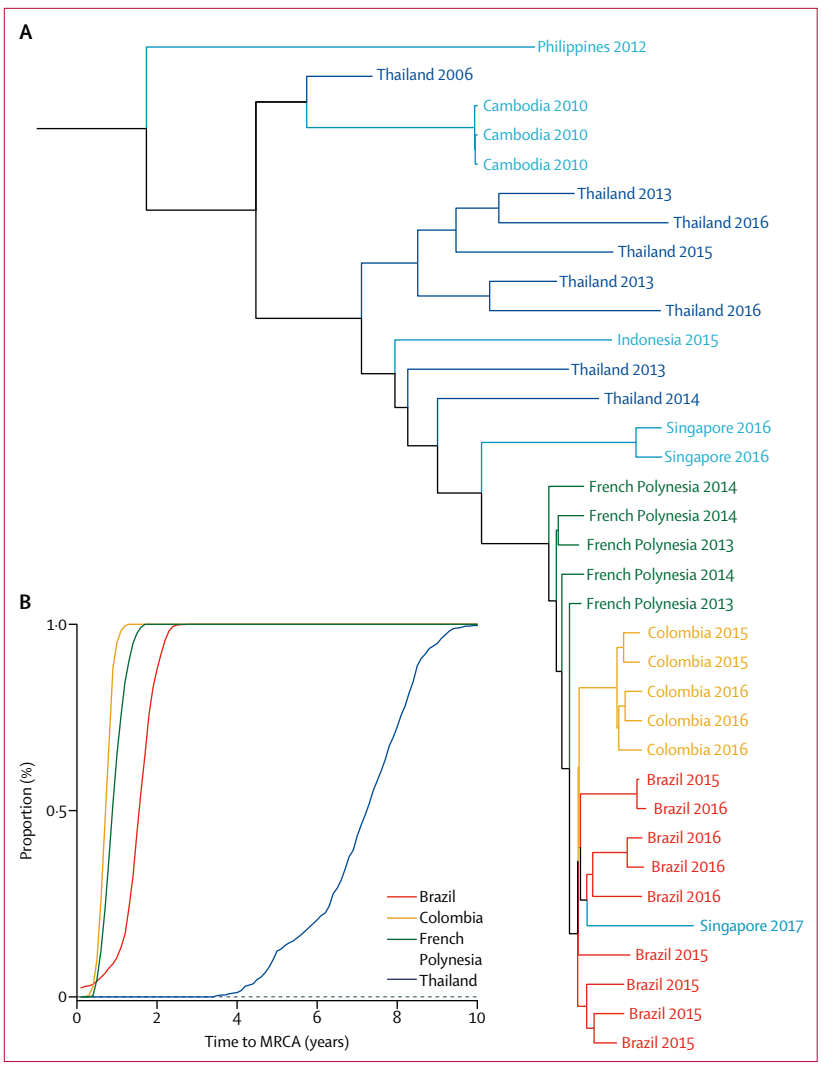


***Figure 4:*** **Genetic analysis**
(A) Time-resolved phylogenetic tree of Zika virus sequences available from GenBank. Two sequences from Malaysia from 1966 were used to root the tree and are not shown. (B) Proportion of pairs of sequences isolated within a year of each other that have their most recent common ancestor (MRCA) within different time limits by country of origin for the pair of viruses. Dotted line indicates zero.

cases of dengue virus in Thailand were strongly concentrated in children, with a mean age of 12 years (SD 9; figure 3). The probability of an individual with dengue virus infection being younger than 10 years was 4·01 times (95% CI 3·85–4·24) greater than the probability of being in that age range in the underlying population. Individuals with dengue virus infection were 0·04 times (0·01–0·05) as likely to be older than 50 years than the underlying population.

Using time-resolved phylogenetic trees, we found that the Thai sequences were ancestral to the viral strains from French Polynesia, Brazil, and Colombia (figure 4). Among these sequences, we estimated that the emergence of Zika virus occurred in October, 2002 (January, 2001, to April, 2004). We also found that the viruses were more diverse in Thailand than in the other locations. In Brazil, if we only considered pairs of viruses isolated within a year of one another, we found that 88% had an ancestor in common within the previous 2 years (figure 4). We found similar values for Colombia and French Polynesia. By contrast, no viral pairs from Thailand that came from individuals sick within a year of one another had an ancestor in common within the previous 2 years. It is only when we considered ancestors in the previous 8 years that this value rose to 73%.

## Discussion

We show that within the first few months of surveillance being initiated, Zika virus was found throughout Thailand. In individual locations, cases exhibited strong spatial dependence, lasting for at least 1 year. These findings are consistent with sustained focal transmission with occasional long-distance transmission events, as has previously been shown with dengue virus.[24] These findings are also consistent with sporadic case reports between 2012 and 2014 of Zika virus infection occurring throughout the country.[10] Although we could not identify the exact timepoint at which it first entered the country, Zika virus appears to have circulated since at least 2002, many years before the commencement of surveillance. This long-term circulation has not yet been enough to result in sufficient immunity to shift transmission to the youngest members of the population, as has occurred with dengue virus.

Where viruses shift from epidemic to endemic transmission, a resultant increase in the genetic diversity among circulating lineages occurs, as individual lineages establish sustained transmission chains. For example, for dengue virus, which has circulated endemically in Thailand for decades, fewer than 1% of pairs of viruses isolated in Bangkok at around the same time by the same serotype have a common ancestor within the previous 6 months.[24] Our findings suggest that Zika virus, in Thailand at least, has shifted towards endemic circulation. It remains unclear whether Zika virus can also make the transition to endemic circulation in South America, as dengue virus did in the 1990s.[25]

The hypothesis that Zika virus transmission will burn itself out is based on assumptions that large-scale population immunity will drive the disease to extinction.[15] Serosurveys done after the 2015–16 outbreak in South America have found high seroprevalence and are therefore consistent with this hypothesis.[26,27] Although serosurveys will be ultimately necessary to properly quantify the level of population immunity in Thailand, the observed similarity between the age-specific incidence patterns in Thailand as compared with those in Colombia and Puerto Rico, where Zika virus has only recently emerged, suggest limited population immunity.[20,28] Thus, our findings from Thailand suggest that Zika virus might have found a middle ground—sufficient transmission to maintain itself but not at high enough levels to result in

widespread immunity. Understanding why Zika virus exhibits such different transmission dynamics in Thailand compared with the Americas will require additional studies.

Our findings are also consistent with age-specific differences in symptomatic infection risk or health seeking behaviour. We find a reduced risk of being infected with Zika virus among individuals older than 50 years, and an increased risk in individuals aged 21–30 years. Similar patterns have been described in the American outbreak.[20,28]

Our findings highlight the key insight that phylogenetic approaches provide, even with only a handful of sequences. Although we cannot rule out that the individual sequences represent the recent offspring from independent introductions into the country, this would necessitate a diverse viral reservoir in the wider region, which has not been observed. This finding is also inconsistent with the spatially widespread nature of sporadic case reports within Thailand, before surveillance initiation.[10] Our study also shows the key sensitivity of phylogenetic analyses to the underlying sampling of isolates. The use of all available sequences from Thailand provides an estimate of the most common recent ancestor in the country being from around 2002. However, this estimate is largely reliant on a single isolate from 2006.[12] Had this isolate not been present, this estimate would have shifted to 2006 (13 years of circulation before 2019). It seems probable that additional sampling would shift the estimated date of emergence further back in time.

Our findings have implications for the ongoing surveillance of Zika virus-related severe health complications. Between January, 2016, and August, 2018, there were four cases of Guillain Barré syndrome in individuals with PCR-confirmed Zika virus infection in Thailand.[29] Over the same period, 130 pregnant women were identified with Zika virus infection, of whom two had subsequent abortions because of fetal Zika virus infection. A further 119 of these women have since given birth, four of whom to babies with microcephaly, although none had signs of fetal Zika virus infection. Over the same period, 285 microcephaly cases in which Zika virus infection had not previously been identified in the mother were reported; congenital Zika virus syndrome was identified in three of these cases. Our findings suggest that should Zika virus continue to circulate endemically these severe complications will occur regularly, and surveillance needs to be able to reliably identify and follow up pregnant women.

We do not know why individuals sought care or had their samples sent to the central laboratory for processing. If older individuals were less likely to be identified, this would imply even less immunity in the population, whereas reduced probability of detection in the youngest individuals would indicate greater immunity. However, there was no age-specific guidance for testing, so any differences are likely to be minor. Furthermore, none of these findings change our inference of long-term circulation of the virus. Population-representative seroprevalence studies, with serological assays that can distinguish between antibody responses to different flaviviruses (particularly Zika virus, dengue virus, and Japanese encephalitis virus) are needed to help quantify the level of circulation and immunity in the population.

Our findings provide strong evidence of the long-term and wide spread of Zika virus in Thailand, suggesting that Zika virus can transition to endemic transmission. These results support the development of long-term sustained interventions and surveillance efforts to tackle both the spread of the virus and the regular occurrences of severe Zika virus-related health outcomes.

#### Contributors

HS conceived of the study, did the data analysis, and wrote the first draft of the Article. KR, PW, AP, and SS did the underlying data collection, interpretation of the assays, and contributed to revising the Article. IR-B and DATC contributed to data analysis, data interpretation, and revision of the Article.

#### Declaration of interests

We declare no competing interests.

#### Acknowledgments

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