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Epidemiology of dengue and the effect of seasonal climate variation on its dynamics in the Chao-Shan area, on China's southeastern coast

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4 **Epidemiology of dengue and the effect of seasonal climate variation on its**
5 **dynamics in the Chao-Shan area, on China's southeastern coast**
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Abstract

Objectives: Dengue is a mosquito-transmitted virus infection that remains rampant across the tropical and subtropical areas worldwide. However, the spatial and temporal dynamics of dengue transmission are poorly understood in Chao-Shan area, one of the most densely-populated regions on China's southeastern coast, limiting disease control efforts. Therefore, we characterized the epidemiology of dengue and assess the effect of seasonal climate variation on its dynamics in the area. **Methods:** Data of dengue cases of three cities including Shantou, Chaozhou and Jieyang in Chao-Shan area during 2014-2017 were extracted. Data for climatic variables including mean temperature, relative humidity, and rainfall were also compiled. We initially described the epidemiology and dynamics of dengue, and then assessed temporal epidemic dynamics with a specific periodicity related to climatic drivers using a wavelet analysis method. Furthermore, a generalized additive model for location, scale and shape model was performed to study the relationship between seasonal dynamics of dengue and climatic drivers. **Results:** Among the cities, the number of notified dengue cases in Chaozhou was greatest, accounting for 78.25%. The median age for the notified cases was 43 years (interquartile range (IQR): 31 years). Two main regions located in Xixin and Chengxi streets of Chaozhou with a high risk of infection were observed, indicating that there was substantial spatial heterogeneity in intensity. We detected an annual attack activity of dengue peaking in autumn across the region, most markedly in 2015. This study reveals that periods of elevated temperatures can drive the occurrence of dengue epidemics across the region, and the risk of transmission is most when the temperature is reached to around 25 °C. **Conclusions:** Our study contributes to a better understanding of dengue dynamics, and a better deployment of control and prevention strategies for local government in Chao-Shan area.

Keywords: China; climate; dengue; seasonal variation; spatial; wavelet analysis

Strengths and limitations of this study

- There was substantial spatial heterogeneity in intensity of dengue incidence across Chao-Shan area.
- This study reveals that periods of elevated temperatures can drive the occurrence of dengue epidemics across Chao-Shan area.
- The risk of dengue transmission is most when the temperature is reached to around 25 °C.

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Introduction

Dengue is characterized as a serious infectious disease with a variety of clinical symptoms, from mild fever to potentially fatal dengue shock syndrome, and remains rampant across the tropical and subtropical areas in the world [1]. There is a sharp increase in the number of dengue infections over the past few decades, and the disease not only has a great influence on population health, but also brings a heavy economic burden to patients, society and government [2]. In China, the areas affected by dengue have expanded and there has been a gradual increase in the incidence over the recent years [3, 4]. In particular, an extensive outbreak of dengue hit China in 2014, and during this outbreak a succession of epidemic fluctuation of dengue occurred in several provinces including Guangdong, Guangxi and Yunnan of southern China [5]. In fact, during this 2014 outbreak, the number of dengue infections in China reached the highest level over the past 25 years [6].

In China, dengue cases are notified to the China Center for Disease Control and Prevention (CDC) for the surveillance of spread pattern of dengue epidemic. We previously observed a remarkable spatial-temporal heterogeneity of dengue incidence in Guangdong, the most developed province of southern China, and showed that the dengue epidemic had noticeable seasonal and annual variability [5, 6]. In fact, recent studies suggest that dengue may now be endemic to China [7], and the dengue outbreaks are strongly related to climatic factors including temperature, rainfall and humidity which have direct impacts on mosquito abundance [8]. Enjoying a subtropical humid monsoon climate and frequent economic and cultural communication with the Southeast Asian countries [9, 10], Guangdong has a potential high-risk opportunity for transmission of dengue. In particular, dengue poses a great burden of disease in the Pearl River Delta Region of Guangdong province, and the epidemic characteristics have been well documented [5-8].

However, the impact of seasonal climatic variation on dengue dynamics in the

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4 Chao-Shan area (Figure 1), the eastern part of Guangdong province is poorly
5 quantified. The Chao-Shan area is one of the special regions on China's southeastern
6 coast. The region has a jurisdiction area of around 10918 square kilometers, but a very
7 high population density. In addition, it is situated in the intersection part of the Tropic
8 of Cancer and the coastline of the mainland China, and has a subtropical oceanic
9 monsoon climate with a mean annual temperature of around 22 °C and an abundant
10 precipitation per year. With the special climatic conditions and the increasingly
11 frequent cultural contact with Southeast Asian countries, the Chao-Shan area itself has
12 a potential high risk of spread of mosquito-borne infectious diseases.
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23 Unfortunately there are few comprehensive reports on the spatial-temporal epidemic
24 pattern for dengue in the Chao-Shan area. The effects of seasonal climate variation on
25 the dynamics of dengue and the associated disease burden in the region are still
26 unclear, highlights gaps in our knowledge to the current status of dengue epidemic
27 and the deployment of prevention and control measures. Therefore, this study aimed
28 to assess the epidemic characteristics of dengue in the Chao-Shan area, and the effect
29 of seasonal climate variation on the dynamics of dengue for the period 1 January 2014
30 to 31 December 2017.
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41 **Material and methods**

42 **Patient and Public Involvement**

43 All information on individual privacy of patients with dengue fever infection is kept
44 confidential in this study. Only the data for number of dengue cases and
45 meteorological parameters were used to construct time series for assessing their
46 associations.
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54 **Dengue Case Data**

55 The Chao-Shan area including Shantou, Chaozhou and Jieyang cities is one of the
56 most densely-populated regions on China's southeastern coast. In addition, this area
57 has special climatic conditions, geographical position, and the frequent culture and
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4 economic communions with Southeast Asian countries. However, the epidemiological
5 characteristics of dengue and disease burden associated with dengue infections are
6 historically unclear. Therefore, the area was selected as the study site.
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11 From September 1, 2008, all probable, clinic-and laboratory-confirmed cases of
12 dengue were reported to the Chinese Center for Disease Control and Prevention
13 (China CDC), and diagnosed according to the Diagnostic Criteria for Dengue [11]
14 released by the National Health Commission of China. Probable and confirmed cases
15 were reported online to the China CDC within 24 hours of diagnosis, by use of a
16 standardized form including basic demographic information (sex, date of birth, and
17 residential address), case classification (probable, clinic-confirmed or
18 laboratory-confirmed), date of symptoms onset, and date of diagnosis. Data for
19 dengue cases of Shantou, Chaozhou and Jieyang cities in the Chao-Shan area from
20 January 1, 2014 to December 31, 2017 were available from the Guangdong Provincial
21 CDC. Only indigenous dengue cases were included, and weekly number of dengue
22 cases was calculated for statistical analysis.
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37 **Climate Surveillance Data**

38 Data for climatic variables including mean relative humidity, mean rainfall
39 (millimeters), and mean temperature (°C) of the three cities during the study period
40 from January 1, 2014 to December 31, 2017 were obtained from the Guangdong
41 Provincial Climate Bureau. Climatic variables were aggregated into a weekly basis to
42 show the seasonal peaks of dengue in surveillance data, and used to assess the
43 potential effect of seasonal variation on the dengue dynamics.
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52 **Statistical Analysis**

53 First, basic characteristics (number, median age, sex ratio, and season and year of
54 diagnosis) of dengue cases reported during the study period were described by city.
55 The probability density distributions of the onset-to-diagnosis of dengue cases for the
56 three cities were calculated to estimate median duration from onset to diagnosis and
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4 test the reaction ability of the surveillance system. Second, according to the residential
5 address registered for each dengue case, we created a map of the number of dengue
6 cases in a spatial scale of street to assess the spatial distribution pattern of dengue
7 epidemic in the Chao-Shan area.
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13 Third, to quantify seasonal patterns of dengue epidemic, time series of weekly case
14 counts were plotted by city, and type of cases (probable or confirmed cases),
15 respectively. The periodicity of the time series of dengue case counts, mean
16 temperature, mean rainfall and mean relative humidity was assessed using
17 time-dependent wavelet analysis [12]. A continuous Morlet wavelet transform on the
18 time series was performed to extract specific frequency components, which were
19 visualized with a wavelet plot in this study. A statistical significance test was
20 performed to test the null hypothesis that a period was irrelevant at a certain time with
21 significance level of 95%.
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33 Fourth, in view of the presence of overdispersion in the dengue case data set, a
34 generalized additive model for location, scale and shape (GAMLSS) model [13] with
35 the distribution of the response variable obeying negative binomial distribution was
36 performed to study the association between seasonal characteristics of dengue and
37 climatic variables, including mean temperature, mean rainfall and mean relative
38 humidity. We implemented the Rigby and Stasinopoulos algorithm [13] to estimate
39 the parameters of the models, and the number of cycles of the algorithm was set to
40 2000 to insure the convergence of the analysis results.
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50 In order to assess the effects of climatic variables on seasonal dynamics of dengue, we
51 established a set of GAMLSS models where climatic variables were modeled as
52 smooth terms with different degrees of freedom (df). This was performed by changing
53 the df for the smooth terms of 3 to 0 during model selection, where the $df=0$ means
54 the distribution of the response variable was directly modeled as a linear parametric
55 function of explanatory variables. We used the smoothing splines for the smooth
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4 terms in the GAMLSS models. As model selection criterion, the Schwartz Bayesian
5 Criterion (SBC) [14] was used here. Models with lower value of SBC are preferred. In
6 addition, we also added the autoregressive term, $\ln(1 + Count_{local\ cases})$ in the previous
7 month, to account for the autoregressive effect in this study [8]. All analyses were
8 conducted using R software (version 3.4.3).
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13 Results

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15 Basic characteristics of dengue cases notified during 2014-17 were shown in Table 1.
16 In total, 2,193 probable and confirmed cases of dengue in the Chao-Shan area were
17 reported to the China CDC system, of which 2,148 (97.95%) were confirmed (clinic-
18 or laboratory-confirmed) and 45 (2.05%) were probable, respectively. Among all the
19 reported cases, the proportion (78.25%) of dengue cases reported in Chaozhou was
20 higher than those among Shantou (12.95%), and Jieyang (8.80%). The median age for
21 all notified cases was 43 years (interquartile range (IQR): 31 years), for Shantou cases
22 35 years (IQR: 26 years), Chaozhou cases 46 years (IQR: 30 years), and Jieyang cases
23 34 years (IQR: 25 years), respectively. The age distributions among probable and
24 confirmed cases for Shantou and Jieyang cities were similar. The male to female ratio
25 was 1:1.06 for all notified dengue cases. The sex distributions among cases for
26 Jieyang were different from those for the other two cities.
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41 For the most recent year 2017 observed, most of the patients was male patients,
42 irrespectively of subgroup analysis results according to total patients, the younger
43 (<35 years old) and elder (≥ 35 years old) (Figure 2, A-C). The median time from
44 illness onset to diagnosis was 6 days (IQR 2.75-8) for Shantou, 3 days (3-5) for
45 Chaozhou, and 3 day (1-5) for Jieyang. The plot of probability density distributions
46 also demonstrated that there was a longer onset-to-diagnosis interval for dengue cases
47 in Shantou (Figure 2, D).
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57 Geographical distribution of dengue incidence in the Chao-Shan area was shown in
58 Figure 3. Radiuses of circles indicate incidence of dengue. We identified two main
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4 geographical regions with a high risk of infection with dengue during the 4-year study
5 period: Xixin and Chengxi streets in Chaozhou city. Overall, dengue showed annual
6 peaks of activity, a major peak in autumn, consistent among time series of dengue
7 cases in Shantou, Chaozhou and Jieyang (Figure 4). Although Chao-Shan area had a
8 seasonal attack of dengue peaking between September and October of each year, we
9 observed that the number of dengue cases increased sharply in 2015 in Chaozhou.
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17 Results of wavelet analysis for time series of notifications of dengue and climatic
18 variables including mean temperature, mean rainfall and mean relative humidity in
19 Chao-Shan area during the study period were shown in Figure 5. It showed that the
20 estimations of local powers for weekly time step examined were the largest at the
21 period of 1 year, suggesting dengue cases showed a significant annual periodicity
22 (Figure 5, A). In particular, based on the strongest power of the annual periodicity
23 estimated for the time series of climatic variables (mean temperature, rainfall and
24 relative humidity) throughout the study period (Figure 5, B-D), these factors broadly
25 followed similar periodicity with that of dengue cases.
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37 Based on the results of wavelet analysis, we further analyzed the effects of climatic
38 variables on seasonal dynamics of dengue using a framework of GAMLSS by testing
39 different degrees of freedom for the smooth terms during model selection to
40 determine an optimal model (Table S1). We found that the model with the variable of
41 mean temperature modeled as a smoothing spline term with $df=1$ had the smallest
42 value of SBC and the best model fit among the 70 kinds of candidate models,
43 suggesting that the effect of mean temperature on dengue dynamics in Chao-Shan
44 area was most obvious. In addition, according to the GAMLSS framework, a
45 significant nonlinear partial effect of mean temperature on dengue dynamics have
46 been observed, and the risk of dengue transmission was most when the temperature
47 was reached to around 25 °C (Figure 6).
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Discussion

Our study of probable and confirmed cases of dengue reported to the national CDC surveillance system during 2014-17 gives the first comprehensive estimation of the local burden and epidemiology of the disease in the study sites. Among the three cities covering a jurisdiction area of around 10918 square kilometers, Chaozhou is responsible for the largest proportion of all the notified dengue cases. We recorded a median age of infection at 43 years (IQR: 31 years) for all notified dengue cases, and the male to female ratio was 1:1.06. Our study suggests that cases of dengue tend to arise in autumn season of the year, presenting a significant annual periodicity of epidemics. In particular, we found that the effect of temperature on dengue epidemics was most obvious among the climatic factors assessed.

This present analysis of spatial distribution patterns of dengue identified two main epidemiological regions corresponding to Xixin and Chengxi streets in Chaozhou city, where dengue peaks in autumn. Our results indicate that dengue cases showed a significant annual periodicity in Chao-Shan area. Findings from Asia-Pacific countries including Thailand, Laos, and the Philippines have shown localized traveling waves of multiannual dengue epidemic cycles [15]. By contrast, data of dengue virus isolate counts from Puerto Rico, a major population center in the Caribbean, indicate interannual variation in transmission across multiple dengue serotypes [16]. An epidemiological study from Machala, Ecuador, provided evidence of significant 1-year and 2-year cycles in dengue [17]. In fact, our data were in agreement with another study indicating that significant coherences were observed between dengue incidence and temperature for the annual periodicity from 2005 to 2009 from Hanoi, Vietnam [18]. Our wavelet analysis revealed that dengue transmission co-varied with temperature at annual cycle, and we inferred that this acted as an important driver for the periodicity of dengue dynamics in Chao-Shan area.

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4 This study built on potential links between dengue dynamics and climatic variables,
5 and quantified their relationships based on a kind of state-of-the-art flexible
6 regression and smoothing technique, GAMLSS model, which enables us to handle
7 adequately the problem of the presence of overdispersion in the data. The GAMLSS is
8 a general framework for fitting semi-parametric regression type models where the
9 distribution of the response variable includes highly skew and kurtotic continuous and
10 discrete distribution, and allows all the parameters of the distribution of the response
11 variable to be modeled as smooth functions of the explanatory variables [13]. Unlike
12 traditional linear regression, the approach enables to find a nonlinear relationship
13 between the dependent variable and a set of independent variables by specifying an
14 appropriate smooth function term that describes the relationship [19]. By inspecting a
15 scatterplot (data was not shown) between dengue incidence and temperature, we
16 found the relationship was nonlinear, so it required the special estimation methods of
17 the nonlinear regression procedure. Additionally, the choice of df values is very
18 important when constructing the GAMLSS model. Therefore, we performed a
19 sensitivity analysis by trying different df in the smooth term of the model, and
20 determined an optimal model according to the assessment of model fit. Overall, our
21 results revealed a powerful capability with GAMLSS model for catching the
22 appropriate relationship between dengue incidence and temperature.
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42 The associations between dengue seasonality and climatic variables such as
43 temperature, rainfall, relative humidity, sunshine and ENSO indices have been
44 documented in previous studies [20]. In this study, our results reveal that temperature
45 has significantly nonlinear effects on epidemics risk and intensity of dengue, with the
46 greatest attributable risk due to temperature estimated at around 25 °C. For detecting
47 the significantly associated climatic factors with dengue epidemics, we repeated
48 model selection, using different climatic factors with different df in the smooth terms
49 of the GAMLSS framework. We found that no additional climatic factors, expect for
50 mean temperature, were related to dengue epidemics, and thus needed to explain these
51 seasonal peaks in dengue incidence in Chao-Shan area. The association between
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4 temperature and seasonal dynamics of dengue in our study was also reported in
5 previous studies in Guangzhou [8] and Taiwan [21], which has a similar subtropical
6 humid monsoon climate. The estimated effects of temperature driving the seasonal
7 dynamics of dengue can be partly explained by some mechanisms, for example,
8 temperature effects on the biting rate of mosquitoes [22], incubation period of
9 pathogens [23], and human exposure to mosquitoes.
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17 Several limitations of this study should be mentioned. First, we did not consider
18 potentially relevant factors, such as mosquito density and other environment elements
19 including vegetation cover, distance to water bodies and so on. The variables may
20 partly influence the relationship between temperature and dengue epidemics estimated
21 in this work. In addition, although the quantitative results derived from this study
22 pointed out that temperature was associated with increased risk of dengue
23 transmission, other factors such as human activities during urbanization and
24 globalization were also suggested to be very important in driving the long-term trends
25 [8, 24]. Furthermore, our results faced a common challenge for studies using data
26 from surveillance with possible under-reporting or variations in diagnosis practices of
27 cases. This was also suggested from a previous study [25].
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41 **Conclusions**

42 In summary, this study represented an initial step to analyze the epidemiological
43 characteristics of dengue in Chao-Shan area, one of the most densely-populated
44 regions on China's southeastern coast, and use a statistically rigorous approach for
45 assessing the effects of climatic factors on dengue epidemics. Overall, our results are
46 helpful toward advancing our understanding of the pattern of how climate influences
47 dengue, and useful for the control and prevention of the disease for local government
48 in the study sites.
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58 **Contributors**

59 QZ, PG and WJM conceived the study, undertook statistical analysis and drafted the
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manuscript. YLC, LW, YHZ, YF, TL and QYZ collected the data and assisted in the statistical analysis. QZ, PG, TL, QYZ and WJM interpreted the results. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Data sharing statement

No additional data are available.

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Figure Legends

Figure 1 Geographical location of Chao-Shan area including Shantou, Chaozhou and Jieyang cities, on China's southeastern coast.

Figure 2 Proportions of gender-specific cases of dengue fever by age groups and estimates of onset-to-diagnosis distributions of dengue fever cases in Chao-Shan area, 2014-2017. (A) Based on total cases. (B) Based on cases aged less than 35 years. (C) Based on cases aged greater than or equal to 35 years. (D) Onset-to-diagnosis distribution by city.

Figure 3 Geographical distribution of dengue incidence in Chao-Shan area from the beginning of 2014 to the end of 2017. Radiuses of circles indicate incidence of dengue. Red circles represent dengue in Chao-Shan area.

Figure 4 Temporal dynamics of dengue in Chao-Shan area from the beginning of 2014 to the end of 2017. (A) Weekly time series of number of probable and confirmed dengue cases in Chao-Shan area. (B) Weekly time series of number of probable and confirmed dengue cases by city in Chao-Shan area.

Figure 5 Wavelet analyses for time series of notifications of dengue and climatic variables including mean temperature, rainfall and relative humidity in Chao-Shan area, 2014-2017. Local wavelet power spectrum for dengue cases (A), mean temperature (B), rainfall (C) and relative humidity (D). Solid and bold lines indicate boundary of statistical significance.

Figure 6 Analysis of potentially nonlinear effects of mean temperature on seasonal dynamics of dengue in Chao-Shan area using a generalized additive model for location, scale and shape (GAMLSS) model based on data from years 2014-2017. Initially, climatic variables including mean temperature, rainfall and

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4 relative humidity were assessed using the GAMLSS model with different degrees of
5 freedom for the smooth terms during model selection. Then, the optimal model with
6 the statistically significant variable (mean temperature) was determined. This plot
7 shows the significant nonlinear partial effect of mean temperature on dengue
8 dynamics, and the risk of dengue transmission is most when the temperature is
9 reached to around 25 °C.
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Tables

Table 1 Basic characteristics of dengue cases by city reported during the study period of 2014-2017.

Characteristics	City		
	Shantou	Chaozhou	Jieyang
Number of cases, number (%)	284 (12.95)	1,716 (78.25)	193 (8.80)
Type of cases, number (%)			
<i>Probable cases</i>	0 (0.00)	45 (2.62)	0 (0.00)
<i>Confirmed cases</i>	284 (100.00)	1,671 (97.38)	193 (100.00)
Median age, years (IQR)	35.00 (26.00)	46.00 (30.00)	34.00 (25.00)
Sex, number (%)			
<i>Male</i>	137 (48.24)	819 (47.73)	110 (56.99)
<i>Female</i>	147 (51.76)	897 (52.27)	83 (43.01)
Season of diagnosis, number (%)			
<i>Spring</i>	0 (0.00)	0 (0.00)	0 (0.00)
<i>Summer</i>	0 (0.00)	33 (1.92)	0 (0.00)
<i>Autumn</i>	281 (98.94)	1,680 (97.90)	192 (99.48)
<i>Winter</i>	3 (1.06)	3 (0.18)	1 (0.52)
Year of diagnosis, number (%)			
2014	247 (86.97)	135 (7.87)	86 (44.56)
2015	14 (4.93)	1380 (80.42)	6 (3.11)
2016	5 (1.76)	194 (11.31)	0 (0.00)
2017	18 (6.34)	7 (0.41)	101 (52.33)

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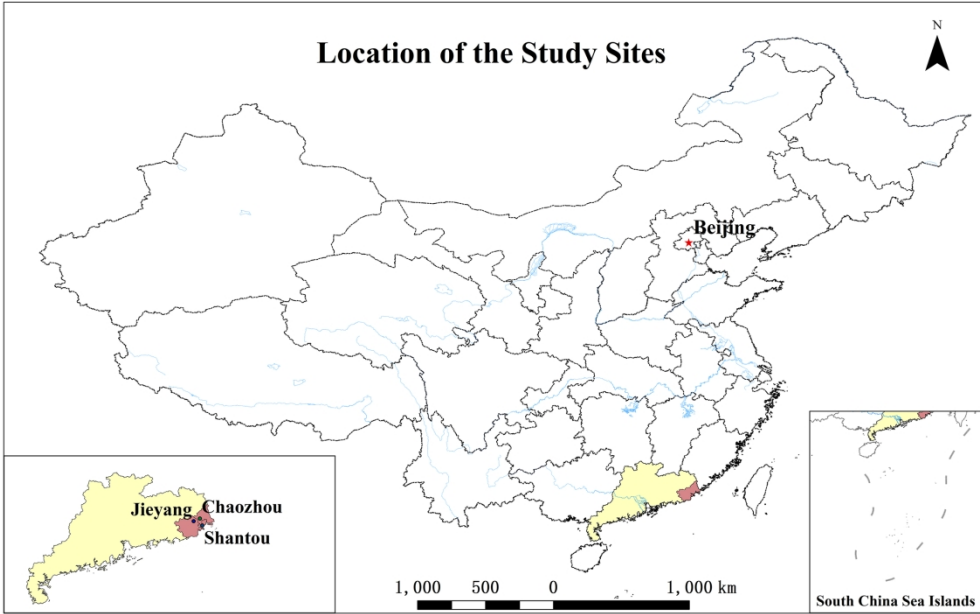


Fig 1

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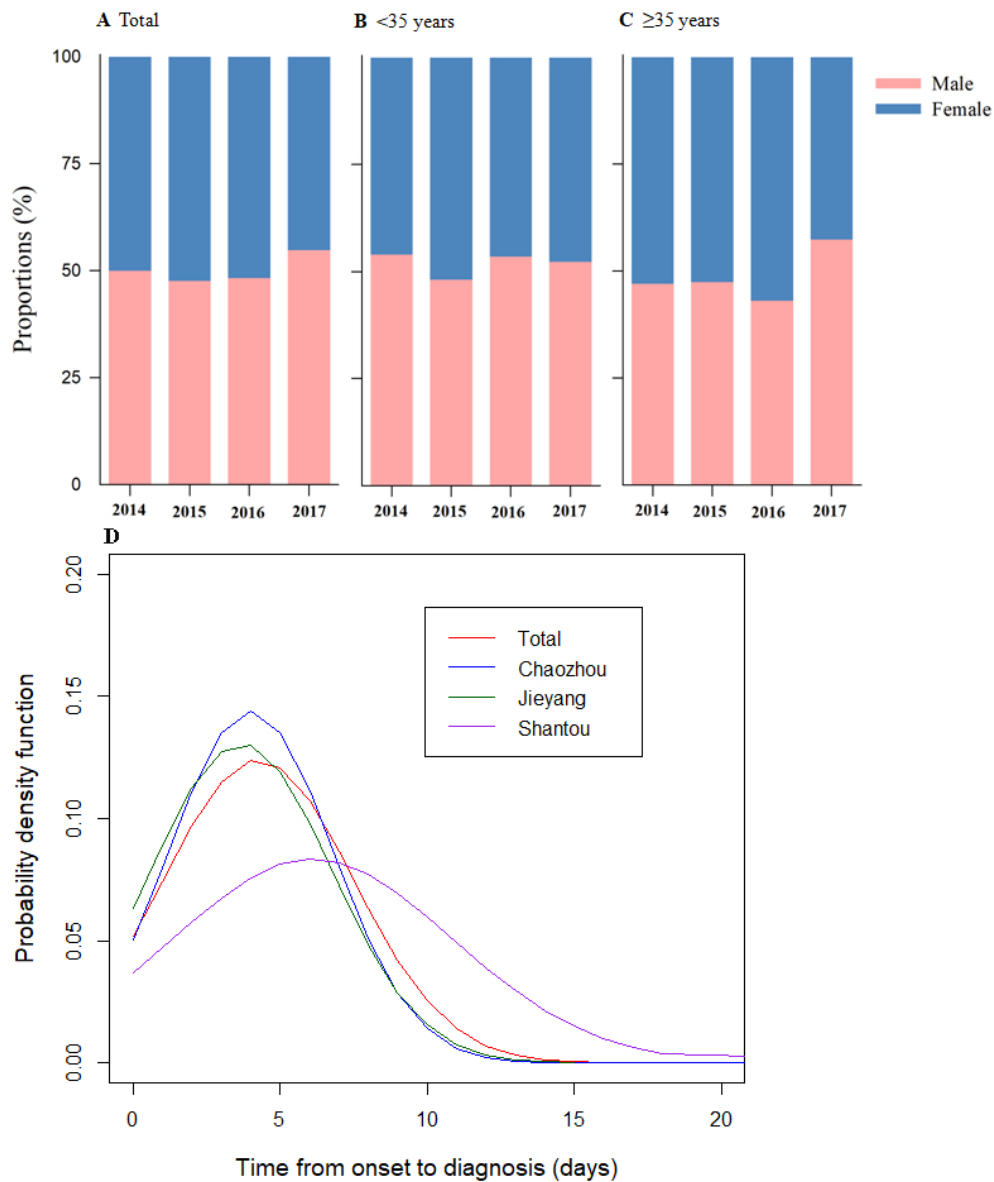


Fig 2

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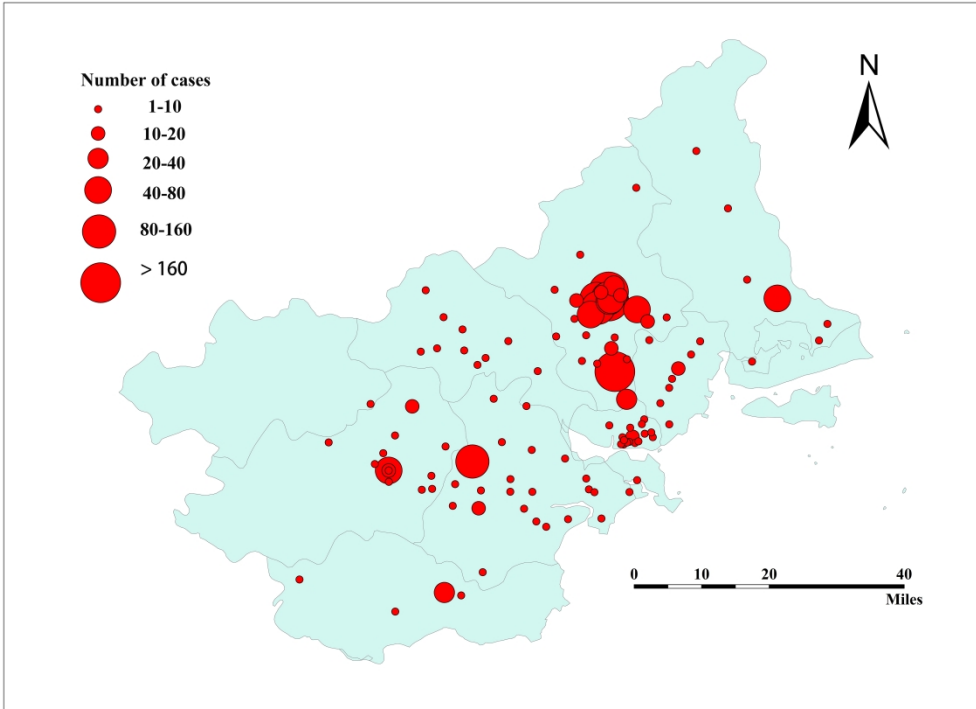


Fig 3

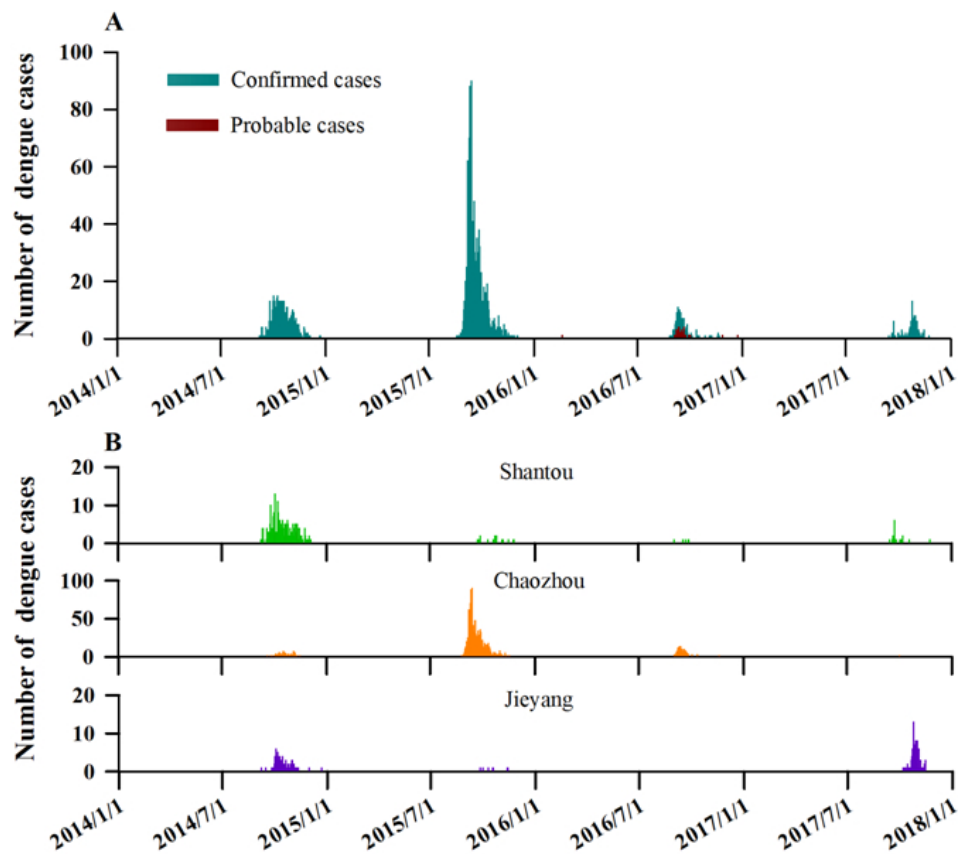


Fig 4

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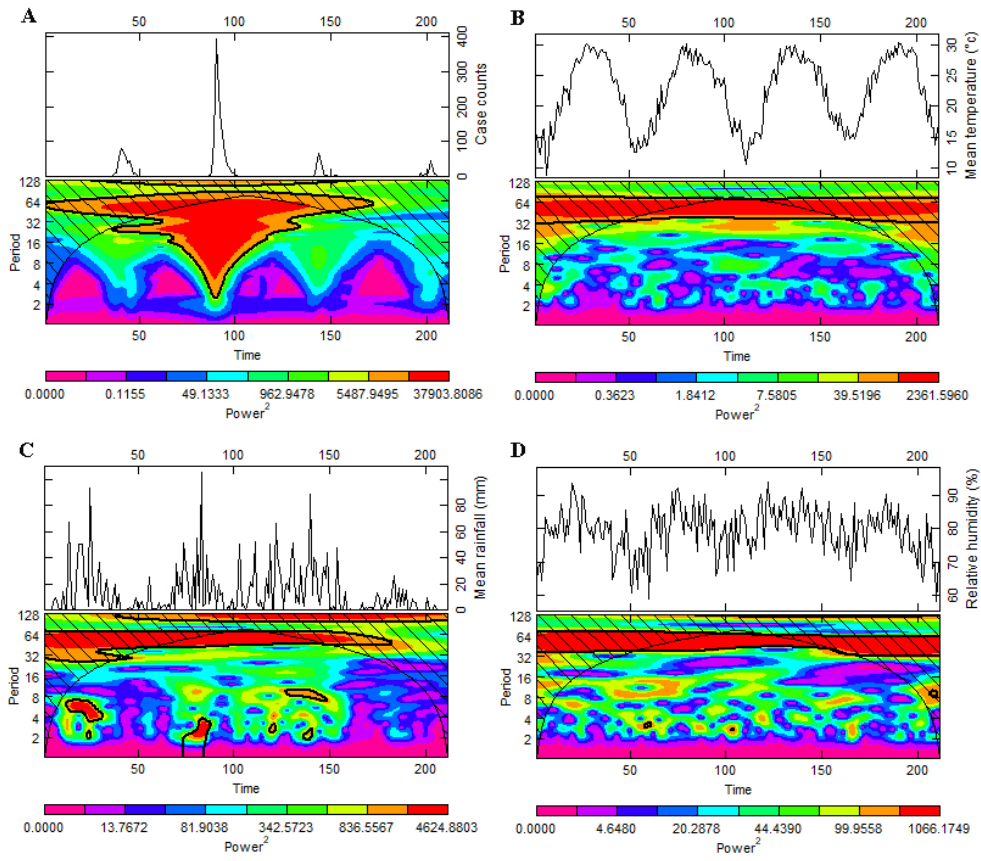


Fig 5

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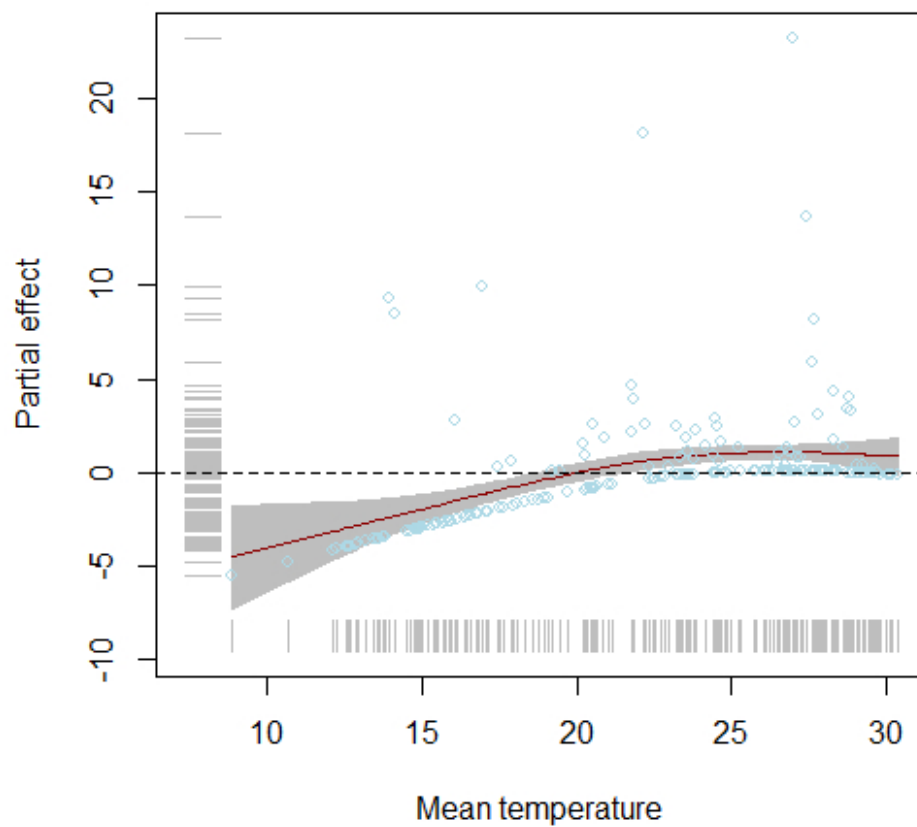


Fig 6

Supplementary File

Table S1 Model fit assessment of a set of generalized additive model for location, scale and shape (GAMLSS) models where climatic variables including mean temperature (MeanTemp), rainfall (Rainfall) and relative humidity (RH) were modeled as smooth terms with different degrees of freedom (*df*). The criterion, Schwartz Bayesian Criterion (SBC), was used for model selection, where models with lower value of SBC are preferred. 0, 1, 2 and 3 were considered for the values of the parameter *df* in the GAMLSS models, respectively. Cubic spline (CS) function was used as the smooth function term.

Rank of model	Model formula	SBC
1	~ CS(MeanTemp, 1)	686.105
2	~ CS(MeanTemp, 2)	686.871
3	~ CS(MeanTemp, 3)	689.207
4	~ MeanTemp	690.681
5	~ CS(MeanTemp, 1) + CS(Rainfall, 1)	691.759
6	~ CS(MeanTemp, 2) + CS(Rainfall, 1)	691.811
7	~ CS(MeanTemp, 3) + CS(Rainfall, 1)	692.740
8	~ CS(MeanTemp, 1) + CS(Rainfall, 2)	693.124
9	~ CS(MeanTemp, 2) + CS(Rainfall, 2)	693.623
10	~ MeanTemp + Rainfall	694.608
11	~ MeanTemp + RH	694.628
12	~ CS(MeanTemp, 1) + CS(RH, 1)	694.790
13	~ CS(MeanTemp, 2) + CS(RH, 1)	695.267
14	~ CS(MeanTemp, 3) + CS(Rainfall, 2)	695.459
15	~ CS(MeanTemp, 3) + CS(RH, 1)	695.533
16	~ CS(MeanTemp, 1) + CS(Rainfall, 3)	696.474
17	~ CS(MeanTemp, 2) + CS(Rainfall, 3)	697.546
18	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 1)	699.055
19	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 1)	699.193
20	~ CS(MeanTemp, 1) + CS(RH, 2)	699.303
21	~ CS(MeanTemp, 3) + CS(Rainfall, 3)	699.363
22	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 2)	699.565
23	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 1)	699.655
24	~ MeanTemp + RH + Rainfall	699.672
25	~ CS(MeanTemp, 2) + CS(RH, 2)	699.851
26	~ CS(MeanTemp, 3) + CS(RH, 2)	700.121
27	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 2)	700.460
28	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 2)	700.535
29	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 3)	703.064

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30	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 1)	703.307
31	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 1)	703.395
32	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 3)	703.790
33	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 2)	703.836
34	~ CS(MeanTemp, 1) + CS(RH, 3)	703.955
35	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 1)	704.037
36	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 3)	704.168
37	~ CS(MeanTemp, 2) + CS(RH, 3)	704.432
38	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 2)	704.519
39	~ CS(MeanTemp, 3) + CS(RH, 3)	704.845
40	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 2)	705.177
41	~ RH	707.331
42	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 3)	707.364
43	~ CS(Rainfall, 1)	707.472
44	~ CS(Rainfall, 2)	707.558
45	~ Rainfall	707.656
46	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 1)	707.991
47	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 1)	708.093
48	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 3)	708.267
49	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 3)	708.330
50	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 2)	708.505
51	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 1)	708.792
52	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 2)	709.107
53	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 2)	709.867
54	~ CS(Rainfall, 3)	710.239
55	~ CS(RH, 1)	711.324
56	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 3)	712.016
57	~ RH + Rainfall	712.666
58	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 3)	712.849
59	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 3)	713.085
60	~ CS(RH, 2)	715.945
61	~ CS(RH, 1) + CS(Rainfall, 1)	717.272
62	~ CS(RH, 1) + CS(Rainfall, 2)	717.727
63	~ CS(RH, 3)	720.343
64	~ CS(RH, 1) + CS(Rainfall, 3)	721.098
65	~ CS(RH, 2) + CS(Rainfall, 1)	721.843
66	~ CS(RH, 2) + CS(Rainfall, 2)	722.342
67	~ CS(RH, 3) + CS(Rainfall, 1)	725.910
68	~ CS(RH, 2) + CS(Rainfall, 3)	725.926
69	~ CS(RH, 3) + CS(Rainfall, 2)	726.601
70	~ CS(RH, 3) + CS(Rainfall, 3)	730.204

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Epidemiology of dengue and the effect of seasonal climate variation on its dynamics: a spatio-temporal descriptive analysis in the Chao-Shan area, on China's southeastern coast

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5 **dynamics: a spatio-temporal descriptive analysis in the Chao-Shan area, on**
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7 **China's southeastern coast**
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Abstract

Objective Dengue is a mosquito-transmitted virus infection that remains rampant across the tropical and subtropical areas worldwide. However, the spatial and temporal dynamics of dengue transmission are poorly understood in Chao-Shan area, one of the most densely-populated regions on China's southeastern coast, limiting disease control efforts. We aimed to characterize the epidemiology of dengue and assessed the effect of seasonal climate variation on its dynamics in the area.

Design A spatio-temporal descriptive analysis was performed in three cities including Shantou, Chaozhou and Jiayang in Chao-Shan area, during the period of 2014-2017.

Setting Data of dengue cases of three cities including Shantou, Chaozhou and Jiayang in Chao-Shan area during 2014-2017 were extracted. Data for climatic variables including mean temperature, relative humidity, and rainfall were also compiled.

Methodology The epidemiology and dynamics of dengue were initially depicted, and then the temporal dynamics related to climatic drivers was assessed by a wavelet analysis method. Furthermore, a generalized additive model for location, scale and shape model was performed to study the relationship between seasonal dynamics of dengue and climatic drivers.

Results Among the cities, the number of notified dengue cases in Chaozhou was greatest, accounting for 78.3%. The median age for the notified cases was 43 years (interquartile range (IQR): 27.0-58.0 years). Two main regions located in Xixin and Chengxi streets of Chaozhou with a high risk of infection were observed, indicating that there was substantial spatial heterogeneity in intensity. We found an annual peak incidence occurred in autumn across the region, most markedly in 2015. This study reveals that periods of elevated temperatures can drive the occurrence of dengue epidemics across the region, and the risk of transmission is highest when the temperature is between 25 °C~ 28 °C.

Conclusion Our study contributes to a better understanding of dengue dynamics in Chao-Shan area.

Keywords: China; climate; dengue; seasonal variation; spatial; wavelet analysis

Strengths and limitations of this study

- There was substantial spatial heterogeneity in intensity of dengue incidence across Chao-Shan area.
- This study reveals that periods of elevated temperatures can drive the occurrence of dengue epidemics across Chao-Shan area.
- The risk of dengue transmission is most when the temperature is between 25 °C~28 °C.
- Some potentially relevant factors such as human activities, mosquito density, vegetation cover, distance to water bodies and so on were not included, and may partly influence the relationship identified in this work.
- The epidemical features of dengue cases caused by different viruses in the areas are in need of further study.

Introduction

Dengue is characterized as a serious infectious disease with a variety of clinical symptoms, from mild fever to potentially fatal dengue shock syndrome, and remains rampant across the tropical and subtropical areas in the world [1]. Dengue viruses are arthropod-borne flaviviruses which cause dengue shock syndrome in humans, and previous studies suggest that dengue virus production is immunologically regulated [2]. At present, endemic dengue virus transmission is reported in the Eastern Mediterranean, American, South-East Asian, Western Pacific and African regions [3]. Accordingly, *Aedes* mosquitoes, including *Aedes aegypti* and *Aedes albopictus*, serve as the main transmission vector of dengue viruses [4]. Studies have documented that the variability in ambient temperature and precipitation have an impact on development rates and habitat availability for *Aedes aegypti* and *Aedes albopictus* larvae and pupae [5]. There has been sharp increase in the number of dengue infections over the past few decades, and the disease not only has a great influence on population health, but also brings a heavy economic burden to patients, society and government [6]. In China, the areas affected by dengue have expanded and there has been a gradual increase in the incidence over the recent years [7, 8]. In particular, an extensive outbreak of dengue hit China in 2014, and high risk areas for dengue outbreaks were concentrated in neighboring provinces including Guangdong, Guangxi and Yunnan of southern China [9]. In fact, during this 2014 outbreak, the number of dengue infections in China reached the highest level over the past 25 years [10].

In China, dengue cases are notified to the China Center for Disease Control and Prevention (CDC) for the surveillance of spread pattern of dengue epidemic. We previously observed a remarkable spatial-temporal heterogeneity of dengue incidence in Guangdong, the most developed province of southern China, and showed that the dengue epidemic had noticeable seasonal and annual variability [9, 10]. In fact, recent studies suggest that dengue may now be endemic to China [11], and the dengue outbreaks are strongly related to climatic factors including temperature, rainfall and

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4 humidity which have direct impacts on mosquito abundance [12]. Enjoying a
5 subtropical humid monsoon climate and frequent economic and cultural
6 communication with the Southeast Asian countries [13, 14], Guangdong has a
7 potential high-risk opportunity for transmission of dengue. In particular, dengue poses
8 a great burden of disease in the Pearl River Delta Region of Guangdong province, and
9 the epidemic characteristics have been well documented [9-12].
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17 The impact of seasonal climatic variation on dengue dynamics in the Chao-Shan area
18 (Figure 1), the eastern part of Guangdong province is poorly quantified. The
19 Chao-Shan area is one of the special regions on China's southeastern coast. The
20 region has a jurisdiction area of around 10918 square kilometers, with a very high
21 population density. In addition, it is situated in the intersection part of the Tropic of
22 Cancer and the coastline of the mainland China, and has a subtropical oceanic
23 monsoon climate with a mean annual temperature of around 22 °C and an abundant
24 precipitation per year [15]. The subtropical oceanic monsoon climate conditions and
25 the increasingly frequent cultural contact with Southeast Asian countries in the
26 Chao-Shan area make it a high risk area for mosquito-borne infectious diseases. In
27 particular, mosquito vectors such as *Aedes albopictus* are reported as the major vector
28 of dengue virus in the area [16].
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43 Unfortunately there are few comprehensive reports on the spatial-temporal epidemic
44 pattern for dengue in the Chao-Shan area. The effects of seasonal climate variation on
45 the dynamics of dengue and the associated disease burden in the region are still
46 unclear. There is an urgent need to understand the association between dengue
47 epidemics and seasonal climatic conditions in order to take corresponding protective
48 measures. Therefore, this study aimed to assess the epidemic characteristics of dengue
49 in the Chao-Shan area, and the effect of seasonal climate variation on the dynamics of
50 dengue for the period January 1, 2014 to December 31, 2017.
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Material and methods

Patient and Public Involvement

All private information on cases diagnosed as dengue is kept confidential in this study. Patients and or public were not involved because this present study only used the data of the number of dengue cases to construct time series for assessing their associations with meteorological factors.

Dengue Case Data

The Chao-Shan area including Shantou, Chaozhou and Jieyang cities is one of the most densely-populated regions on China's southeastern coast. In addition, this area has a subtropical oceanic monsoon climate, geographical position, and the frequent culture, economic and trade exchanges with Southeast Asian countries. However, the epidemiological characteristics of dengue and disease burden associated with dengue infections are historically unclear. Therefore, the area was selected as the study site.

From September 1, 2008, all probable, clinic-and laboratory-confirmed cases of dengue were reported to the Chinese Center for Disease Control and Prevention (China CDC), and diagnosed according to the Diagnostic Criteria for Dengue [17] released by the National Health Commission of China. Probable and confirmed cases were reported online to the China CDC within 24 hours of diagnosis, by use of a standardized form including basic demographic information (sex, date of birth, and residential address), case classification (probable, clinic-confirmed or laboratory-confirmed), date of symptoms onset, and date of diagnosis. Data for dengue cases of Shantou, Chaozhou and Jieyang cities in the Chao-Shan area from January 1, 2014 to December 31, 2017 were available from the Guangdong Provincial CDC. Only indigenous dengue cases were included, and weekly number of dengue cases was calculated for statistical analysis.

Climate Surveillance Data

Daily average values for climatic variables including relative humidity, rainfall

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4 (millimeters), and temperature ($^{\circ}\text{C}$) in the three cities during the study period from
5 January 1, 2014 to December 31, 2017 were obtained from the Guangdong Provincial
6 Climate Bureau. Weekly values of climatic variables were calculated as simple
7 arithmetic means to show the seasonal peaks of dengue in surveillance data, and used
8 to assess the potential effect of seasonal variation on the dengue dynamics.
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15 **Statistical Analysis**

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17 First, basic characteristics (number, median age, sex ratio, and season and year of
18 diagnosis) of dengue cases reported during the study period were described by city.
19 By calculating the interval between the date of onset and the diagnosed date for each
20 dengue case, we estimated the probability density function of the continuous variable
21 of the 'interval' and then plot corresponding probability density distributions of the
22 onset-to-diagnosis of dengue cases for the three cities. The median duration from
23 onset to diagnosis by city was estimated. Each case of dengue has registered current
24 address information. To identify high-risk areas of dengue in the Chao-Shan area, we
25 applied a kernel smoothing method [18] to plot the geographical distribution of cases
26 by street or town. A total of 231 streets or towns in the Chao-Shan area covering the
27 three cities were included in our dataset. ArcGIS 10.2 (ESRI, Redlands, CA, USA)
28 was used to plot the geographical distribution.
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43 Third, to quantify seasonal patterns of dengue epidemic, time series of weekly case
44 counts were plotted by city, and type of cases (probable or confirmed cases),
45 respectively. The periodicity of the time series of dengue case counts, mean
46 temperature, mean rainfall and mean relative humidity was assessed using
47 time-dependent wavelet analysis [19]. The time series data of dengue case counts and
48 climatic factors were analyzed by wavelet analysis, and then the wavelet
49 decomposition results were used to calculate power spectrum. By decomposing the
50 time series into the time-frequency domain, wavelet analysis can obtain the significant
51 fluctuation pattern of the time series, that is, the periodic dynamic time pattern. A
52 continuous Morlet wavelet transform on the time series was performed to extract
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4 specific frequency components. Then, the temporal periodicity in a time series was
5 inspected with a wavelet plot. A statistical significance test was performed to test the
6 null hypothesis that the signal in a wavelet plot is generated by a stable process of a
7 given background power spectrum (usually white noise) at a certain time with
8 significance level of 95%. The *dplr* package within R software was used for the
9 Morlet wavelet analysis in this study.
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17 Fourth, in view of the presence of overdispersion in the dengue case data set, a
18 generalized additive model for location, scale and shape (GAMLSS) model [20] with
19 the distribution of the response variable obeying negative binomial distribution was
20 performed to study the association between seasonal characteristics of dengue and
21 climatic variables, including mean temperature, mean rainfall and mean relative
22 humidity. We implemented the Rigby and Stasinopoulos algorithm [20] to estimate
23 the parameters of the models, and the number of cycles of the algorithm was set to
24 2000 to insure the convergence of the analysis results.
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35 In order to assess the effects of climatic variables on seasonal dynamics of dengue, we
36 established a set of GAMLSS models where climatic variables were modeled as
37 smooth terms with different degrees of freedom (*df*). This was performed by changing
38 the *df* for the smooth terms of 3 to 1 during model selection. We used the smoothing
39 splines for the smooth terms in the GAMLSS models. We considered temperature,
40 relative humidity and rainfall separately, then with both in model, then with all in
41 model, yielding 63 models. In addition, some sensitivity analyses by setting *df*=0 in
42 the smooth terms, where the *df*=0 means the distribution of the response variable is
43 directly modeled as a linear parametric function of explanatory variables, for the three
44 climatic factors considered separately, then both in model, then all in model, yielding
45 7 models. As model selection criterion, the Schwartz Bayesian Criterion (SBC) [21]
46 was used here. Models with lower value of SBC are preferred. In addition, we also
47 added the autoregressive term, $\ln(1 + Count_{local\ cases})$ in the previous month, to account
48 for the autoregressive effect in this study [10]. The *gamlss* package within R software
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was used to establish the GAMLSS model. The values of partial effect function from a smoothing effect term in a GAMLSS model were extracted and plot against their predictors. We can identify the nonlinear relationship between the dependent variable and the independent variables from this plot. R software (version 3.4.3) was used for the statistical analyses.

Results

Basic characteristics of dengue cases notified during 2014-17 were shown in Table 1. In total, 2,193 probable and confirmed cases of dengue in the Chao-Shan area were reported to the China CDC system, of which 2,148 (98.0%) were confirmed (clinic- or laboratory-confirmed) and 45 (2.1%) were probable, respectively. Among all the reported cases, the proportion (78.3%) of dengue cases reported in Chaozhou was higher than those among Shantou (13.0%), and Jieyang (8.8%). The median age for all notified cases was 43 years (interquartile range (IQR): 27.0-58.0 years), for Shantou cases 35 years (IQR: 23.0-49.0 years), Chaozhou cases 46 years (IQR: 29.0-59.0 years), and Jieyang cases 34 years (IQR: 25.0-50.0 years), respectively. The age distributions among probable and confirmed cases for Shantou and Jieyang cities were similar. The male to female ratio was 1:1.1 for all notified dengue cases. The sex distributions among cases for Jieyang were different from those for the other two cities.

For the most recent year 2017 observed, most of the patients were male, irrespective of subgroup analysis results (Figure 2, A-C). The median time from illness onset to diagnosis was 6 days (IQR 2.8-8), 3 days (IQR 3-5) and 3 days (IQR 1-5) for Shantou, Chaozhou and Jieyang, respectively. The plot of probability density distributions also demonstrated that there was a longer onset-to-diagnosis interval for dengue cases in Shantou (Figure 2, D).

In Figure 3, a kernel density estimate map from dengue case data with

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4 latitude/longitude coordinates shows geographical distributions of dengue cases in the
5 Chao-Shan area. Spatial smoothing with the kernel density estimation produced
6 dengue risk hotspots suggested that the geographical regions with the greatest number
7 of dengue cases were Xixin and Chengxi streets in Chaozhou city. Overall, dengue
8 showed annual peaks of activity, a major peak in autumn, consistent among time
9 series of dengue cases in Shantou, Chaozhou and Jieyang (Figure 4). Although
10 Chao-Shan area had a seasonal attack of dengue peaking between September and
11 October of each year, we observed that the number of dengue cases increased sharply
12 in 2015 in Chaozhou.
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23 Results of wavelet analysis for time series of notifications of dengue and climatic
24 variables including mean temperature, mean rainfall and mean relative humidity in
25 Chao-Shan area during the study period were shown in Figure 5. It showed that the
26 estimations of local powers for weekly time step examined were the largest at the
27 period of 1 year, suggesting dengue cases showed a significant annual periodicity
28 (Figure 5, A). In particular, based on the strongest power of the annual periodicity
29 estimated for the time series of climatic variables (mean temperature, rainfall and
30 relative humidity) throughout the study period (Figure 5, B-D), these factors broadly
31 followed similar periodicity with that of dengue cases.
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43 Based on the results of wavelet analysis, we further analyzed the effects of climatic
44 variables on seasonal dynamics of dengue using a framework of GAMLSS by testing
45 different degrees of freedom for the smooth terms during model selection to
46 determine an optimal model (Table S1). We found that the model with the variable of
47 mean temperature modeled as a smoothing spline term with $df=1$ had the smallest
48 value of SBC and the best model fit among the 70 kinds of candidate models,
49 suggesting that the effect of mean temperature on dengue dynamics in Chao-Shan
50 area was most obvious. In addition, according to the GAMLSS framework, a
51 significant nonlinear partial effect of mean temperature on dengue dynamics have
52 been observed, and the risk of dengue transmission was most when the temperature
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4 was between 25 °C~ 28 °C (Figure 6).
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8 **Discussion**

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10 Our study of probable and confirmed cases of dengue reported to the national CDC
11 surveillance system during 2014-17 gives the first comprehensive estimation of the
12 local burden and epidemiology of the disease in the study sites. Among the three
13 cities covering a jurisdiction area of around 10918 square kilometers, Chaozhou is
14 responsible for the largest proportion of all the notified dengue cases. We recorded a
15 median age of infection at 43 years (IQR 27.0-58.0 years) for all notified dengue
16 cases, and the male to female ratio was 1:1.1. Our study suggests that cases of dengue
17 tend to arise in autumn season of the year, presenting a significant annual periodicity
18 of epidemics. In particular, we found that the effect of temperature on dengue
19 epidemics was most obvious among the climatic factors assessed.
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31 This present analysis of spatial distribution patterns of dengue identified two main
32 epidemiological regions corresponding to Xixin and Chengxi streets in Chaozhou city,
33 where dengue peaks in autumn. We found that Chaozhou accounted for around 78%
34 of the total number of dengue cases reported during the study period. We speculated
35 that this may owe to the combined effect of multiple factors including its subtropical
36 monsoonal climate, its industries producing abandoned ceramics that could provide
37 abundant of breeding places for mosquitos after raining, and local residents' habit of
38 planting flowers at home providing breeding environment for mosquito vectors [22].
39 Our results indicate that dengue cases showed a significant annual periodicity in
40 Chao-Shan area. Findings from Asia-Pacific countries including Thailand, Laos, and
41 the Philippines have shown localized traveling waves of multiannual dengue epidemic
42 cycles [23]. By contrast, data of dengue virus isolate counts from Puerto Rico, a major
43 population center in the Caribbean, indicate interannual variation in transmission
44 across multiple dengue serotypes [24]. An epidemiological study from Machala,
45 Ecuador, provided evidence of significant 1-year and 2-year cycles in dengue [25]. In
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4 fact, our data were in agreement with another study indicating that significant
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6 coherences were observed between dengue incidence and temperature for the annual
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8 periodicity from 2005 to 2009 from Hanoi, Vietnam [26]. Our wavelet analysis
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10 revealed that dengue transmission co-varied with temperature at annual cycle, and we
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12 inferred that this acted as an important driver for the periodicity of dengue dynamics
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14 in Chao-Shan area.

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17 This study built on potential links between dengue dynamics and climatic variables,
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19 and quantified their relationships based on a kind of state-of-the-art flexible
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21 regression and smoothing technique, GAMLSS model, which enables us to handle
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23 adequately the problem of the presence of overdispersion in the data. The GAMLSS is
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25 a general framework for fitting semi-parametric regression type models where the
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27 distribution of the response variable includes highly skew and kurtotic continuous and
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29 discrete distribution, and allows all the parameters of the distribution of the response
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31 variable to be modeled as smooth functions of the explanatory variables [20]. Unlike
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33 traditional linear regression, the approach enables to find a nonlinear relationship
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35 between the dependent variable and a set of independent variables by specifying an
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37 appropriate smooth function term that describes the relationship [27]. By inspecting a
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39 scatterplot (data was not shown) between dengue incidence and temperature, we
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41 found the relationship was nonlinear, so it required the special estimation methods of
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43 the nonlinear regression procedure. Additionally, the choice of df values is very
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45 important when constructing the GAMLSS model. Therefore, we performed a
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47 sensitivity analysis by trying different df in the smooth term of the model, and
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49 determined an optimal model according to the assessment of model fit. Overall, our
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51 results revealed a powerful capability with GAMLSS model for catching the
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53 appropriate relationship between dengue incidence and temperature.

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56 The associations between dengue seasonality and climatic variables such as
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58 temperature, rainfall, relative humidity, sunshine and ENSO indices have been
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60 documented in previous studies [28]. In this study, our results reveal that temperature

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4 has significantly nonlinear effects on epidemics risk and intensity of dengue, with the
5 greatest attributable risk due to temperature estimated at around 25 °C. To identify the
6 significantly associated climatic factors with dengue epidemics, we repeated model
7 selections and adopted different climatic factors with different df in the smooth terms
8 of the GAMLSS framework. We found that the variation in daily mean temperature
9 partly drives dengue dynamics, and this climatic factor is closely related to the
10 seasonal fluctuation of dengue incidence in Chao-Shan area. The association between
11 temperature and seasonal dynamics of dengue observed in our study was also reported
12 in previous studies in Guangzhou [12] and Taiwan [29], which has a similar
13 subtropical humid monsoon climate. The estimated effects of temperature driving the
14 seasonal dynamics of dengue can be partly explained by some mechanisms, for
15 example, temperature effects on the biting rate of mosquitoes [30], incubation period
16 of pathogens [31], and human exposure to mosquitoes.

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31 Several limitations of this study should be mentioned. First, we did not consider
32 potentially relevant factors, such as mosquito density and other environment elements
33 including vegetation cover, distance to water bodies and so on. The variables may
34 partly influence the relationship between temperature and dengue epidemics estimated
35 in this work. In addition, although the quantitative results derived from this study
36 pointed out that temperature was associated with increased risk of dengue
37 transmission, other factors such as human activities during urbanization and
38 globalization were also suggested to be very important in driving the long-term trends
39 [8, 32]. At present, we can't obtain the data on laboratory test results for various kinds
40 of dengue viruses. Therefore, we can't study the epidemical features of dengue cases
41 caused by different viruses as well as the age-specific dengue antibody prevalence in
42 the population. Furthermore, our results faced a common challenge for studies using
43 data from surveillance with possible under-reporting or variations in diagnosis
44 practices of cases. This was also suggested from a previous study [33].
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Conclusions

In summary, this study represented an initial step to analyze the epidemiological characteristics of dengue in Chao-Shan area, one of the most densely-populated regions on China's southeastern coast, and use a statistically rigorous approach for assessing the effects of climatic factors on dengue epidemics. Overall, our results are helpful toward advancing our understanding of the pattern of how climate influences dengue, and useful for the control and prevention of the disease for local government in the study sites.

Contributors

QZ, PG and WJM conceived the study, undertook statistical analysis and drafted the manuscript. YLC, YF, TL and QYZ collected the data and assisted in the statistical analysis. QZ, PG, TL, QYZ and WJM interpreted the results. QZ, PG and TL wrote and revised the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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Data Availability Statement

Data are not publicly available because of personal privacy preservation. The dengue surveillance data are available from the corresponding authors on request. Requests for materials should be addressed to P.G. (Email: pguo@stu.edu.cn) or T.L. (Email: gztt_2002@163.com).

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Figure Legends

Figure 1 Geographical location of Chao-Shan area including Shantou, Chaozhou and Jieyang cities, on China's southeastern coast.

Figure 2 Proportions of gender-specific cases of dengue fever by age groups and estimates of onset-to-diagnosis distributions of dengue fever cases in Chao-Shan area, 2014-2017. (A) Based on total cases. (B) Based on cases aged less than 35 years. (C) Based on cases aged greater than or equal to 35 years. (D) Onset-to-diagnosis distribution by city.

Figure 3 Geographical distributions of dengue cases in Chao-Shan area from the beginning of 2014 to the end of 2017. Spatial smoothing with the kernel density estimation produced dengue risk hotspots suggested that the geographical regions with the greatest number of dengue cases were Xixin and Chengxi streets in Chaozhou city.

Figure 4 Temporal dynamics of dengue in Chao-Shan area from the beginning of 2014 to the end of 2017. (A) Weekly time series of number of probable and confirmed dengue cases in Chao-Shan area. (B) Weekly time series of number of probable and confirmed dengue cases by city in Chao-Shan area.

Figure 5 Wavelet analyses for time series of notifications of dengue and climatic variables including mean temperature, rainfall and relative humidity in Chao-Shan area, 2014-2017. Local wavelet power spectrum for dengue cases (A), mean temperature (B), rainfall (C) and relative humidity (D). Solid and bold lines indicate boundary of statistical significance.

Figure 6 Analysis of potentially nonlinear effects of mean temperature on seasonal dynamics of dengue in Chao-Shan area using a generalized additive model for location, scale and shape (GAMLSS) model based on data from years

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4 **2014-2017.** Initially, climatic variables including mean temperature, rainfall and
5 relative humidity were assessed using the GAMLSS model with different degrees of
6 freedom for the smooth terms during model selection. Then, the optimal model with
7 the statistically significant variable (mean temperature) was determined. The values of
8 partial effect function from a smoothing effect term in a GAMLSS model were
9 extracted and plot against their predictors. This plot shows the significant nonlinear
10 partial effect of mean temperature on dengue dynamics, and the risk of dengue
11 transmission is most when the temperature is between 25 °C~28 °C. The lightblue
12 dots denote partial residuals of the model, which were added in the plot to see how the
13 model fits. From the dots of partial residuals, we can there are no outliers in the data.
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Table 1 Basic characteristics of dengue cases reported in the study areas during the period from 2014 to 2017.

Characteristics	City			Total
	Shantou	Chaozhou	Jieyang	
Number of cases, number (%)	284 (13.0)	1716 (78.3)	193 (8.8)	2193 (100%)
Type of cases, number (%)				
<i>Probable cases</i>	0 (0.0)	45 (2.6)	0 (0.0)	45 (2.1)
<i>Confirmed cases</i>	284 (100.0)	1,671 (97.4)	193 (100.0)	2148 (97.9)
Median age, years (IQR)	35.0 (23.0-49.0)	46.0 (29.0-59.0)	34.0 (25.0-50.0)	43.0 (27.0-58.0)
Sex, number (%)				
<i>Male</i>	137 (48.2)	819 (47.7)	110 (57.0)	1066 (48.6)
<i>Female</i>	147 (51.8)	897 (52.3)	83 (43.0)	1127 (51.4)
Season of diagnosis, number (%)				
<i>Spring</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Summer</i>	0 (0.0)	33 (1.9)	0 (0.0)	33 (1.5)
<i>Autumn</i>	281 (98.9)	1,680 (97.9)	192 (99.5)	2153 (98.2)
<i>Winter</i>	3 (1.1)	3 (0.2)	1 (0.5)	7 (0.3)
Year of diagnosis, number (%)				
2014	247 (87.0)	135 (7.9)	86 (44.6)	468 (21.3)
2015	14 (4.9)	1380 (80.4)	6 (3.1)	1400 (63.8)
2016	5 (1.8)	194 (11.3)	0 (0.0)	199 (9.1)
2017	18 (6.3)	7 (0.4)	101 (52.3)	126 (5.8)

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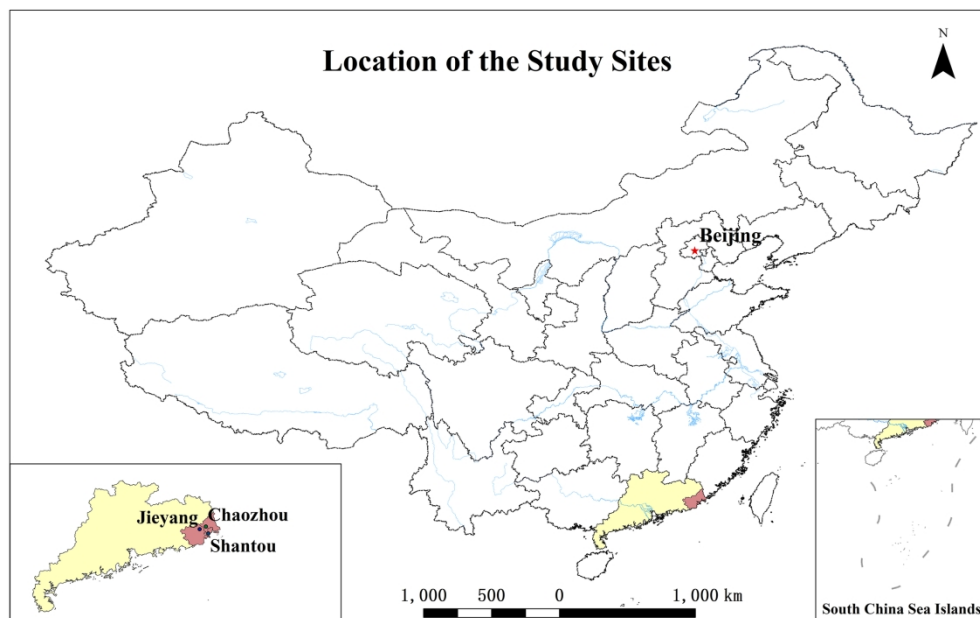


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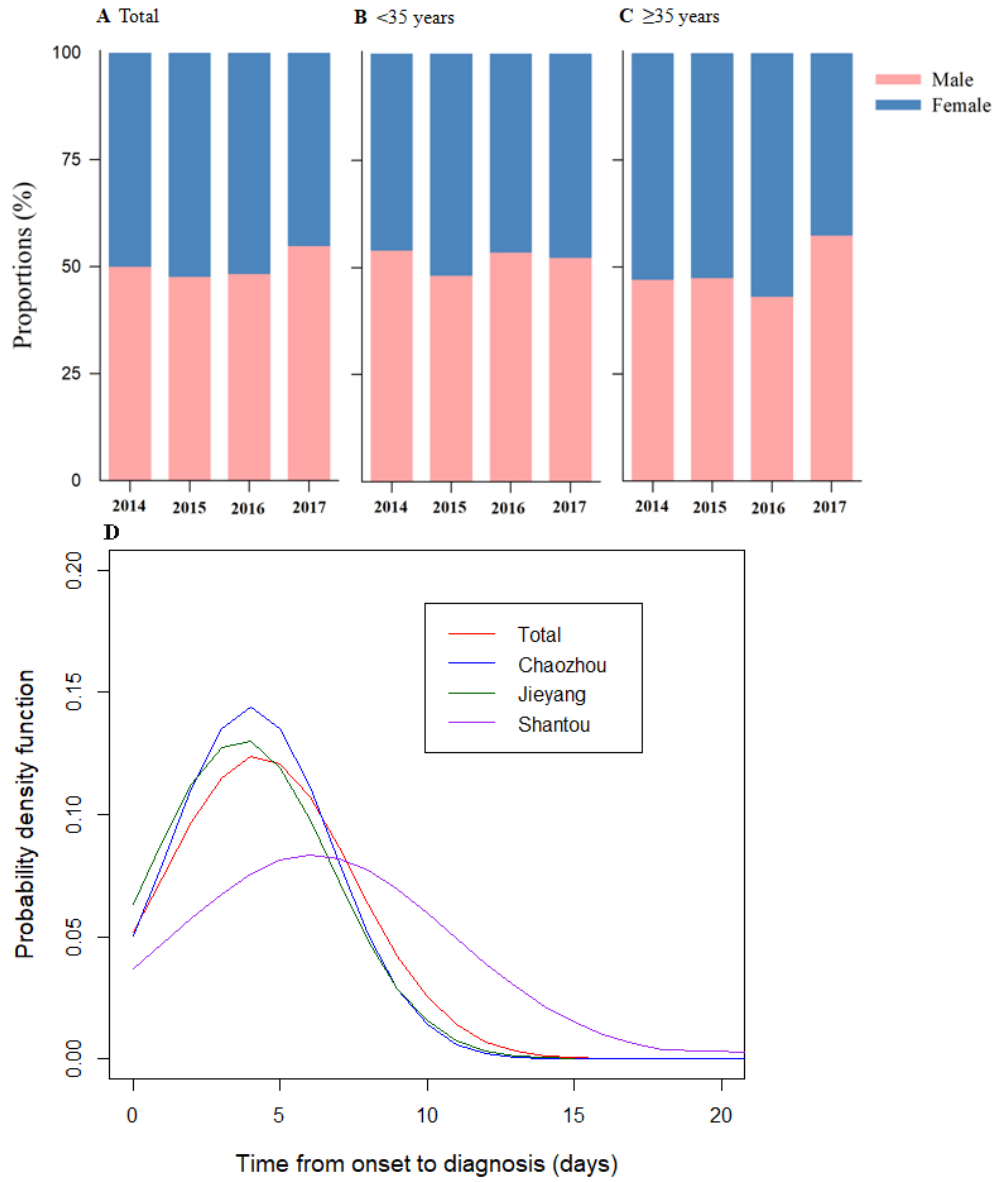


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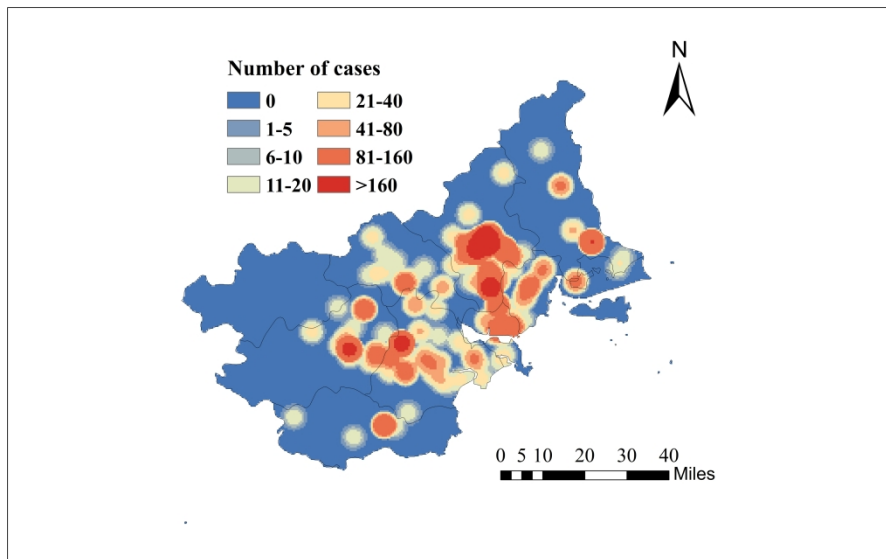


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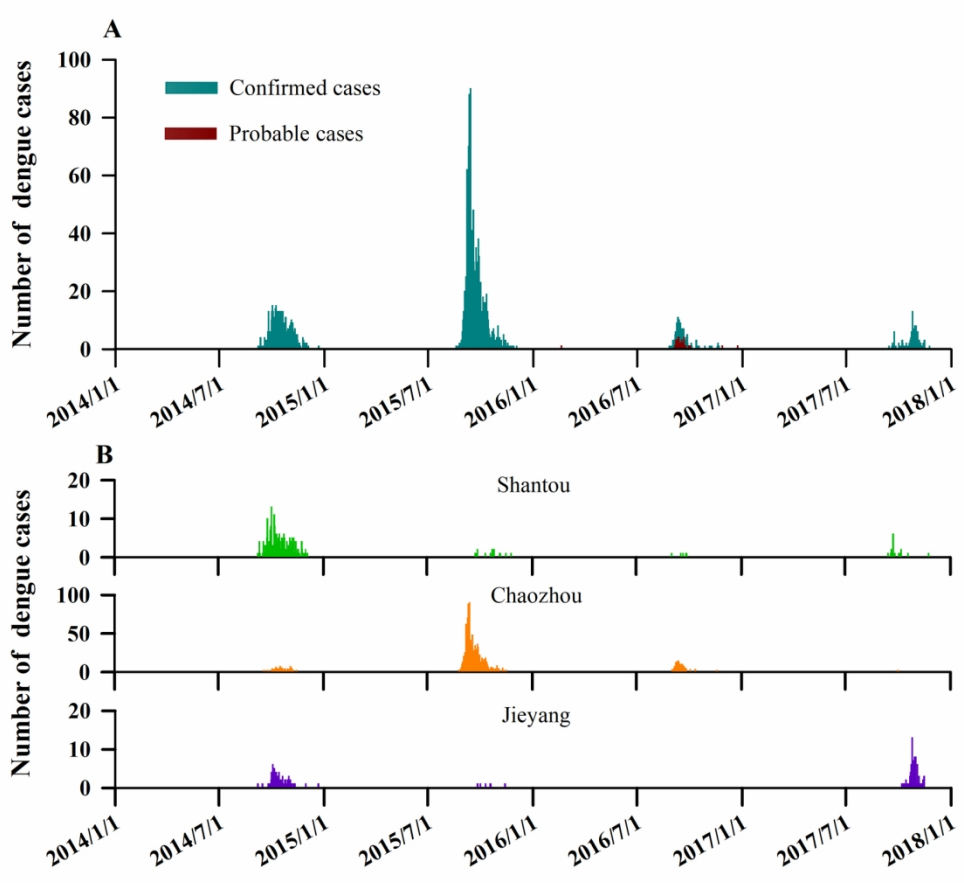


Fig 4

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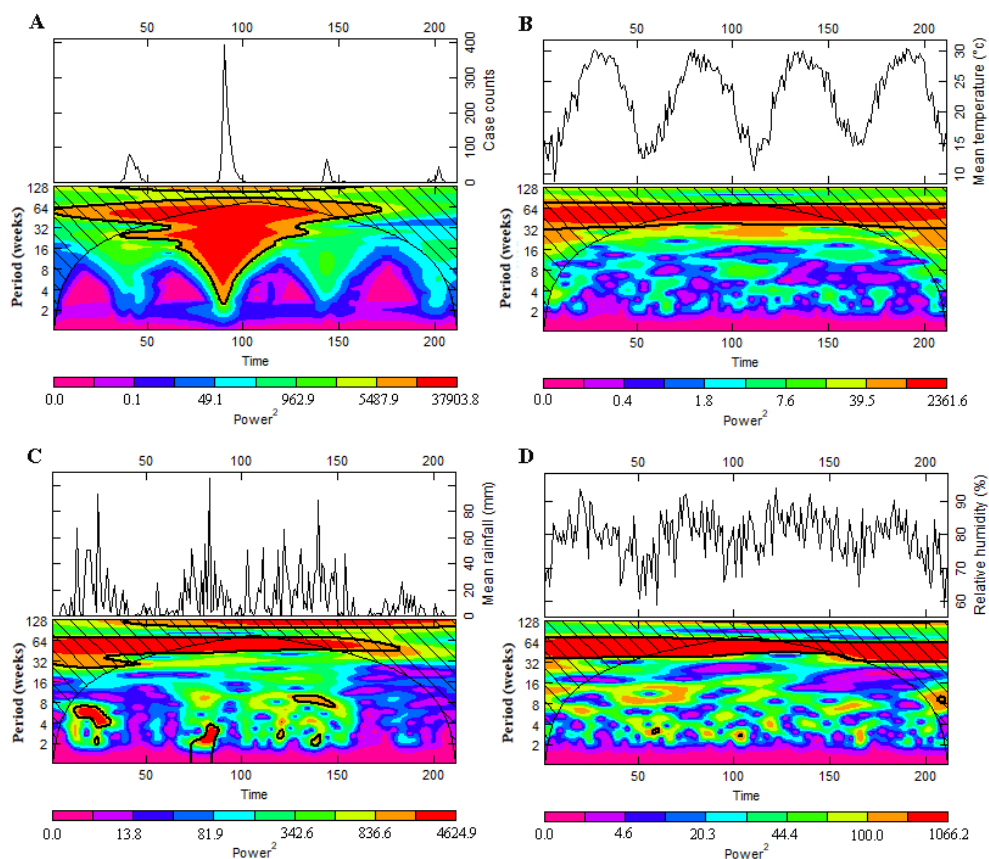


Fig 5

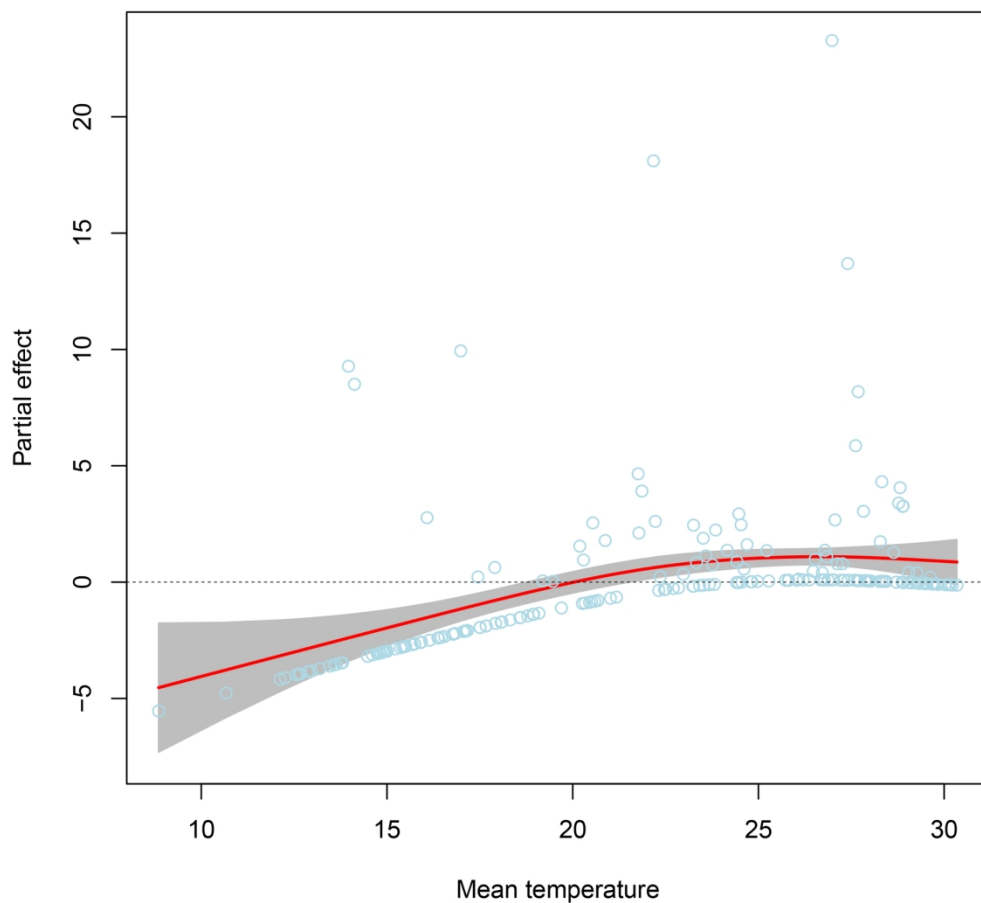


Fig 6

169x156mm (300 x 300 DPI)

Supplementary File

Table S1. Model fit assessment of a set of generalized additive model for location, scale and shape (GAMLSS) models where climatic variables including mean temperature (MeanTemp), rainfall (Rainfall) and relative humidity (RH) were modeled as smooth terms with different degrees of freedom (*df*). The criterion, Schwartz Bayesian Criterion (SBC), was used for model selection, where models with lower value of SBC are preferred. 0, 1, 2 and 3 were considered for the values of the parameter *df* in the GAMLSS models, respectively. Cubic spline (CS) function was used as the smooth function term.

Rank of model	Model formula	SBC
1	~ CS(MeanTemp, 1)	686.105
2	~ CS(MeanTemp, 2)	686.871
3	~ CS(MeanTemp, 3)	689.207
4	~ MeanTemp	690.681
5	~ CS(MeanTemp, 1) + CS(Rainfall, 1)	691.759
6	~ CS(MeanTemp, 2) + CS(Rainfall, 1)	691.811
7	~ CS(MeanTemp, 3) + CS(Rainfall, 1)	692.740
8	~ CS(MeanTemp, 1) + CS(Rainfall, 2)	693.124
9	~ CS(MeanTemp, 2) + CS(Rainfall, 2)	693.623
10	~ MeanTemp + Rainfall	694.608
11	~ MeanTemp + RH	694.628
12	~ CS(MeanTemp, 1) + CS(RH, 1)	694.790
13	~ CS(MeanTemp, 2) + CS(RH, 1)	695.267
14	~ CS(MeanTemp, 3) + CS(Rainfall, 2)	695.459
15	~ CS(MeanTemp, 3) + CS(RH, 1)	695.533
16	~ CS(MeanTemp, 1) + CS(Rainfall, 3)	696.474
17	~ CS(MeanTemp, 2) + CS(Rainfall, 3)	697.546
18	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 1)	699.055
19	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 1)	699.193
20	~ CS(MeanTemp, 1) + CS(RH, 2)	699.303
21	~ CS(MeanTemp, 3) + CS(Rainfall, 3)	699.363
22	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 2)	699.565
23	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 1)	699.655
24	~ MeanTemp + RH + Rainfall	699.672
25	~ CS(MeanTemp, 2) + CS(RH, 2)	699.851
26	~ CS(MeanTemp, 3) + CS(RH, 2)	700.121
27	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 2)	700.460
28	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 2)	700.535
29	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 3)	703.064

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3	30	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 1)	703.307
4	31	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 1)	703.395
5	32	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 3)	703.790
6	33	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 2)	703.836
7	34	~ CS(MeanTemp, 1) + CS(RH, 3)	703.955
8	35	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 1)	704.037
9	36	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 3)	704.168
10	37	~ CS(MeanTemp, 2) + CS(RH, 3)	704.432
11	38	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 2)	704.519
12	39	~ CS(MeanTemp, 3) + CS(RH, 3)	704.845
13	40	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 2)	705.177
14	41	~ RH	707.331
15	42	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 3)	707.364
16	43	~ CS(Rainfall, 1)	707.472
17	44	~ CS(Rainfall, 2)	707.558
18	45	~ Rainfall	707.656
19	46	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 1)	707.991
20	47	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 1)	708.093
21	48	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 3)	708.267
22	49	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 3)	708.330
23	50	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 2)	708.505
24	51	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 1)	708.792
25	52	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 2)	709.107
26	53	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 2)	709.867
27	54	~ CS(Rainfall, 3)	710.239
28	55	~ CS(RH, 1)	711.324
29	56	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 3)	712.016
30	57	~ RH + Rainfall	712.666
31	58	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 3)	712.849
32	59	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 3)	713.085
33	60	~ CS(RH, 2)	715.945
34	61	~ CS(RH, 1) + CS(Rainfall, 1)	717.272
35	62	~ CS(RH, 1) + CS(Rainfall, 2)	717.727
36	63	~ CS(RH, 3)	720.343
37	64	~ CS(RH, 1) + CS(Rainfall, 3)	721.098
38	65	~ CS(RH, 2) + CS(Rainfall, 1)	721.843
39	66	~ CS(RH, 2) + CS(Rainfall, 2)	722.342
40	67	~ CS(RH, 3) + CS(Rainfall, 1)	725.910
41	68	~ CS(RH, 2) + CS(Rainfall, 3)	725.926
42	69	~ CS(RH, 3) + CS(Rainfall, 2)	726.601
43	70	~ CS(RH, 3) + CS(Rainfall, 3)	730.204
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Table S2. STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6, 7
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	6, 7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	7, 8

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Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7, 8
		(b) Describe any methods used to examine subgroups and interactions	7, 8
		(c) Explain how missing data were addressed	7, 8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	7, 8
		(e) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9, 10
		(b) Report category boundaries when continuous variables were categorized	9, 10

		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9, 10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11, 12, 13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13, 14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiology of dengue and the effect of seasonal climate variation on its dynamics: a spatio-temporal descriptive analysis in the Chao-Shan area, on China's southeastern coast

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4 **Epidemiology of dengue and the effect of seasonal climate variation on its**
5 **dynamics: a spatio-temporal descriptive analysis in the Chao-Shan area, on**
6 **China's southeastern coast**
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Abstract

Objective Dengue is a mosquito-transmitted virus infection that remains rampant across the tropical and subtropical areas worldwide. However, the spatial and temporal dynamics of dengue transmission are poorly understood in Chao-Shan area, one of the most densely-populated regions on China's southeastern coast, limiting disease control efforts. We aimed to characterize the epidemiology of dengue and assessed the effect of seasonal climate variation on its dynamics in the area.

Design A spatio-temporal descriptive analysis was performed in three cities including Shantou, Chaozhou and Jieyang in Chao-Shan area, during the period of 2014-2017.

Setting Data of dengue cases of three cities including Shantou, Chaozhou and Jieyang in Chao-Shan area during 2014-2017 were extracted. Data for climatic variables including mean temperature, relative humidity, and rainfall were also compiled.

Methodology The epidemiology and dynamics of dengue were initially depicted, and then the temporal dynamics related to climatic drivers was assessed by a wavelet analysis method. Furthermore, a generalized additive model for location, scale and shape model was performed to study the relationship between seasonal dynamics of dengue and climatic drivers.

Results Among the cities, the number of notified dengue cases in Chaozhou was greatest, accounting for 78.3%. The median age for the notified cases was 43 years (interquartile range (IQR): 27.0-58.0 years). Two main regions located in Xixin and Chengxi streets of Chaozhou with a high risk of infection were observed, indicating that there was substantial spatial heterogeneity in intensity. We found an annual peak incidence occurred in autumn across the region, most markedly in 2015. This study reveals that periods of elevated temperatures can drive the occurrence of dengue epidemics across the region, and the risk of transmission is highest when the temperature is between 25 °C~ 28 °C.

Conclusion Our study contributes to a better understanding of dengue dynamics in Chao-Shan area.

Keywords: China; climate; dengue; seasonal variation; spatial; wavelet analysis

Strengths and limitations of this study

- This study represented an initial step to use a statistically rigorous approach for assessing the effects of climatic factors on dengue epidemics in Chao-Shan area.
- Our dengue surveillance may be not complete, as the patient may not seek regular treatment or have not been diagnosed and reported.
- Some potentially relevant factors such as human activities, mosquito density, vegetation cover, distance to water bodies and so on were not included, and may partly influence the relationship identified in this work.
- The epidemical features of dengue cases caused by different viruses in the areas are in need of further study.

Introduction

Dengue is characterized as a serious infectious disease with a variety of clinical symptoms, from mild fever to potentially fatal dengue shock syndrome, and remains rampant across the tropical and subtropical areas in the world [1]. Dengue viruses are arthropod-borne flaviviruses which cause dengue shock syndrome in humans, and previous studies suggest that dengue virus production is immunologically regulated [2]. At present, endemic dengue virus transmission is reported in the Eastern Mediterranean, American, South-East Asian, Western Pacific and African regions [3]. Accordingly, *Aedes* mosquitoes, including *Aedes aegypti* and *Aedes albopictus*, serve as the main transmission vector of dengue viruses [4]. Studies have documented that the variability in ambient temperature and precipitation have an impact on development rates and habitat availability for *Aedes aegypti* and *Aedes albopictus* larvae and pupae [5]. There has been sharp increase in the number of dengue infections over the past few decades, and the disease not only has a great influence on population health, but also brings a heavy economic burden to patients, society and government [6]. In China, the areas affected by dengue have expanded and there has been a gradual increase in the incidence over the recent years [7, 8]. In particular, an extensive outbreak of dengue hit China in 2014, and high risk areas for dengue outbreaks were concentrated in neighboring provinces including Guangdong, Guangxi and Yunnan of southern China [9]. In fact, during this 2014 outbreak, the number of dengue infections in China reached the highest level over the past 25 years [10].

In China, dengue cases are notified to the China Center for Disease Control and Prevention (CDC) for the surveillance of spread pattern of dengue epidemic. We previously observed a remarkable spatial-temporal heterogeneity of dengue incidence in Guangdong, the most developed province of southern China, and showed that the dengue epidemic had noticeable seasonal and annual variability [9, 10]. In fact, recent studies suggest that dengue may now be endemic to China [11], and the dengue outbreaks are strongly related to climatic factors including temperature, rainfall and

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4 humidity which have direct impacts on mosquito abundance [12]. Enjoying a
5 subtropical humid monsoon climate and frequent economic and cultural
6 communication with the Southeast Asian countries [13, 14], Guangdong has a potential
7 high-risk opportunity for transmission of dengue. In particular, dengue poses a great
8 burden of disease in the Pearl River Delta Region of Guangdong province, and the
9 epidemic characteristics have been well documented [9-12].
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17 The impact of seasonal climatic variation on dengue dynamics in the Chao-Shan area
18 (Figure 1), the eastern part of Guangdong province is poorly quantified. The Chao-Shan
19 area is one of the special regions on China's southeastern coast. The region has a
20 jurisdiction area of around 10918 square kilometers, with a very high population density.
21 In addition, it is situated in the intersection part of the Tropic of Cancer and the coastline
22 of the mainland China, and has a subtropical oceanic monsoon climate with a mean
23 annual temperature of around 22 °C and an abundant precipitation per year [15]. The
24 subtropical oceanic monsoon climate conditions and the increasingly frequent cultural
25 contact with Southeast Asian countries in the Chao-Shan area make it a high risk area
26 for mosquito-borne infectious diseases. In particular, mosquito vectors such as *Aedes*
27 *albopictus* are reported as the major vector of dengue virus in the area [16].
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41 Unfortunately there are few comprehensive reports on the spatial-temporal epidemic
42 pattern for dengue in the Chao-Shan area. The effects of seasonal climate variation on
43 the dynamics of dengue and the associated disease burden in the region are still unclear.
44 There is an urgent need to understand the association between dengue epidemics and
45 seasonal climatic conditions in order to take corresponding protective measures.
46 Therefore, this study aimed to assess the epidemic characteristics of dengue in the
47 Chao-Shan area, and the effect of seasonal climate variation on the dynamics of dengue
48 for the period January 1, 2014 to December 31, 2017.
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58 **Material and methods**

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Patient and Public Involvement

This time series study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Table S1). We obtained the surveillance data of dengue in the study sites and only used the count data for statistical modeling. Patients and or public were not involved because this present study only used the data of the number of dengue cases to construct time series for assessing their associations with meteorological factors. Therefore, no ethics committee approval is required to obtain the data since the count data is presented.

Dengue Case Data

The Chao-Shan area including Shantou, Chaozhou and Jieyang cities is one of the most densely-populated regions on China's southeastern coast. In addition, this area has a subtropical oceanic monsoon climate, geographical position, and the frequent culture, economic and trade exchanges with Southeast Asian countries. However, the epidemiological characteristics of dengue and disease burden associated with dengue infections are historically unclear. Therefore, the area was selected as the study site.

From September 1, 2008, all probable, clinic-and laboratory-confirmed cases of dengue were reported to the Chinese Center for Disease Control and Prevention (China CDC), and diagnosed according to the Diagnostic Criteria for Dengue [17] released by the National Health Commission of China. Probable and confirmed cases were reported online to the China CDC within 24 hours of diagnosis, by use of a standardized form including basic demographic information (sex, date of birth, and residential address), case classification (probable, clinic-confirmed or laboratory-confirmed), date of symptoms onset, and date of diagnosis. Data for dengue cases of Shantou, Chaozhou and Jieyang cities in the Chao-Shan area from January 1, 2014 to December 31, 2017 were available from the Guangdong Provincial CDC. Only indigenous dengue cases were included, and weekly number of dengue cases was calculated for statistical analysis.

Climate Surveillance Data

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4 Daily average values for climatic variables including relative humidity, rainfall
5 (millimeters), and temperature ($^{\circ}\text{C}$) in the three cities during the study period from
6 January 1, 2014 to December 31, 2017 were obtained from the Guangdong Provincial
7 Climate Bureau. Weekly values of climatic variables were calculated as simple
8 arithmetic means to show the seasonal peaks of dengue in surveillance data, and used
9 to assess the potential effect of seasonal variation on the dengue dynamics.
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17 **Statistical Analysis**

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19 First, basic characteristics (number, median age, sex ratio, and season and year of
20 diagnosis) of dengue cases reported during the study period were described by city. By
21 calculating the interval between the date of onset and the diagnosed date for each
22 dengue case, we estimated the probability density function of the continuous variable
23 of the 'interval' and then plot corresponding probability density distributions of the
24 onset-to-diagnosis of dengue cases for the three cities. The median duration from onset
25 to diagnosis by city was estimated. Each case of dengue has registered current address
26 information. To identify high-risk areas of dengue in the Chao-Shan area, we applied a
27 kernel smoothing method [18] to plot the geographical distribution of cases by street or
28 town. A total of 231 streets or towns in the Chao-Shan area covering the three cities
29 were included in our dataset. ArcGIS 10.2 (ESRI, Redlands, CA, USA) was used to
30 plot the geographical distribution.
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45 Third, to quantify seasonal patterns of dengue epidemic, time series of weekly case
46 counts were plotted by city, and type of cases (probable or confirmed cases),
47 respectively. The periodicity of the time series of dengue case counts, mean temperature,
48 mean rainfall and mean relative humidity was assessed using time-dependent wavelet
49 analysis [19]. The time series data of dengue case counts and climatic factors were
50 analyzed by wavelet analysis, and then the wavelet decomposition results were used to
51 calculate power spectrum. By decomposing the time series into the time-frequency
52 domain, wavelet analysis can obtain the significant fluctuation pattern of the time series,
53 that is, the periodic dynamic time pattern. A continuous Morlet wavelet transform on
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4 the time series was performed to extract specific frequency components. Then, the
5 temporal periodicity in a time series was inspected with a wavelet plot. A statistical
6 significance test was performed to test the null hypothesis that the signal in a wavelet
7 plot is generated by a stable process of a given background power spectrum (usually
8 white noise) at a certain time with significance level of 95%. The *dplR* package within
9 R software was used for the Morlet wavelet analysis in this study.
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17 Fourth, in view of the presence of overdispersion in the dengue case data set, a
18 generalized additive model for location, scale and shape (GAMLSS) model [20] with
19 the distribution of the response variable obeying negative binomial distribution was
20 performed to study the association between seasonal characteristics of dengue and
21 climatic variables, including mean temperature, mean rainfall and mean relative
22 humidity. We implemented the Rigby and Stasinopoulos algorithm [20] to estimate the
23 parameters of the models, and the number of cycles of the algorithm was set to 2000 to
24 insure the convergence of the analysis results.
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35 In order to assess the effects of climatic variables on seasonal dynamics of dengue, we
36 established a set of GAMLSS models where climatic variables were modeled as smooth
37 terms with different degrees of freedom (*df*). This was performed by changing the *df*
38 for the smooth terms of 3 to 1 during model selection. We used the smoothing splines
39 for the smooth terms in the GAMLSS models. We considered temperature, relative
40 humidity and rainfall separately, then with both in model, then with all in model. In
41 addition, some sensitivity analyses by setting $df=0$ in the smooth terms, where the $df=0$
42 means the distribution of the response variable is directly modeled as a linear parametric
43 function of explanatory variables, for the three climatic factors considered separately,
44 then both in model, then all in model. As a result, a total of 70 models were generated
45 for comparison and evaluation. As model selection criterion, the Schwartz Bayesian
46 Criterion (SBC) [21] was used here. Models with lower value of SBC are preferred. In
47 addition, we also added the autoregressive term, $\ln(1 + Count_{local\ cases})$ in the previous
48 month, to account for the autoregressive effect in this study [10]. The *gamlss* package
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4 within R software was used to establish the GAMLSS model. The values of partial
5 effect function from a smoothing effect term in a GAMLSS model were extracted and
6 plot against their predictors. We can identify the nonlinear relationship between the
7 dependent variable and the independent variables from this plot. R software (version
8 3.4.3) was used for the statistical analyses. R codes were provided to show how to
9 employ the time-dependent wavelet analysis to assess the periodicity of the time series,
10 and GAMLSS model to study the association between seasonal characteristics of
11 dengue and climatic variables (see Supplementary Material, “R codes”).
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22 **Results**

23 Basic characteristics of dengue cases notified during 2014-17 were shown in Table 1.
24 In total, 2,193 probable and confirmed cases of dengue in the Chao-Shan area were
25 reported to the China CDC system, of which 2,148 (98.0%) were confirmed (clinic- or
26 laboratory-confirmed) and 45 (2.1%) were probable, respectively. Among all the
27 reported cases, the proportion (78.3%) of dengue cases reported in Chaozhou was
28 higher than those among Shantou (13.0%), and Jieyang (8.8%). The median age for all
29 notified cases was 43 years (interquartile range (IQR): 27.0-58.0 years), for Shantou
30 cases 35 years (IQR: 23.0-49.0 years), Chaozhou cases 46 years (IQR: 29.0-59.0 years),
31 and Jieyang cases 34 years (IQR: 25.0-50.0 years), respectively. The age distributions
32 among probable and confirmed cases for Shantou and Jieyang cities were similar. The
33 male to female ratio was 1:1.1 for all notified dengue cases. The sex distributions
34 among cases for Jieyang were different from those for the other two cities.
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49 For the most recent year 2017 observed, most of the patients were male, irrespective of
50 subgroup analysis results (Figure 2, A-C). The median time from illness onset to
51 diagnosis was 6 days (IQR 2.8-8), 3 days (IQR 3-5) and 3 days (IQR 1-5) for Shantou,
52 Chaozhou and Jieyang, respectively. The plot of probability density distributions also
53 demonstrated that there was a longer onset-to-diagnosis interval for dengue cases in
54 Shantou (Figure 2, D).
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6 In Figure 3, a kernel density estimate map from dengue case data with latitude/longitude
7 coordinates shows geographical distributions of dengue cases in the Chao-Shan area.
8 Spatial smoothing with the kernel density estimation produced dengue risk hotspots
9 suggesting that the geographical regions with the greatest number of dengue cases were
10 around Xixin and Chengxi streets in Chaozhou city. Overall, dengue showed annual
11 peaks of activity, a major peak in autumn, consistent among time series of dengue cases
12 in Shantou, Chaozhou and Jieyang (Figure 4). Although Chao-Shan area had a seasonal
13 attack of dengue peaking between September and October of each year, we observed
14 that the number of dengue cases increased sharply in 2015 in Chaozhou.
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25 Results of wavelet analysis for time series of notifications of dengue and climatic
26 variables including mean temperature, mean rainfall and mean relative humidity in
27 Chao-Shan area during the study period were shown in Figure 5. It showed that the
28 estimations of local powers for weekly time step examined were the largest at the period
29 of 1 year, suggesting dengue cases showed a significant annual periodicity (Figure 5,
30 A). In particular, based on the strongest power of the annual periodicity estimated for
31 the time series of climatic variables (mean temperature, rainfall and relative humidity)
32 throughout the study period (Figure 5, B-D), these factors broadly followed similar
33 periodicity with that of dengue cases.
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45 Based on the results of wavelet analysis, we further analyzed the effects of climatic
46 variables on seasonal dynamics of dengue using a framework of GAMLSS by testing
47 different degrees of freedom for the smooth terms during model selection to determine
48 an optimal model (Table S2). We found that the model with the variable of mean
49 temperature modeled as a smoothing spline term with $df=1$ had the smallest value of
50 SBC and the best model fit among the 70 kinds of candidate models, suggesting that
51 the effect of mean temperature on dengue dynamics in Chao-Shan area was most
52 obvious. In addition, according to the GAMLSS framework, a significant nonlinear
53 partial effect of mean temperature on dengue dynamics have been observed, and the
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4 risk of dengue transmission was most when the temperature was between 25 °C~ 28 °C
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6 (Figure 6).
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10 **Discussion**

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12 Our study of probable and confirmed cases of dengue reported to the national CDC
13 surveillance system during 2014-17 gives the first comprehensive estimation of the
14 local burden and epidemiology of the disease in the study sites. Among the three cities
15 covering a jurisdiction area of around 10918 square kilometers, Chaozhou is
16 responsible for the largest proportion of all the notified dengue cases. We recorded a
17 median age of infection at 43 years (IQR 27.0-58.0 years) for all notified dengue cases,
18 and the male to female ratio was 1:1.1. Our study suggests that cases of dengue tend to
19 arise in autumn season of the year, presenting a significant annual periodicity of
20 epidemics. In particular, we found that the effect of temperature on dengue epidemics
21 was most obvious among the climatic factors assessed.
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33 This present analysis of spatial distribution patterns of dengue identified two main
34 epidemiological regions corresponding to Xixin and Chengxi streets in Chaozhou city,
35 where dengue peaks in autumn. We found that Chaozhou accounted for around 78% of
36 the total number of dengue cases reported during the study period. We speculated that
37 this may owe to the combined effect of multiple factors including its subtropical
38 monsoonal climate, its industries producing abandoned ceramics that could provide
39 abundant of breeding places for mosquitos after raining, and local residents' habit of
40 planting flowers at home providing breeding environment for mosquito vectors [22].
41 Our results indicate that dengue cases showed a significant annual periodicity in Chao-
42 Shan area. Findings from Asia-Pacific countries including Thailand, Laos, and the
43 Philippines have shown localized traveling waves of multiannual dengue epidemic
44 cycles [23]. By contrast, data of dengue virus isolate counts from Puerto Rico, a major
45 population center in the Caribbean, indicate interannual variation in transmission across
46 multiple dengue serotypes [24]. An epidemiological study from Machala, Ecuador,
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4 provided evidence of significant 1-year and 2-year cycles in dengue [25]. In fact, our
5 data were in agreement with another study indicating that significant coherences were
6 observed between dengue incidence and temperature for the annual periodicity from
7 2005 to 2009 from Hanoi, Vietnam [26]. Our wavelet analysis revealed that dengue
8 transmission co-varied with temperature at annual cycle, and we inferred that this acted
9 as an important driver for the periodicity of dengue dynamics in Chao-Shan area.
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17 This study built on potential links between dengue dynamics and climatic variables,
18 and quantified their relationships based on a kind of state-of-the-art flexible regression
19 and smoothing technique, GAMLSS model, which enables us to handle adequately the
20 problem of the presence of overdispersion in the data. The GAMLSS is a general
21 framework for fitting semi-parametric regression type models where the distribution of
22 the response variable includes highly skew and kurtotic continuous and discrete
23 distribution, and allows all the parameters of the distribution of the response variable
24 to be modeled as smooth functions of the explanatory variables [20]. There is more
25 flexibility in identifying those non-linearities using a GAM-based approach than
26 traditional linear regression models [27]. By inspecting a scatterplot (data was not
27 shown) between dengue incidence and temperature, we found the relationship was
28 nonlinear, so it required the special estimation methods of the nonlinear regression
29 procedure. Additionally, the choice of df values is very important when constructing
30 the GAMLSS model. Therefore, we performed a sensitivity analysis by trying different
31 df in the smooth term of the model, and determined an optimal model according to the
32 assessment of model fit. Overall, our results revealed a powerful capability with
33 GAMLSS model for catching the appropriate relationship between dengue incidence
34 and temperature.
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54 The associations between dengue seasonality and climatic variables such as
55 temperature, rainfall, relative humidity, sunshine and ENSO indices have been
56 documented in previous studies [28]. In this study, our results reveal that temperature
57 has significantly nonlinear effects on epidemics risk and intensity of dengue, with the
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4 greatest attributable risk due to temperature estimated at around 25 °C. To identify the
5 significantly associated climatic factors with dengue epidemics, we repeated model
6 selections and adopted different climatic factors with different df in the smooth terms
7 of the GAMLSS framework. We found that the variation in daily mean temperature
8 partly drives dengue dynamics, and this climatic factor is closely related to the seasonal
9 fluctuation of dengue incidence in Chao-Shan area. The association between
10 temperature and seasonal dynamics of dengue observed in our study was also reported
11 in previous studies in Guangzhou [12] and Taiwan [29], which has a similar subtropical
12 humid monsoon climate. The estimated effects of temperature driving the seasonal
13 dynamics of dengue can be partly explained by some mechanisms, for example,
14 temperature effects on the biting rate of mosquitoes [30], incubation period of
15 pathogens [31], and human exposure to mosquitoes.

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29 Several limitations of this study should be mentioned. First, we did not consider
30 potentially relevant factors, such as mosquito density and other environment elements
31 including vegetation cover, distance to water bodies and so on. The variables may partly
32 influence the relationship between temperature and dengue epidemics estimated in this
33 work. In addition, although the quantitative results derived from this study pointed out
34 that temperature was associated with increased risk of dengue transmission, other
35 factors such as human activities during urbanization and globalization were also
36 suggested to be very important in driving the long-term trends [8, 32]. At present, we
37 can't obtain the data on laboratory test results for various kinds of dengue viruses.
38 Therefore, we can't study the epidemical features of dengue cases caused by different
39 viruses as well as the age-specific dengue antibody prevalence in the population.
40 Furthermore, our results faced a common challenge for studies using data from
41 surveillance with possible under-reporting or variations in diagnosis practices of cases.
42 This was also suggested from a previous study [33].

43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 **Conclusions**

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4 In summary, this study represented an initial step to analyze the epidemiological
5 characteristics of dengue in Chao-Shan area, one of the most densely-populated regions
6 on China's southeastern coast, and use a statistically rigorous approach for assessing
7 the effects of climatic factors on dengue epidemics. Overall, our results are helpful
8 toward advancing our understanding of the pattern of how climate influences dengue,
9 and useful for the control and prevention of the disease for local government in the
10 study sites.
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20 **Contributors**

21 QZ, PG and WJM conceived the study, undertook statistical analysis and drafted the
22 manuscript. YLC, YF, TL and QYZ collected the data and assisted in the statistical
23 analysis. QZ, PG, TL, QYZ and WJM interpreted the results. QZ, PG and TL wrote and
24 revised the manuscript. All authors read and approved the final manuscript.
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32 **Competing Interests**

33 The authors declare that they have no competing interests.
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40 81703323 and 81773497), and Top-tier University Construction Project under
41 Department of Education of Guangdong Government (No. 2015022 and 2015023).
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49 **Data Availability Statement**

50 Data are not publicly available because of personal privacy preservation. The dengue
51 surveillance data are available from the corresponding authors on request. Requests for
52 materials should be addressed to P.G. (Email: pguo@stu.edu.cn) or T.L. (Email:
53 gztt_2002@163.com).
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50 Figure Legends

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52 **Figure 1 Geographical location of Chao-Shan area including Shantou, Chaozhou**
53 **and Jieyang cities, on China's southeastern coast.**
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58 **Figure 2 Proportions of gender-specific cases of dengue fever by age groups and**
59 **estimates of onset-to-diagnosis distributions of dengue fever cases in Chao-Shan**
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4 **area, 2014-2017.** (A) Based on total cases. (B) Based on cases aged less than 35 years.
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6 (C) Based on cases aged greater than or equal to 35 years. (D) Onset-to-diagnosis
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8 distribution by city.
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11 **Figure 3 Geographical distributions of dengue cases in Chao-Shan area from the**
12 **beginning of 2014 to the end of 2017.** Spatial smoothing with the kernel density
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14 estimation produced dengue risk hotspots suggested that the geographical regions with
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16 the greatest number of dengue cases were around Xixin and Chengxi streets in
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18 Chaozhou city.
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23 **Figure 4 Temporal dynamics of dengue in Chao-Shan area from the beginning of**
24 **2014 to the end of 2017.** (A) Weekly time series of number of probable and confirmed
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26 dengue cases in Chao-Shan area. (B) Weekly time series of number of probable and
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28 confirmed dengue cases by city in Chao-Shan area.
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33 **Figure 5 Wavelet analyses for time series of notifications of dengue and climatic**
34 **variables including mean temperature, rainfall and relative humidity in Chao-**
35 **Shan area, 2014-2017.** Local wavelet power spectrum for dengue cases (A), mean
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37 temperature (B), rainfall (C) and relative humidity (D). Solid and bold lines indicate
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39 boundary of statistical significance.
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44 **Figure 6 Analysis of potentially nonlinear effects of mean temperature on seasonal**
45 **dynamics of dengue in Chao-Shan area using a generalized additive model for**
46 **location, scale and shape (GAMLSS) model based on data from years 2014-2017.**
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48 Initially, climatic variables including mean temperature, rainfall and relative humidity
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50 were assessed using the GAMLSS model with different degrees of freedom for the
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52 smooth terms during model selection. Then, the optimal model with the statistically
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54 significant variable (mean temperature) was determined. The values of partial effect
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56 function from a smoothing effect term in a GAMLSS model were extracted and plot
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58 against their predictors. This plot shows the significant nonlinear partial effect of mean
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4 temperature on dengue dynamics, and the risk of dengue transmission is most when the
5 temperature is between 25 °C~28 °C. The lightblue dots denote partial residuals of the
6 model, which were added in the plot to see how the model fits. From the dots of partial
7 residuals, we can there are no outliers in the data.
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50 Tables

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52 **Table 1 Basic characteristics of dengue cases reported in the study areas during**
53 **the period from 2014 to 2017.**
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56 Characteristics	57 City			58 Total
	59 Shantou	60 Chaozhou	Jieyang	
Number of cases, number (%)	284 (13.0)	1716 (78.3)	193 (8.8)	2193 (100)
Type of cases, number (%)				

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3	<i>Probable cases</i>	0 (0.0)	45 (2.6)	0 (0.0)	45 (2.1)
4	<i>Confirmed cases</i>	284 (100.0)	1,671 (97.4)	193 (100.0)	2148 (97.9)
5	Median age, years (IQR)	35.0 (23.0-49.0)	46.0 (29.0-59.0)	34.0 (25.0-50.0)	43.0 (27.0-58.0)
6	Sex, number (%)				
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8	<i>Male</i>	137 (48.2)	819 (47.7)	110 (57.0)	1066 (48.6)
9	<i>Female</i>	147 (51.8)	897 (52.3)	83 (43.0)	1127 (51.4)
10	Season of diagnosis, number (%)				
11					
12	<i>Spring</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
13	<i>Summer</i>	0 (0.0)	33 (1.9)	0 (0.0)	33 (1.5)
14	<i>Autumn</i>	281 (98.9)	1,680 (97.9)	192 (99.5)	2153 (98.2)
15	<i>Winter</i>	3 (1.1)	3 (0.2)	1 (0.5)	7 (0.3)
16	Year of diagnosis, number (%)				
17					
18	2014	247 (87.0)	135 (7.9)	86 (44.6)	468 (21.3)
19	2015	14 (4.9)	1380 (80.4)	6 (3.1)	1400 (63.8)
20	2016	5 (1.8)	194 (11.3)	0 (0.0)	199 (9.1)
21	2017	18 (6.3)	7 (0.4)	101 (52.3)	126 (5.8)
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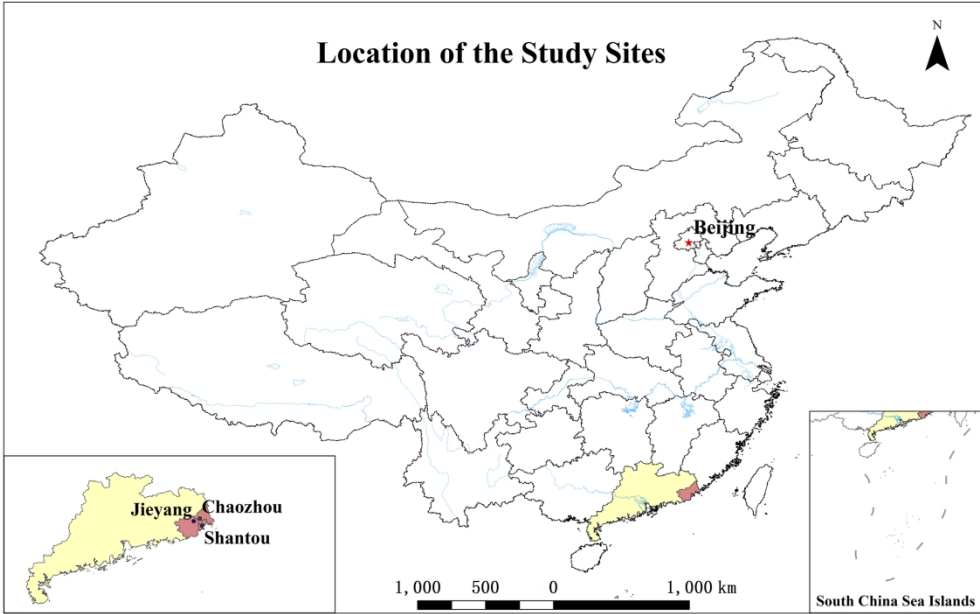


Fig 1

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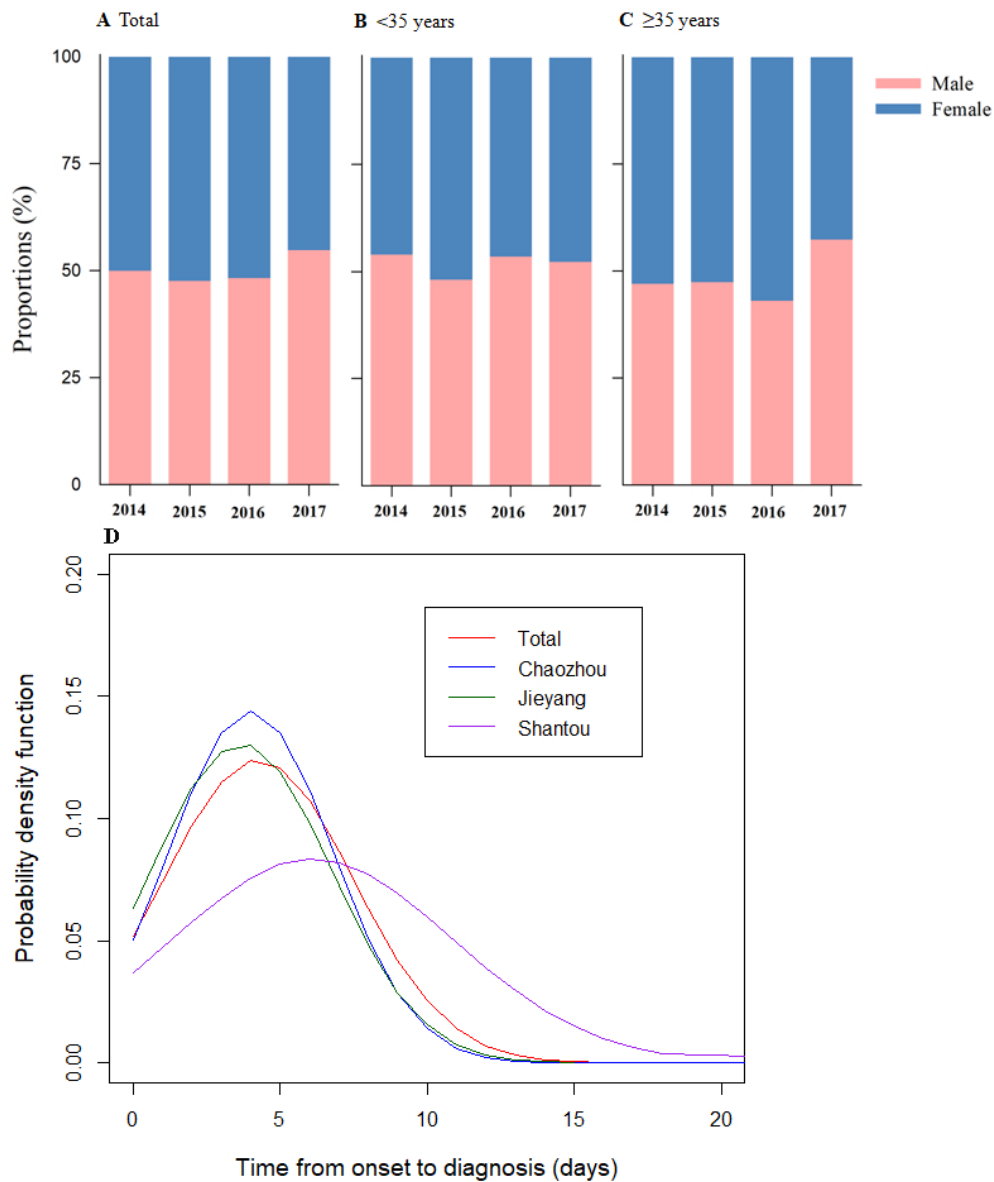


Fig 2

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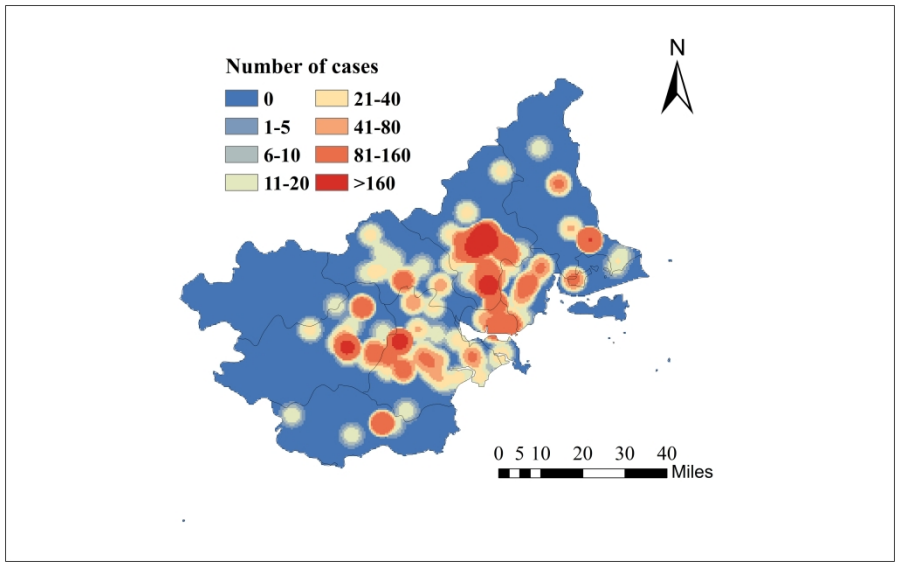


Fig 3

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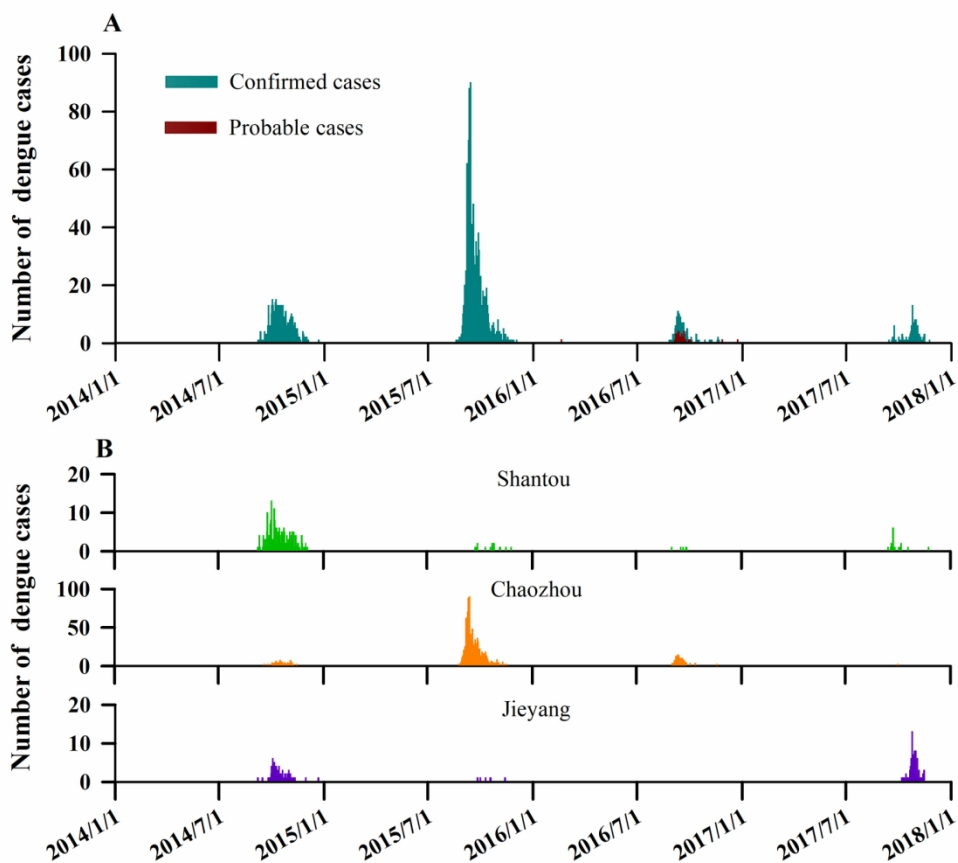


Fig 4

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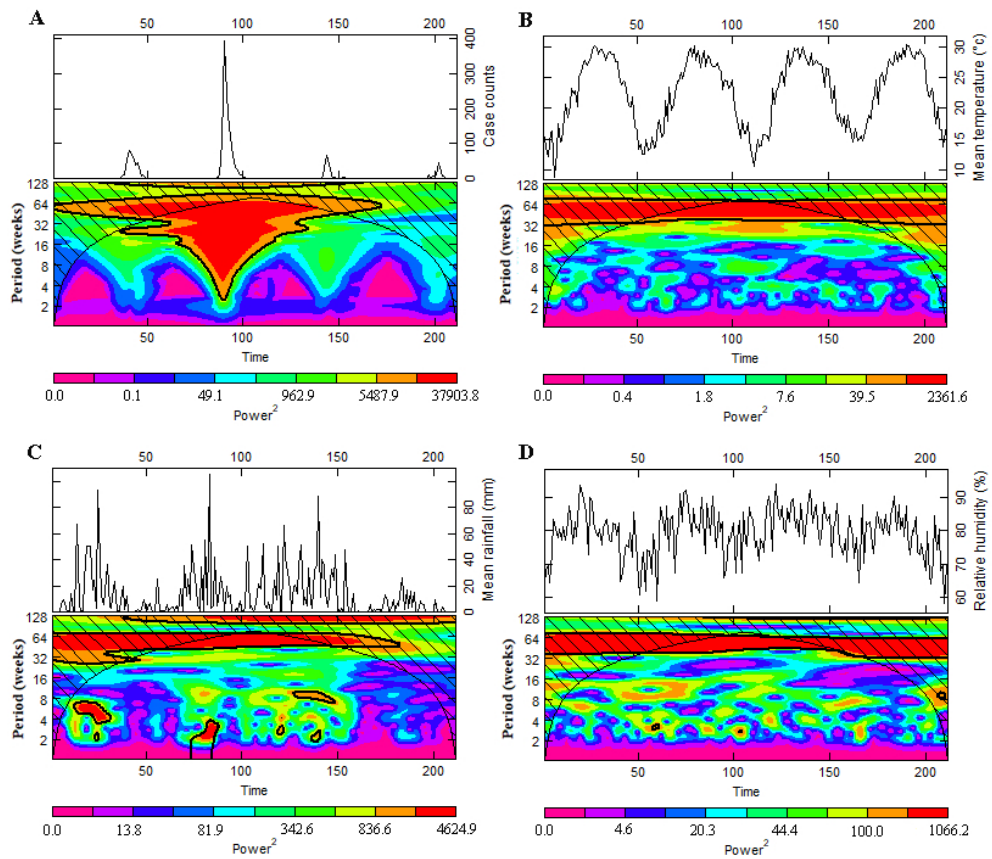


Fig 5

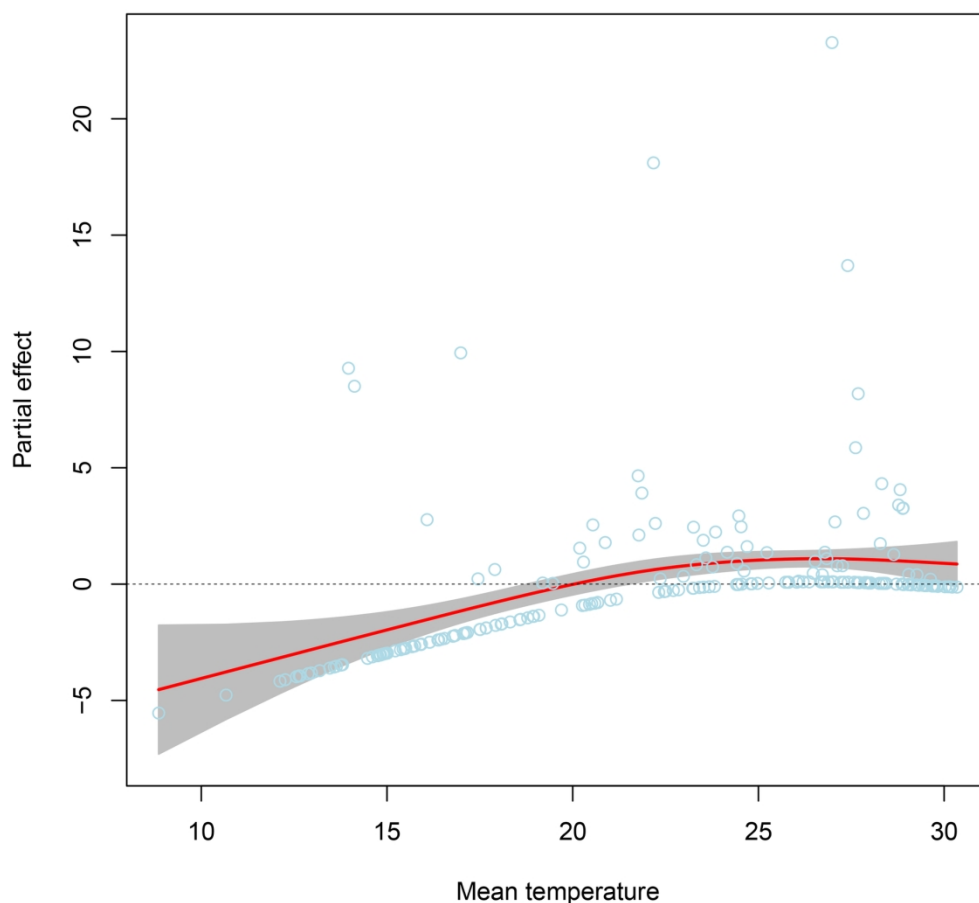


Fig 6

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Supplementary File

Table S1. STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6, 7
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	6, 7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7

Bias	9	Describe any efforts to address potential sources of bias	7, 8
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7, 8
		(b) Describe any methods used to examine subgroups and interactions	7, 8
		(c) Explain how missing data were addressed	7, 8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	7, 8
		(e) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	9
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9, 10

		(b) Report category boundaries when continuous variables were categorized	9, 10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9, 10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11, 12, 13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13, 14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

Table S2. Model fit assessment of a set of generalized additive model for location, scale and shape (GAMLSS) models where climatic variables including mean temperature (MeanTemp), rainfall (Rainfall) and relative humidity (RH) were modeled as smooth terms with different degrees of freedom (*df*). The criterion, Schwartz Bayesian Criterion (SBC), was used for model selection, where models with lower value of SBC are preferred. 0, 1, 2 and 3 were considered for the values of the parameter *df* in the GAMLSS models, respectively. Cubic spline (CS) function was used as the smooth function term.

Rank of model	Model formula	SBC
1	~ CS(MeanTemp, 1)	686.105
2	~ CS(MeanTemp, 2)	686.871
3	~ CS(MeanTemp, 3)	689.207
4	~ MeanTemp	690.681
5	~ CS(MeanTemp, 1) + CS(Rainfall, 1)	691.759
6	~ CS(MeanTemp, 2) + CS(Rainfall, 1)	691.811
7	~ CS(MeanTemp, 3) + CS(Rainfall, 1)	692.740
8	~ CS(MeanTemp, 1) + CS(Rainfall, 2)	693.124
9	~ CS(MeanTemp, 2) + CS(Rainfall, 2)	693.623
10	~ MeanTemp + Rainfall	694.608
11	~ MeanTemp + RH	694.628
12	~ CS(MeanTemp, 1) + CS(RH, 1)	694.790
13	~ CS(MeanTemp, 2) + CS(RH, 1)	695.267
14	~ CS(MeanTemp, 3) + CS(Rainfall, 2)	695.459
15	~ CS(MeanTemp, 3) + CS(RH, 1)	695.533
16	~ CS(MeanTemp, 1) + CS(Rainfall, 3)	696.474
17	~ CS(MeanTemp, 2) + CS(Rainfall, 3)	697.546
18	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 1)	699.055
19	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 1)	699.193
20	~ CS(MeanTemp, 1) + CS(RH, 2)	699.303
21	~ CS(MeanTemp, 3) + CS(Rainfall, 3)	699.363
22	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 2)	699.565
23	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 1)	699.655
24	~ MeanTemp + RH + Rainfall	699.672
25	~ CS(MeanTemp, 2) + CS(RH, 2)	699.851
26	~ CS(MeanTemp, 3) + CS(RH, 2)	700.121
27	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 2)	700.460
28	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 2)	700.535
29	~ CS(MeanTemp, 2) + CS(RH, 1) + CS(Rainfall, 3)	703.064
30	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 1)	703.307

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31	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 1)	703.395
32	~ CS(MeanTemp, 1) + CS(RH, 1) + CS(Rainfall, 3)	703.790
33	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 2)	703.836
34	~ CS(MeanTemp, 1) + CS(RH, 3)	703.955
35	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 1)	704.037
36	~ CS(MeanTemp, 3) + CS(RH, 1) + CS(Rainfall, 3)	704.168
37	~ CS(MeanTemp, 2) + CS(RH, 3)	704.432
38	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 2)	704.519
39	~ CS(MeanTemp, 3) + CS(RH, 3)	704.845
40	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 2)	705.177
41	~ RH	707.331
42	~ CS(MeanTemp, 2) + CS(RH, 2) + CS(Rainfall, 3)	707.364
43	~ CS(Rainfall, 1)	707.472
44	~ CS(Rainfall, 2)	707.558
45	~ Rainfall	707.656
46	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 1)	707.991
47	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 1)	708.093
48	~ CS(MeanTemp, 3) + CS(RH, 2) + CS(Rainfall, 3)	708.267
49	~ CS(MeanTemp, 1) + CS(RH, 2) + CS(Rainfall, 3)	708.330
50	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 2)	708.505
51	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 1)	708.792
52	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 2)	709.107
53	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 2)	709.867
54	~ CS(Rainfall, 3)	710.239
55	~ CS(RH, 1)	711.324
56	~ CS(MeanTemp, 2) + CS(RH, 3) + CS(Rainfall, 3)	712.016
57	~ RH + Rainfall	712.666
58	~ CS(MeanTemp, 3) + CS(RH, 3) + CS(Rainfall, 3)	712.849
59	~ CS(MeanTemp, 1) + CS(RH, 3) + CS(Rainfall, 3)	713.085
60	~ CS(RH, 2)	715.945
61	~ CS(RH, 1) + CS(Rainfall, 1)	717.272
62	~ CS(RH, 1) + CS(Rainfall, 2)	717.727
63	~ CS(RH, 3)	720.343
64	~ CS(RH, 1) + CS(Rainfall, 3)	721.098
65	~ CS(RH, 2) + CS(Rainfall, 1)	721.843
66	~ CS(RH, 2) + CS(Rainfall, 2)	722.342
67	~ CS(RH, 3) + CS(Rainfall, 1)	725.910
68	~ CS(RH, 2) + CS(Rainfall, 3)	725.926
69	~ CS(RH, 3) + CS(Rainfall, 2)	726.601
70	~ CS(RH, 3) + CS(Rainfall, 3)	730.204

R codes. Code examples show how to employ the time-dependent wavelet analysis to assess the periodicity of the time series, and GAMLSS model to study the association between seasonal characteristics of dengue and climatic variables in this study.

```
##### Part I: Code example for generating Figure 5 in this study #####  
  
# Wavelet analysis  
  
# Install R package  
install.packages("dplR")  
  
# Load the package  
library(dplR)  
  
# Read the dataset 'mydata.csv' into R  
my.data <- read.csv("mydata .csv", head=TRUE)  
  
  
my.morlet <- morlet(y1=x, x1=my.data $week, p2=7, dj=0.1, siglvl=0.95)  
  
# y1 denotes the series to be transformed  
# x1 is a vector of values giving the years for the plot  
# p2 represents the number of power of two to be computed for the wavelet transform  
# siglvl is the level for the significance test  
my.morlet$period # The period  
my.morlet$coi # The cone of influence  
my.morlet$Signif # The significant values  
my.morlet$Power # The squared power  
  
  
# The wavelet.plot function creates a filled.contour plot of a continuous wavelet transform as  
output from the morlet function.  
wavelet.plot(my.morlet, useRaster=T, add.spline=T, reverse.y=T, add.coi=T, add.sig=T)  
  
##### Part II: Code example for generating Figure 6 in this study #####  
  
# Generalized additive model for location, scale and shape model (GAMLSS) analysis  
# Install R package
```

```
1
2
3
4 install.packages("gamlss")
5
6 # Load the package
7
8 library(gamlss)
9
10 # Read the dataset 'mydata.csv' into R
11
12 my.data <- read.csv("mydata .csv", head=TRUE)
13
14 head(my.data)
15
16 # Establish a GAMLSS model using the gamlss.fit function
17
18 gamlss.fit <- gamlss(case_num ~ cs(avg_tem_mean, 3) + cs(avg_rh, 1) + cs(avg_rainfall, 2) ,
19 data= my.data, family=NBI, n.cyc=2000, trace=FALSE)
20
21 # The setting "family=NBI" denotes the negative binomial distribution used for the
22
23 corresponding link function for over-dispersed count data
24
25 gamlss.fit
26
27 # Calculate the Generalised Akaike information criterion for a fitted GAMLSS object
28
29 GAIC(gamlss.fit)
30
31 # Plot residual diagnostics for a fitted GAMLSS Object
32
33 plot(gamlss.fit$residuals)
34
35 # Plot regression terms for a specified parameter of a fitted GAMLSS object
36
37 term.plot(gamlss.fit, se=TRUE, partial=TRUE)
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