BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024197
Article Type:	Research
Date Submitted by the Author:	15-May-2018
Complete List of Authors:	Zhang, Qin; Cancer Hospital of Shantou University Medical College, Good Clinical Practice Office Chen, Yuliang Wang, Li Zhang, Yanhong Fu, Yu Zhang, Qingying Liu, Tao Guo, Pi Ma, Wenjun
Keywords:	China, Climate, Dengue, Seasonal variation, Spatial, Wavelet analysis

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

Authors

Qin Zhang [1], Yuliang Chen [2], Li Wang [3], Yanhong Zhang [4], Yu Fu [5], Tao Liu [6,*], Qingying Zhang [7,*], Pi Guo [2,*], Wenjun Ma [6]

[1] Good Clinical Practice Office, Cancer Hospital of Shantou University Medical College, Shantou 515041, China

[2] Department of Preventive Medicine, Shantou University Medical College, Shantou 515041, China

[3] Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen University Town, Shenzhen 518055, China

[4] Shantou University Medical College, Shantou 515041, China

[5] Department of Finance, Zhongnan University of Economics and Law, Wuhan 430074, China

[6] Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, China

[7] Department of Preventive Medicine, Guangdong Provincial Key Laboratory for Breast Cancer Diagnosis and Treatment, Shantou University Medical College, Shantou 515041, China

[*] **These authors are co-corresponding authors in this work.**

Dr. Pi Guo

Department of Preventive Medicine, Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, China

E-mail: pguo@stu.edu.cn

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.

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

Chao-Shan area (Figure 1), the eastern part of Guangdong province is poorly quantified. The Chao-Shan area is one of the special regions on China's southeastern coast. The region has a jurisdiction area of around 10918 square kilometers, but a very high population density. In addition, it is situated in the intersection part of the Tropic of Cancer and the coastline of the mainland China, and has a subtropical oceanic monsoon climate with a mean annual temperature of around 22 °C and an abundant precipitation per year. With the special climatic conditions and the increasingly frequent cultural contact with Southeast Asian countries, the Chao-Shan area itself has a potential high risk of spread of mosquito-borne infectious diseases.

Unfortunately there are few comprehensive reports on the spatial-temporal epidemic pattern for dengue in the Chao-Shan area. The effects of seasonal climate variation on the dynamics of dengue and the associated disease burden in the region are still unclear, highlights gaps in our knowledge to the current status of dengue epidemic and the deployment of prevention and control measures. Therefore, this study aimed to assess the epidemic characteristics of dengue in the Chao-Shan area, and the effect of seasonal climate variation on the dynamics of dengue for the period 1 January 2014 to 31 December 2017.

Material and methods

Patient and Public Involvement

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

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 special climatic conditions, geographical position, and the frequent culture and economic communions 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 [11] 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

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

Statistical Analysis

First, basic characteristics (number, median age, sex ratio, and season and year of diagnosis) of dengue cases reported during the study period were described by city. The probability density distributions of the onset-to-diagnosis of dengue cases for the three cities were calculated to estimate median duration from onset to diagnosis and test the reaction ability of the surveillance system. Second, according to the residential address registered for each dengue case, we created a map of the number of dengue cases in a spatial scale of street to assess the spatial distribution pattern of dengue epidemic in the Chao-Shan area.

Third, to quantify seasonal patterns of dengue epidemic, time series of weekly case counts were plotted by city, and type of cases (probable or confirmed cases), respectively. The periodicity of the time series of dengue case counts, mean temperature, mean rainfall and mean relative humidity was assessed using time-dependent wavelet analysis [12]. A continuous Morlet wavelet transform on the time series was performed to extract specific frequency components, which were visualized with a wavelet plot in this study. A statistical significance test was performed to test the null hypothesis that a period was irrelevant at a certain time with significance level of 95%.

Fourth, in view of the presence of overdispersion in the dengue case data set, a generalized additive model for location, scale and shape (GAMLSS) model [13] with the distribution of the response variable obeying negative binomial distribution was performed to study the association between seasonal characteristics of dengue and climatic variables, including mean temperature, mean rainfall and mean relative humidity. We implemented the Rigby and Stasinopoulos algorithm [13] to estimate the parameters of the models, and the number of cycles of the algorithm was set to 2000 to insure the convergence of the analysis results.

In order to assess the effects of climatic variables on seasonal dynamics of dengue, we established a set of GAMLSS models where climatic variables were modeled as smooth terms with different degrees of freedom (*df*). This was performed by changing the *df* for the smooth terms of 3 to 0 during model selection, where the *df*=0 means the distribution of the response variable was directly modeled as a linear parametric function of explanatory variables. We used the smoothing splines for the smooth terms in the GAMLSS models. As model selection criterion, the Schwatz Bayesian Criterion (SBC) [14] was used here. Models with lower value of SBC are preferred. In addition, we also added the autoregressive term, $\ln(1 + Count_{local\ cases})$ in the previous month, to account for the autoregressive effect in this study [8]. All analyses were conducted using R software (version 3.4.3).

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 (97.95%) were confirmed (clinic- or laboratory-confirmed) and 45 (2.05%) were probable, respectively. Among all the reported cases, the proportion (78.25%) of dengue cases reported in Chaozhou was higher than those among Shantou (12.95%), and Jieyang (8.80%). The median age for all notified cases was 43 years (interquartile range (IQR): 31 years), for Shantou cases 35 years (IQR: 26 years), Chaozhou cases 46 years (IQR: 30 years), and Jieyang cases 34 years (IQR: 25 years), respectively. The age distributions among probable and confirmed cases for Shantou and Jieyang cities were similar. The male to female ratio was 1:1.06 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 was male patients, irrespectively of subgroup analysis results according to total patients, the younger (<35 years old) and elder (≥35 years old) (Figure 2, A-C). The median time from illness onset to diagnosis was 6 days (IQR 2.75-8) for Shantou, 3 days (3-5) for Chaozhou, and 3 day (1-5) for Jieyang. 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).

Geographical distribution of dengue incidence in the Chao-Shan area was shown in Figure 3. Radiuses of circles indicate incidence of dengue. We identified two main geographical regions with a high risk of infection with dengue during the 4-year study period: Xixin and Chengxi streets in Chaozhou city. Overall, dengue showed annual peaks of activity, a major peak in autumn, consistent among time series of dengue cases in Shantou, Chaozhou and Jieyang (Figure 4). Although Chao-Shan area had a seasonal attack of dengue peaking between September and October of each year, we observed that the number of dengue cases increased sharply in 2015 in Chaozhou.

Results of wavelet analysis for time series of notifications of dengue and climatic variables including mean temperature, mean rainfall and mean relative humidity in Chao-Shan area during the study period were shown in Figure 5. It showed that the estimations of local powers for weekly time step examined were the largest at the period of 1 year, suggesting dengue cases showed a significant annual periodicity (Figure 5, A). In particular, based on the strongest power of the annual periodicity estimated for the time series of climatic variables (mean temperature, rainfall and relative humidity) throughout the study period (Figure 5, B-D), these factors broadly followed similar periodicity with that of dengue cases.

Based on the results of wavelet analysis, we further analyzed the effects of climatic variables on seasonal dynamics of dengue using a framework of GAMLSS by testing different degrees of freedom for the smooth terms during model selection to determine an optimal model (Table S1). We found that the model with the variable of mean temperature modeled as a smoothing spline term with df=1 had the smallest value of SBC and the best model fit among the 70 kinds of candidate models, suggesting that the effect of mean temperature on dengue dynamics in Chao-Shan area was most obvious. In addition, according to the GAMLSS framework, a significant nonlinear partial effect of mean temperature on dengue dynamics have been observed, and the risk of dengue transmission was most when the temperature was reached to around 25 °C (Figure 6).

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.

This study built on potential links between dengue dynamics and climatic variables, and quantified their relationships based on a kind of state-of-the-art flexible regression and smoothing technique, GAMLSS model, which enables us to handle adequately the problem of the presence of overdispersion in the data. The GAMLSS is a general framework for fitting semi-parametric regression type models where the distribution of the response variable includes highly skew and kurtotic continuous and discrete distribution, and allows all the parameters of the distribution of the response variable to be modeled as smooth functions of the explanatory variables [13]. Unlike traditional linear regression, the approach enables to find a nonlinear relationship between the dependent variable and a set of independent variables by specifying an appropriate smooth function term that describes the relationship [19]. By inspecting a scatterplot (data was not shown) between dengue incidence and temperature, we found the relationship was nonlinear, so it required the special estimation methods of the nonlinear regression procedure. Additionally, the choice of *df* values is very important when constructing the GAMLSS model. Therefore, we performed a sensitivity analysis by trying different *df* in the smooth term of the model, and determined an optimal model according to the assessment of model fit. Overall, our results revealed a powerful capability with GAMLSS model for catching the appropriate relationship between dengue incidence and temperature.

The associations between dengue seasonality and climatic variables such as temperature, rainfall, relative humidity, sunshine and ENSO indices have been documented in previous studies [20]. In this study, our results reveal that temperature has significantly nonlinear effects on epidemics risk and intensity of dengue, with the greatest attributable risk due to temperature estimated at around 25 °C. For detecting the significantly associated climatic factors with dengue epidemics, we repeated model selection, using different climatic factors with different *df* in the smooth terms of the GAMLSS framework. We found that no additional climatic factors, expect for mean temperature, were related to dengue epidemics, and thus needed to explain these seasonal peaks in dengue incidence in Chao-Shan area. The association between temperature and seasonal dynamics of dengue in our study was also reported in previous studies in Guangzhou [8] and Taiwan [21], which has a similar subtropical humid monsoon climate. The estimated effects of temperature driving the seasonal dynamics of dengue can be partly explained by some mechanisms, for example, temperature effects on the biting rate of mosquitoes [22], incubation period of pathogens [23], and human exposure to mosquitoes.

Several limitations of this study should be mentioned. First, we did not consider potentially relevant factors, such as mosquito density and other environment elements including vegetation cover, distance to water bodies and so on. The variables may partly influence the relationship between temperature and dengue epidemics estimated in this work. In addition, although the quantitative results derived from this study pointed out that temperature was associated with increased risk of dengue transmission, other factors such as human activities during urbanization and globalization were also suggested to be very important in driving the long-term trends [8, 24]. Furthermore, our results faced a common challenge for studies using data from surveillance with possible under-reporting or variations in diagnosis practices of cases. This was also suggested from a previous study [25].

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, 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.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81703323 and 81773497), Guangdong Science and Technology Department Funding (No. 2014A040401041), National Key R&D Program of China (No. 2016YFC1304000), and Top-tier University Construction Project under Department of Education of Guangdong Government (No. 2015022 and 2015023).

Data sharing statement

No additional data are available.

References

1. World Health Organization, 2009. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva World Health Organization6, 990.
2. Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., et al., 2013. The global distribution and burden of dengue. Nature 496,504-507.
3. Lai, S., Huang, Z., Zhou, H., Anders, K.L., Perkins, T.A., et al., 2015. The changing epidemiology of dengue in China, 1990-2014: a descriptive analysis of 25 years of nationwide surveillance data. BMC Med 13, 100.
4. Chen, B., Liu, Q., 2015. Dengue fever in China. Lancet 385, 1621-1622.
5. Guo, P., Liu, T., Zhang, Q., Wang, L., Xiao, J., et al., 2017. Developing a dengue forecast model using machine learning: A case study in China. PLoS Neglected Tropical Diseases 11, e0005973.

6. Xiao, J.P., He, J.F., Deng, A.P., Lin, H.L., Song, T., et al., 2016. Characterizing a large outbreak of dengue fever in Guangdong Province, China. Infectious Diseases of Poverty 5, 44.

7. Lin, Y.P., Luo, Y., Chen, Y., Lamers, M.M., Zhou, Q., et al., 2016. Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. BMC Infectious Diseases 16, 102.

8. Xu, L., Stige, L.C., Chan, K.S., Zhou, J., Yang, J., et al., 2017. Climate variation drives dengue dynamics. Proceedings of the National Academy of Sciences of the United States of America 114, 113-118.

9. Shepard, D.S., Undurraga, E.A., Halasa, Y.A., 2013. Economic and Disease Burden of Dengue in Southeast Asia. PLoS neglected tropical diseases 7, e2055.

10. Halstead, S.B., 2006. Dengue in the Americas and Southeast Asia: do they differ? RevistaPanamericana de SaludPública = Pan American journal of public health. 20, 407-415.

11. National Health Commission of the People's Republic of China, 2008. Diagnostic criteria for dengue fever. http://www.nhfpc.gov.cn/zhuz/s9491/200802/38819.shtml (accessed 11 April 2018).

12. Cazelles, B., Chavez, M., Magny, G.C., Guégan, J.F., Hales, S., 2007. Time-dependent spectral analysis of epidemiological time-series with wavelets. Journal of the Royal Society Interface 4, 625-636.

13. Rigby, R.A., Stasinopoulos, D.M., 2005. Generalized additive models for location, scale and shape. Applied Statistics 54, 507-554.

14. Stasinopoulos, M., Rigby, R.A., 2007. Generalized Additive Models for Location Scale and Shape (GAMLSS) in R. Journal of Statistical Software 223, 1-46.

15. van Panhuis, W.G., Choisy, M., Xiong, X., Chok, N.S., Akarasewi, P., et al., 2015. Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia. Proceedings of the National Academy of Sciences 112, 13069-13074.

16. Bennett, S.N., Drummond, A.J., Kapan, D.D., Suchard, M.A., Munoz-Jordán, J.L., et al., 2010. Epidemic dynamics revealed in dengue evolution. Molecular Biology

and Evolution 27, 811–818.

17. Zielinski, A., Rudowska, J., Ying, Z., Ning, L.D., Xin, L., et al., 2015. Spatiotemporal clustering, climate periodicity, and social-ecological risk factors for dengue during an outbreak in Machala, Ecuador, in 2010. BMC Public Health 11, 37.

18. Toan Do, T.T., Martens, P., Luu, N.H., Wright, P., Choisy, M., 2014. Climatic-driven seasonality of emerging dengue fever in Hanoi, Vietnam. BMC Public Health 14, 1078.

19. Stasinopoulos, D., Rigby, R., Heller, G., Voudouris, V., De Bastiani, F., 2017. Flexible Regression and Smoothing: Using GAMLSS in R.

20. Naish, S., Dale, P., Mackenzie, J.S., McBride, J., Mengersen, K., et al., 2014. Climate change and dengue: A critical and systematic review of quantitative modelling approaches. BMC Infectious Diseases 14, 167.

21. Wu,C. P., Su, J. H., Guo, H.R., Lung, S.C, Lin, C.Y., 2005. Temperature Can Be An Effective Predictor for Dengue Fever Outbreak. Epidemiology 16, S72.

22. Patz, J.A., Epstein, P.R., Burke, T.A., Balbus, J.M., 1996. Global climate change and emerging infectious diseases. JAMA 275, 217-223.

23. Xiao, F.Z., Zhang, Y., Deng, Y.Q., He, S., Xie, H. G., et al., 2014. The effect of temperature on the extrinsic incubation period and infection rate of dengue virus serotype 2 infection in Aedesalbopictus. Arch Virol 159, 3053-3057.

24. Gubler, D.J., 2011. Dengue, Urbanization and Globalization: The Unholy Trinity of the 21st Century. Tropical Medicine and Health 39, S3-S11.

25. Yang, B., Liu, F., Liao, Q., Wu, P., Chang, Z., et al., 2017. Epidemiology of hand, foot and mouth disease in china, 2008 to 2015 prior to the introduction of EV-A71 vaccine. Eurosurveillance 22.

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

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 (%)			
Probable cases	0 (0.00)	45 (2.62)	0 (0.00)
Confirmed cases	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 (%)			
Male	137 (48.24)	819 (47.73)	110 (56.99)
Female	147 (51.76)	897 (52.27)	83 (43.01)
Season of diagnosis, number (%)			
Spring	0 (0.00)	0 (0.00)	0 (0.00)
Summer	0 (0.00)	33 (1.92)	0 (0.00)
Autumn	281 (98.94)	1,680 (97.90)	192 (99.48)
Winter	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)

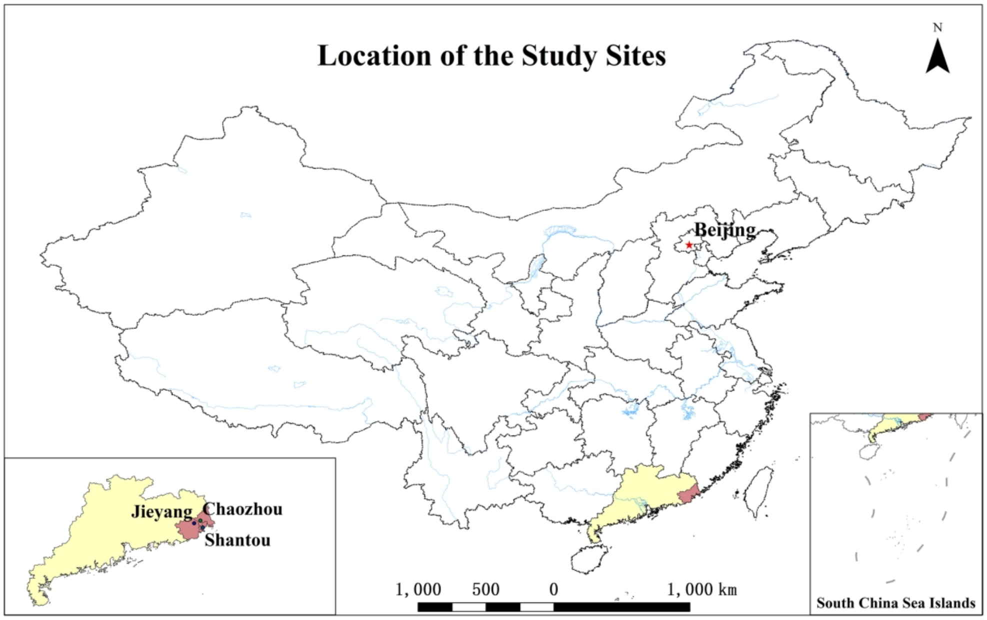

Fig 1

213x134mm (300 x 300 DPI)

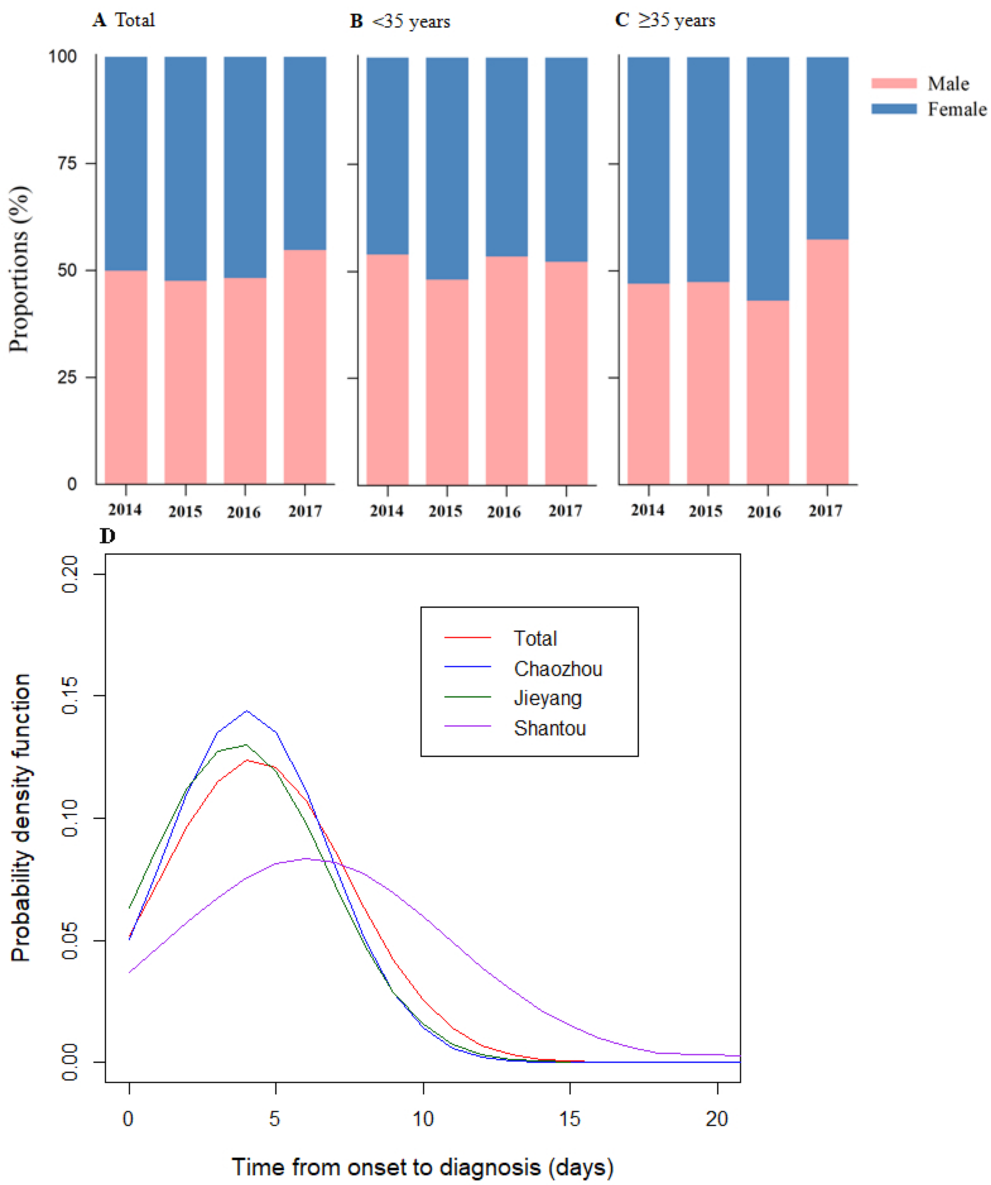
A Total
B <35 years
C ≥35 years
Proportions (%)
100
75
50
25
0
2014
2015
2016
2017
Male
Female
D
Probability density function
0.20
0.15
0.10
0.05
0.00
Total
Chaozhou
Jieyang
Shantou
0
5
10
15
20
Time from onset to diagnosis (days)

Fig 2

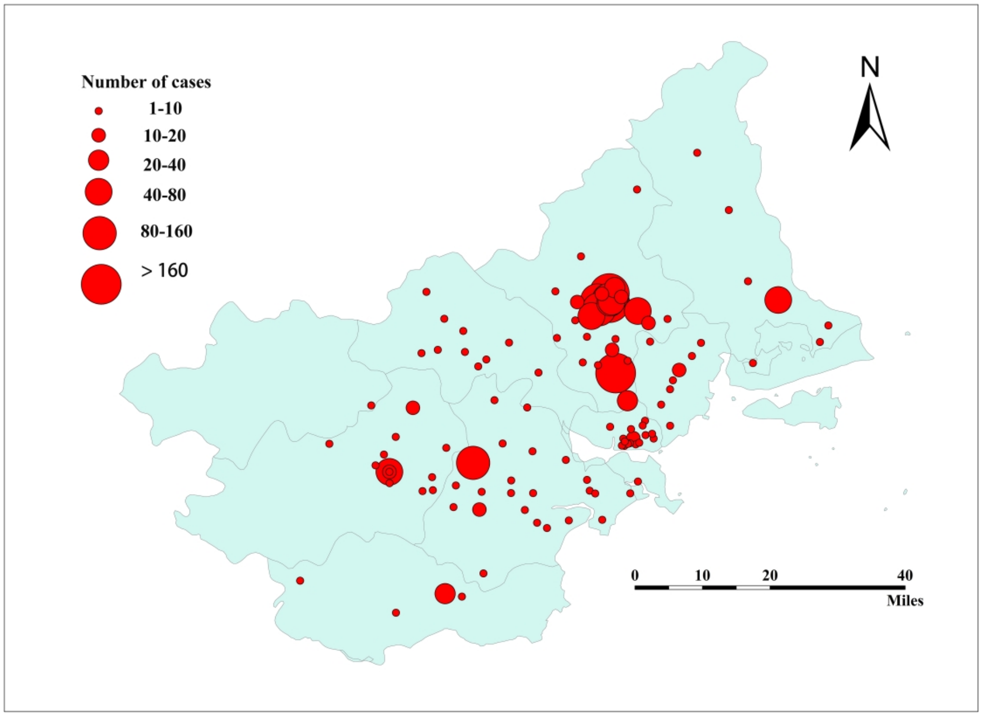

Fig 3

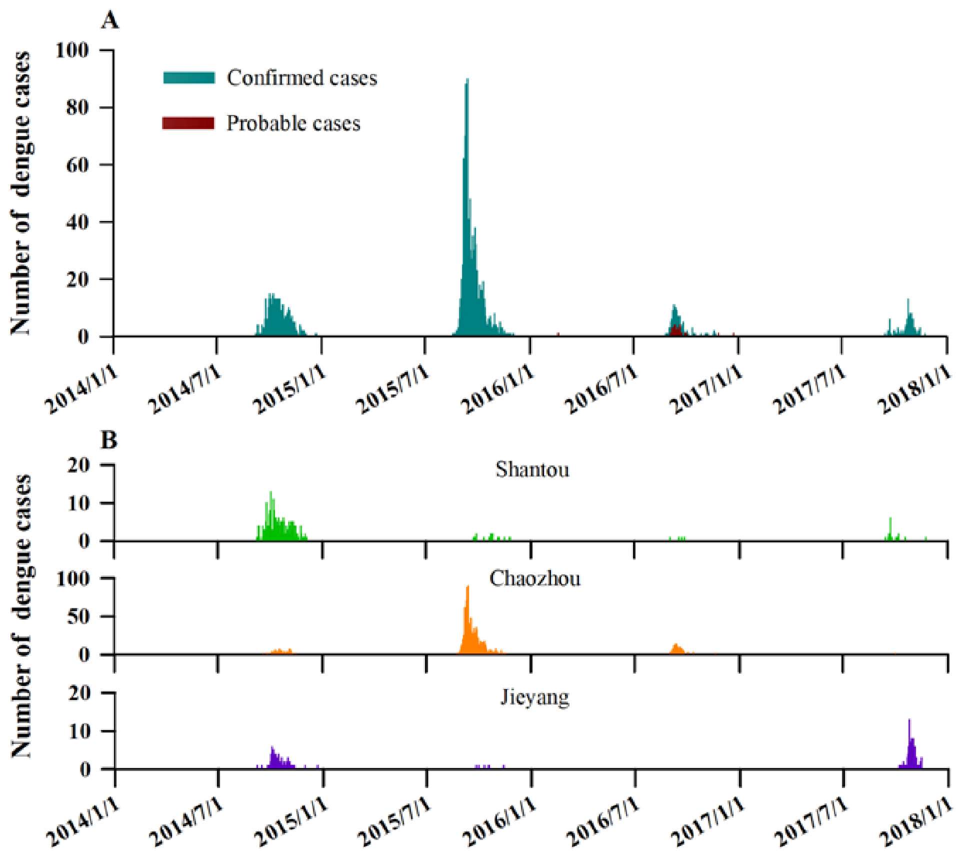

Fig 4

249x215mm (72 x 72 DPI)

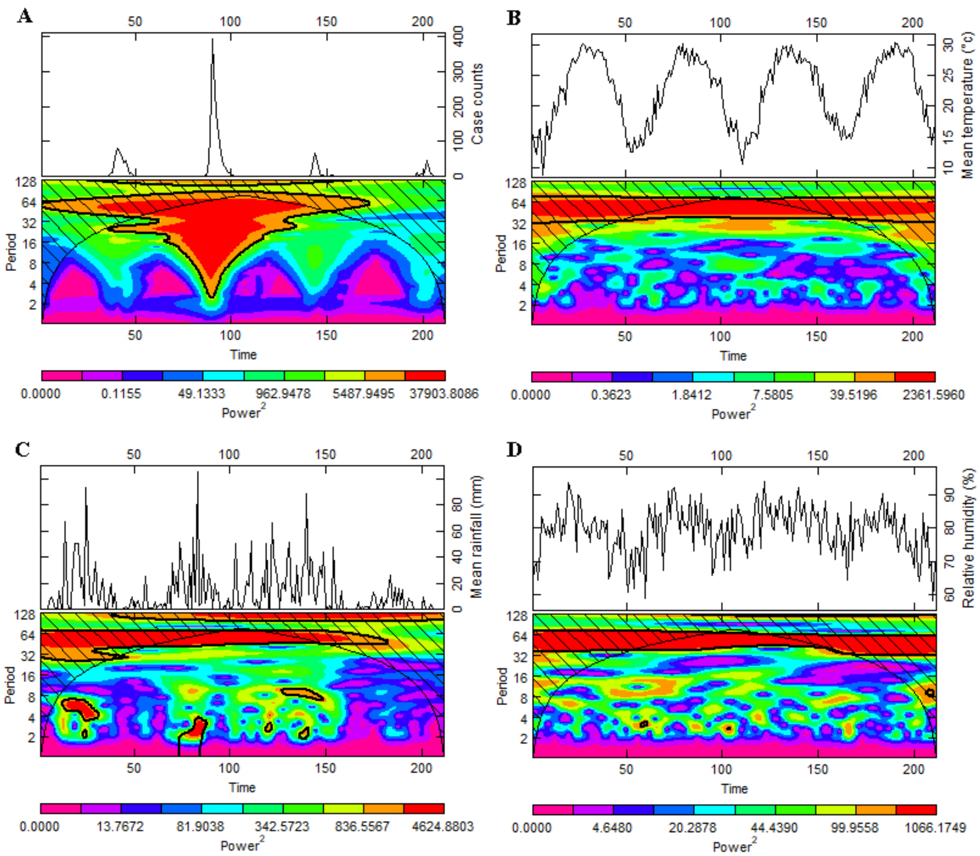

Fig 5

289x254mm (72 x 72 DPI)

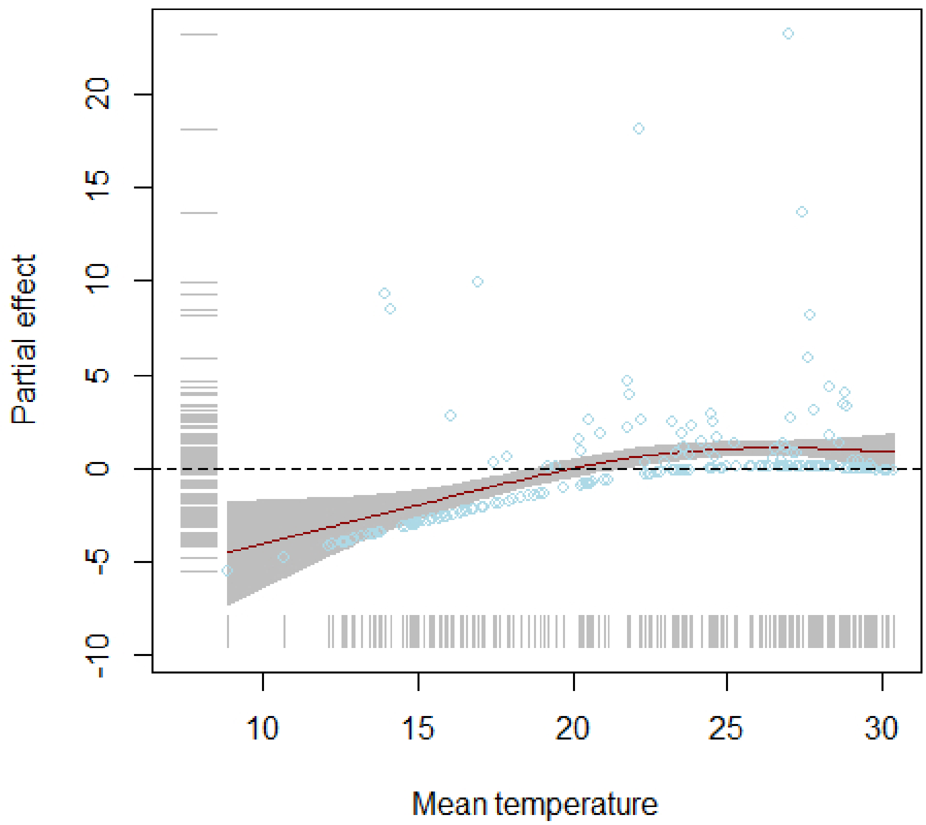

Fig 6

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, Schwatz 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
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

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024197.R1
Article Type:	Research
Date Submitted by the Author:	18-Feb-2019

Complete List of Authors:	Zhang, Qin; Cancer Hospital of Shantou University Medical College, Good Clinical Practice Office Chen, Yuliang; Shantou University Medical College, Department of Preventive Medicine Fu, Yu; Zhongnan University of Economics and Law, Department of Finance Liu, Tao; Guangdong Provincial Center for Disease Control and Prevention, Guangdong Provincial Institute of Public Health Zhang, Qingying; Shantou University Medical College, Department of Preventive Medicine Guo, Pi; Shantou University Medical College, Department of Preventive Medicine Ma, Wenjun; Guangdong Provincial Center for Disease Control and Prevention, Guangdong Provincial Institute of Public Health

Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	China, Climate, Dengue, Seasonal variation, Spatial, Wavelet analysis

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

Authors

Qin Zhang [1], Yuliang Chen [2], Yu Fu [3], Tao Liu [4, 5, *], Qingying Zhang [2, *], Pi Guo [2, *], Wenjun Ma [4]

[1] Good Clinical Practice Office, Cancer Hospital of Shantou University Medical College, Shantou 515041, China

[2] Department of Preventive Medicine, Shantou University Medical College, Shantou 515041, China

[3] Department of Finance, Zhongnan University of Economics and Law, Wuhan 430074, China

[4] Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, China

[5] General Practice Center, Nanhai Hospital, Southern Medical University, Foshan, Guangdong, China

[*] These authors are co-corresponding authors in this work.

Dr. Tao Liu

Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, China; General Practice Center, Nanhai Hospital, Southern Medical University, Foshan, Guangdong, China

E-mail: gztt_2002@163.com

Dr. Qingying Zhang

Department of Preventive Medicine, Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, China

E-mail: qyzhang@stu.edu.cn

Dr. Pi Guo

Department of Preventive Medicine, Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, China

E-mail: pguo@stu.edu.cn

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

- 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 humidity which have direct impacts on mosquito abundance [12]. Enjoying a subtropical humid monsoon climate and frequent economic and cultural communication with the Southeast Asian countries [13, 14], 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 [9-12].

The impact of seasonal climatic variation on dengue dynamics in the Chao-Shan area (Figure 1), the eastern part of Guangdong province is poorly quantified. The Chao-Shan area is one of the special regions on China's southeastern coast. The region has a jurisdiction area of around 10918 square kilometers, with a very high population density. In addition, it is situated in the intersection part of the Tropic of Cancer and the coastline of the mainland China, and has a subtropical oceanic monsoon climate with a mean annual temperature of around 22 °C and an abundant precipitation per year [15]. The subtropical oceanic monsoon climate conditions and the increasingly frequent cultural contact with Southeast Asian countries in the Chao-Shan area make it a high risk area for mosquito-borne infectious diseases. In particular, mosquito vectors such as Aedes albopictus are reported as the major vector of dengue virus in the area [16].

Unfortunately there are few comprehensive reports on the spatial-temporal epidemic pattern for dengue in the Chao-Shan area. The effects of seasonal climate variation on the dynamics of dengue and the associated disease burden in the region are still unclear. There is an urgent need to understand the association between dengue epidemics and seasonal climatic conditions in order to take corresponding protective measures. Therefore, this study aimed to assess the epidemic characteristics of dengue in the Chao-Shan area, and the effect of seasonal climate variation on the dynamics of dengue for the period January 1, 2014 to December 31, 2017.

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 (millimeters), and temperature (°C) in the three cities during the study period from January 1, 2014 to December 31, 2017 were obtained from the Guangdong Provincial Climate Bureau. Weekly values of climatic variables were calculated as simple arithmetic means to show the seasonal peaks of dengue in surveillance data, and used to assess the potential effect of seasonal variation on the dengue dynamics.

Statistical Analysis

First, basic characteristics (number, median age, sex ratio, and season and year of diagnosis) of dengue cases reported during the study period were described by city. By calculating the interval between the date of onset and the diagnosed date for each dengue case, we estimated the probability density function of the continuous variable of the 'interval' and then plot corresponding probability density distributions of the onset-to-diagnosis of dengue cases for the three cities. The median duration from onset to diagnosis by city was estimated. Each case of dengue has registered current address information. To identify high-risk areas of dengue in the Chao-Shan area, we applied a kernel smoothing method [18] to plot the geographical distribution of cases by street or town. A total of 231 streets or towns in the Chao-Shan area covering the three cities were included in our dataset. ArcGIS 10.2 (ESRI, Redlands, CA, USA) was used to plot the geographical distribution.

Third, to quantify seasonal patterns of dengue epidemic, time series of weekly case counts were plotted by city, and type of cases (probable or confirmed cases), respectively. The periodicity of the time series of dengue case counts, mean temperature, mean rainfall and mean relative humidity was assessed using time-dependent wavelet analysis [19]. The time series data of dengue case counts and climatic factors were analyzed by wavelet analysis, and then the wavelet decomposition results were used to calculate power spectrum. By decomposing the time series into the time-frequency domain, wavelet analysis can obtain the significant fluctuation pattern of the time series, that is, the periodic dynamic time pattern. A continuous Morlet wavelet transform on the time series was performed to extract specific frequency components. Then, the temporal periodicity in a time series was inspected with a wavelet plot. A statistical significance test was performed to test the null hypothesis that the signal in a wavelet plot is generated by a stable process of a given background power spectrum (usually white noise) at a certain time with significance level of 95%. The *dplR* package within R software was used for the Morlet wavelet analysis in this study.

Fourth, in view of the presence of overdispersion in the dengue case data set, a generalized additive model for location, scale and shape (GAMLSS) model [20] with the distribution of the response variable obeying negative binomial distribution was performed to study the association between seasonal characteristics of dengue and climatic variables, including mean temperature, mean rainfall and mean relative humidity. We implemented the Rigby and Stasinopoulos algorithm [20] to estimate the parameters of the models, and the number of cycles of the algorithm was set to 2000 to insure the convergence of the analysis results.

In order to assess the effects of climatic variables on seasonal dynamics of dengue, we established a set of GAMLSS models where climatic variables were modeled as smooth terms with different degrees of freedom (*df*). This was performed by changing the *df* for the smooth terms of 3 to 1 during model selection. We used the smoothing splines for the smooth terms in the GAMLSS models. We considered temperature, relative humidity and rainfall separately, then with both in model, then with all in model, yielding 63 models. In addition, some sensitivity analyses by setting *df*=0 in the smooth terms, where the *df*=0 means the distribution of the response variable is directly modeled as a linear parametric function of explanatory variables, for the three climatic factors considered separately, then both in model, then all in model, yielding 7 models. As model selection criterion, the Schwatz Bayesian Criterion (SBC) [21] was used here. Models with lower value of SBC are preferred. In addition, we also added the autoregressive term, $\ln(1 + Count_{local\ cases})$ in the previous month, to account for the autoregressive effect in this study [10]. The *gamlss* package within R software 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 latitude/longitude coordinates shows geographical distributions of dengue cases in the Chao-Shan area. 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. Overall, dengue showed annual peaks of activity, a major peak in autumn, consistent among time series of dengue cases in Shantou, Chaozhou and Jieyang (Figure 4). Although Chao-Shan area had a seasonal attack of dengue peaking between September and October of each year, we observed that the number of dengue cases increased sharply in 2015 in Chaozhou.

Results of wavelet analysis for time series of notifications of dengue and climatic variables including mean temperature, mean rainfall and mean relative humidity in Chao-Shan area during the study period were shown in Figure 5. It showed that the estimations of local powers for weekly time step examined were the largest at the period of 1 year, suggesting dengue cases showed a significant annual periodicity (Figure 5, A). In particular, based on the strongest power of the annual periodicity estimated for the time series of climatic variables (mean temperature, rainfall and relative humidity) throughout the study period (Figure 5, B-D), these factors broadly followed similar periodicity with that of dengue cases.

Based on the results of wavelet analysis, we further analyzed the effects of climatic variables on seasonal dynamics of dengue using a framework of GAMLSS by testing different degrees of freedom for the smooth terms during model selection to determine an optimal model (Table S1). We found that the model with the variable of mean temperature modeled as a smoothing spline term with *df*=1 had the smallest value of SBC and the best model fit among the 70 kinds of candidate models, suggesting that the effect of mean temperature on dengue dynamics in Chao-Shan area was most obvious. In addition, according to the GAMLSS framework, a significant nonlinear partial effect of mean temperature on dengue dynamics have been observed, and the risk of dengue transmission was most when the temperature was between 25 °C~ 28 °C (Figure 6).

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 27.0-58.0 years) for all notified dengue cases, and the male to female ratio was 1:1.1. 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. We found that Chaozhou accounted for around 78% of the total number of dengue cases reported during the study period. We speculated that this may owe to the combined effect of multiple factors including its subtropical monsoonal climate, its industries producing abandoned ceramics that could provide abundant of breeding places for mosquitos after raining, and local residents' habit of planting flowers at home providing breeding environment for mosquito vectors [22]. 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 [23]. 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 [24]. An epidemiological study from Machala, Ecuador, provided evidence of significant 1-year and 2-year cycles in dengue [25]. 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 [26]. 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.

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

The associations between dengue seasonality and climatic variables such as temperature, rainfall, relative humidity, sunshine and ENSO indices have been documented in previous studies [28]. In this study, our results reveal that temperature has significantly nonlinear effects on epidemics risk and intensity of dengue, with the greatest attributable risk due to temperature estimated at around 25 °C. To identify the significantly associated climatic factors with dengue epidemics, we repeated model selections and adopted different climatic factors with different *df* in the smooth terms of the GAMLSS framework. We found that the variation in daily mean temperature partly drives dengue dynamics, and this climatic factor is closely related to the seasonal fluctuation of dengue incidence in Chao-Shan area. The association between temperature and seasonal dynamics of dengue observed in our study was also reported in previous studies in Guangzhou [12] and Taiwan [29], which has a similar subtropical humid monsoon climate. The estimated effects of temperature driving the seasonal dynamics of dengue can be partly explained by some mechanisms, for example, temperature effects on the biting rate of mosquitoes [30], incubation period of pathogens [31], and human exposure to mosquitoes.

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

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.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81703323 and 81773497), and Top-tier University Construction Project under Department of Education of Guangdong Government (No. 2015022 and 2015023).

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).

References

1. World Health Organization, 2009. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva World Health Organization6, 990.
2. Halstead SB， O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. Nature 1977;265(5596):739-41.
3. Guzman MG, Gubler DJ, Izquierdo A, et al. Dengue infection. Nat Rev Dis Primers2016;2:16055.
4. Guzman MG, Harris E. Dengue. Lancet 2015; 385(9966):453–65.
5. Cory WM, Andrew CC, and Kacey E. Climate and Dengue Transmission: Evidence and Implications. Environ Health Perspect 2013; 121(11–12): 1264–72.
6. Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., et al., 2013. The global distribution and burden of dengue. Nature 496,504-507.
7. Lai, S., Huang, Z., Zhou, H., Anders, K.L., Perkins, T.A., et al., 2015. The changing epidemiology of dengue in China, 1990-2014: a descriptive analysis of 25 years of nationwide surveillance data. BMC Med 13, 100.
8. Chen, B., Liu, Q., 2015. Dengue fever in China. Lancet 385, 1621-1622.
9. Guo, P., Liu, T., Zhang, Q., Wang, L., Xiao, J., et al., 2017. Developing a dengue forecast model using machine learning: A case study in China. PLoS Neglected Tropical Diseases 11, e0005973.
10. Xiao, J.P., He, J.F., Deng, A.P., Lin, H.L., Song, T., et al., 2016. Characterizing a large outbreak of dengue fever in Guangdong Province, China. Infectious Diseases of Poverty 5, 44.
11. Lin, Y.P., Luo, Y., Chen, Y., Lamers, M.M., Zhou, Q., et al., 2016. Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. BMC Infectious Diseases 16, 102.
12. Xu, L., Stige, L.C., Chan, K.S., Zhou, J., Yang, J., et al., 2017. Climate variation drives dengue dynamics. Proceedings of the National Academy of Sciences of the United States of America 114, 113-118.
13. Shepard, D.S., Undurraga, E.A., Halasa, Y.A., 2013. Economic and Disease

Burden of Dengue in Southeast Asia. PLoS neglected tropical diseases 7, e2055.

14. Halstead, S.B., 2006. Dengue in the Americas and Southeast Asia: do they differ? RevistaPanamericana de SaludPública = Pan American journal of public health. 20, 407-415.

15. https://en.wikipedia.org/wiki/Chaoshan(accessed 15Feb2019)

16. Yang S, Liu Q. Trend in global distribution and spread of Aedes Albopictus. Chin J Vector Biol & Control. 2013;24(1):1-4.

17. National Health Commission of the People's Republic of China, 2008. Diagnostic criteria for dengue fever. http://www.nhfpc.gov.cn/zhuz/s9491/200802/38819.shtml (accessed 11 April 2018).

18. Silverman, B. W. Density Estimation for Statistics and Data Analysis. New York: Chapman and Hall, 1986.

19. Cazelles, B., Chavez, M., Magny, G.C., Guégan, J.F., Hales, S., 2007. Time-dependent spectral analysis of epidemiological time-series with wavelets. Journal of the Royal Society Interface 4, 625-636.

20. Rigby, R.A., Stasinopoulos, D.M., 2005. Generalized additive models for location, scale and shape. Applied Statistics 54, 507-554.

21. Stasinopoulos, M., Rigby, R.A., 2007. Generalized Additive Models for Location Scale and Shape (GAMLSS) in R. Journal of Statistical Software 223, 1-46.

22. Liu T, Zhu G, He J, et al.Early rigorous control interventions can largely reduce dengue outbreak magnitude: experience from Chaozhou, China. BMC Public Health 2017;18(1):90.

23. van Panhuis, W.G., Choisy, M., Xiong, X., Chok, N.S., Akarasewi, P., et al., 2015. Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia. Proceedings of the National Academy of Sciences 112, 13069-13074.

24. Bennett, S.N., Drummond, A.J., Kapan, D.D., Suchard, M.A., Munoz-Jordán, J.L., et al., 2010. Epidemic dynamics revealed in dengue evolution. Molecular Biology and Evolution 27, 811–818.

25. Zielinski, A., Rudowska, J., Ying, Z., Ning, L.D., Xin, L., et al., 2015.

Spatiotemporal clustering, climate periodicity, and social-ecological risk factors for dengue during an outbreak in Machala, Ecuador, in 2010. BMC Public Health 11, 37.

26. Toan Do, T.T., Martens, P., Luu, N.H., Wright, P., Choisy, M., 2014. Climatic-driven seasonality of emerging dengue fever in Hanoi, Vietnam. BMC Public Health 14, 1078.

27. Stasinopoulos, D., Rigby, R., Heller, G., Voudouris, V., De Bastiani, F., 2017. Flexible Regression and Smoothing: Using GAMLSS in R.

28. Naish, S., Dale, P., Mackenzie, J.S., McBride, J., Mengersen, K., et al., 2014. Climate change and dengue: A critical and systematic review of quantitative modelling approaches. BMC Infectious Diseases 14, 167.

29. Wu,C. P., Su, J. H., Guo, H.R., Lung, S.C, Lin, C.Y., 2005. Temperature Can Be An Effective Predictor for Dengue Fever Outbreak. Epidemiology 16, S72.

30. Patz, J.A., Epstein, P.R., Burke, T.A., Balbus, J.M., 1996. Global climate change and emerging infectious diseases. JAMA 275, 217-223.

31. Xiao, F.Z., Zhang, Y., Deng, Y.Q., He, S., Xie, H. G., et al., 2014. The effect of temperature on the extrinsic incubation period and infection rate of dengue virus serotype 2 infection in Aedesalbopictus. Arch Virol 159, 3053-3057.

32. Gubler, D.J., 2011. Dengue, Urbanization and Globalization: The Unholy Trinity of the 21st Century. Tropical Medicine and Health 39, S3-S11.

33. Yang, B., Liu, F., Liao, Q., Wu, P., Chang, Z., et al., 2017. Epidemiology of hand, foot and mouth disease in china, 2008 to 2015 prior to the introduction of EV-A71 vaccine. Eurosurveillance 22.

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

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

Tables

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 (%)				
Probable cases	0 (0.0)	45 (2.6)	0 (0.0)	45 (2.1)
Confirmed cases	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 (%)				
Male	137 (48.2)	819 (47.7)	110 (57.0)	1066 (48.6)
Female	147 (51.8)	897 (52.3)	83 (43.0)	1127 (51.4)
Season of diagnosis, number (%)				
Spring	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Summer	0 (0.0)	33 (1.9)	0 (0.0)	33 (1.5)
Autumn	281 (98.9)	1,680 (97.9)	192 (99.5)	2153 (98.2)
Winter	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)

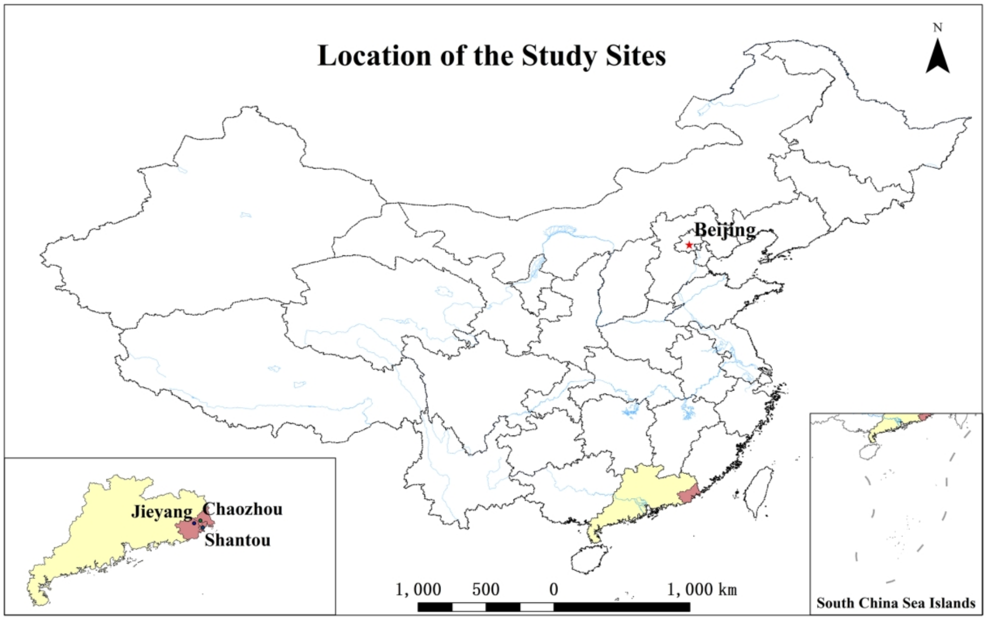

Fig 1

213x134mm (300 x 300 DPI)

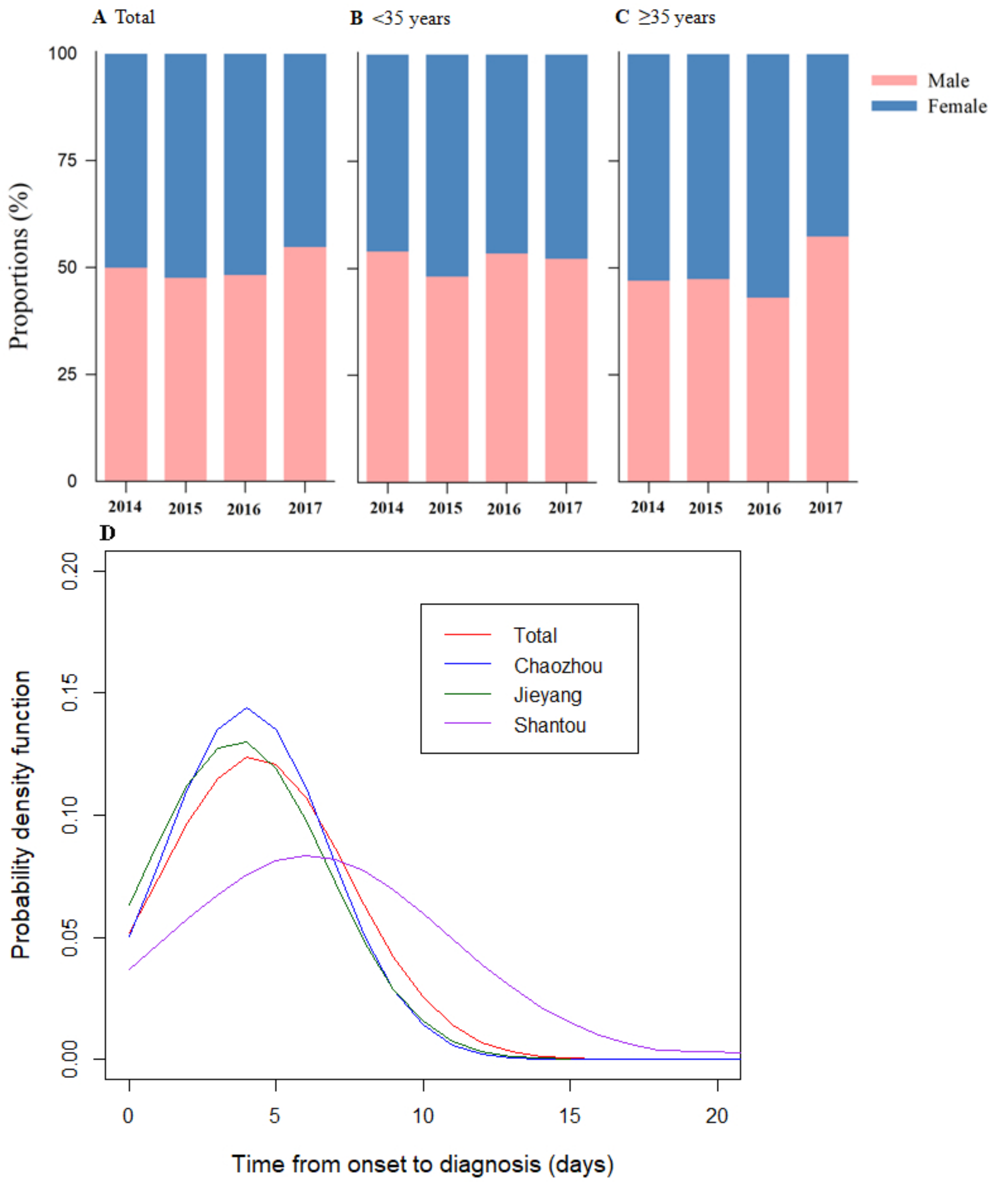
A Total
B <35 years
C ≥35 years
Proportions (%)
100
75
50
25
0
2014
2015
2016
2017
Male
Female
D
Probability density function
0.20
0.15
0.10
0.05
0.00
Total
Chaozhou
Jieyang
Shantou
0
5
10
15
20
Time from onset to diagnosis (days)

Fig 2

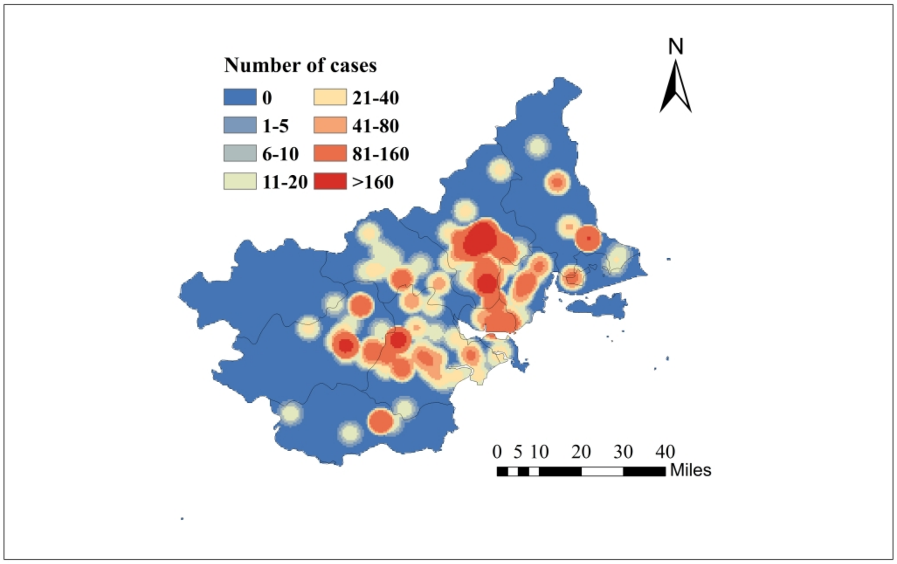

Fig 3

457x304mm (300 x 300 DPI)

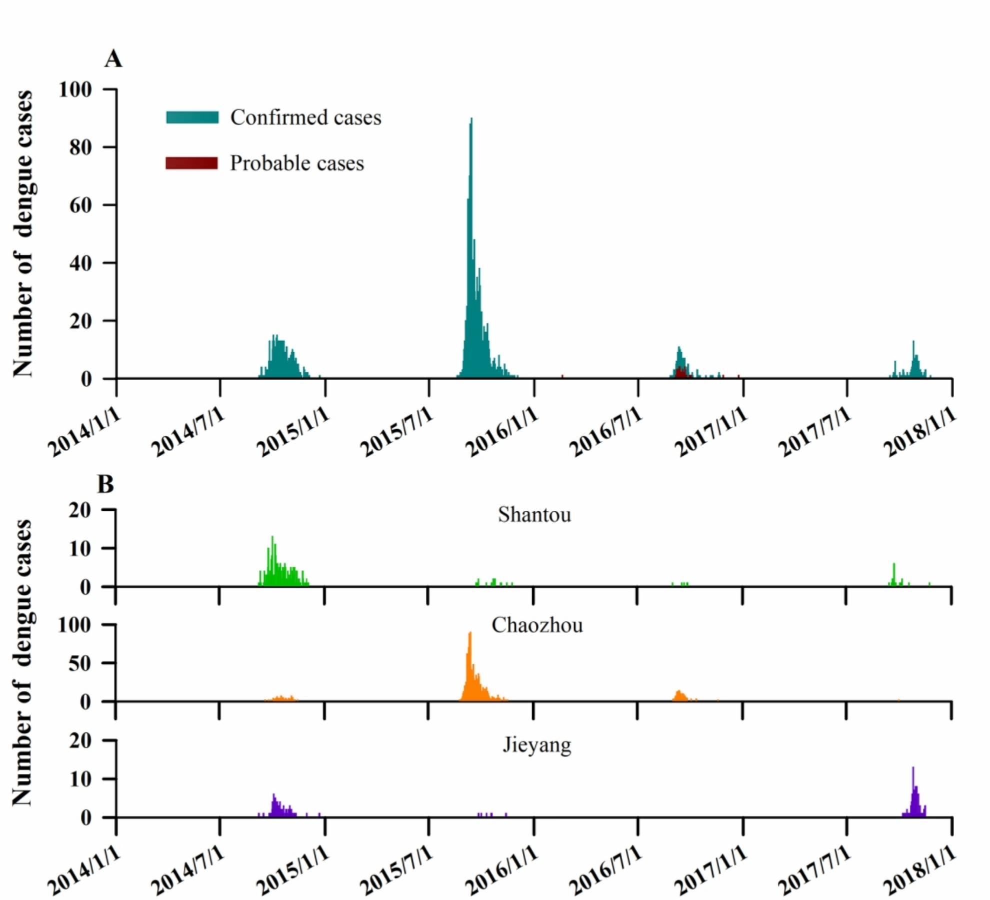

Fig 4

155x141mm (300 x 300 DPI)

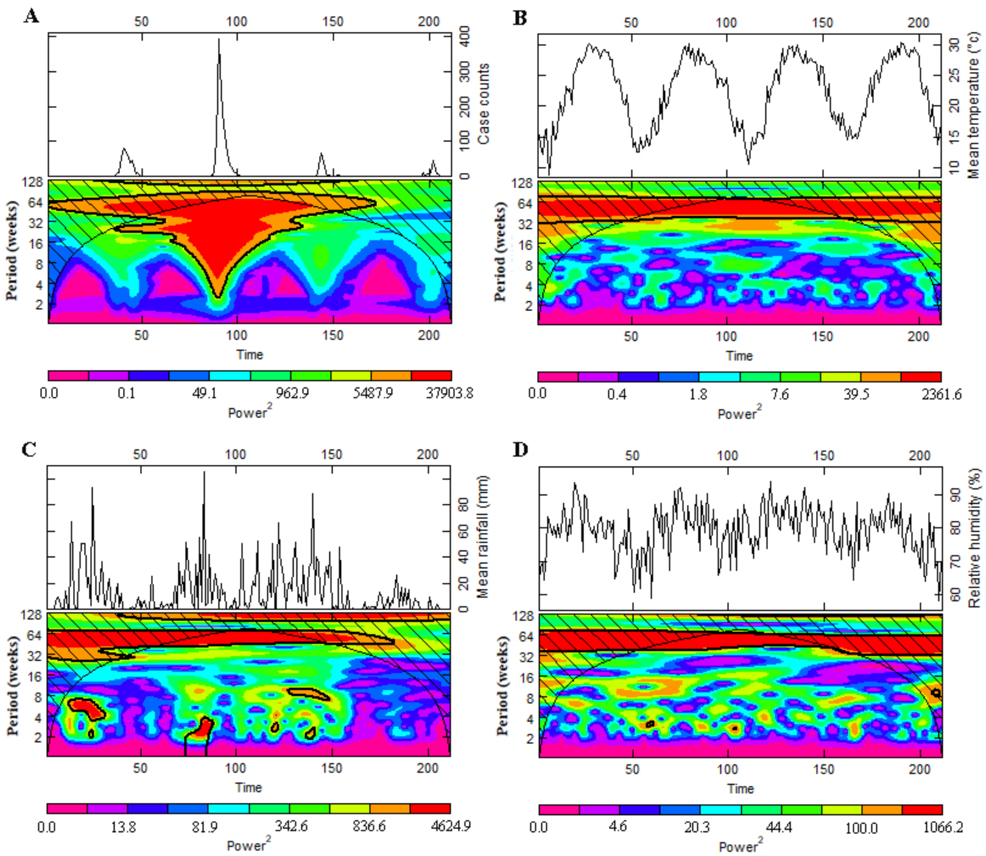
A
B
C
D
Case counts
Mean temperature (°c)
Mean rainfall (mm)
Relative humidity (%)
Period (weeks)
Time
Power^2
0.0
0.1
49.1
962.9
5487.9
37903.8
0.4
1.8
7.6
39.5
2361.6
13.8
81.9
342.6
836.6
4624.9
4.6
20.3
44.4
100.0
1066.2

Fig 5

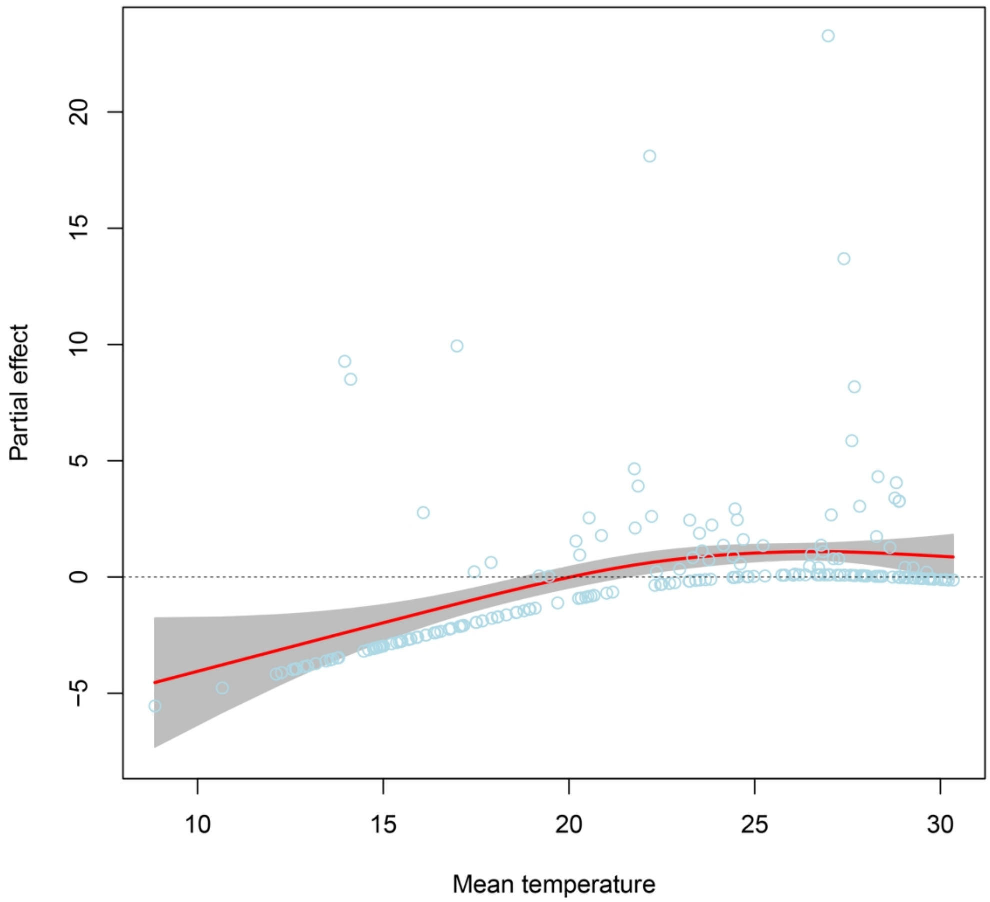

Fig 6

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, Schwatz 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
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

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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6, 7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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.

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024197.R2
Article Type:	Research
Date Submitted by the Author:	18-Mar-2019
Complete List of Authors:	Zhang, Qin; Cancer Hospital of Shantou University Medical College, Good Clinical Practice Office Chen, Yuliang; Shantou University Medical College, Department of Preventive Medicine Fu, Yu; Zhongnan University of Economics and Law, Department of Finance Liu, Tao; Guangdong Provincial Center for Disease Control and Prevention, Guangdong Provincial Institute of Public Health Zhang, Qingying; Shantou University Medical College, Department of Preventive Medicine Guo, Pi; Shantou University Medical College, Department of Preventive Medicine Ma, Wenjun; Guangdong Provincial Center for Disease Control and Prevention, Guangdong Provincial Institute of Public Health
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	China, Climate, Dengue, Seasonal variation, Spatial, Wavelet analysis

SCHOLARONE™
Manuscripts

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

Authors

Qin Zhang [1], Yuliang Chen [2], Yu Fu [3], Tao Liu [4, 5, *], Qingying Zhang [2, *], Pi Guo [2, *], Wenjun Ma [4]

[1] Good Clinical Practice Office, Cancer Hospital of Shantou University Medical College, Shantou 515041, China

[2] Department of Preventive Medicine, Shantou University Medical College, Shantou 515041, China

[3] Department of Finance, Zhongnan University of Economics and Law, Wuhan 430074, China

[4] Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, China

[5] General Practice Center, Nanhai Hospital, Southern Medical University, Foshan, Guangdong, China

[*] These authors are co-corresponding authors in this work.

Dr. Qingying Zhang

Department of Preventive Medicine, Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, China

E-mail: qyzhang@stu.edu.cn

Dr. Pi Guo

Department of Preventive Medicine, Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, China

E-mail: pguo@stu.edu.cn

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 humidity which have direct impacts on mosquito abundance [12]. Enjoying a subtropical humid monsoon climate and frequent economic and cultural communication with the Southeast Asian countries [13, 14], 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 [9-12].

The impact of seasonal climatic variation on dengue dynamics in the Chao-Shan area (Figure 1), the eastern part of Guangdong province is poorly quantified. The Chao-Shan area is one of the special regions on China's southeastern coast. The region has a jurisdiction area of around 10918 square kilometers, with a very high population density. In addition, it is situated in the intersection part of the Tropic of Cancer and the coastline of the mainland China, and has a subtropical oceanic monsoon climate with a mean annual temperature of around 22 °C and an abundant precipitation per year [15]. The subtropical oceanic monsoon climate conditions and the increasingly frequent cultural contact with Southeast Asian countries in the Chao-Shan area make it a high risk area for mosquito-borne infectious diseases. In particular, mosquito vectors such as Aedes albopictus are reported as the major vector of dengue virus in the area [16].

Unfortunately there are few comprehensive reports on the spatial-temporal epidemic pattern for dengue in the Chao-Shan area. The effects of seasonal climate variation on the dynamics of dengue and the associated disease burden in the region are still unclear. There is an urgent need to understand the association between dengue epidemics and seasonal climatic conditions in order to take corresponding protective measures. Therefore, this study aimed to assess the epidemic characteristics of dengue in the Chao-Shan area, and the effect of seasonal climate variation on the dynamics of dengue for the period January 1, 2014 to December 31, 2017.

Material and methods

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

Daily average values for climatic variables including relative humidity, rainfall (millimeters), and temperature (°C) in the three cities during the study period from January 1, 2014 to December 31, 2017 were obtained from the Guangdong Provincial Climate Bureau. Weekly values of climatic variables were calculated as simple arithmetic means to show the seasonal peaks of dengue in surveillance data, and used to assess the potential effect of seasonal variation on the dengue dynamics.

Statistical Analysis

First, basic characteristics (number, median age, sex ratio, and season and year of diagnosis) of dengue cases reported during the study period were described by city. By calculating the interval between the date of onset and the diagnosed date for each dengue case, we estimated the probability density function of the continuous variable of the 'interval' and then plot corresponding probability density distributions of the onset-to-diagnosis of dengue cases for the three cities. The median duration from onset to diagnosis by city was estimated. Each case of dengue has registered current address information. To identify high-risk areas of dengue in the Chao-Shan area, we applied a kernel smoothing method [18] to plot the geographical distribution of cases by street or town. A total of 231 streets or towns in the Chao-Shan area covering the three cities were included in our dataset. ArcGIS 10.2 (ESRI, Redlands, CA, USA) was used to plot the geographical distribution.

Third, to quantify seasonal patterns of dengue epidemic, time series of weekly case counts were plotted by city, and type of cases (probable or confirmed cases), respectively. The periodicity of the time series of dengue case counts, mean temperature, mean rainfall and mean relative humidity was assessed using time-dependent wavelet analysis [19]. The time series data of dengue case counts and climatic factors were analyzed by wavelet analysis, and then the wavelet decomposition results were used to calculate power spectrum. By decomposing the time series into the time-frequency domain, wavelet analysis can obtain the significant fluctuation pattern of the time series, that is, the periodic dynamic time pattern. A continuous Morlet wavelet transform on the time series was performed to extract specific frequency components. Then, the temporal periodicity in a time series was inspected with a wavelet plot. A statistical significance test was performed to test the null hypothesis that the signal in a wavelet plot is generated by a stable process of a given background power spectrum (usually white noise) at a certain time with significance level of 95%. The *dplR* package within R software was used for the Morlet wavelet analysis in this study.

Fourth, in view of the presence of overdispersion in the dengue case data set, a generalized additive model for location, scale and shape (GAMLSS) model [20] with the distribution of the response variable obeying negative binomial distribution was performed to study the association between seasonal characteristics of dengue and climatic variables, including mean temperature, mean rainfall and mean relative humidity. We implemented the Rigby and Stasinopoulos algorithm [20] to estimate the parameters of the models, and the number of cycles of the algorithm was set to 2000 to insure the convergence of the analysis results.

In order to assess the effects of climatic variables on seasonal dynamics of dengue, we established a set of GAMLSS models where climatic variables were modeled as smooth terms with different degrees of freedom (*df*). This was performed by changing the *df* for the smooth terms of 3 to 1 during model selection. We used the smoothing splines for the smooth terms in the GAMLSS models. We considered temperature, relative humidity and rainfall separately, then with both in model, then with all in model. In addition, some sensitivity analyses by setting *df*=0 in the smooth terms, where the *df*=0 means the distribution of the response variable is directly modeled as a linear parametric function of explanatory variables, for the three climatic factors considered separately, then both in model, then all in model. As a result, a total of 70 models were generated for comparison and evaluation. As model selection criterion, the Schwatz Bayesian Criterion (SBC) [21] was used here. Models with lower value of SBC are preferred. In addition, we also added the autoregressive term, $\ln(1 + Count_{local\ cases})$ in the previous month, to account for the autoregressive effect in this study [10]. The *gamlss* package within R software 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. R codes were provided to 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 (see Supplementary Material, "R codes").

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 latitude/longitude coordinates shows geographical distributions of dengue cases in the Chao-Shan area. Spatial smoothing with the kernel density estimation produced dengue risk hotspots suggesting that the geographical regions with the greatest number of dengue cases were around Xixin and Chengxi streets in Chaozhou city. Overall, dengue showed annual peaks of activity, a major peak in autumn, consistent among time series of dengue cases in Shantou, Chaozhou and Jieyang (Figure 4). Although Chao-Shan area had a seasonal attack of dengue peaking between September and October of each year, we observed that the number of dengue cases increased sharply in 2015 in Chaozhou.

Results of wavelet analysis for time series of notifications of dengue and climatic variables including mean temperature, mean rainfall and mean relative humidity in Chao-Shan area during the study period were shown in Figure 5. It showed that the estimations of local powers for weekly time step examined were the largest at the period of 1 year, suggesting dengue cases showed a significant annual periodicity (Figure 5, A). In particular, based on the strongest power of the annual periodicity estimated for the time series of climatic variables (mean temperature, rainfall and relative humidity) throughout the study period (Figure 5, B-D), these factors broadly followed similar periodicity with that of dengue cases.

Based on the results of wavelet analysis, we further analyzed the effects of climatic variables on seasonal dynamics of dengue using a framework of GAMLSS by testing different degrees of freedom for the smooth terms during model selection to determine an optimal model (Table S2). We found that the model with the variable of mean temperature modeled as a smoothing spline term with df=1 had the smallest value of SBC and the best model fit among the 70 kinds of candidate models, suggesting that the effect of mean temperature on dengue dynamics in Chao-Shan area was most obvious. In addition, according to the GAMLSS framework, a significant nonlinear partial effect of mean temperature on dengue dynamics have been observed, and the risk of dengue transmission was most when the temperature was between 25 °C~ 28 °C (Figure 6).

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 27.0-58.0 years) for all notified dengue cases, and the male to female ratio was 1:1.1. 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. We found that Chaozhou accounted for around 78% of the total number of dengue cases reported during the study period. We speculated that this may owe to the combined effect of multiple factors including its subtropical monsoonal climate, its industries producing abandoned ceramics that could provide abundant of breeding places for mosquitos after raining, and local residents' habit of planting flowers at home providing breeding environment for mosquito vectors [22]. 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 [23]. 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 [24]. An epidemiological study from Machala, Ecuador, provided evidence of significant 1-year and 2-year cycles in dengue [25]. 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 [26]. 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.

This study built on potential links between dengue dynamics and climatic variables, and quantified their relationships based on a kind of state-of-the-art flexible regression and smoothing technique, GAMLSS model, which enables us to handle adequately the problem of the presence of overdispersion in the data. The GAMLSS is a general framework for fitting semi-parametric regression type models where the distribution of the response variable includes highly skew and kurtotic continuous and discrete distribution, and allows all the parameters of the distribution of the response variable to be modeled as smooth functions of the explanatory variables [20]. There is more flexibility in identifying those non-linearities using a GAM-based approach than traditional linear regression models [27]. By inspecting a scatterplot (data was not shown) between dengue incidence and temperature, we found the relationship was nonlinear, so it required the special estimation methods of the nonlinear regression procedure. Additionally, the choice of *df* values is very important when constructing the GAMLSS model. Therefore, we performed a sensitivity analysis by trying different *df* in the smooth term of the model, and determined an optimal model according to the assessment of model fit. Overall, our results revealed a powerful capability with GAMLSS model for catching the appropriate relationship between dengue incidence and temperature.

The associations between dengue seasonality and climatic variables such as temperature, rainfall, relative humidity, sunshine and ENSO indices have been documented in previous studies [28]. In this study, our results reveal that temperature has significantly nonlinear effects on epidemics risk and intensity of dengue, with the greatest attributable risk due to temperature estimated at around 25 °C. To identify the significantly associated climatic factors with dengue epidemics, we repeated model selections and adopted different climatic factors with different *df* in the smooth terms of the GAMLSS framework. We found that the variation in daily mean temperature partly drives dengue dynamics, and this climatic factor is closely related to the seasonal fluctuation of dengue incidence in Chao-Shan area. The association between temperature and seasonal dynamics of dengue observed in our study was also reported in previous studies in Guangzhou [12] and Taiwan [29], which has a similar subtropical humid monsoon climate. The estimated effects of temperature driving the seasonal dynamics of dengue can be partly explained by some mechanisms, for example, temperature effects on the biting rate of mosquitoes [30], incubation period of pathogens [31], and human exposure to mosquitoes.

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

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.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81703323 and 81773497), and Top-tier University Construction Project under Department of Education of Guangdong Government (No. 2015022 and 2015023).

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).

References

1. World Health Organization, 2009. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva World Health Organization6, 990.

2. Halstead SB， O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. Nature 1977;265(5596):739-41.

3. Guzman MG, Gubler DJ, Izquierdo A, et al. Dengue infection. Nat Rev Dis Primers2016;2:16055.

4. Guzman MG, Harris E. Dengue. Lancet 2015; 385(9966):453–65.

5. Cory WM, Andrew CC, and Kacey E. Climate and Dengue Transmission: Evidence and Implications. Environ Health Perspect 2013; 121(11–12): 1264–72.

6. Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., et al., 2013. The global distribution and burden of dengue. Nature 496,504-507.

7. Lai, S., Huang, Z., Zhou, H., Anders, K.L., Perkins, T.A., et al., 2015. The changing epidemiology of dengue in China, 1990-2014: a descriptive analysis of 25 years of nationwide surveillance data. BMC Med 13, 100.

8. Chen, B., Liu, Q., 2015. Dengue fever in China. Lancet 385, 1621-1622.

9. Guo, P., Liu, T., Zhang, Q., Wang, L., Xiao, J., et al., 2017. Developing a dengue forecast model using machine learning: A case study in China. PLoS Neglected Tropical Diseases 11, e0005973.

10. Xiao, J.P., He, J.F., Deng, A.P., Lin, H.L., Song, T., et al., 2016. Characterizing a large outbreak of dengue fever in Guangdong Province, China. Infectious Diseases of Poverty 5, 44.

11. Lin, Y.P., Luo, Y., Chen, Y., Lamers, M.M., Zhou, Q., et al., 2016. Clinical and epidemiological features of the 2014 large-scale dengue outbreak in Guangzhou city, China. BMC Infectious Diseases 16, 102.

12. Xu, L., Stige, L.C., Chan, K.S., Zhou, J., Yang, J., et al., 2017. Climate variation drives dengue dynamics. Proceedings of the National Academy of Sciences of the United States of America 114, 113-118.

13. Shepard, D.S., Undurraga, E.A., Halasa, Y.A., 2013. Economic and Disease Burden

of Dengue in Southeast Asia. PLoS neglected tropical diseases 7, e2055.

14. Halstead, S.B., 2006. Dengue in the Americas and Southeast Asia: do they differ? RevistaPanamericana de SaludPública = Pan American journal of public health. 20, 407-415.

15. https://en.wikipedia.org/wiki/Chaoshan(accessed 15Feb2019)

16. Yang S, Liu Q. Trend in global distribution and spread of Aedes Albopictus. Chin J Vector Biol & Control. 2013;24(1):1-4.

17. National Health Commission of the People's Republic of China, 2008. Diagnostic criteria for dengue fever. http://www.nhfpc.gov.cn/zhuz/s9491/200802/38819.shtml (accessed 11 April 2018).

18. Silverman, B. W. Density Estimation for Statistics and Data Analysis. New York: Chapman and Hall, 1986.

19. Cazelles, B., Chavez, M., Magny, G.C., Guégan, J.F., Hales, S., 2007. Time-dependent spectral analysis of epidemiological time-series with wavelets. Journal of the Royal Society Interface 4, 625-636.

20. Rigby, R.A., Stasinopoulos, D.M., 2005. Generalized additive models for location, scale and shape. Applied Statistics 54, 507-554.

21. Stasinopoulos, M., Rigby, R.A., 2007. Generalized Additive Models for Location Scale and Shape (GAMLSS) in R. Journal of Statistical Software 223, 1-46.

22. Liu T, Zhu G, He J, et al.Early rigorous control interventions can largely reduce dengue outbreak magnitude: experience from Chaozhou, China. BMC Public Health 2017;18(1):90.

23. van Panhuis, W.G., Choisy, M., Xiong, X., Chok, N.S., Akarasewi, P., et al., 2015. Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia. Proceedings of the National Academy of Sciences 112, 13069-13074.

24. Bennett, S.N., Drummond, A.J., Kapan, D.D., Suchard, M.A., Munoz-Jordán, J.L., et al., 2010. Epidemic dynamics revealed in dengue evolution. Molecular Biology and Evolution 27, 811–818.

25. Zielinski, A., Rudowska, J., Ying, Z., Ning, L.D., Xin, L., et al., 2015. Spatiotemporal clustering, climate periodicity, and social-ecological risk factors for

dengue during an outbreak in Machala, Ecuador, in 2010. BMC Public Health 11, 37.

26. Toan Do, T.T., Martens, P., Luu, N.H., Wright, P., Choisy, M., 2014. Climatic-driven seasonality of emerging dengue fever in Hanoi, Vietnam. BMC Public Health 14, 1078.

27. Stasinopoulos, D., Rigby, R., Heller, G., Voudouris, V., De Bastiani, F., 2017. Flexible Regression and Smoothing: Using GAMLSS in R.

28. Naish, S., Dale, P., Mackenzie, J.S., McBride, J., Mengersen, K., et al., 2014. Climate change and dengue: A critical and systematic review of quantitative modelling approaches. BMC Infectious Diseases 14, 167.

29. Wu,C. P., Su, J. H., Guo, H.R., Lung, S.C, Lin, C.Y., 2005. Temperature Can Be An Effective Predictor for Dengue Fever Outbreak. Epidemiology 16, S72.

30. Patz, J.A., Epstein, P.R., Burke, T.A., Balbus, J.M., 1996. Global climate change and emerging infectious diseases. JAMA 275, 217-223.

31. Xiao, F.Z., Zhang, Y., Deng, Y.Q., He, S., Xie, H. G., et al., 2014. The effect of temperature on the extrinsic incubation period and infection rate of dengue virus serotype 2 infection in Aedesalbopictus. Arch Virol 159, 3053-3057.

32. Gubler, D.J., 2011. Dengue, Urbanization and Globalization: The Unholy Trinity of the 21st Century. Tropical Medicine and Health 39, S3-S11.

33. Yang, B., Liu, F., Liao, Q., Wu, P., Chang, Z., et al., 2017. Epidemiology of hand, foot and mouth disease in china, 2008 to 2015 prior to the introduction of EV-A71 vaccine. Eurosurveillance 22.

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

Tables

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 (%)				

Probable cases	0 (0.0)	45 (2.6)	0 (0.0)	45 (2.1)
Confirmed cases	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 (%)				
Male	137 (48.2)	819 (47.7)	110 (57.0)	1066 (48.6)
Female	147 (51.8)	897 (52.3)	83 (43.0)	1127 (51.4)
Season of diagnosis, number (%)				
Spring	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Summer	0 (0.0)	33 (1.9)	0 (0.0)	33 (1.5)
Autumn	281 (98.9)	1,680 (97.9)	192 (99.5)	2153 (98.2)
Winter	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)

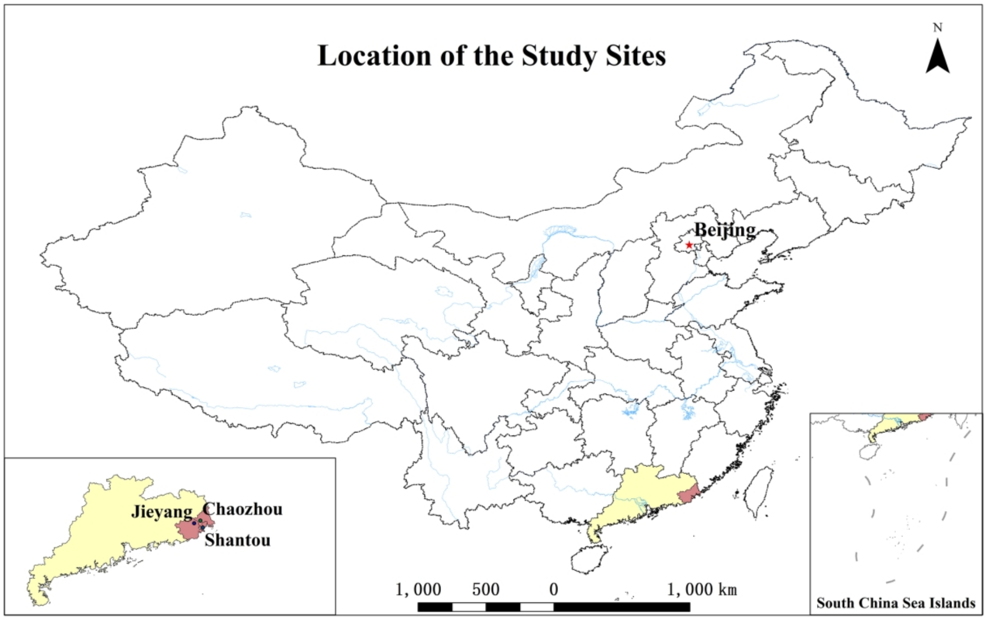

Fig 1

213x134mm (300 x 300 DPI)

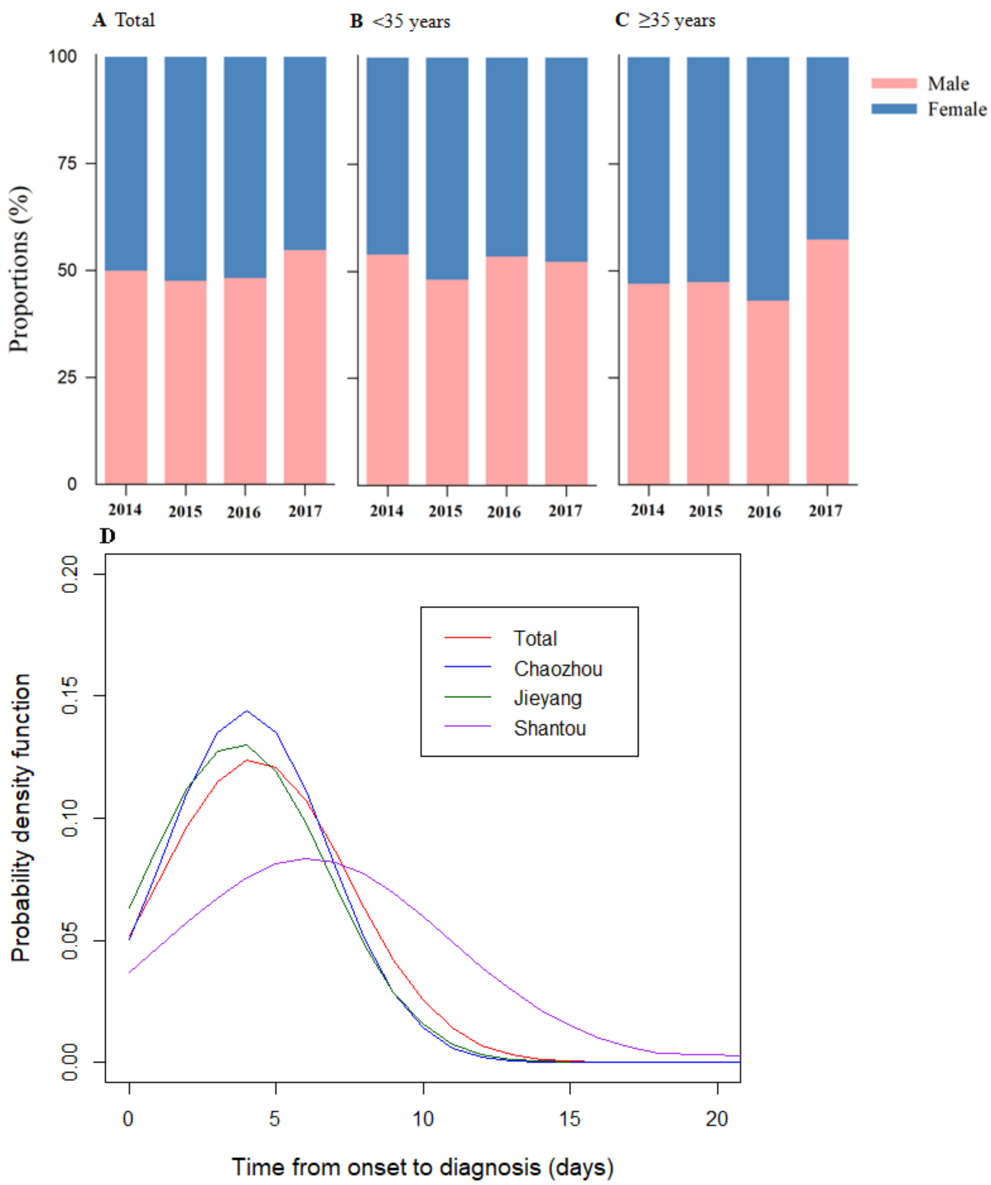
A Total
B <35 years
C ≥35 years
Male
Female
Proportions (%)
100
75
50
25
0
2014
2015
2016
2017
D
Total
Chaozhou
Jieyang
Shantou
Probability density function
0.00
0.05
0.10
0.15
0.20
0
5
10
15
20
Time from onset to diagnosis (days)

Fig 2

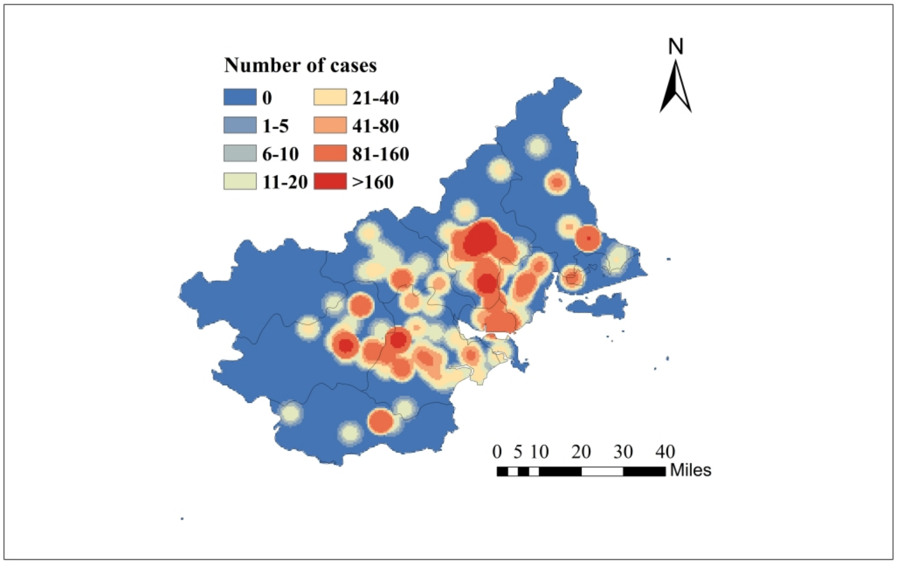

Fig 3

457x304mm (300 x 300 DPI)

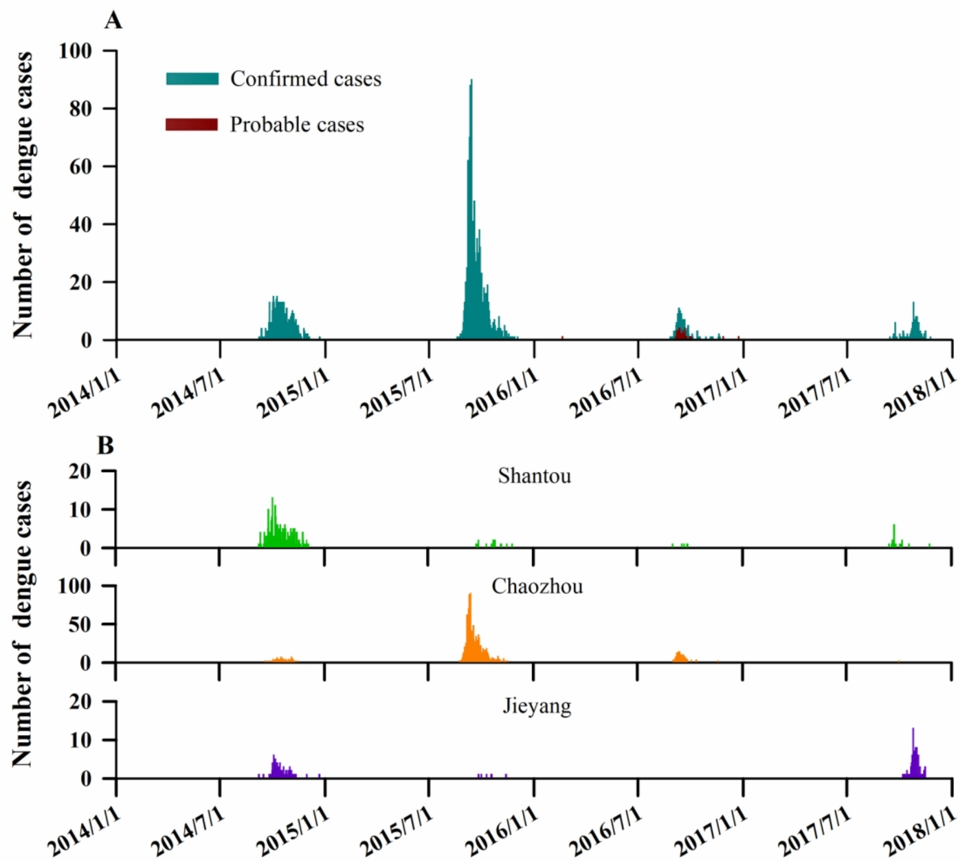

Fig 4

155x141mm (300 x 300 DPI)

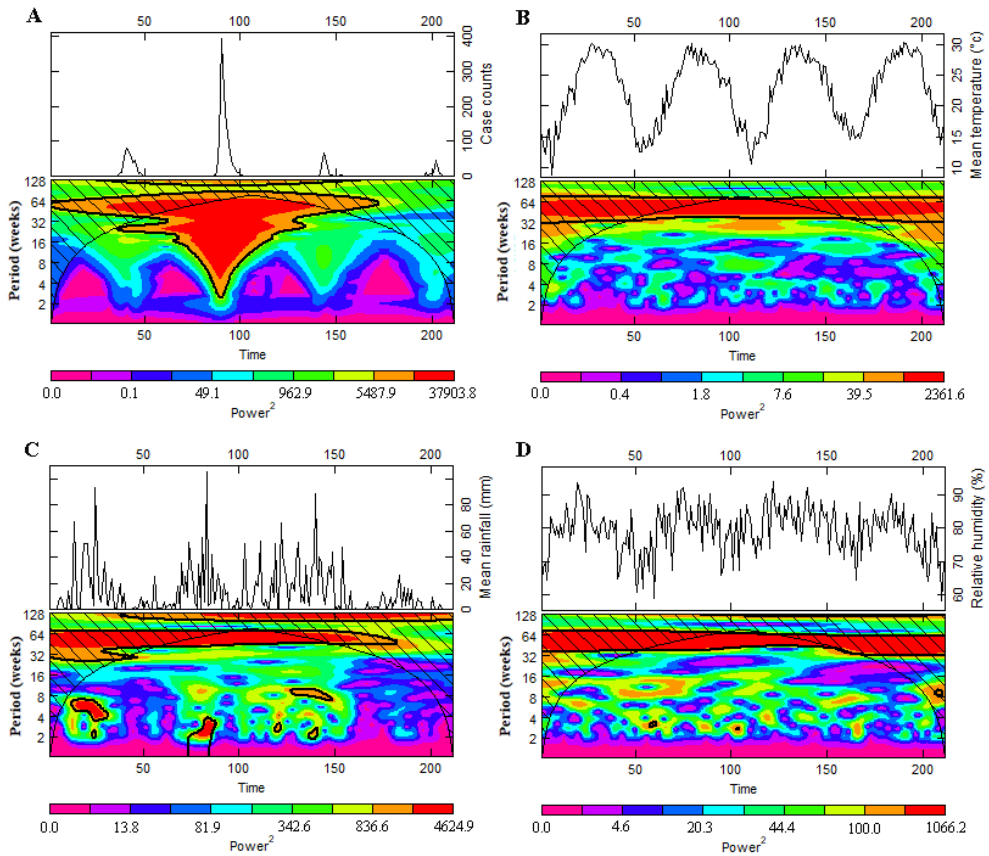
A
B
C
D
Case counts
Mean temperature (°c)
Mean rainfall (mm)
Relative humidity (%)
Period (weeks)
Time
Power²
0.0 0.1 49.1 962.9 5487.9 37903.8
0.0 0.4 1.8 7.6 39.5 2361.6
0.0 13.8 81.9 342.6 836.6 4624.9
0.0 4.6 20.3 44.4 100.0 1066.2

Fig 5

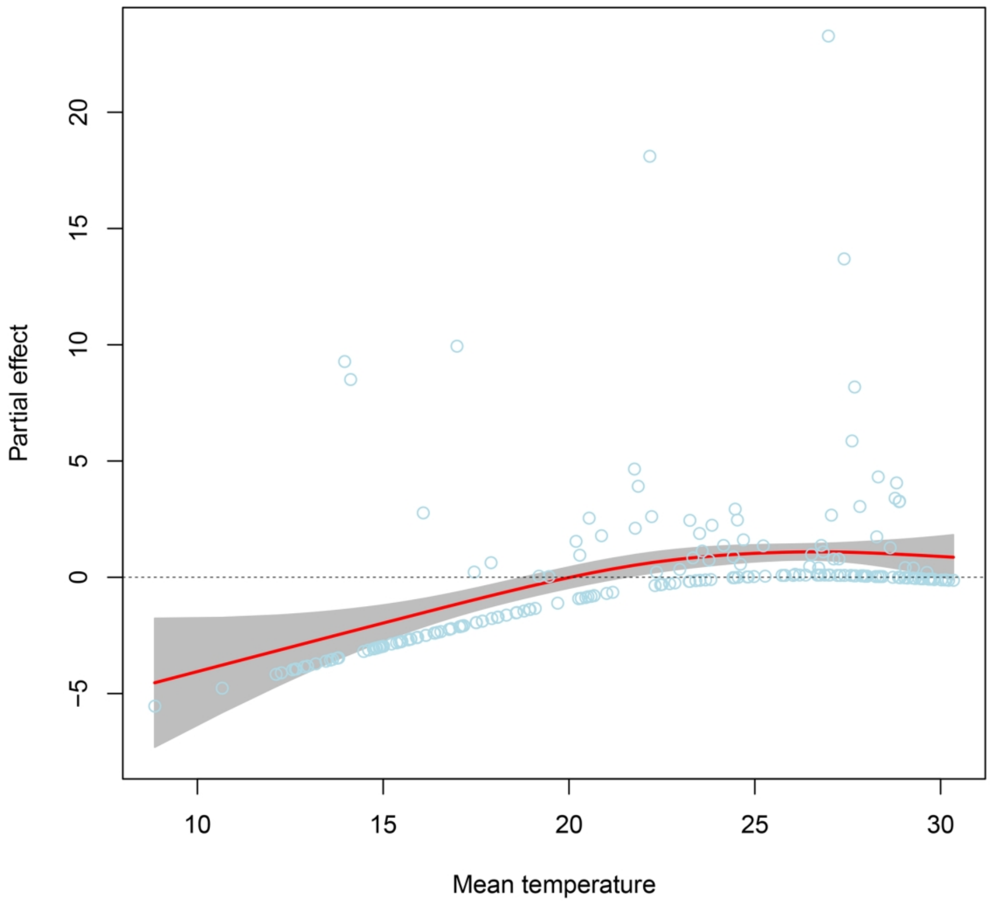

Fig 6

169x156mm (300 x 300 DPI)

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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6, 7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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, Schwatz 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

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
```

```
install.packages("gamlss")

# Load the package

library(gamlss)

# Read the dataset 'mydata.csv' into R

my.data <- read.csv("mydata .csv", head=TRUE)

head(my.data)

# Establish a GAMLSS model using the gamlss.fit function

gamlss.fit <- gamlss(case_num ~ cs(avg_tem_mean, 3) + cs(avg_rh, 1) + cs(avg_rainfall, 2) ,
data= my.data, family=NBI, n.cyc=2000, trace=FALSE)

# The setting "family=NBI" denotes the negative binomial distribution used for the
corresponding link function for over-dispersed count data

gamlss.fit

# Calculate the Generalised Akaike information criterion for a fitted GAMLSS object

GAIC(gamlss.fit)

# Plot residual diagnostics for a fitted GAMLSS Object

plot(gamlss.fit$residuals)

# Plot regression terms for a specified parameter of a fitted GAMLSS object
term.plot(gamlss.fit, se=TRUE, partial=TRUE)
```