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Epidemiological characteristics of malaria in Ningbo City, China 2000-2011

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Abstract. The objective of this study was to explore the trend and the epidemiological characteristics of malaria in Ningbo from 2000 through 2011 and provide scientific evidence for the prevention and control. Using the retrospective study, data relating to malaria epidemics during 2000-2011 in Ningbo were collected through the questionnaires of the cases and other important surveillance data. Adult mosquitoes were collected using CDC light traps baited with CO_2. Results showed that *Plasmodium vivax* was the main species, and *Anopheles sinensis* was the only vector species of malaria. The annual incidence rates (per 100000) ranged from 0.156 to 3.224, and about 95.41% of the cases were imported. The cases occurred mainly between May and November and the incidence showed two peaks, and the local transmission of malaria was determined by vector density. Considering the vast majority of these infections were imported vivax malaria cases from domestic endemic areas (particularly Anhui province), and may lead to limited local transmission, strengthening the routine monitoring of imported malaria, ensuring timely diagnosis, improving case detection rate, giving a standardized treatment to patients and intensifying public health education on malaria prevention are extremely important to malaria prevention and control activities.

INTRODUCTION

Malaria is the world's most devastating parasitic disease that causes more than 1 million deaths per year, and about 50% of the world's population are exposed to the risk of malaria (WHO, 2008; Abdulsalam *et al*., 2010). In China, malaria was one of the most serious public health problems in the past. The prevalence gradually decreased from south to north. The meso-endemic regions were located to the south of latitude 25° North, where falciparum malaria was widely present. The hypo-endemic regions were from latitude 25° North to latitude 33° North, where vivax malaria was predominant, though falciparum malaria also existed and focal outbreaks often occurred. Northern parts of latitude 33° North used to be the lowest malaria epidemic regions and *Plasmodium vivax* was the only species present (Tang, 2000; Bi *et al*., 2003).

Ningbo city (120.55° ~ 122.16°E, 28.51° ~30.33°N), a coastal city in the Zhejiang province of China, is located in the middle of the mainland coast-line and the south of Yangze River Delta, bordering on the east China sea. The city is on the northern subtropical belt and is influenced by a maritime monsoon climate, the annual average temperature is 16.4ºC. Ningbo can be classified as an unstable malaria-endemic area of China, *Anopheles sinensis* is the main vector (Jiang *et al*.,1995). Malaria prevailed throughout this city until the 1960s. Decades of intensive control efforts have, however, resulted in a significant decrease of malaria incidence and mortality. By 1989, malaria had been nearly eliminated.

In the early 2000s, a malaria resurgence has occurred in some areas of China, like Anhui, Henan, Jiangsu and Hubei provinces were the most seriously affected areas where malaria incidence was previously at a low level (Huang *et al*., 2007; Zhou *et al*., 2007). According to official statistics, the total number of reported malaria cases in China showed a steep increase from about 24000 in 2000 to 64988 (18 deaths) in 2007 (Xiao *et al*., 2010). Following the annual incidence of malaria came down to extremely low levels (0.1/100000) in the late 1980s and 1990s, epidemiologic data from the Center for Disease Control and Prevention (CDC) show the number of cases of malaria reported in Ningbo has risen sharply since the early 2000s, locally transmitted malaria has re-emerged and even caused an outbreak in this city. In this paper, we carried out a review of the malaria situation in Ningbo during the 2000-2011 period to explore the trend and the epidemiological characteristics, and provide scientific evidence for the prevention and control.

MATERIALS AND METHODS

Routine laboratory confirmation of malaria diagnosis

In Ningbo, three types of fever cases (including clinically diagnosed malaria cases, suspected malaria cases, and cases with fever of unknown origin) require laboratory examination and confirmation. Parasitological confirmation is done by thin-thick blood smear microscopy examination or dipstick (Rapid Diagnostic Test [RDT]). The subjects were divided into two groups, floating population group and local residents group. The positive rate for each group were calculated using the total number of subjects of the corresponding groups as denominator and the number of confirmed malaria cases as numerator.

Malaria infections

Studied population was all patients with laboratory-confirmed infections of malaria reported between January 2000 and December 2011. The malaria cases were diagnosed in the medical and health units of each county and reported to the Ningbo CDC through the *China Information System For Diseases Control And Prevention*. Demographic data from 2000 to 2011 was obtained from the Ningbo municipal public security bureau.

Data on malaria cases were obtained from the malaria questionnaires. The main parameters were as follows: the *plasmodium* species (*Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* or co-infection), demographical (age, gender, occupation, place of domicile/nationality), travel information (most likely region/country of infection), date of onset of symptoms, primary infection or relapse, outdoor sleeping behavior, and personal anti-mosquito measures (bed nets, window screen or mosquito-repellents).

The studied cases were classified into two groups (Lee *et al*., 2009; Lin *et al*., 2009; Rey *et al*., 2010; van Rijckevorsel *et al*., 2010): 1. Imported malaria: as shown by tracing the malaria case to its origin in an endemic region outside Ningbo. Such as local residents who have recently traveled to endemic regions and get infected with malaria (travel-acquired infections), or floating population (overseas travelers included) who had acquired infection in endemic regions but diagnosed in Ningbo. 2. Autochthonous malaria: an infection which has been proved or cannot be disproved to be due to recent local transmission, in both local residents and floating population.

Vector surveillance

Adult mosquitoes were collected monthly in fixed sampling sites (parks, residential areas, hospitals, farmhouses, and stockyards) with CO_2-baited Centers for Disease Control (CDC) light traps, operating overnight from before dusk to after the sunrise (18:00 – 6:00). All traps were suspended 1.5m above the ground, either in trees or human-made structures. Captured mosquitoes were sorted by species and sex. Adult mosquito densities were calculated as mean mosquitoes per lamp·night.

Meteorological data

The data of monthly mean temperature, relative humidity and accumulated rainfall from 2000 to 2011 were acquired from Ningbo weather bureau.

Investigation of a local malaria outbreak
From July 6th - August 19th 2008, a localized malaria outbreak occurred in Daxu town in Xiangshan county. An investigation was performed to confirm the outbreak, describe the timeliness of case detection and treatment, and recommend control measures.

A case of malaria was defined as any slide-confirmed parasitaemia and/or a positive RDT test in a resident of Daxu town. Surveillance for adult mosquito populations was conducted in the outbreak area. In addition, an inventory of suspected mosquito breeding sites had been done.

Data analysis
A descriptive analysis was performed, presenting the percentages to describe the qualitative values, quantitative data were expressed as Mean±SD. The associations between proportions were tested using the chisquare test and 95% confidence interval. Spearman's correlation analysis, and multivariate stepwise linear regression analysis were used to explore the relationship between malaria vector densities and climatic parameters, and the relationship between monthly number of autochthonous malaria cases and vector densities. Differences were considered statistically significant if $p<0.05$ in two-tailed tests. All statistic analyzes were performed by SPSS 10.0 software.

RESULTS

Parasitological confirmation of fever cases
During the last 12 years, 224020 blood samples from febrile patients were tested by Microscopy or RDT, and the positive rate of laboratory examination in local febrile patients was 0.04% (61/140780) but 0.82% (680/83240) in floating population. There were significant differences between these two groups (χ^2=949.53, P <0.001).

Epidemiological Situation
From 2000 through 2011, a total of 741 malaria cases were reported in Ningbo and no death was recorded. Of whom 481 (64.91%) were males and 260 (35.09%)were females. Most infections (562 or 75.84%) occurred in adults (18-77), (143 or 19.30%) occurred in children aged 6-17 years, and (36 or 4.86%) in children five years or younger, the mean age of infection was 28.30±14.36 years (age range: 7 months – 77 years). Table 1 shows that, migrant workers accounted for the greatest part (447 or 60.32%) of the total malarial cases, followed by students (89 or 12.01%), peasants (59 or 7.96%), scattered children (38 or 5.13%) and household/ unemployed (36 or 4.86%).

The majority of these infections (712 or 96.09%) were caused by *P. vivax*, the remainder were caused by *P. falciparum* (26 or 3.51%), and *P. malariae* (3 or 0.40%). *P. ovale* and co-infection was not found (Table 2). The annual incidence rate of reported malaria ranged from 0.156 to 3.224 per 100,000 population for the period of 2000-2011. During 2000-2007, a significant increase had been reported in the number of malaria cases: from 22 in 2000 to 182 in 2007, followed by a rapidly decreasing trend, only 9 cases were recorded in 2011 (Figure 1). The majority of these cases occurred between May and November, from December to April the following year only a few cases of malaria were reported, but thereafter the incidence of malaria started rising and reaching a bigger peak in July and a smaller peak in October (Figure 2).

Based on the data of all reported malaria cases, (707 or 95.41%) patients were diagnosed with imported malaria, including 33 cases of travel-acquired infections. Only (34 or 4.59%) infections were regarded as autochthonous malaria (28 local residents and 6 floating population) (Table 1). A large portion of imported malaria (671 or 94.91%) could be confirmed as domestic infections, and most of these infections originated from Anhui province. Thirty six malaria cases were infected abroad, and mainly from Africa, especially Nigeria (Table 2).

Cases with relapses occupied (219 or 30.76%) of the total *P. vivax* infections and all of them had not taken a full course of anti-relapse treatment during their primary infection. Most of the relapses occurred among floating population, the relapse rates of cases between local (5 out of 41) and non-

Table 1. Distribution of the malaria cases by population groups, and by occupation, 2000-2011

Occupation	Local residents		Floating population		Total
	Autochthonous malaria	Imported malaria	Autochthonous malaria	Imported malaria	
Migrant worker	4	0	4	439	447
Student	3	0	0	86	89
Peasant	14	6	0	39	59
Scattered children	0	0	1	37	38
Household/unemployed	4	0	0	32	36
Commercial service	0	12	1	14	27
Worker	2	12	0	1	15
Seafarers and Long-distance drivers	0	1	0	5	6
Catering and food service industry	0	0	0	5	5
Kindergarten children	0	0	0	2	2
Teacher	0	0	0	1	1
Soldier	0	0	0	1	1
Medical staff	1	0	0	0	1
Others	0	2	0	12	14
Total	28	33	6	674	741

Table 2. Distribution of reported imported malaria cases by country of origin, and by species, 2000-2011

Region	P. vivax	P. falciparum	P. malariae	Total
Domestic infection	667 (98.38%)	1 (3.85%)	3 (100%)	671 (94.91%)
Anhui	562	0	1	563
Hubei	25	0	0	25
Henan	16	0	0	16
Sichuan	14	0	0	14
Guizhou	13	0	1	14
Yunnan	8	1	1	10
Hunan	6	0	0	6
Zhejiang	4	0	0	4
Jiangsu	3	0	0	3
Hainan	2	0	0	2
Shanghai	2	0	0	2
Shandong	1	0	0	1
Other Regions	11	0	0	11
Infected abroad	11 (1.62%)	25 (96.15%)	0	36 (5.09%)
Nigeria	5	16	0	21
Gabon	0	2	0	2
Ghana	0	2	0	2
Sierra Leone	0	1	0	1
Liberia	0	1	0	1
Bangui	0	1	0	1
Sudan	1	0	0	1
Abyssinian	1	0	0	1
Region of infection unknown (Africa)	0	1	0	1
Burma	4	1	0	5
Total	678 (100%)	26 (100%)	3 (100%)	707 (100%)

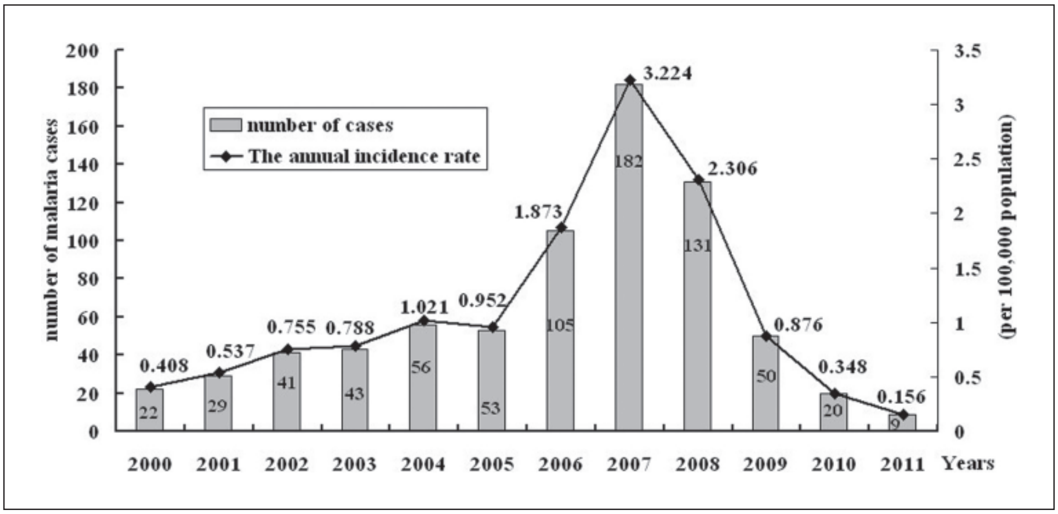

Figure 1. The annual incidence of malaria in the period of 2000-2011

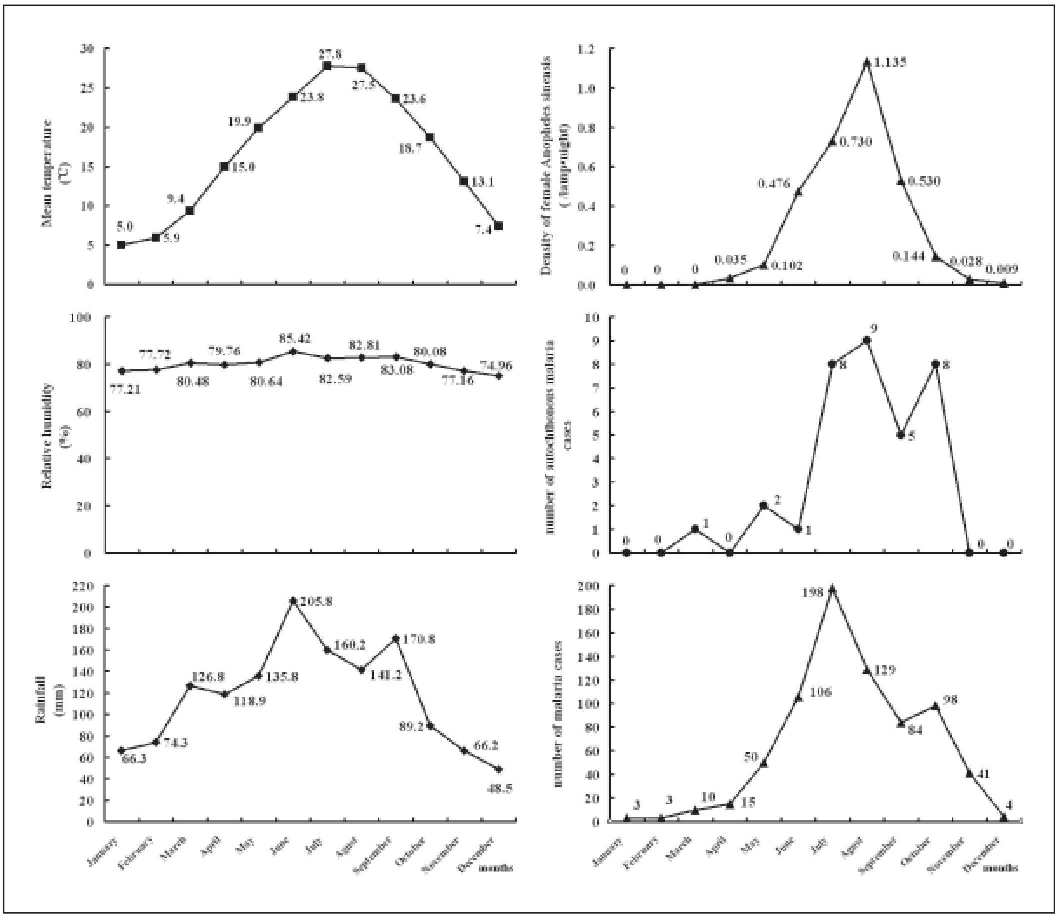

Figure 2. Monthly climatic factors, vector density and number of malaria cases in Ningbo, 2000-2011

local residents (214 out of 671) has significant differences (χ^2=7.039, P<0.01). Furthermore, more than half of the cases (386 or 52.09%) did not take any anti-mosquito measures, and at least 70 (9.45%) had outdoor sleeping behavior.

Investigation on malaria vectors

A total of 221,136 adult mosquitoes were collected and identified, with the average mosquito density of 14.80/trap-night. Among them, *Culex pipiens pallens*, *Culex tritaeniorhynchus*, *Anopheles sinensis* and *Aedes albopictus* occupied 47.63%, 45.26%, 3.27%, and 3.14% respectively. *Anopheles sinensis* was the only malaria vector found.

Spearman correlation analyses were conducted relating monthly density of female *An. sinensis* to climatic variables (Figure 2). Table 3 shows that monthly mean temperature, monthly mean relative humidity and monthly mean rainfall had positive correlation to monthly density of female *An. sinensis*. The multi-liner stepwise regression analysis showed that monthly mean temperature was the key factor which affected the monthly density of female *An. sinensis* (R=0.844, R^2=0.713, β=0.038, P=0.001).

Furthermore, monthly density of female *An. sinensis* was positively correlated with monthly number of autochthonous malaria cases, with a one-month lagged effect (r=0.748, P=0.005).

Local malaria outbreak

The index case was a local female peasant, aged 73 years, who had no past history of malaria and no history of travel to malaria-endemic areas during the 2 months prior to the onset of symptoms. On July 6th 2008, she suffered from the first attack of malaria but did not seek medical treatment until 8 days later. When other residents in the area were subsequently diagnosed with *P. vivax* malaria, local transmission was suspected since all these cases did not have a past malaria attack and they had no history of recent travel outside of Ningbo. A total of 9 cases of *P. vivax* malaria were confirmed between 6 July and 19 August 2008. Of these, 7 were local residents who were living in the area, and the rest were floating population.

Vector surveillance was carried out in the outbreak area using light traps and labour hour method. The data showed that, the highest mosquito density in the outbreak area was 4.36/trap-hour, the average density index was 32.33/lamp-night and 52/man-hour. *Anopheles sinensis* accounted for 30.57% of the captured mosquitoes. Moreover, 51 out of 208 potential mosquito breeding sites in the outbreak area were positive for the immature forms of mosquitoes.

Control activities were organized around surveillance and epidemiology, infection source management, vector control, and social education and mobilization. An active case surveillance system was established to determine the extent and magnitude of the outbreak, identify and localize foci of disease activity, and detect cases early. Eight days radical treatment: chloroquine 1200 mg (divided into 3 days) + primaquine 180 mg (divided into 8 days) was used to cure patients. Chemoprophylaxis was conducted by giving chloroquine 1200 mg and primaquine 180 mg to those living in the outbreak area. All existing and potential mosquito breeding sites within a three-kilometer radius of the focus were eliminated immediately by intensifying anti-larval measures, and indoor residue sprayings (IRS) with deltamethrin were done three times in the outbreak area. After implementation of the above control measures, the outbreak was promptly brought under control.

Table 3. Correlation between climatic variables and monthly density of female *An. sinensis*

	monthly density of female *An. sinensis*	P-value
Temperature	0.958	<0.001
Humidity	0.746	0.005
Rainfall	0.725	0.008

DISCUSSION

Malaria epidemic characteristics in Ningbo were as follows: The large majority of reported cases were imported malaria and mainly occurred among floating population. *Plasmodium vivax* was the prevalent malaria species, although *P. falciparum* infections also occurred occasionally. *Anopheles sinensis* was the only vector species of malaria. Most cases were young and middle-aged, the male group, migrant workers (floating population group), students (floating population group) and peasants (local residents group) were the high-risk groups of infection. The cases occurred mainly during summer and autumn and the incidence showed two peaks, and the local transmission of malaria was determined by vector density.

Our results showed that, until 2011, more than 95 percent of reported cases were imported malaria, and the number of cases of imported malaria was 20.79 times greater than that of autochthonous malaria. Moreover, there is already evidence that *An. sinensis* is refractory to *P. falciparum*, but it is still considered an important vector of *P. vivax* malaria (Sinka *et al.*, 2011). Therefore, imported vivax malaria was a major threat to malaria prevention and control in Ningbo.

Migration between malaria-endemic and non-endemic regions can promote the transmission and spread of malaria, even cause some outbreaks in economically developed regions (Xu *et al.*, 2006). Because the floating population in Ningbo were mainly from Anhui, Sichuan, Guizhou and Jiangxi provinces, which accounted for approximately 24%, 19%, 12% and 11% of the total floating population, respectively. And the infections acquired in Anhui were for the greater part of imported malaria cases (563/707), the increasing incidence of malaria during 2000-2007 in Ningbo may be explained by the outbreak of malaria in this area: the reported incidence of malaria in Anhui was 1.32/100,000 in 1999, but after 2000 the incidence of malaria increased year by year and reaching 57.16/100,000 in 2006 (Wang *et al.*, 2008; Zhang *et al.*, 2008).

Malaria cases occurred relatively concentrated in July, August and October, showed a marked seasonal distribution with two peaks: the first coinciding with the summer holiday period and the second coinciding with the autumn harvest period. Analysis predicts that, during these period, the migration of the population will become more frequent, such as migrant workers or their children (students) move back and forth between Ningbo and their home town (malaria-endemic regions) for harvest or visit relatives and friends.

Moreover, it has been acknowledged that temperature and rainfall played the determinant role of climatic factors in the transmission of malaria by affecting both malaria parasites and vectors directly or indirectly (Zhou *et al.*, 2010). Especially, ambient temperature plays a significant role in the life cycle and the daily survival of the malaria vector. And the sporogonic development of the malaria parasite within the mosquito is also dependent on temperature. Rainfall provides breeding sites for mosquitoes and ensures a suitable relative humidity, which enhances their survival (Snow *et al.*, 1999; Bi *et al.*, 2003; Alemu *et al.*, 2011). In current study, most of the autochthonous malaria cases were focused in the high temperature and moist season, which was fit for mosquito breeding.

Occupational distribution also showed a concentration of cases in migrant workers, students and peasants, the main reasons for this situation are based on the bad living situation and the lack of self-protective consciousness of these people living in rural/suburban areas. The investigation data showed that although personal anti-mosquito measures were used by some patients, these people had not been fully protected. This could be mainly due to the fact that outdoor activities in the early morning or after dusk and sleeping in open spaces are still widespread in some poor areas, and the feeding behavior of some vector mosquitoes has changed by either feeding predominantly outdoors or in the early part of the evening (Govella *et al.*, 2010).

The higher relapse rate in floating population may be caused by many malaria patients had not taken a full course of antimalarials and some village doctors failed to ensure compliance. In some poor malarious areas of China, the usual practice among village doctors is to give suspected malaria cases a single dose of chloroquine or sometimes to use quinine injection as an antipyretic (Liu *et al.*, 1996).

Clustering of cases in an area should also be considered as an outbreak even if the total number has not reached threshold level. Although the annual incidence of malaria of local residents remained at low levels and *An. sinensis* was not an effective transmission vector (Paik, 1990), the potential threat of malaria outbreak cannot be ignored. It is a remarkable fact that after attaining the standard of the basic malaria elimination in 1988, there were no autochthonous malaria cases recorded until July 6th 2008 in Daxu town, all the reported malaria cases were imported. Therefore, this outbreak probably occurred due to the low immunity of local residents, the presence of malaria vector, suitable breeding sites, high mosquito density, and the floating population from epidemic regions offers a sufficient number of gametocyte carriers for *An. sinensis* to feed on (Baomar *et al.*, 2000).

These findings should be considered in future malaria prevention and control strategies (Uneka, 2009; Danis *et al.*, 2011; Askling *et al.*, 2012): Local disease control and prevention departments and hospitals should have plans for diagnosing and managing patients with malaria, and medical staff should receive enhanced training on the diagnosis and treatment of malaria. Surveillance on people returning from malaria-endemic regions should be strengthened, obtaining a recent travel and mosquito exposure history is important for all patients with fever. Laboratory confirmation of malaria should be performed immediately on suspicion of malaria and smear microscopy is considered the gold standard for diagnosis. Patients diagnosed with malaria should take the full course of the appropriate antimalarials and anti-relapse treatment (*P. vivax*), and all patients must be followed up until clinical and parasitological cure. Surveillance of mosquitoes should be strengthened in the affected areas, intensified vector control activities should be implemented using larviciding and IRS. All households in the area should be visited fortnightly to detect secondary cases early. Furthermore, dissemination of information on malaria prevention and treatment to floating population and travelers should also be strengthened.

The present study is not without its limitations. Firstly, data on the annual number of floating population were unavailable in Ningbo during the study period, although they might have a significant impact on the incidence of malaria. Thus, future studies should determine whether changes in the number of floating population cause changes in incidence of malaria in Ningbo. Secondly, the current study failed to show rainfall as a precipitating factor for the monthly density of female *An. sinensis*. This is consistent with other studies which found a negative or neutral effect of rainfall (Tian *et al.*, 2008). Suppose that rainfall has a linear effect on the monthly density of female *An. sinensis*, this effect would emerge after removing the seasonal component.

Taken together, this study described the epidemiology of malaria in Ningbo, which had a large floating population, including a high number of people from malaria-endemic regions. This population pattern offers a realistic overview of the epidemiological characteristics to this area and may also reveal the current status and characteristics of malaria epidemics in most economically developed coastal cities of China: a very small number of autochthonous malaria infections; the vast majority of malaria infections are imported and mainly occurred among floating population; because of the vector *Anopheles* mosquitoes are still present, imported malaria can even cause occasional localized outbreaks of malaria. Therefore, strengthening the routine monitoring of imported malaria, ensuring timely diagnosis, improving case detection rate, giving a standardized treatment to patients and intensifying public health education on malaria prevention are extremely important to current malaria prevention and control activities in Ningbo.

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