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Vector capacity of *Anopheles sinensis* in malaria outbreak areas of central China

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Abstract

Background: Both falciparum and vivax malaria were historically prevalent in China with high incidence. With the control efforts, the annual incidence in the whole country has reduced to 0.0001% except in some areas in the southern borders after 2000. Despite this, the re-emergence or outbreak of malaria was unavoidable in central China during 2005–2007. In order to understand the role of the vector in the transmission of malaria during the outbreak period, the vector capacity of *An. sinensis* in Huanghuai valley of central China was investigated.

Findings: The study was undertaken in two sites, namely Huaiyuan county of Anhui province and Yongcheng county of Henan province. In each county, malaria cases were recorded for recent years, and transmission risk factors for each study village including anti-mosquito facilities and total number of livestock were recorded by visiting each household in the study sites. The specimens of mosquitoes were collected in two villages, and population density and species in each study site were recorded after the identification of different species, and the blood-fed mosquitoes were tested by ring precipitation test. Finally, various indicators were calculated to estimate vector capacity or dynamics, including mosquito biting rate (MBR), human blood index (HBI), and the parous rates (M). Finally, the vector capacity, as an important indicator of malaria transmission to predict the potential recurrence of malaria, was estimated and compared in each study site.
About 93.0% of 80 households in Huaiyuan and 89.3% of 192 households in Yongcheng had anti-mosquito facilities. No cattle or pigs were found, only less than 10 sheep were found in each study village. A total of 94 and 107 *Anopheles* spp. mosquitos were captured in two study sites, respectively, and all of *An. sinensis* were morphologically identified. It was found that mosquito blood-feeding peak was between 9:00 pm and 12:00 pm. Man biting rate of *An. sinensis* was 6.0957 and 5.8621 (mosquitoes/people/night) estimated by using half-night human bait trap method and full-capture method, respectively. Human blood indexes (HBI) were 0.6667 (6/9) and 0.6429 (18/28), and man-biting habits were 0.2667 and 0.2572 in two sites, respectively. Therefore, the expectation of infective life and vector capacity of *An. sinensis* was 0.3649-0.4761 and 0.5502-0.7740, respectively, in Huanhuai valley of central China where the outbreak occurred, which is much higher than that in the previous years without malaria outbreak.

Conclusions: This study suggests that vivax malaria outbreak in Huanhuai valley is highly related to the enhancement in vector capacity of *An. sinensis* for *P. vivax*, which is attributed to the local residents' habits and the remarkable drop in the number of large livestock leading to disappearance of traditional biological barriers.

Keywords: Vector capacity, *Anopheles sinensis*, Outbreak, Malaria, *Plasmodium vivax*, China

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Background

Malaria is one of the most important parasitic diseases in People's Republic of China [1-3]. Historically, a higher incidence of malaria, caused by infection of both *Plasmodium vivax* and *P. falciparum*, was observed among human populations in central China, such as Anhui, Henan, Hubei, Shangdong and Jiangsu provinces, located in Huanghuai valley [2,4]. Two big epidemics of malaria occurred at the beginning of the 1960s and the 1970s, of which the incidence rate was 1.55% and 2.96%, respectively [5]. Following the great control efforts, the incidences of both species of malaria had reduced remarkably. For instance, only 0.1% to 0.0017% of annual malaria incidence was reported during the 1980s and the 1990s in the country, respectively [6]. While, the incidence of vivax malaria transmitted by *Anopheles sinensis* was increased after 2000, due to the social and environment changes in macro scenario, such as population mobility increase due to the development of the economy, global warming, leading to the longer transmission season for *P. vivax*, and so on [4,7-10]. About 86% of malaria cases in the country were located in the Huanghuai valley of central China where the predominant vector mosquito was *An. sinensis* [2,11,12]. In this region, most of the outbreaks that occurred were localized at village level or township level of 4 provinces in central China, including Anhui, Henan, Hubei and Jiangsu provinces [13,14]. Previous studies in central China have shown that vector capacity is one of most important factors contributing to the transmission of vivax malaria, which could accelerate re-emergence of malaria outbreaks [2,15-18], but only a few relevant field-epidemiological investigations on the re-emergence of vivax malaria have been reported in China as well as in the Greater Mekong Subregion [19,20].

China launched the National Malaria Elimination Program (NMEP) in 2010, with its goal to eliminate malaria in China by 2020 [21,22]. In the NMEP, vector control is one of the important components in rapid response to the malaria transmission in outbreak foci as well as to improve the efficiency of case management, which has been promoted by both World Health Organization (WHO) and Roll Back Malaria Partnership (RBM) for reduction of malaria transmission [23,24]. One of the most difficult issues in the elimination process is to have real-time surveillance and response systems to monitor the changes of transmission patterns in order to guide the elimination efforts in the high risk areas [8]. It is believed that transmission intensity of human malaria is highly dependent on the vector capacity and competence of local mosquitoes. Recent research aimed at understanding the relationship between vector capacity and the transmission patterns of malaria is of significant concern [25,26]. Therefore, it is important to understand the changes of

Figure 1 Location of the two study sites. One study site was located in the Zhao village, Zhuji township, Huaiyuan county (33°14'N and 116°51'E), Anhui province (site A), the other one was located in Pengguanyao village, Peiqiao township, Yongcheng county (33°48'N and 116°12'E), Henan province.

An. sinensis vector capacity in the high risk areas or in outbreak foci. But few investigations have explored the changes in vector capacity of *An. sinensis* at the micro settings, although it is believed that the vector capacity of mosquitoes is a good indicator to assess the transmission level of vivax malaria [26-28]. It is worthwhile understanding the role of the anopheles mosquitoes in the transmission of vivax malaria in Huanghuai valley during outbreaks, which will guide the formulation of elimination strategy and establishment of surveillance and response systems in the NMEP in China.

The investigation on vector capacity of *An. sinensis* in two outbreak areas of Anhui and Henan provinces was carried out during summer of 2007 when vivax malaria sporadic outbreaks were still taking place, in order to clearly understand the change patterns for vector capacity of *An. sinensis* which will promote the surveillance-response strategy [26].

Methods

Area and time of investigation

The study was undertaken in two sites, including Zhao village, Zhuji township, Huaiyuan county (33°14'N and 116°51'E), Anhui province, and Pengguanyao village, Peiqiao township, Yongcheng county (33°48'N and 116° 12'E), Henan province (Figure 1). Both counties are located in the plain region, surrounded with rice pads, and planted with crops, such as beans, rice etc., and chemical compounds are rarely used as insecticides. The drainage system is not adequate, facilitated with more open water around villages in Summer time, thus providing more suitable habitats for the development of *An. sinensis* larvae.

The investigation was carried out at two time points, e.g. early August and late August, 2007, respectively, with an interval of 10 days, during the peak transmission period of vivax malaria and its sporadic outbreaks which still occurred in the study region. Half-night mosquito biting experiments were undertaken twice in Huaiyuan county and Yongcheng county, respectively. Full-capture method was used to collect *Anopheles* spp. mosquito specimens in the early morning inside and outside of mosquito-nets in human living rooms, as well as in livestock rooms in the study sites.

Malaria incidence and risk factors

All reported data on malaria cases recorded by the Center for Disease Control and Prevention in each county was collected, and the malaria incidence in each study county was estimated in 2007 and compared with reported data from the region in previous years. Malaria transmission-related risk factors for each study site, including usage of anti-mosquitofacilities and total number of livestock, were recorded by visiting each household of the study villages.

Vector capacity

The specimens of mosquito were collected in two villages, and population density and species in each study site were recorded after the identification of different species, based on morphology of mosquitoes, and the saturated mosquitoes were tested by ring precipitation test [29]. Finally, various indicators were calculated to estimate vector capacity, including mosquito biting rate (MBR), human blood index (HBI), and the parous rates (M) [30,31] using the following details of methodology.

The vector capacity was estimated from the calculation of three indices [32]. Firstly, the investigation on mosquito biting rate was conducted by two approaches. One approach is the half-night bite rate, which was carried out during 19:30 pm to 24:00 pm by the human attraction method, this was carried out 4 times to capture as many anopheles mosquitoes as possible [29]. Briefly, a person was inside the half-opened mosquito-net, and *Anopheles* spp. mosquitoes were continuously collected over the time period [12]. The number of *An. sinensis* collected was recorded. The half-night man biting rate was calculated by following formula:

$$\text{Half}-\text{night man biting rate (No. mosquitoes/people)} = \frac{\text{number of mosquitoes captured inside and outside the net}}{\text{number of people for bait trap inside net}}$$

The other approach used to investigate mosquito biting rate was performed in the same villages using a full-capture method [33]. Briefly, *Anopheles* spp. mosquitoes were searched for and captured in the early morning during 6:00 am to 7:00 am. All *Anopheles* spp. mosquitoes were captured inside each mosquito-net in houses, and barnyards in the investigation village, covering a population of 300–450 people for each village. The number of *An. sinensis* captured was recorded, and the mosquito man-biting rate per night was calculated by the following formula:

$$\text{Man biting density inside net whole night (No. mosquitoes/people)} = \frac{\text{the number of mosquitoes captured from nets}}{\text{the number of people in the nets surveyed}}$$

And then,

Man-biting rate (ma) (No. mosquitoes/ people/ night) = half-night man biting rate + Man biting density inside net whole night.

Secondly, the human blood index (HBI) was estimated using the method of ring precipitation test [29]. All stomach blood specimens were examined from the captured *Anopheles* spp. mosquitoes engorged with blood after these mosquitoes had been morphologically identified. A serologic ring precipitation test was conducted to estimate the HBI, both HBI and man-biting habit (a) were calculated by using the following formula [33]:

$$\mathrm{HBI} = \frac{\text{No. mosquitoes of the human blood}}{\text{No. mosquitoes saturated}}$$

$$\text{Man} - \text{biting habit(a)} = \frac{\mathrm{HBI}}{\text{Gonotrophic cycle of } \textit{An. sinensis}\ (\mathrm{x} = 2.5 \text{ days})}$$

Thirdly, the parous rate (M) was estimated. Briefly, egg-laying mosquitoes were distinguished by dissecting ovaries to see the morpho-rami of ovarial trachea, and x stands for the period in days from human biting to laying eggs, which will be easily considered as the gonotrophic cycle period, e.g. *An. sinensis* will take about 2.5 days to finish the gonotrophic cycle. Then, the parous rate (M) and daily survival rate ($p = M^{1/x}$) were estimated. Given the local average temperatures and the sporgenous cycle (n) of 10 days [31,34], we estimated the expectation of infective life of *An. sinensis* ($p^n/{-\ln P}$).

Finally, the vector capacity (C) [35], an important indicator and measure of malaria transmission predicting recurrence of transmission [29,35-37], was estimated by using the following formula [29]:

$$\text{Vector capacity (C)} = \frac{ma^2P^n}{-\ln P}$$

Where P is survival rate of mosquitoes, n is the mean days for reproduction of *Plasmodium* sporozoites in mosquitoes under the local temperature.

Ethical considerations

The study protocol was approved by the institutional review board of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention in Shanghai. The objectives, procedures, and potential risks of the mosquito biting experiment using the human attraction method were carefully explained to each participant. Interested individuals provided written informed consent in person before participation in the study.

Results

The malaria incidence and risk factors

The incidence of malaria in 2007 were $9.1/10^4$ (1167/1287000) and $22.2/10^4$ (2883/1297000) in Huaiyuan county, Anhui province and Yongcheng county, Henan province, respectively.

A total of 343 residents from 86 households in Huaiyuan, and 860 residents from 215 households in Yongcheng were visited. About 93.0% of the households (80/86) in Huaiyuan and 89.3% of the households (192/215) in Yongcheng had anti-mosquito facilities, such as mosquito nets, screened windows, screened doors and mosquito repellent. On average, about 1.3 (51/39) and 1.7 (219/129) people were used for one mosquito-net, and their mosquito-net coverage rates per household were 45.34% and 60.0% in Huaiyuan and Yongcheng, respectively. Neither cattle nor pigs were found in the study villages of two counties, only 9 and 7 sheep free-roaming were found in Huaiyuan and Yongcheng, respectively.

Vector capacity

Anopheles populations and its blood-sucking behavior

The half-night human bait trap was performed twice in Huaiyuan county and Yongcheng county, respectively. A total of 201 *Anopheles* spp. mosquitoes were captured in

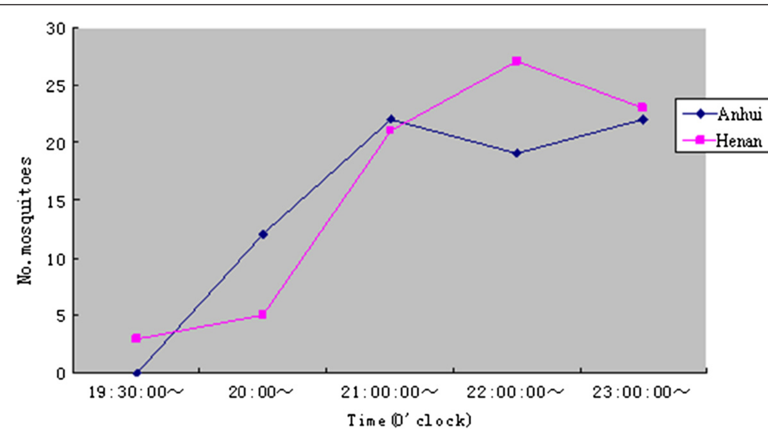

Figure 2 Patterns of blood feeding activities of *Anopheles sinesis* at night. Both curves indicating patterns of mosquito blood feeding activities from Anhui (blue line) and Henan (pink line) provinces.

study sites, among them 94 were found in Huaiyuan and 107 were collected in Yongcheng. Morphological analysis showed that they all belonged to species of *An.sinensis*. Results from the half-night human bait trap showed that *An. sinensis* activity for taking blood meals occurred from 7:30 pm to 12:00 pm, and the blood-feeding peak was between 9:00 pm and 12:00 pm (Figure 2).

The mosquito specimens were collected by the full-capture method 3 times in total in the study sites. A total of 16 and 3 *An. sinensis* mosquitoes were obtained from 39 mosquito-nets and 3 livestock rooms in Huaiyuan county, respectively. 16 and 12 *An. sinensis* mosquitoes were collected from 42 mosquito-nets and 3 livestock rooms in Yongcheng, respectively. It was estimated that the man biting rate from the study sites was 6.0957 (mosquitoes/people/night) and 5.8621 (mosquitoes/people/night) by using the half-night human bait trap method and full-capture method, respectively (Table 1, Figure 3).

Figure 3 A photograph of *Anopheles sinesis* mosquito capture at night, using half-night human bait trap method inside mosquito net.

Vector capacity

A total of 47 *An. sinensis* mosquitoes were captured inside the houses, mosquito-nets and sheep yards in the study sites. Among them, 9 out of 19 *An. sinensis* mosquitoes engorged with blood were captured in Huaiyuan, and 28 out of 28 *An. sinensis* mosquitoes engorged with blood were captured in Yongcheng. Ring precipitation tests showed that HBI of these two study sites were 0.6667(6/9)and 0.6429(18/28), thus the man-biting habits (a) were 0.2667 and 0.2572 in Huaiyuan and Yongcheng, respectively.

A total of 93 *An.sinensis* were captured and dissected for examination of ovaries. The parous rates (M) were 57.14% (28/49) and 54.55% (24/44) from Huaiyuan and Yongcheng, respectively. Given the local average temperatures, the sporgenous cycle of *An. sinensis* was 10 days and the gonotrophic cycle was 2.5 days [38], we estimated that the expectations of infective life of *An. sinensis* were 0.4761 and 0.3649 from Huaiyuan and Yongcheng, respectively. Therefore, the vectorial capacities of *An. sinensis* in Huaiyuan and Yongcheng were 0.7740 and 0.5502, respectively (Table 2). When compared with the vector capacity of *An. sinensis* from the reported data in the previous years (1984–1994), the three indicators including the man-biting habit, human blood index and vector capacity were changed in consistency with the fluctuations of malaria incidence during the same period. The significant changes in man-biting habit reflected that the number of livestock as sources of infection changed during the study period as well, indicating that this change in pattern is closely related to the reduction of livestock numbers in the study sites.

Discussion

Vector control has been one of the important components for the malaria control program leading to elimination [39]. There are various methods of vector control that are used in blocking the transmission of *P. vivax* [28]. While the level of *P. vivax* transmission is significantly related to two main factors, such as vector capacity and the number of infectious sources or human cases [25]. When the environmental conditions are appropriate, malaria outbreaks could occur, involving large numbers of human cases [4,40].

In terms of infectious sources, human cases infected with *P. vivax* have been reduced significantly in the Huanghuai valley of China in the new millennium, although it was more than 10 million cases per year in the 1950s. In particular, following the large-scale efforts of the NMCP in China since the 1970s, malaria incidence in the areas where *An. sinensis* was the only vector had been reduced significantly to a low level in the 1990s. According to the national data on malaria transmission, the average annual incidences of vivax malaria in 1998 in Anhui and Henan province with *An. sinensis* being

Table 1 Man-biting rate estimation in study sites

Location	Bait method	Number of bait	Number of Capture	Half-night bite rate *	Biting rate in the mosquito nets§	Man-biting rate#
Huaiyuan, Anhui province	Human bait outdoor	13	75	5.7692	0.3265(16/49)	6.0957
Yongcheng, Henan province	Human bait indoor and outdoor	14	79	5.6429	0.2192(16/73)	5.8621

Note: * Half-night bite rate was measured in mosquitoes/people; §Biting rate in the mosquito nets was measured in mosquitoes/people/night; and #Biting rate was measured in mosquito/people/night).

Table 2 Comparison of vector capacity of *An. sinensis* between outbreak time period and non outbreak time period in areas where only species of *An. sinensis* served as vectors of vivax malaria

Time (Location)	Incidence rate (%)	Man-biting rate (ma)	Human blood index (HBI)	Man-biting habit (a)	Expectation infective life (p^n/-lnp)*	Vector capacity (C)Ma × a × (p^n/-lnp)
1984§	0.02	20.8	0.0300	0.0120	0.2424	0.0605
1994#	0.01	4.7	0.128	0.051	0.7275	0.1744
2007 (Huaiyuan)	0.091	6.0957	0.600	0.2667	0.4761	0.7740
2007 (Yongcheng)	0.222	5.8621	0.666	0.2572	0.3649	0.5502

*: Expectation of infective life was measured in days. §: Reported data in Huanghuai valley, China. #: Reported data in Henan provinces, China.

the only vector, were below $1/10^5$ and $0.03/10^5$, respectively. However, the incidence of vivax malaria had a resurgence in Huanghuai valley after 2003 [4,41]. For example, the annual incidence of malaria from 2004 to 2006 was $101.22/10^5$, $95.38/10^5$ and $90.67/10^5$ in Anhui province, and was $12.29/10^5$, $54.99/10^5$ and $222.82/10^5$ in Henan province, respectively, which were significantly higher than those recorded in the 1990s [42]. Serological investigation of malaria antibodies in these provinces in 2006 and 2007 showed that about 87.83% of the positive samples were asymptomatic cases [43], indicating that existing control measures were failing to protect these populations, since then the infection source of vivax malaria has been accumulating and increasing significantly.

For the vector capacity, human blood index (HBI) is an important and quantitative indicator, which showed the contact relationship between mosquitoes and humans [17]. HBI of the areas where *An. sinensis* was present was about 5% in the 1990s, which was attributed to the reduction in the incidence of vivax malaria in the region [44]. Reviewing the data during the 1990s, the number of pigs and farm cattle reared by each family increased, which caused the mosquitoes to feed more on blood from livestock instead of human blood. But since the development of the economy and popularity of agricultural mechanization, the farmers no longer keep pigs in their houses, and the number of farm cattle has also been greatly reduced. The increased probability of *An. sinensis* feeding on human blood indicates that the mosquitoes change their behavior caused by changes in number of livestock [45,46]. In addition, our study also revealed that the HBIs in Huaiyuan and Yongcheng were 0.6667 and 0.6429, and the HBI values were more than 12 times higher than that in the historical records. Results in the study also indicated that the increasing HBI value was due to the changes of human activities or living customs [25,47].

It has been observed that the local villagers generally used mosquito-nets and mosquito-repellent incense when asleep, and most families have screened-doors, and/or screened-windows. Only 7 out of 81 mosquito nets had *Anopheles* spp. mosquitoes inside nets in the morning of the investigation, which indicated that the chance of contact between mosquitoes and humans might occur. However, the mosquito-biting was inevitable because the villagers had a habit of enjoying cool outdoor fresh air before midnight in the Summer time [2].

The average man-biting rate observed in the 1990s in the areas where *An. sinensis* was the only vector was 2.36 [48], however, the rates in the study sites were 6.0957 and 5.8621 according to this investigation, which is more than 2.5 times higher than before. From the history data, the average vector capacity was 0.331 in the 1990s in Huanghuai valley where *An. sinensis* was mostly predominately distributed [48], and was 0.1686 during 1996–1998 in Henan province [2,38]. However, our findings showed that the vector capacity was 0.7740 and 0.5502 in Anhui and Henan province, respectively, about 2.3 and 1.7 times higher than that in the 1990s, and were 4.6 and 3.3 times higher than that in Henan during 1996–1998, respectively. All these results demonstrated that the ability of *An. sinensis* to transmit *P. vivax* had been obviously enhanced; therefore, more effective and practical control measures need to be implemented in Huanghuai valley, particularly in the mosquito control and reduction of the residual carriers with *P. vivax*. Our findings illustrated that the vector capacity is a good indicator to assess the malaria transmission capability, which contributes to changes in transmission patterns of vivax malaria.

Conclusions

This study suggests that vivax malaria outbreaks in Huanghuai valley is highly related to the enhancement in transmission ability for *P. vivax* through *An. sinensis*, which is attributed to the habits of local residents and the remarkable drop in the number of large livestock leading to disappearance of traditional biological barriers.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Conceived and designed the experiments: JYP, SSZ, XZ, FH. Performed the experiments: XZ, FH, DQW, MZS, YPS, GCZ, JJJ. Analyzed the data: JYP, SSZ, XZ. Contributed reagents/materials/analysis tools: JYP, SSZ, XZ, FH. Wrote the paper: JYP, SSZ, XZ. All authors read and approved the final version of the manuscript.

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