Host preferences and feeding patterns of *Anopheles sinensis* Wiedemann in three sites of Shandong province, China

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**ABSTRACT**

*Background & objectives:* *Anopheles sinensis* Wiedemann is a major vector of malaria and is among the dominant species in Shandong province of China. Knowledge of the blood-feeding patterns of mosquitoes is crucial for elimination of malaria vectors. However, little information is available on the blood-feeding behaviour of *An. sinensis* mosquitoes in Shandong province. This study was carried out to compare the blood-feeding behaviour of *An. sinensis* in malaria-endemic areas of Shandong province China.

*Methods:* Adult *Anopheles* mosquitoes were collected from three malaria-endemic areas (Jimo, Yinan and Shanxian) during the peak months of mosquito population (August and September) from 2014 to 2015. Indoor-resting mosquitoes and outdoor-resting blood-fed females were sampled in the morning hours (0600 to 0900 hrs) from 10 randomly selected houses using pyrethrum spray catch method, and sweeping with an insect net. ELISA was used for the identification of blood meal. The blood meal of each mosquito was tested against antisera specific to human, pig, dog, cow, goat, horse (mule) and fowl.

*Results:* At all indoor study locations of Jimo, Yinan and Shanxian, 59.4, 68.1 and 98.8% blood-engorged female *An. sinensis* collected from cattle sheds fed almost exclusively on bovines, respectively. For outdoor locations, at Jimo site, 27.27 and 49.55% *An. sinensis* fed on cattle and pigs; at Yinan, 30.42% fed on cattle and 36.88% fed both on cattle and goats, while no pig antibodies were detected. At Shanxian, percent of *An. sinensis* that fed on cattle, pigs and cattle-goat was 20.72, 27.62 and 21.78%, respectively.

*Interpretation & conclusion:* The analysis of *An. sinensis* blood meals in all the three studied areas from human houses, cattle sheds, pig sheds and mixed dwellings revealed that *An. sinensis* prefers cattle hosts, and can feed on other available animal hosts if the cattle hosts are absent, and the mosquitoes readily feed on humans when domestic animals (cattle and pigs) are not nearby for feeding. The analysis of blood meal revealed that *An. sinensis* follow opportunistic feeding in Shandong province, China.

**Key words** *Anopheles sinensis* Wiedemann; ELISA; host preference; malaria

**INTRODUCTION**

*Anopheles sinensis* Wiedemann is a major vector of human malaria in southeast Asia1–3 and is among the dominant mosquito species in Shandong province, China4–6. Shandong province was once an endemic region for malaria; in the early 1960s and 1970s3, two large-scale outbreaks occurred with annual infections of six and four million people, respectively. Due to the large-scale surveys and antimalarial campaigns, the disease burden sharply reduced. At present, the reported malaria incidence has decreased to low levels and imported malaria has become the greatest challenge in Shandong6. Recent malaria outbreaks and re-emergences have been reported only in areas prevalent with *An. sinensis*, with peak incidence of 0.019 per 10,000 in the year 2007 in the province9.

Anophelines exhibit a wide range of host preferences including humans, livestock, birds, and reptiles10–12. The risk of malaria transmission and prevalence of malaria are influenced by anopheline mosquitoes’ host selection13–14. Host preferences, especially the degree of anthropophily, affect the efficacy of the disease transmission15. To combat malaria, vector control is indispensable in endemic foci16. Hence, vector surveillance is essential to prevent recrudescence of malaria in low-transmission regions17. Identification of blood meals in mosquitoes is an important step in malaria epidemiological studies18–19. This is a measure of human-vector contact20 and is useful in the estimation of vectorial capacity.

Extensive studies have been done on the feeding behaviour and blood meal origins of *An. sinensis* mosquitoes in China (Anhui, Zhejiang, Jiangsu, Henan provinces and...
Chongqing city), Japan, Korea and Thailand21-30. Though vectorial capacity of An. sinensis have been studied during 1975–84 and 1996 in Jining district of Shandong31-32, and in 2012 in Huang-Huai Valley of China33; little research has been conducted on the transit period of host preferences and blood-feeding behaviour of An. sinensis species from the control phase to the elimination phase in Shandong province, China. Therefore, the present study was conducted to determine the blood feeding behaviour and host preference of the malaria vector, An. sinensis inhabiting the malaria endemic areas of Shandong province for planning appropriate vector control strategy.

MATERIAL & METHODS

Study area

The present study was conducted in three malaria-endemic areas in Shandong province of China, characterized by different levels of past incidence of P. vivax malaria. These included a high-risk village, Dachen (34° 49′ 10″ N, 116° 02′ 15″ E) (Shanxian county, annual average incidence rate >100/100,000), an intermediate risk village, Gegou (35° 21′ 05″ N, 118° 29′ 00″ E) (Yinan county, annual average incidence rate ~10 to ~100/100,000) and a low-risk village, Xiazhuang (36° 14′ 27″ N, 120° 27′ 14″ E) (Jimo county, annual average incidence rate <10/100,000)34. Altitude of this region is ~70 m. The climate is warm from May to October, and the annual average temperature is 14.3 °C. The range of annual rainfall is 550–1548 mm, and the rainfall concentration is higher from June to September months. The main crops of these villages are wheat, soybean, corn, cotton and potato.

The inhabitants of the villages live in houses that are made of bricks. Additionally, most families in these villages keep domestic animals in their compound such as bovines, dogs, pigs, goats, chickens and others. A typical compound consists of a human residence and a shed for animals. The average distance between domestic animal sheds and human dwellings ranged from 15–20 m. Each of the three villages is surrounded by dense vegetation, which provides potential resting and oviposition sites for mosquitoes. Doors and windows are normally kept open until people go to bed.

The presence of waterbodies distribution and amount of appropriate breeding habitat for An. sinensis larvae was characteristically higher in Dachen village than that in Gegou and Xiazhuang villages. During the study period, there were many ponds and canals that contained endogenous lotus, and a large number of anopheline species larvae were observed in Dachen village.

Ethical approval

The study was initiated after obtaining approved ethical clearance from the Institutional Ethics Committee of the Shandong Institute of Parasitic Diseases (SIPD), Shandong Academy of Medical Sciences, Shandong. The area chief and villagers were sensitized on the study activities planned, and written consent of authorizing for mosquito collection was obtained.

Mosquito collection

Freshly engorged adult An. sinensis mosquitoes were collected (indoor and outdoor collections at resting places) from three malaria-endemic areas (Jimo, Yinan and Shanxian counties of Shandong province), during the peak months of the mosquito population (August and September) of the years 2014 and 2015. Indoor-resting mosquitoes were sampled during the morning hours (0600 to 0900 hrs) from 10 randomly selected houses by the pyrethrum spray catch method35. At the same time, to study unbiased host feeding patterns of engorged female mosquitoes from vegetation and bushes in the three study areas, outdoor-resting blood-fed females were collected by sweeping with an insect net36. The collection sites were confined to places 0–250 m away from the houses and the animal sheds around the villages37. The low vegetation that constitutes resting sites was stirred up to disturb mosquitoes, and flying females were caught. Each capture was performed by a single collector and lasted for 30 min. Fully-fed to half-fed An. sinensis females were used for blood meal identification.

Collected female mosquitoes were individually transferred to 10 ml glass vials, placed on ice using a mouth aspirator, brought to the laboratory, and identified morphologically as An. sinensis species38. Mosquitoes were stored at –20°C for blood meal analysis.

Detection of blood meal sources

The stomach contents of freshly engorged An. sinensis females were dissected out and used as a template for blood-meal analysis. ELISA as previously described by Beier et al39 and modified by Loyola et al40 was used to identify the source of the mosquito’s blood meal. The blood meal of each mosquito was tested against antisera specific to humans, cattle, goats, horses, pigs, dogs, and fowl. All the antisera were obtained from Chinese Center for Disease Control and Prevention, Government of China.

Statistical analysis

Changes in the host preferences and host feeding patterns of An. sinensis on selected host species
and study sites were analyzed by chi-square ($\chi^2$) analysis, to determine differences using Python (version 3.5) for Windows. The level of significance was set at $p<0.05$).

**RESULTS**

**Study area**

According to the data shared by the village doctors, Dachen village covers approximately 2400 acres of arable land, and has a population of 1290 people, 30 calves, and 150 goats in 480 households; Gegou village covers approximately 2500 acres of arable land, with a population of 1675 people, 60 calves, 70 pigs, and 180 goats in 470 households, and Xiazhuang village covers approximately 1300 acres of arable land, with a population of 950 people, five calves, 45 pigs, and 70 goats in 170 households.

**Blood meal analysis result of mosquitoes**

The numbers of *An. sinensis* collected indoors vs outdoors were recorded during the study period (Table 1). A total of 354 (134 indoors vs 220 outdoors), 417 (154 indoors vs 263 outdoors) and 1487 (734 indoors vs 753 outdoors) blood-engorged *An. sinensis* females were collected from Jimo, Yinan and Shanxian, respectively.

The results indicated that the proportion of blood-fed mosquitoes did not differ significantly between those collected indoors and outdoors in Shanxian county. Significantly more mosquitoes were collected outdoors than indoors in Yinan and Jimo counties. At all indoor study locations of Jimo, Yinan and Shanxian, 1.4, 23.2 and 83.3% *An. sinensis* females collected from human houses fed on humans, respectively. This was statistically significant ($\chi^2 = 177.111, p<0.001$). Similarly, 59.4, 68.1 and 98.8% *An. sinensis* females collected from cattlesheds of Jimo, Yinan and Shanxian fed almost exclusively on bovines, respectively; which was statistically significant ($\chi^2 = 305.772, p<0.001$). For outdoor locations, at Jimo site, 27.27 and 49.55% *An. sinensis* fed on cattle and pigs, respectively. At Yinan, 30.42% *An. sinensis* females fed on cattle, 36.88% fed on both cattle and goats; while no pig antibodies were detected. At Shanxian, 20.72 and 27.62% *An. sinensis* fed on cattle and pigs, respectively, and the percent for feeding cattle-goat was 21.78% (Table 2). For both indoor and outdoor collections, *An. sinensis* preferentially fed on domestic animals rather than humans.

**DISCUSSION**

The determination of a mosquito blood meal helps us to understand the pathogen’s life cycle and its potential hosts, and to identify suitable control strategies. Host feeding pattern is influenced by numerous factors such as host preference, host availability and host irritability. Epidemiologically, it is quite important to know the conditions of host availability under which species with fixed

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**Table 1. Ingested-blood meal analysis in *An. sinensis* collected from human houses and animals’ sheds**

<table>
<thead>
<tr>
<th>Areas</th>
<th>Location</th>
<th>No. tested</th>
<th>Number of blood-meal sources analyzed</th>
<th>No-call*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Human</td>
<td>Cattle</td>
</tr>
<tr>
<td>Jimo</td>
<td>Human houses</td>
<td>58</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cattlesheds</td>
<td>69</td>
<td>1 (1.4)</td>
<td>41 (59.4)</td>
</tr>
<tr>
<td></td>
<td>Donkey sheds</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>134</td>
<td>9 (6.7)</td>
<td>42</td>
</tr>
<tr>
<td>Yinan</td>
<td>Human houses</td>
<td>56</td>
<td>13</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>Cattlesheds</td>
<td>91</td>
<td>1 (1.1)</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Donkey sheds</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>154</td>
<td>14 (9.9)</td>
<td>63</td>
</tr>
<tr>
<td>Shanxian</td>
<td>Human houses</td>
<td>149</td>
<td>124 (83.3)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Cattlesheds</td>
<td>166</td>
<td>1 (0.6)</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Pig herds</td>
<td>189</td>
<td>0</td>
<td>2 (1.05)</td>
</tr>
<tr>
<td></td>
<td>Shed with Cattle, Horse, Mule, Goat</td>
<td>230</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>734</td>
<td>125 (17)</td>
<td>195</td>
</tr>
</tbody>
</table>

*No reaction with antibodies to any of the tested hosts; Figures in parentheses indicate percent positives.*
feeding patterns may deviate from their normal hosts. In a study in Japan, Sasa showed that An. hyrcanus was strongly attracted to large animals and very few fed on humans. Similarly, study by Otsuru and Ohmori showed that cows were the most attractive animal to An. sinensis, but they fed persistently on humans as well. Other studies carried out in Korea have shown that An. sinensis fed exclusively on bovines, though they also fed on dogs, chicken and cats in small numbers, with almost no feeding on humans, hence human blood meal rate was very low (0.7–1.7%). In Thailand, An. sinensis was almost entirely zoophilic; almost none of the mosquitoes were attracted to humans in comparative biting tests involving humans and cows. In another study conducted in Chongqing, China, indicated that An. sinensis fed mainly on cows and rarely on humans. Studies in Anhui, Zhejiang and Jiangsu provinces of China have shown that An. sinensis feeds both on humans and cattle, but has a strong preference for cattle blood. Pigs, goats and calves were more attractive to An. sinensis than humans in Yongcheng city of Henan province.

Indoor collections of this study, at Shanxian site showed that 83.3% of the An. sinensis mosquitoes collected from households were positive for human blood, while at Jimo and Yinan, only 1.4 and 23.2% of blood-engorged An. sinensis females mosquitoes showed blood meals positive for human blood, respectively. At Jimo, Yinan and Shanxian sites, 59.4, 68.1 and 98.8% An. sinensis females collected from cattle sheds showed blood meal positives for cattle, respectively. Similarly, 27.27, 30.42 and 20.72% female An. sinensis mosquitoes collected outdoors from Jimo, Yinan, Shanxian fed on bovines, respectively. Outdoor collections revealed that 3.98% (Shanxian), 2.29% (Yinan) and 3.6% (Jimo) mosquitoes were positive for human blood. No pig antibodies were detected at Yinan which might be due to limited number of pig presence. At Shanxian, higher percent of An. sinensis fed on humans than at Jimo and Yinan, the reason could be presence of more waterbodies and appropriate An. sinensis larvae breeding habitats at Dachen village (Shanxian) than those at Gegou village (Yinan) and Xiazhuang village (Jimo). Thus, host preference of An. sinensis in Shandong province was consistent with other studies in Japan, Korea, Thailand and other provinces in China.

**CONCLUSION**

The analysis of mosquito blood meals from human households, cattle and pig sheds and mixed dwellings revealed that An. sinensis prefers cattle than pigs, dogs, goats and humans in Shandong province. Additionally, the present study indicates that An. sinensis in the study area is preferentially zoophilic in nature. The results also suggest that the vector can feed on other available hosts, if the cattle host is absent. Though human blood meal rates were low, An. sinensis readily feeds on humans in great numbers when domestic animals (cows and pigs) are not nearby for feeding. This phenomenon could be explained by opportunistic feeding behaviour present in this species. Though An. sinensis is the most widely distributed species in China, due to its exophilic and exophagic host preferences, the species had been judged not to be the efficiency vector for malaria for decades.

**Conflict of interest**

The authors declare that they have no competing interests.

**ACKNOWLEDGEMENTS**

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