JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2014. Vol. **8**(Spl. Edn. 1), p. 677-680

Host-feeding Habits Study of *Anopheles sinensis* with Fluorescent Pigment

Chongxing Zhang, Gongzhen Liu, Xiaodan Huang, Peng Cheng, Haifang Wang, Xiuxia Guo, Lijuan Liu and Maoqing Gong*

Department of Medical Entomology, Vector Biology Key Laboratory of Medicine and Health Shandong Province, Shandong Institute of Parasitic Diseases, Shandong Academy of Medical Sciences, Jining, Shandong 272033, P.R. China.

(Received: 12 April 2014; accepted: 09 May 2014)

Fluorescent dyes experiments were conducted to study host-feeding habits with wild collected, newly emerged first generation and laboratory reared domesticated the thirtieth generation adult *Anopheles sinensis* Wiedemann (*An. sinensis*) at Tangkou Township, Shandong Province, China. Of 5942 wild collected in the field, 10027 newly emerged first generation and 2761 domesticated the thirtieth generation adult *An. sinensis* was released, according to the recaptured rate by human landing collections and cattle bait collections, showed that host-feeding habits of *An. sinensis* were not random, most wild collected *An. sinensis* were still tend to seek their original host, first generation *An. sinensis* were still had feeding habits as their parents do, the original host tropism weakened for the thirtieth generation of *An. sinensis.*

Key words: Host-feeding habits, *Anopheles sinensis*, Fluorescent dyes.

For mosquito vectors, the blood-host foraging activity is an important behavioral characteristic[1]. In order to design and implement efficient strategies for vector control, understanding of the host-feeding habits of a mosquito species is critical important because it can provides important insight into the dynamics of virus transmission[2,3]. So, for cutting off the route of transmission, to detect potential source of infection, host-feeding habits of mosquitoes should studied[4]. Under natural conditions, blood feeding choice is conditioned by regional factors. Additionally, multiple other factors also influenced the contact between the mosquito and the potential host, such as re-feeding, attempts to blood feed and reaction behavior to the host[5]. However, little is known about these important life history traits of *Anopheles* mosquitoes in nature. Fluorescent dyes technique is one of the methods most commonly used to obtain information on mosquito populations, which has been carried out widely with populations of *Anopheles* and *Aedes* mosquitoes. Service lists 150 such studies[6]. The majority of blood-feeding data on *An. sinensis* is from studies conducted in Sichun, Jiangsu, Fujian Provinces[7,8,9,10]. In Shandong Province, however, little information was available regarding the host preferences of *An. sinensis*. Therefore, in this present study, we compared the feeding habits of wild caught *An. sinensis*, first generation and the thirtieth generation in the laboratory by applying fluorescent dyes.

MATERIALS AND METHODS

Research areas

The present study was conducted at Tangkou Township, Jining District, which is about

* To whom all correspondence should be addressed. Tel: +86 0537 2321859; Fax: +86 0537 2353277; E-mail: maoqingg@yahoo.com

1 km far away from other villages, located in the southwest of Shandong Province. Geographically, the village is in the delta between ancient and modern Yellow River, belongs to the Huang-Huai River region, and adjacent to Anhui, Henan and Jiangsu provinces.

Survivorship of *An. sinensis* effect with fluorescent powder

The survivorship of *An. sinensis* effect with fluorescent powder based on methods described by Liu *et al.*,[11], but has some modifications. i.e., 30 three-day-old females *An. sinensis* was used, and then aspirated into a waxed, 200 mm diameter custom made cylinder with gauze tops, gauze bottoms and metal bracket. Mix 3 ml acetone with 2 g of Day-Glo fluorescent pigment powder, then add distilled water to 100ml. We use a 5 ml syringe with a 22 gauge needle as a powder atomization device. To produce atomizing, the syringe filled with fluorescent pigment was pushed quickly. 30 minutes later, all the experimental group 30 *An. sinensis* were marked with fluorescent pigment. 30 three-day-old females *An. sinensis* without marking was also set a control group. All these mosquitoes were provided with 10% sucrose, kept at 28!, relative humidity was 70~80%. In the following 14 days, the number of live mosquitoes was counted daily, the existence of fluorescent powder on the body surface of *An. sinensis* was observed under dissecting microscope.

Collected Wild *An. sinensis* in the field

Wild adult *An. sinensis* was collected by human landing collections (HLC) and cattle bait collections (CBC) outdoors at Cuitang village, Tangkou Township, Jining District with handheld, battery-operated aspirators after sunset. The outdoors HLC involved a mosquito net measuring 1.2 m× 1.2 m× 1.5 m (L:W:H) with half-opened, four corners were fixed, the lower edge 30 cm above the ground level, human serving as bait inside. Outdoors CBC was carried out following methods described by Sungvornyothin *et al.*,[12], that is, placing one adult cow under a clean nylon mosquito net (measuring 2.5 m x 2.0 m x 2.0 m (L:W:H)) with the net suspended 30 cm above the ground level to allow mosquitoes access inside. All *An. sinensis* were collected within three days, placed into mosquito cages (45 cm×45 cm×45 cm), and then transported alive to the laboratory, transferred to a large custom made cylinder mentioned above[13].

First generation and domesticate thirtieth generation of *An. sinensis* in the Laboratory

As described by Methods in *Anopheles* Research[14], wild *An. sinensis* caught by HLC and CBC were mass reared to collect first generation and thirtieth generation of *An. sinensis* in the laboratory, respectively.

Fluorescent dyes Marking and Releasing

The outdoors fluorescent dyes mark, releasing experiment for wild and first generation adult *An. sinensis* carried out 16th – 25th September 2011, the release point located at 300m southeast from Cuitang village, surrounded by rice paddies and vegetable without human residents and animal sheds within 250 m. Wild *An. sinensis* collected by HLC, CBC and first generation reared in the laboratory were marked with red, yellow fluorescent pigment powder, respectively. 2646 HLC and 3296 CBC wild *An. sinensis* was released. 6372 HLC and 3655 CBC first generation adult of *An. sinensis* was released. At the same place, the outdoors fluorescent dyes mark, releasing experiment for the thirtieth generation of *An. sinensis* was conducted from 15 to 24 September 2012, 1777 HLC and 984 CBC domesticate the thirtieth generation of *An. sinensis* was released.

Mosquitoes recaptured

To recapture the marked *An. sinensis*, taking the release point as the radius, 4 human landing collections (2 person/trap) and 2 cattle bait collections (1 cow/trap) were set up outdoors. For HLC, mosquitoes were continuously collected for 2 hours with battery-powered aspirator. For CBC, uninterrupted for one hour, the cow was exposed to mosquitoes entering the net. At the time of collection, mosquitoes, either resting inside the net or on the cow were captured at an interval of 10 minutes. At the same time, one human landing collection (3 person / trap) at a distance of 7.8 meters from cattle bait collections (1 cow/trap) were set up indoors, respectively. In the same day after releasing, marked *An. sinensis* were recaptured, recaptured for 2 successive nights. For the presence or absence of fluorescent pigment, all the *An. sinensis* mosquitoes recaptured were examined under dissecting microscope.

Statistical methods

The number of marked *An. sinensis* recaptured over the total number of originally

released were calculated as the recapture rates. By using Chi-square analysis, the mortality rate of marked *An. sinensis* and untreated *An. sinensis* in the laboratory, wild, newly emerged first generation and domesticate the thirtieth generation of *An. sinensis* were compared. By regressing the number of recaptured *An. sinensis* transformed into ln (y+1) as a function of time in days post-release the calculation was done. Statistical analysis was conducted using SPSS software (Version 11.5 for Microsoft Windows, SPSS Inc., Chicago, USA).

Results

Survivorship of *An. sinensis* Effect with Fluorescent Pigment

In mortality rates, there was no difference between marked and unmarked *An. sinensis* in the laboratory ($x^2 = 4.23$, $P>0.05$). During the 14 day bioassay period, in all replicates, on the body surface of marked *An. sinensis*, fluorescent pigment was detected under a dissecting microscope. According to the results, if sprayed correctly, fluorescent pigments may have little effect on the survivorship of *An. sinensis* and could be used to conduct fluorescent dyes experiment[15,16].

Release and recapture

At Cuitang village, 5942 wild *An. sinensis* were released, 147 were recaptured, and the recapture rate was 2.47%. 2646 human landing collections wild *An. sinensis* was released, 33 and 20 were recaptured by HLC and CBC, respectively, accounting for 62.3% and 37.7%. 3296 cattle bait collections wild *An. sinensis* was released, 39 and 55 were recaptured by HLC and CBC, respectively, accounting for 41.5% and 58.5%. This indicates that most of *An. sinensis* were still tend to seek the original host ($X^2 = 5.05$, $P <0.05$).

6372 human landing and 3655 cattle bait collections first generation adult of *An. sinensis* were released. For first generation adult *An. sinensis* of human landing collections, 12 was recaptured by HLC, accounting for 70% of all the recapture marked *An. sinensis*. For first generation adult *An. sinensis* of cattle bait collections, 47 was recaptured by CBC, accounting for 69.1% of all the recapture marked *An. sinensis*. The distribution was non-random ($X^2 =7.43$, $P<0.01$).

1777 domesticated human landing collections *An. sinensis* the thirtieth generation was released, 194 and 158 *An. sinensis* was recaptured by HLC and CBC, respectively, accounting for 55.4% and 44.6%. 984 domesticated cattle bait collections the thirtieth generation were released; 89 and 98 were recaptured by HLC and CBC, accounting for 47.6% and 52.4%, respectively. The results showed that after 30 generation of domesticated in the laboratory, offspring of human landing and cattle bait collections *An. sinensis* almost have equally blood feeding patterns ($X^2 = 2.48$, $P> 0.05$).

DISCUSSION

For the first time, this study describes fluorescent dyes experiment using wild, newly emerged first generation and domesticated the thirtieth generation *An. sinensis* to determine the recapture rate, blood feeding patterns at one villages of Jining City, a representative region of unstable *Plasmodium vivax* malaria transmission in the southwest of Shandong Province, China. The results showed that feeding patterns of *An. sinensis* was non-random, most still tend to seek their original host, first generation adult *An. sinensis* still had feeding habits as their parent did, but after 30 generations reared in the laboratory, the original host tropism decreased.

The results indicated that the blood-feeding pattern of wild caught, first generation *An. sinensis* species were consistent with experiments in Sichun, Jiangsu, Fujian Provinces[7,8,9,10]. They prefer cattle to human, but all of these authors only study the wild caught *An. sinensis*, but they do not study blood-feeding pattern of their offspring.

In conclusion, we have investigated the blood feeding pattern of *An. sinensis* by fluorescent dyes mark, releasing experiments. Based on results, we suggest that there is a need to conduct more fluorescent dyes mark, releasing experiments by using this method and to compare results with the approach where naturally caught females. Information on the blood feeding pattern of mosquitoes is important, especially where malaria control by reducing human-vector contact is a priority. This information was also necessary for malaria vector control programs that focusing on integrated vector management methods.

ACKNOWLEDGMENTS

The present study was supported by the National Natural Science Foundation of China (NSFC) (Grant No. 81271877) and the development of medical science and technology project of Shandong Province (Grant No. 2013WS0353).

REFERENCES

1. Chaves, L.F., Harrington, L.C., Keogh, C.L., Nguyen, A.M., Kitron, U.D. Blood feeding patterns of mosquitoes: random or structured? *Front. Zool*., 2010; **7**: 3.
2. Tempelis, C.H. Host feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. *J. Med. Entomol.*, 1975; **11**(6): 635-653.
3. Vinogradova, E.B., *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control, 1st edition. Pensoft Publishers, 2000.
4. Liu, Q.Y., Liu, X.B., Zhou, G.C., Jiang, J.Y., Guo, Y.H., Ren, D.S., Zheng, C.J., Wu, H.X., Yang, S.R., Liu, H.S., Li, H.Z., Li, Q., Li, W.Z., Chu, C. Dispersal Range of *Anopheles sinensis* in Yongcheng City, China by Mark-Release-Recapture Methods. *PLoS ONE*, 2012; 7(11): e51209. doi:10.1371/journal.pone.0051209.
5. DeFoliart, G.R., Grimstad, P.R., Watts, D.M. Advances in mosquito-borne arbovirus/vector research. *Ann. Rev. Entomol*., 1987; **32**: 497-505.
6. Service, M.W., Mosquito ecology: field sampling methods. Vol. 2nd ed.. London, United Kingdom: Elsevier Applied Science Publishers Ltd, 1993.
7. Yang, W., Xu, G.J., Chen, H.L., Yan, J.C., Feng, S.Z., Liu, S.P., Xu, Z.Z. Survey on Resting and Sucking Habits of *Anopheles Anthropophagus* and *Anopheles sinensis* in Sichuan. *Chin. J. Parasit. Dis. Con.*, 2003; **16**(5): 278-280
8. Wang, H.W., Hu, Y.X., Sun, C.H., Zhen, T.M., Yan, G. Host-feeding Habits Investigation of *Anopheles sinensis* by Mark-Release-Recapture Methods. *Chin. J. Parasit. Dis. Con.*, 1990; **3**(2): 278-280.
9. Zheng, Z.J., Xu, B.H., Liu, Q.S., Wu, J.L., Huang, B.F. Survey on the Blood Preference of *Anopheles sinensis* by the Net-Bait Men and Domestic Animals. *Chin J Vector Biol Con*., 1996; **7**(5):324-326.
10. Qiu, Z.M., Zheng, L.X., Xie, H.G. Survey on the Blood Preference of *Anopheles sinensis* in Ximen. *Chine J Zoonoses*., 2010; **26**(1): 91-92
11. Liu, X.B., Liu, Q.Y., Guo, Y.H., Jiang, J.Y., Ren, D.S., Zhou, G.C., Zheng, C.J., Liu, J.L., Chen, Y., Li, H.S., Li, H.Z., Li, Q. Random repeated cross sectional study on breeding site characterization of *Anopheles sinensis* larvae in distinct villages of Yongcheng City, People's Republic of China. *Parasit. Vectors*., 2012; **5**: 58.
12. Sungvornyothin, S., Kongmee, M., Muenvorn, V., Polsomboon, S., Bangs, M.J., Prabaripai, A., Tantakom, S., Chareonviriyaphap, T. Seasonal Abundance and Bloodfeeding Activity of *Anopheles dirus* Sensu Lato in Western Thailand. *J. Am. Mosq. Control. Assoc.* 2009; **25**(4):425-430.
13. Midega, J.T., Mbogo, C.M., Mwnambi, H., Wilson, M.D., Ojwang, G., Mwangangi, J.M., Nzovu, J.G., Githure, J.I., Yan, G., Beier, J.C. Estimating dispersal and survival of *Anopheles gambiae* and *Anopheles funestus* along the Kenyan coast by using mark-release-recapture methods. *J. Med. Entomol.*, 2007; **44**(6): 923-929.
14. Methods in Anopheles Research Second Edition. 2011; pp 57-88. http://www.mr4.org/AnophelesProgram/TrainingMethods.aspx.
15. Tsuda, Y., Takagi, M., Suwonkerd, W. A mark-release-recapture study on the spatial distribution of host-seeking anophelines in northern Thailand. *J. Vector Ecol.*, 2000; **25**(1): 16-22.
16. Jaal, Z., MacDonald, W.W. A mark-release-recapture experiment with *Anopheles lesteri* paraliae in northwest Peninsular Malaysia. *Am. Trop. Med. Parasitol*., 1992; **86**(4): 419-424.