

Mosquito Species Composition and *Plasmodium vivax* Infection Rates on Baengnyeong-do (Island), Republic of Korea

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Abstract: Vivax malaria is a significant military and civilian health threat in the north of the Republic of Korea (ROK). The island of Baengnyeong-do is the westernmost point of the ROK and is located close to the southwestern coast of the Democratic People's Republic of Korea (DPRK). Mosquitoes were collected using a black light trap on Baengnyeong-do, and *Anopheles* spp. were assayed by PCR, to identify the species, and screened for sporozoites of *Plasmodium vivax*. Of a subsample of 257 mosquitoes, *Anopheles lesteri* was the most frequently collected (49.8%), followed by *Anopheles sinensis* (22.6%), *Anopheles pullus* (18.7%), *Anopheles kleini* (7.8%), and *Anopheles belenrae* (1.2%). The overall sporozoite rate was 3.1%, with the highest rates observed in *An. kleini* (15.0%), *An. sinensis* (5.2%), and *An. lesteri* (1.6%). No sporozoite positive *An. pullus* or *An. belenrae* were observed. The results extend our knowledge of the distribution and potential role in malaria transmission of *An. kleini*, *An. lesteri*, and *An. sinensis*, for an area previously considered to be at a low risk for contracting vivax malaria.

Key words: *Anopheles lesteri*, *Anopheles kleini*, *Anopheles sinensis*, *Plasmodium vivax*, malaria, sporozoite, Baengnyeong-do (Island)

Prior to being eradicated from the Republic of Korea (ROK) in 1979, *Plasmodium vivax* Grassi and Feletti, the causative agent of vivax malaria, was endemic and widespread throughout the ROK [1,2]. In 1993, vivax malaria reemerged on the Korean peninsula [2,3], and in the ROK, most malaria cases have since been contracted near the demilitarized zone (DMZ) that borders North Korea (Democratic People's Republic of Korea, DPRK) [4-6]. Unique conditions in the vicinity of the DMZ, such as the exposure of large numbers of soldiers to malaria parasites and the unmanaged nature of the environment that encourages mosquito breeding, are thought to exacerbate the malaria situation [2,7]. Little information is available as to how widespread malaria is in the DPRK, what mosquito species are involved in malaria transmission, and the relative abundance

and distribution of potential malaria vector populations. Ongoing mosquito surveillance data is available for the ROK [5,6] and parasite rates for different species have been reported [8,9], but few data of a similar nature are available for the DPRK. *Anopheles belenrae* Rueda, *Anopheles pullus* Yamada, and *Anopheles sinensis* Wiedemann have been recorded in the DPRK in 2 provinces (Hwanghae and Pyongyang) by Rueda and Gao [10]. Foley et al. [11] produced ecological niche models for 8 anopheline species using collection location data from the ROK that predicted suitable habitat in the ROK and southern DPRK. *An. sinensis* is the predominant species collected south of Seoul; however, in malaria risk areas in the north, other species are frequently more common [4,12] and may play a greater role in malaria transmission.

Baengnyeong-do is a 45.8 km² island located near the Northern Limit Line (Fig. 1). This island is the westernmost point of the ROK, and is 16 km from the DPRK coast. As such, entomological conditions on Baengnyeong-do may more closely reflect those in the southwestern DPRK than the rest of the ROK. We collected mosquitoes from Baengnyeong-do in 2007

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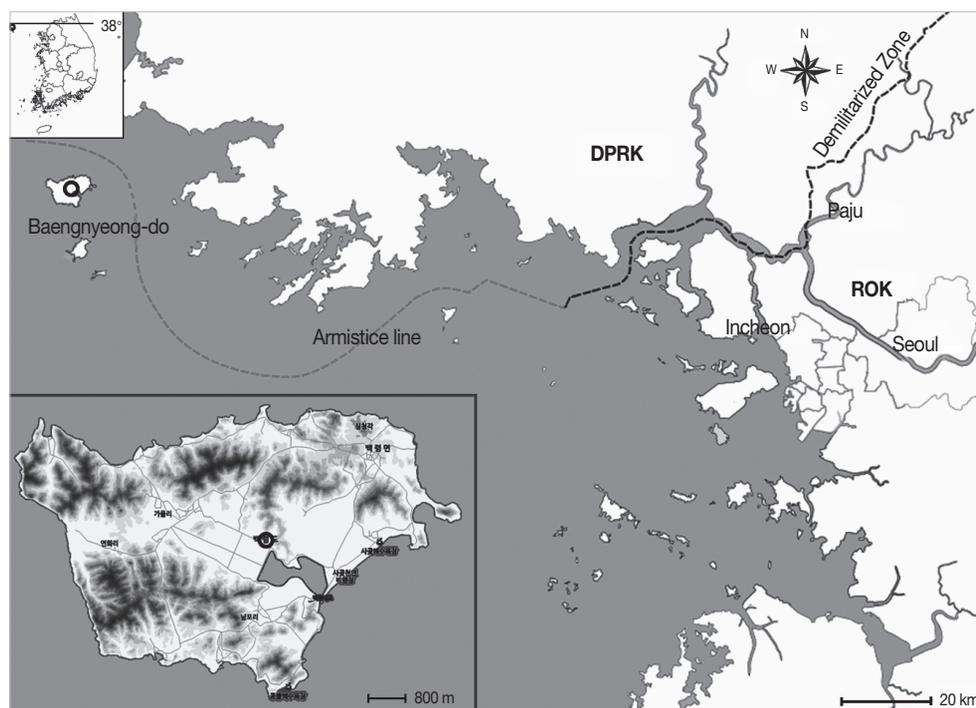


Fig. 1. Mosquito collection site (O) at a cowshed on Baengnyeong-do in the northwest part of the Republic of Korea.

to better understand the composition of mosquito species and their potential role in malaria transmission in locations where ROK military are deployed.

Mosquitoes were collected using a black light trap near a cowshed on 15 July 2007 at Baengnyeong-do, Jinchon-2ri, Baengnyeong-myeon, Ongjin-Gun, Incheon, 37° 57' 24.5" N 124° 41' 55.5" E. Specimens were transported to the 5th Medical Detachment, Yongsan Army Garrison, Seoul, ROK, where they were identified to species or Subgroup using a dissecting microscope and standard keys [13,14]. It was impossible to identify members of the *An. sinensis* Subgroup (*An. belenrae*, *Anopheles kleini*, *Anopheles lesteri* Baisas and Hu, *An. pullus*, and *An. sinensis sensu stricto*) by morphological techniques. For members of the *An. sinensis* Subgroup, the abdomen was separated from the thorax and head of the mosquito to reduce the probability of detecting DNA from parasite oocysts in the midgut. The head and thorax of a subsample of 257 mosquitoes were identified by PCR for species [15,16], and by single step and semi-nested multiplex-PCR to identify *P. vivax* sporozoite infections [17].

A total of 9,971 female mosquitoes were collected (Table 1), 79.0% of which were *Aedes vexans nipponii* Theobald, and 8.8% *Culex tritaeniorhynchus* Giles, the primary vector of Japanese encephalitis virus (JEV) in the ROK. Overall, *An. sinensis* Subgroup

Table 1. Total numbers of mosquitoes collected by black-light trap near a cowshed in Baengnyeong-do (Island), Incheon, Republic of Korea, on 15 July 2007

Species	Female (%)	Male	Total
<i>Ochlerotatus dorsalis</i> (Meigen)	27 (0.3)	0	27
<i>Aedes vexans nipponii</i> Theobald	7,877 (79.0)	88	7,965
<i>Anopheles sinensis</i> Subgroup	1,174 (11.8)	42	1,216
<i>Culex bitaeniorhynchus</i> Giles	2 (<0.1)	0	2
<i>Culex pipiens</i> Linnaeus	16 (0.2)	6	22
<i>Culex tritaeniorhynchus</i> Giles	873 (8.8)	2	875
<i>Mansonia uniformis</i> (Theobald)	2 (<0.1)	0	2
Total	9,971 (100.0)	138	10,109

species made up 11.8% of the collections, of which *An. lesteri* predominated (49.8%), followed by *An. sinensis* (22.6%), *An. pullus* (18.7%), *An. kleini* (7.8%), and *An. belenrae* (1.2%). Overall, the sporozoite rate for members of the *An. sinensis* Subgroup was 3.1%, with highest rates in *An. kleini* (15.0%), followed by *An. sinensis* (5.2%), and *An. lesteri* (1.6%). Sporozoite positive *An. pullus* and *An. belenrae* were not observed (Table 2). *Ae. vexans nipponii*, is considered a nuisance mosquito that is not readily attracted to humans. It is an early-season floodwater mosquito whose populations rapidly increase with the flooding of rice paddies or heavy rains [5]. *Ae. vexans nipponii* is usually collected less frequently than members of the *An. sinensis* Sub-

Table 2. Total numbers of malaria infected mosquitoes collected and identified by black-light trap near a cowshed in Baengnyeong-do (Island), Incheon, Republic of Korea, on 15 July 2007

Species	Number identified (%)	Sporozoite rate (%) ^a
<i>Anopheles belenrae</i>	3 (1.2)	0.0
<i>Anopheles kleini</i>	20 (7.8)	15.0
<i>Anopheles lesteri</i>	128 (49.7)	1.6
<i>Anopheles pullus</i>	48 (18.7)	0.0
<i>Anopheles sinensis</i>	58 (22.6)	5.2
Total	257 (100.0)	3.1

^aSporozoite rate (%) = (Malaria infected/number identified) × 100.

group in New Jersey light trap catches throughout most of the ROK [4,12,18], but was the most commonly collected species trapped at Baengnyeong-do.

An. lesteri was the most frequently collected anopheline, but is infrequently collected in north Gyeonggi and southern provinces throughout the ROK [9,19]. This species is considered an important malaria vector in China [20], and was found to be infected with *P. vivax*, albeit at relatively low rates, in the present study. The highest sporozoite rate was observed in *An. kleini*, and we have found this species infected also in northern Gyeonggi province of the ROK (unpublished data). Laboratory experiments found that *An. lesteri* and *An. kleini* produced many sporozoites, whereas *An. sinensis* and *An. pullus* produced few sporozoites [21]. However, in another study, *An. pullus* was observed by ELISA to have sporozoite infections of >1,000 sporozoites (unpublished data). Although no sporozoite positive *An. belenrae* were found in this study, this species has been reported as positive in other studies [9,18]. Taking into consideration of the extrinsic incubation period of *P. vivax*, the fact that *An. sinensis*, *An. lesteri*, and *An. kleini* were positive for sporozoites, and the remoteness of the island, these species were biting infected humans on Baengnyeong-do, probably 10 days or more beforehand.

As only one trap catch is reported, these results cannot be assumed to reflect conditions for other times of the year, especially during the latter part of summer when normally higher malaria infection rates are observed. For example, relative species abundance and demographic factors, such as longevity, which may predispose mosquitoes for the growth of sporozoites, varies throughout the mosquito season in the ROK [8] (unpublished data). Also, the presence of sporozoites, while necessary for infection of humans, may not translate to new cases, if the number of sporozoites is low [22]. Data provided by the Korea Centers for Disease Control and Prevention for

years 2001-2010 showed that Incheon Metropolitan city, which includes the remote island Banengnyeong-do, had the second highest overall mean number of malaria cases reported [23], but with low numbers of malaria cases (7 cases) for 2001-2010 reported in Baengnyeong-do [23]. Baengnyeong-do and possibly nearby areas of the DPRK may be suitable for malaria transmission, although it differs from most of the rest of the ROK in the high frequency of *An. lesteri*, limited human population movements, and island environmental conditions.

The Incheon Metropolitan city government has plans to increase access and facilities on the island to attract tourists year-round [24]. The likelihood of local malaria transmission suggests that active malaria surveillance to reduce asymptomatic cases, vigilance regarding anti-malarial prophylaxis, and vector control is needed, to minimize the potential for the spread of malaria to visitors and residents of Baengnyeong-do.

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REFERENCES

1. World Health Organization. Synopsis of the world malaria situation, 1979. WHO Weekly Epidemiol Rec 1981; 56: 145-149.
2. Ree HI. Unstable vivax malaria in Korea. Korean J Parasitol 2000; 38: 119-138.
3. World Health Organization Regional Office for Southeast Asia. Malaria situation in SEAR countries: DPR Korea. WHO SEARO, New Delhi Publishing Ltd. 2003.
4. Lee WJ, Klein TA, Kim HC, Choi YM, Yoon SH, Chang KS, Chong ST, Lee IY, Jones JW, Jacobs JS, Sattabongkot J, Park JS. *Anopheles kleini*, *Anopheles pullus*, and *Anopheles sinensis*: Potential vectors *Plasmodium vivax* in the Republic of Korea. J Med Entomol 2007; 44: 1086-1090.
5. Kim HC, Chong ST, Collier BW, Klein TA. Seasonal prevalence

- of mosquitoes collected from light traps in the Republic of Korea, 2005. *Entomol Res* 2009; 39: 70-77.
6. Kim HC, Chong ST, Nunn PV, McNemee RB, Klein TA. Seasonal prevalence of mosquitoes collected from light traps in the Republic of Korea, 2006. *Entomol Res* 2009; 39: 248-256.
 7. Klein TA, Pacha IA, Lee HC, Kim HC, Lee WJ, Lee JK, Jeung GG, Sames WJ, Gaydos JC. *Plasmodium vivax* malaria among U.S. Forces Korea in the Republic of Korea, 1993-2007. *Mil Med* 2009; 174: 412-418.
 8. Oh SS, Hur MJ, Joo GS, Kim ST, Go JM, Kim YH, Lee WG, Shin EH. Malaria vector surveillance in Ganghwa-do, a malaria-endemic area in the Republic of Korea. *Korean J Parasitol* 2010; 48: 35-41.
 9. Rueda LM, Li C, Kim HC, Klein TA, Foley DH, Wilkerson RC. *Anopheles belenrae*, a potential vector of *Plasmodium vivax* in the Republic of Korea. *J Am Mosq Control Assoc* 2010; 26: 430-432.
 10. Rueda LM, Gao Q. New records of *Anopheles belenrae* Rueda (Diptera: Culicidae) in North Korea. *Proc Entomol Soc Wash* 2008; 110: 523-524.
 11. Foley DH, Klein TA, Kim HC, Sames WJ, Wilkerson RC, Rueda LM. Geographic distribution and ecology of potential malaria vectors in the Republic of Korea. *J Med Entomol* 2009; 46: 680-692.
 12. Kim HC, Klein TA, Lee WJ, Collier BW, Chong ST, Sames WJ, Lee IY, Lee YJ, Lee DK. Mosquito species distribution and larval breeding habitats with taxonomic identification of anopheline mosquitoes in Korea. *Entomol Res* 2007; 37: 29-35.
 13. Tanaka K, Mizusawa K, Saugstad ES. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib Am Entomol Inst* 1979; 16: 1-987.
 14. Lee KW. A revision of the illustrated taxonomic keys to genera and species of female mosquitoes of Korea. Department of the Army, 5th Medical Detachment, 168th Medical Battalion, 18th Medical Command, 1998, p 40.
 15. Wilkerson, RC, Li C, Rueda LM, Kim HC, Klein TA, Song GH, Strickman D. Molecular confirmation of *Anopheles (Anopheles) lesteri* from the Republic of South Korea and its genetic identity with *An. (Ano.) anthropophagus* from China (Diptera: Culicidae). *Zootaxa* 2003; (378): 1-14.
 16. Li C, Lee JS, Groebner JL, Kim HC, Klein TA, O'Guinn ML, Wilkerson RC. A newly recognized species in the *Anopheles* Hyrcanus group and molecular identification of related species from the Republic of South Korea (Diptera: Culicidae). *Zootaxa* 2005; (939): 1-8.
 17. Rubio JM, Benito A, Roche J, Berzosa PJ, Garcia ML, Mico M, Edu M, Alvar J. Semi-nested, multiplex polymerase chain reaction for detection of human malaria parasites and evidence of *Plasmodium vivax* infection in Equatorial Guinea. *Am J Trop Med Hyg* 1999; 60: 183-187.
 18. Jeong KY, Un S, Lee J, Lee IY, Yong TS, Ree HI. Population dynamics of five *Anopheles* species of the Hyrcanus group in northern Gyeonggi-do, Korea. *Korean J Parasitol* 2010; 48: 351-353.
 19. Kim HC, Rueda LM, Wilkerson RC, Foley DH, Sames WJ, Chong ST, Nunn PV, Klein TA. Distribution and larval habitats of *Anopheles* species in northern Gyeonggi Province, Republic of Korea. *J Vector Ecol* 2011; 36: 124-134.
 20. Liu C. Comparative studies on the role of *Anopheles anthropophagus* and *Anopheles sinensis* in malaria transmission in China. *Zhonghua Liu Xing Bing Xue Za Zhi* 1990; 11: 360-363.
 21. Joshi D, Choochote W, Park MH, Kim JY, Kim TS, Suwonkerd W, Min GS. The susceptibility of *Anopheles lesteri* to infection with Korean strain of *Plasmodium vivax*. *Malaria J* 2009; 8: 42.
 22. Golenda CE, Klein T, Coleman R, Burge R, Ward RA, Seeley DC. Depletion of total salivary gland protein in blood-fed *Anopheles* mosquitoes. *J Med Entomol* 1995; 32: 300-305.
 23. Korea Centers for Disease Control and Prevention (K-CDC). Diseases Web Statistics System. Accessed at <http://cdc.go.kr/kcdcho-me/jsp/observation/stat/rgt/STATRGT0003List.jsp> on 15 March 2011.
 24. Arirang News/Oct. 05, 2009 10:46 KST http://english.chosun.com/site/data/html_dir/2009/10/05/2009100500466.html