ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol. Vol. 48, No. 4: 351-353, December 2010
DOI: 10.3347/kjp.2010.48.4.351

▣ **BRIEF COMMUNICATION**

Population Dynamics of Five *Anopheles* Species of the Hyrcanus Group in Northern Gyeonggi-do, Korea

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Abstract: To investigate the population densities of potential malaria vectors, *Anopheles* species were collected by light traps in malaria endemic areas, Paju and Gimpo, Gyeonggi-do of Korea. Five *Anopheles* Hyrcanus sibling species (*An. sinensis*, *An. pullus*, *An. lesteri*, *An. kleini*, and *An. belenrae*) were identified by PCR. The predominant species, *An. pullus* was collected during the late spring and mid-summer, while higher population consists of *An. sinensis* were collected from late summer to early autumn. These 2 species accounted for 92.1% of all *Anopheles* mosquitoes collected, while the other 3 species accounted for 7.9%. Taking into account of these population densities, late seasonal prevalence, and long-term incubation period (9-13 months) of the Korean *Plasmodium vivax* strain, *An. sinensis* s.s is thought to play an important role in the transmission of vivax malaria in the study areas.

Key words: *Anopheles sinensis, Anopheles pullus, Anopheles lesteri, Anopheles kleini, Anopheles belenrae, Plasmodium vivax,* malaria, vector, population dynamics

Malaria re-emerged in the Republic of Korea in 1993, despite being declared malaria free in 1979. Over 1,000 cases of vivax malaria are diagnosed annually, with a peak number of cases (4,142) reported in 2000 [1]. Most of the patients reported are from northern Gyeonggi-do (Province) and the north-west Kangwon-do, near the 4 km wide demilitarized zone (DMZ) separating South and North Korea [2].

Eight species of *Anopheles* mosquitoes have been described in Korea; *Anopheles sinensis* sensu stricto (s.s.) Wiedemann, *An. pullus* Yamada (= *An. yatsushiroensis*), *An. lesteri* Baisas & Hu (= *An. anthropophagus*), *An. sineroides* Yamada, *An. kleini* Rueda, *An. belenrae* Rueda, *An. lindesayi japonicus* Yamada, and *An. koreicus* Yamada & Watanabe [3,4]. All of these species, with the exceptions of *An. lindesayi japonicus* and *An. koreicus*, belong to the Hyrcanus group. The 5 sibling species (*An. sinensis*, *An. pullus*, *An. lesteri*, *An. kleini*, and *An. belenrae*) are impossible to distinguish by morphological identification, and therefore identification by PCR was developed to differentiate these species [5-7].

• Received 9 June 2010, revised 19 August 2010, accepted 28 August 2010.
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There is still some debate regarding the primary vector species for malaria in Korea. *An. sinensis* used to be considered the most important vivax malaria vector in Korea, but recently, *An. pullus* and *An. kleini* have been proposed to play important roles in malaria transmission. Lee et al. [1] suggested that *An. kleini*, *An. pullus*, and *An. sinensis* are vectors of malaria in Korea based on the finding that higher proportions of *An. kleini* and *An. pullus* were captured using Mosquito Magnet traps near the base camps of United States forces in Korea, and on detection rates of circumsporozoite protein by ELISA [1]. Joshi et al. [8] suggested that *An. lesteri* is a highly competent vector based on its ability to develop oocysts in the midgut and sporozoites in salivary glands of experimentally infected mosquitoes with a Korean isolate of *Plasmodium vivax* [8]. In this study, we have investigated the population dynamics of members of the *Anopheles* Hyrcanus group collected by light traps at cow sheds in Paju (City) and Gimpo (City) in 2005.

Mosquitoes were collected on 5 occasions in Paju (latitude 37°48′, longitude 126°42′) (Jun 2, Jun 6, Jul 5, Jul 26, and Aug 17, 2005) and twice in Gimpo (latitude 37°43′, longitude 126°39′) (Jun 22 and Sep 21, 2005). Both locations are close to the DMZ, a malaria high-risk area. Light traps were set up at cowsheds and operated throughout the night from 18:00 to 06:00 the following morning. Female mosquitoes were identified using morphological taxonomic keys and descriptions [9-11].

Dry female mosquitoes of the Hyrcanus group were preserved in 75% ethanol for DNA extraction. Genomic DNA from individual mosquitoes was isolated with GeneAll Exgene™ Tissue SV kit (GeneAll, Seoul, Korea). PCR analysis was performed as described by Li et al. [7]. The amplified DNA fragments were separated on 2% agarose gels, and species were identified by size estimation of PCR products with a size marker (øX174/Hae III digest, TaKaRa, Shiga, Japan).

Among the total 1,115 female Hyrcanus group mosquitoes identified by PCR, *An. pullus* was the most commonly collected species (52.4%), followed by *An. sinensis* (39.7%), *An. lesteri* (3.5%), *An. belenrae* (2.3%), and *An. kleini* (2.1%). A total of 22 mosquitoes that produced multiple bands were not included. *An. pullus* was most frequently collected at Paju during June (79.0%) and July (66.7%), whereas *An. sinensis* was most frequently collected during August and September, accounting for 57.2% and 79.0% of the total collected at Paju and Gimpo, respectively. The other 3 species, *An. lesteri*, *An. belenrae*, and *An. kleini* made up less than 10% of the total mosquitoes collected during any collection period (Table 1).

In summary, *An. sinensis* populations increased through the summer until early September, whereas *An. pullus* numbers were highest in late May-June and decreased sharply in August. *An. lesteri*, *An. belenrae*, and *An. kleini* maintained low numbers throughout the season, without having any particular period of prevalence (Fig. 1).

Primary malaria vector species are determined by relative population densities, human feeding habits, susceptibility to malaria parasites, longevity, and other factors. The fact that relative high densities of *An. sinensis* s.s. or *An. pullus* were present and both are susceptible to malaria infection and sporozoite development to the salivary glands implicate these 2 species as primary malaria vectors in the study area. Joshi et al. [8] carried out an experimental infection study and reported that vivax malaria sporozoites were not detected in *An. pullus*, but found in 11% and 64% of *An. sinensis* and *An. lesteri*, respectively [8]. Tiburskaja and Vrublevskaja (1977) experimentally infected 77 humans with the North Korean strain of *P. vivax* through bites of the infected mosquitoes, and resulted that 24.7% of them became ill after a short incubation period (14-22 days) and 75.3% after a long incubation period (1.3% after 1 month, 14.3% after 8 months, 18.2% after 9 months, 18.2% after 10

Table 1. The number of female *Anopheles* mosquitoes captured by light trap in 2005

Site	Date	*sinensis*	*pullus*	*lesteri*	*belenrae*	*kleini*	Total
Paju	June	28 (15.4)	144 (79.1)	4 (2.2)	6 (3.3)	0 (0.0)	182
	July	94 (26.3)	238 (66.7)	3 (0.8)	13 (3.6)	9 (2.5)	357
	August	215 (57.2)	138 (36.7)	14 (3.7)	6 (1.6)	3 (0.8)	376
	Subtotal	337 (36.8)	520 (56.8)	21 (2.3)	25 (2.7)	12 (1.3)	915
Gimpo	June	27 (27.0)	58 (58.0)	8 (8.0)	1 (1.0)	6 (6.0)	100
	September	79 (79.0)	6 (6.0)	10 (10.0)	0 (0.0)	5 (5.0)	100
	Subtotal	106 (53.0)	64 (32.0)	18 (9.0)	1 (0.5)	11 (5.5)	200
	Total	443 (39.7)	584 (52.4)	39 (3.5)	26 (2.3)	23 (2.1)	1,115

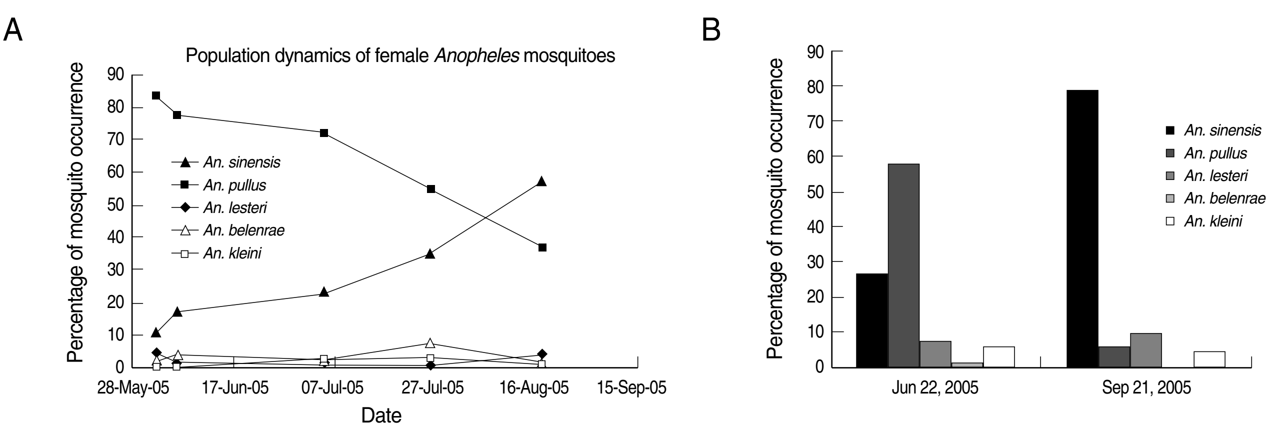

Fig. 1. Population trends of *Anopheles* mosquitoes collected by light trap from Paju (A) and Gimpo (B), Korea.

months, 16.9% after 11 months, 3.9% after 12 months, and 2.6% after 13 months) [12]. Though it is extremely difficult to study the correlation between seasonal occurrences of human cases and vector mosquitoes, most malaria cases of June-August would be infected in the previous year by *An. sinensis* s.s. rather than *An. pullus*. The population densities of *An. lesteri*, *An. belenrae*, and *An. kleini* were too low for these species to be considered important vectors in the study areas, accounting only for 3.5%, 2.3%, and 2.1% of the total mosquitoes examined. Lee et al. [1] studied population densities of the Hyrcanus group using methods similar to ours, and reported that *An. sinensis* s.s. was dominant both in Paju and Gimpo areas, but that *An. kleini* also occurred with considerably high density, making up 40% of totals at Paju and 14.2% at Gimpo [1]. Our studies, in contrast, identified this species in only 1.3% of totals at Paju and 5.5% at Gimpo. Rueda et al. [13] also carried out similar studies with larvae in Munsan and reported that *An. kleini* was most abundant in early summer (May to July), whereas *An. sinensis* was the most frequently collected species in August, September, and October, and *An. pullus* peaked in May [13], which is a similar result with ours, except for the population density of *An. kleini*. More comprehensive and detailed geographic studies throughout the Korean Peninsula are required to better understand the dynamics of malaria transmission.

ACKNOWLEDGEMENTS

This study was supported by a research grant from Health and Medical Technology R&D Program (A030075), Ministry of Health and Welfare, Korea.

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