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 northern Gyeonggi-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 Biais & Hu (= An. anthropophagus), An. sinereoides 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].

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].

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 identi-
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

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**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 |

A

Population dynamics of female *Anopheles* mosquitoes

B

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.

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