

Malaria Vector Surveillance in Ganghwa-do, a Malaria-Endemic Area in the Republic of Korea

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Abstract: We investigated the seasonality of *Anopheles* mosquitoes, including its species composition, density, parity, and population densities of mosquitoes infected with the parasite in Ganghwa-do (Island), a vivax malaria endemic area in the Republic of Korea. Mosquitoes were collected periodically with a dry-ice-tent trap and a blacklight trap during the mosquito season (April-October) in 2008. *Anopheles sinensis* (94.9%) was the most abundant species collected, followed by *Anopheles belenrae* (3.8%), *Anopheles pullus* (1.2%), and *Anopheles lesteri* (0.1%). Hibernating *Anopheles* mosquitoes were also collected from December 2007 to March 2008. *An. pullus* (72.1%) was the most frequently collected, followed by *An. sinensis* (18.4%) and *An. belenrae* (9.5%). The composition of *Anopheles* species differed between the mosquito season and hibernation seasons. The parous rate fluctuated from 0% to 92.9%, and the highest rate was recorded on 10 September 2008. Sporozoite infections were detected by PCR in the head and thorax of female *Anopheles* mosquitoes. The annual sporozoite rate of mosquitoes was 0.11% (2 of 1,845 mosquitoes). The 2 mosquitoes that tested positive for sporozoites were *An. sinensis*. Malarial infections in anopheline mosquitoes from a population pool were also tried irrespective of the mosquito species. Nine of 2,331 pools of *Anopheles* mosquitoes were positive. From our study, it can be concluded that *An. sinensis*, which was the predominant vector species and confirmed as sporozoite-infected, plays an important role in malaria transmission in Ganghwa-do.

Key words: *Anopheles* mosquito, *Plasmodium vivax*, sporozoite rate, parous rate, PCR, Ganghwa-do

INTRODUCTION

Malaria is a vector-borne infectious disease caused by several species of protozoan parasites. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Only vivax malaria is endemic to the Republic of Korea (ROK). The outbreak area is confined to the northern part of Gyeonggi-do (province), Gangwon-do (province), and Incheon city (including Ganghwa-do), near the demilitarized zone (DMZ; the border between ROK and the Democratic People's Republic of Korea, DPRK). Ganghwa-do (Island) has the highest reported incidence of malaria among these areas [1].

Eight species of *Anopheles* mosquitoes are found in ROK; *Anopheles sinensis* Wiedemann*, *An. pullus* M. Yamada*, *An. lesteri* Baisas and Hu*, *An. kleini* Rueda*, *An. belenrae* Rueda, *An. sineoides* Yamada, *An. koreicus* Yamada and Watanabe, and *An. lindesayi japonicus* Yamada [2-4]. The 4 *Anopheles* species with an

astrix (*) have been confirmed as vectors of vivax malaria in KOK [4-6]. Among these species, *An. sinensis* is the most widespread, whereas the others are variably distributed in each area [7]. Factors that affect the *Anopheles* species' ability to transmit malaria include its innate susceptibility to *Plasmodium*, its host choice, and the longevity. There are also variations in these parameters within a same mosquito species [8].

Malaria transmission ultimately depends upon the sporozoite positive rate of vector mosquitoes. Therefore, determining the presence of sporozoites in *Anopheles* mosquitoes caught in the wild is an important factor in epidemiological studies in malaria endemic areas [9]. The standard method for detecting sporozoites in mosquito salivary glands is based on light microscopy. However, the salivary gland dissection is impractical when large numbers of mosquitoes are to be processed. In recent years, the PCR method has been used to amplify specific DNA sequences of *Plasmodium* for detection of parasites in mosquitoes [10,11].

Despite efforts of previous studies on malaria in ROK, our knowledge of the vector systems and their contribution to malaria transmission remains incomplete. More information on the vector systems will help the study of malaria epidemiology and

• Received 10 July 2009, revised 26 October 2009, accepted 19 December 2009.

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benefit the vector control programs. In the present study, in view of the seasonal incidence of malaria in ROK, we investigated a survey on the seasonal composition, density, longevity, and parasite infection rate of *Anopheles* species, to determine the sporozoite rates and to confirm the main vector in Ganghwa-do.

MATERIALS AND METHODS

Study sites and mosquito collection

The study was conducted in Ganghwa-do, where the incidence of malaria was 1.2 cases/1,000 persons per year during the study period of 2008 [1]. Ganghwa-do has a total population of 65,510 and is consisted with an area of 411.189 km². It is located at the west end of the central Korean peninsula (37°-31'-37°45' N, 125°33'-126°2' W), and the main Ganghwa-do Island shares a border with Yeonbaek-gun and Gaepung-gun of DPRK. Mosquitoes were collected from April to October 2008 at 9 study sites.

Mosquitoes were periodically collected at 3 study sites, i.e., Gyosan-ri (Yangsa-myeon, S.1), Namsan-ri (Ganghwa-eup, S.2), and Chogi-ri (Gilsang-myeon, S.3), using a dry-ice-tent trap and a blacklight trap (Nozawa type; Shinyoung Korea Co., Seoul, Korea). The traps were placed around the villages, and collections began at 19 : 00 and ended at 06 : 00 the next morning. The captured mosquitoes were transported to the laboratory within 2 hr and stored at -18°C until processing. The female anopheline mosquitoes were determined parity, identified, and individually detected parasites.

Mosquitoes were collected at another 6 study sites, i.e., Dae-ryong-ri (Gyodong-myeon, S.4), Seokmo-ri (Samsan-myeon, S.5), Seogom-ri (Samsan-myeon, S.6), Sungro-ri (Songhae-myeon, S.7), Daesan-ri (Ganghwa-eup, S.8), and Geumwol-ri (Seonwon-myeon, S.9), using a blacklight trap. Mosquito collection at these sites has been conducted by the Korea Centers for Disease Control and Prevention (KCDC) during a research for National Vector Control and Surveillance. The traps were set every night throughout the study period (30 weeks). At each of the 6 study sites, the dried bodies of the captured mosquitoes were transported weekly to the laboratory. The female anopheline mosquitoes were counted and detected parasites in pools because of the large numbers collected.

Parous rate, probability of daily survival, and P¹¹

At each of the collection times, the anopheline mosquitoes collected at the 3 study sites (S.1-S.3) were dissected to deter-

mine their parity according to the method of World Health Organization (WHO) [12]. Mosquitoes stored at -18°C were dissected within 5 days to avoid freeze-drying. The probability of daily survival was calculated as the cube root of the proportion of parous females in the population sample, because the gonotrophic cycle takes 3 days in Korea [13]. P¹¹ was calculated as the 11th root of the proportion of parous females in the population sample, because *Plasmodium vivax* requires 10.7 days to mature and to migrate to the salivary gland at 25°C [14].

Identification of mosquitoes

Anopheline mosquitoes were identified to the species level using a PCR method with multiplex primers. The genomic DNA was extracted from single legs of individual mosquitoes [2,4], according to the manufacturer's instructions of DNAzol (Invitrogen, USA).

Extraction of *Plasmodium* DNA from mosquitoes

At each of the 3 study sites (S.1-S.3), DNA was extracted from individual mosquitoes after the wings, legs, and abdomens were removed. The head and thorax were placed in individual 1.5 ml microcentrifuge tubes (Axygen, USA) containing 100 μ l of DNAzol.

At each of the 6 study sites (S.4-S.9), DNA was extracted from pooled mosquitoes. *Anopheles* mosquitoes were grouped into pools of up to 10-20 in number, irrespective of the species. To each pool was added 500 μ l of DNAzol in 1.5 ml tubes. The tissues, prepared as described above, were ground with a pellet pestle (Kimble/Kontes, USA). To overcome PCR inhibitors in the mosquito extracts [15,16], we added 500 μ l of sterile water and incubated the samples at 37°C overnight [17]. DNA extraction was performed according to the manufacturer's instructions of DNAzol. The eluted DNA was stored at 4°C until required.

Detection of *P. vivax* in mosquitoes by PCR

Nested PCR was used to detect the presence of malarial parasites in the mosquitoes with *P. vivax* species-specific small subunit (SSU) rRNA primers [18]. The reaction mixture included the Maxime PCR premix kit (iNtRON Biotechnology, Seoul, Korea), to which was added the template, 1 μ l of each primer (20 pmol/ μ l), and distilled water to a total volume of 20 μ l. The reactions were subjected to 30 cycles (nest-1) of the following conditions: predenaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. Nest-

2 was the same as nest-1, except that 38 cycles were performed and the annealing temperature was 50°C. The DNA of *P. vivax* from clinical patients was used as the positive control and sterile water was used as the negative control.

Survey of hibernation

Hibernating mosquitoes were collected from December 2007 to March 2008 at 6 study sites, i.e., Gogu-ri (Gyodong-myeon, H.S.1), Gyosan-ri (Yangsa-myeon, H.S.2), Sangdo-ri (Songhae-myeon, H.S.3), Changhu-ri (Hajeom-myeon, H.S.4), Daesan-ri (Ganghwa-eup, H.S.5), Chogi-ri (Gilsang-myeon, H.S.6), using a sunlit tent trap, which covered the dried heavy grasses on the bank around a rice field (Fig. 1). When the temperature inside the tent was increased by sunlight and a portable heater, the hibernating mosquitoes flew out from grasses in a few minutes. The mosquitoes, collected using a mouth aspirator, were placed in screen-topped pint cartons, provided with a 5% sugar solution on cotton wool, and transported to the laboratory. The mosquitoes were stored at -18°C until they were processed for DNA extraction.

RESULTS

Mosquito species and seasonal prevalence

Mosquitoes collected periodically from April to October at 3

study sites (S.1-S.3) in Ganghwa-do showed seasonal prevalence of the *Anopheles* species (Table 1). The density of anopheline mosquitoes was high in July, August, and early September. *Anopheles sinensis* (94.9%) was the most abundant species, followed by *An. belenrae* (3.8%), *An. pullus* (1.2%), and *An. lesteri* (0.1%). The nocturnal activities of the mosquitoes were confirmed between April and October (Table 2).

Detection of *P. vivax* in mosquitoes

Details of the malarial infection rates of the mosquitoes are shown in Tables 1 and 2. We analyzed 1,845 individual *Anopheles* mosquitoes collected at sites S.1-S.3. In total, 2 *Anopheles* mosquitoes were found to be infected. Therefore, the annual sporozoite rate was 0.11% in Ganghwa-do. The infected mosquitoes were collected on July 8 in Gyosan-ri (S.1) and on September 10 in Namsan-ri (S.2). The *Anopheles* mosquitoes positive for the malarial parasite were *An. sinensis*.

At sites S.4-S.9, a total of 29,346 *Anopheles* mosquitoes were captured. Nine of the 2,331 pools of mosquitoes examined were positive for malarial parasites. Of the 9 pools of malaria-positive mosquitoes, 1 was collected in the period 4-10 July, 1 in the period 25-31 July, 4 in the period 4-8 August, 2 in the period 22-28 August, and 1 in the period 26 September to 2 October. The total 11 samples that were positive on PCR were confirmed by DNA sequencing. The sequencing results confirmed that the

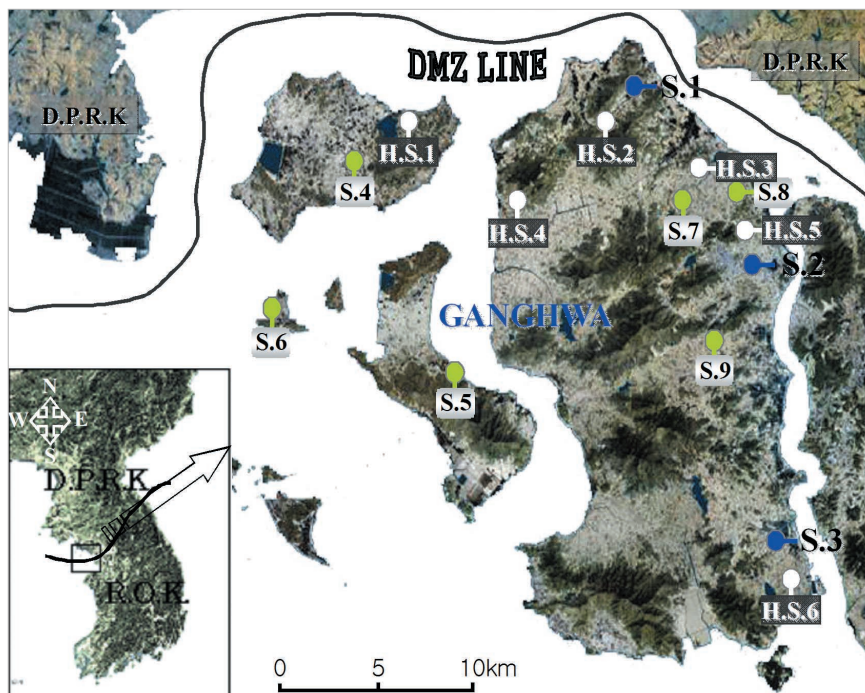


Fig. 1. Map of Ganghwa-do (Republic of Korea) showing the locations of the 15 study sites.

Table 1. The number of *Anopheles* mosquitoes collected in Gangwha-do (2008) which were analyzed by PCR to detect *Plasmodium vivax* infection

Collection date	<i>Anopheles sinensis</i>		<i>An. belenrae</i>		<i>An. pullus</i>		<i>An. lesteri</i>		Total	
	No. of Mosq.	No. of positive	No. of Mosq.	No. of positive	No. of Mosq.	No. of positive	No. of Mosq.	No. of positive	No. of Mosq.	No. of positive
22 May	0	-	0	-	1	0	0	-	1	0
4 Jun	0	-	1	0	1	0	0	-	2	0
12 Jun	11	0	0	-	1	0	0	-	12	0
26 Jun	52	0	4	0	0	-	0	-	56	0
8 Jul	91	1	3	0	0	-	0	-	94	1
4 Aug	206	0	7	0	0	-	0	-	213	0
19 Aug	419	0	32	0	0	-	0	-	451	0
25 Aug	378	0	9	0	0	-	0	-	387	0
3 Sep	211	0	12	0	0	-	0	-	223	0
10 Sep	283	1	15	0	5	0	0	-	303	1
17 Sep	32	0	3	0	1	0	0	-	36	0
23 Sep	20	0	1	0	2	0	0	-	23	0
30 Sep	3	0	1	0	8	0	0	-	12	0
8 Oct	5	0	0	-	2	0	1	0	8	0
15 Oct	3	0	1	0	0	-	0	-	4	0
21 Oct	11	0	6	0	1	0	0	-	18	0
28 Oct	1	0	1	0	0	-	0	-	2	0
Total	1,726	2	96	0	22	0	1	0	1,845	2

amplified fragments were identical to the SSU rDNA fragment of *P. vivax*.

Parous rate, probability of daily survival, and P^{11}

The parous rate was fluctuated from 0.0% to 92.9%, and the highest rate was recorded on 10 September. The probability of daily survival and P^{11} are shown in Table 3. The expectancy of an infective life through 11 days was 0.766 on 10 September, which means that 76.6% of the female mosquitoes may have lived long enough to transmit malaria.

Hibernation of anopheline mosquitoes

Of the 662 hibernating *Anopheles* mosquitoes collected, *An. pullus* (72.1%) was the most frequently collected, followed by *An. sinensis* (18.4%) and *An. belenrae* (9.5%) (Table 4). These results differ from the distribution of *Anopheles* mosquitoes during the mosquito season (April-October).

DISCUSSION

Knowledge of mosquito bionomics, including their seasonal distributions and population densities, is a prerequisite for a comprehensive understanding of mosquito-borne diseases. However, few studies of the malaria vector have been undertaken in the Ganghwa-do region. Eight species of *Anopheles* mosquitoes

are found in ROK. Among them, 2 new species of *Anopheles* mosquitoes have been recently identified (*An. kleini* and *An. belenrae*) [3], while *An. yatsushiroensis* was synonymized with *An. pullus*, and *An. anthropophagus* Baisas and Hu, considered to be a primary malaria vector in China, was synonymized with *An. lesteri* [4]. Therefore, before 2005, there was much confusion concerning the vector status of these species, which can be identified by PCR techniques, but not based on morphology.

In our study, mosquito collection was conducted during the mosquito season (April-October) and the hibernation season (December-March) to investigate the seasonal distributions of malaria vectors. The density of *Anopheles* mosquitoes was high in July, August, and early September. *An. sinensis* (94.9%) was the most abundant *Anopheles* species collected. *An. belenrae* was consistently collected as a small proportion of the mosquitoes and *An. pullus* was collected for a limited period in June, September, and October, at a relatively low temperatures. *An. kleini* is particularly known for its distribution near the DMZ [6,7], but was not identified in Ganghwa-do. Although the 4 species described above are known to be collectable with a light trap and dry-ice trap [6,7], further studies are required to confirm the collection of species by other methods. In the hibernation season, *An. pullus* (72.1%) was the most frequently collected species, followed by *An. sinensis* (18.4%) and *An. belenrae* (9.5%). We confirmed that the composition of *Anopheles* species showed

Table 2. Detection of *Plasmodium vivax* by PCR in pooled anopheline mosquitoes collected in Ganghwa-do, Incheon city, during the mosquito season of 2008

Collection date	Daeryong (S.4)			Seokmo (S.5)			Seogeom (S.6)			Sungroe (S.7)			Daesan (S.8)			Geumwol (S.9)		
	No. of <i>Anopheles</i>	No. of pooling	Malaria positive No.	No. of <i>Anopheles</i>	No. of pooling	Malaria positive No.	No. of <i>Anopheles</i>	No. of pooling	Malaria positive No.	No. of <i>Anopheles</i>	No. of pooling	Malaria positive No.	No. of <i>Anopheles</i>	No. of pooling	Malaria positive No.	No. of <i>Anopheles</i>	No. of pooling	Malaria positive No.
11 Apr-1 May ^a	0	-	-	0	-	-	3	2	0	3	3	0	4	3	0	0	-	-
2-22 May ^a	0	-	-	2	1	0	10	2	0	4	3	0	16	3	0	0	-	-
23-29 May	0	-	-	0	-	-	1	1	0	0	-	-	20	2	0	2	1	0
30 May-4 Jun	0	-	-	8	1	0	0	-	-	10	1	0	7	1	0	6	1	0
5-12 Jun	5	1	0	5	1	0	60	6	0	-	-	-	-	-	-	9	1	0
13-19 Jun	4	1	0	12	2	0	-	-	-	10	1	0	14	2	0	4	1	0
20-26 Jun	1	1	0	132	14	0	132	14	0	32	4	0	120	12	0	44	5	0
27 Jun-3 Jul	19	2	0	310	31	0	247	25	0	50	5	0	200	20	0	36	4	0
4-10 Jul	74	8	0	600	60	0	947	95	0	800	80	0	57	6	1	110	11	0
11-17 Jul	98	10	0	680	68	0	470	47	0	592	60	0	560	56	0	98	10	0
18-24 Jul	160	16	0	276	30	0	97	10	0	35	4	0	40	4	0	61	6	0
25-31 Jul	226	23	0	1,038	104	1	84	9	0	134	14	0	298	30	0	30	3	0
1-7 Aug	90	9	0	1,300	130	0	-	-	-	157	16	0	46	5	0	4	1	0
8-14 Aug	338	34	0	1,720	86 ^b	2	347	35	2	39	4	0	190	19	0	17	2	0
15-21 Aug	4	1	0	2,460	123 ^b	0	335	34	0	45	5	0	820	82	0	51	5	0
22-28 Aug	960	96	2	1,994	100 ^b	0	1,580	79 ^b	0	207	21	0	169	17	0	16	2	0
29 Aug-4 Sep	134	14	0	1,880	94 ^b	0	1,240	62 ^b	0	57	6	0	82	18	0	15	2	0
5-11 Sep	206	21	0	1,012	51 ^b	0	1,468	74 ^b	0	84	9	0	43	5	0	23	3	0
12-18 Sep	8	1	0	86	9	0	84	9	0	30	3	0	30	3	0	13	2	0
19-25 Sep	10	1	0	89	9	0	169	17	0	52	6	0	34	4	0	14	2	0
26 Sep-2 Oct	1	1	0	12	2	0	126	13	1	4	1	0	34	4	0	13	2	0
3-9 Oct	3	1	0	19	2	0	113	12	0	4	1	0	20	2	0	11	1	0
10-16 Oct	0	-	-	-	-	-	-	-	-	8	1	0	7	1	0	6	1	0
17-23 Oct	0	-	-	4	1	0	76	8	0	1	1	0	7	1	0	2	1	0
24-30 Oct	0	-	-	0	-	-	2	1	0	0	-	-	13	2	0	1	1	0
Total	2,341	241	2	13,639	919	3	7,591	555	3	2,358	248	0	2,831	300	1	586	68	0

^aThese data were summed for numbers of mosquitoes collected during 3 weeks, because of small numbers of captured mosquitoes.

^b20 mosquitoes were pooled, whereas the other pools consisted of 10 mosquitoes.

marked difference between seasons. These data suggest that *An. sinensis* is sensitive to low temperatures compared with the other species, and that *An. pullus* is resistant to low temperatures.

Any mosquito that lives for less than the duration of the sporogonic cycle will be unable to transmit infection through sporozoite production. Therefore, mosquito longevity is an important factor in malarial transmission and can be estimated from the parous rate of the mosquito. The parous rates of *Anopheles* mosquitoes in Ganghwa-do fluctuated from 12.5% to 92.9% between June and October. It is noteworthy that there was a high parous rate time with no reduction in the population and thereafter the population of mosquitoes decreased dynamically. This finding indicates that older populations of mosquitoes tend to accumulate with time and that relatively few new adults emerge during this period. This also shows that the environmental conditions for mosquito survivorship deteriorated sud-

denly at this time.

PCR was used to identify sporozoite-infected mosquitoes because false positive results have been recorded with ELISA [19,20]. Vythilingam et al. [21] reported that 20 pools were positive for *P. vivax* according to PCR, whereas only 7 were positive on ELISA. Two pools were positive for *P. falciparum* by both ELISA and PCR [21]. Most malaria prevalence areas were difficult to determine because the sporozoite rate was exceedingly low. Therefore, the highly sensitive and specific PCR is considered the best method for determining the exact sporozoite rate.

In areas of Africa with high malarial transmission, the sporozoite rate generally ranges from 1% to 20% when evaluated by dissection of salivary glands [22]. The sporozoite rate in Bangladesh was determined to be 2.8% using PCR [23]. In ROK, it was 0.09% in Paju-shi in 2000 [24] and 4% in northern Gyeonggi-do (province) in 2005 [6]. However, both these studies

Table 3. The parous rate, probability of daily survival, and P¹¹ of *Anopheles* species collected at 3 study sites (S.1-S.3) in Ganghwa-do (2008)

Collection date	No. of dissected	No. of parous	Parous rate (%)	Probability of daily survival ^a	P ¹¹ (%) ^b
22 May	1	0	0.0		
4 Jun	2	0	0.0		
12 Jun	12	4	33.3	0.691	1.7
26 Jun	49	22	44.9	0.766	5.3
8 Jul	83	34	41.0	0.743	3.8
4 Aug	196	122	62.2	0.854	17.6
19 Aug	435	224	51.5	0.802	8.8
25 Aug	333	176	52.9	0.809	9.7
3 Sep	206	151	73.3	0.902	32.2
10 Sep	285	265	92.9	0.976	76.6
17 Sep	31	20	64.5	0.864	20.0
23 Sep	19	14	73.7	0.903	32.6
30 Sep	12	5	41.7	0.747	4.0
8 Oct	8	1	12.5	0.5	0.05
15 Oct	3	0	0.0		
21 Oct	14	0	0.0		
28 Oct	2	0	0.0		
Total	1,691	1,038	61.4	0.850	

^aThe probability of a mosquito surviving through 1 day.

^bThe probability of a mosquito surviving through 11 days.

detected the malarial parasite by ELISA. In our study, 2 malaria-positive *Anopheles* mosquitoes were detected among 1,845 mosquitoes by PCR, indicating an annual sporozoite rate in Ganghwa-do of 0.11%. The infected mosquitoes were *An. sinensis*. Because the malarial parasite DNA was extracted from the head and thorax and not from the whole mosquito, we confirmed that all the positive *Anopheles* mosquitoes had infective sporozoites.

At the 6 study sites S.4-S.9, we examined malarial parasite infections in population pools of mosquitoes. We also determined the relationships between parasite infection rates in the mosquitoes and the incidence of malaria in patients. Malaria-positive mosquitoes were detected at 4 study sites. These sites had higher incidences of malaria than those of the other areas in Ganghwa-do [1]. Mosquito collection has also been performed annually at these study sites by KCDC. Therefore, a program of continuous monitoring should confirm the annual variations in parasite infection rates in the mosquito.

Lee et al. [6] reported that the infection rate of *Anopheles* species was 9.2% for *An. pullus*, 3.2% for *An. sinensis*, and 1.8% for *An. kleini* in northern Gyeonggi-do (province) in 2005, using ELISA [6]. However, in our study, we found the malarial parasite only in *An. sinensis*, which may be attributable to the low abundance

Table 4. Number (percentage) of *Anopheles* species collected at hibernation sites in Ganghwa-do between December 2007 and March 2008

Collection site	Collection date	No. of collected mosquitoes	<i>Anopheles</i> species (%)		
			<i>Sinensis</i>	<i>Belensrae</i>	<i>Pullus</i>
Gogu (H.S.1)	27 Dec	151	25 (16.4)	9 (5.9)	117 (77.0)
Gyosan (H.S.2)	13 Dec, 3 Mar	85	9 (10.6)	28 (32.9)	48 (56.5)
Songdo (H.S.3)	5 Dec	134	36 (26.9)	16 (11.9)	82 (61.2)
Changhu (H.S.4)	14 Dec, 14 Feb	102	2 (2.0)	1 (1.0)	99 (97.1)
Daesan (H.S.5)	21 Dec	82	8 (9.8)	4 (4.9)	70 (85.4)
Chogji (H.S.6)	26 Dec	108	42 (38.9)	5 (4.6)	61 (56.5)
Total		662	122 (18.4)	63 (9.5)	477 (72.1)

of the other species in Ganghwa-do. Although the difference in the innate susceptibility of the different *Anopheles* species is not well known, our results indicate that the infection rate in *An. pullus* was 2.9-fold higher than that in *An. sinensis* in the field. In Korea, the malarial life cycle is suspended in the mosquito's hibernation season and then resumes in the mosquito season. Therefore, the high density of *An. pullus* in the hibernation season may promote the resumption of the malarial life cycle. To confirm these propositions, further studies are required to investigate the vectorial capacity of *Anopheles* species, including their host preferences and innate susceptibility to *P. vivax* infection.

Because the high density and sporozoite infection of *An. sinensis* were confirmed, we consider that *An. sinensis* is the main vector of *P. vivax* in Ganghwa-do. Our results constitute the first report of the sporozoite rate calculated in individual *Anopheles* mosquitoes in ROK using PCR. Our study should also extend our understanding of the prevalence of malaria, and benefit vector control in Ganghwa-do.

ACKNOWLEDGEMENTS

We thank the staff of the Public Health Centers of Ganghwa-do for their efforts in mosquito collection. We also appreciate the efforts of Yeon Ja Go, Gyeong Ae Kim, Gyu Cheol Lee, Uli Hwa Hwang, and Seong Hui Gwon at the Incheon Research Institute for Public Health and the Environment. This work was funded by the National Vector Control and Surveillance, the Construction of a Regional Diagnostic System, and the Incheon Research Institute for Public Health and the Environment.

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