

Malaria transmission potential by *Anopheles sinensis* in the Republic of Korea

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Abstract: To evaluate the factors that determine the transmission level of vivax malaria using vectorial capacity, entomological surveys were conducted from June to August, 2000. From 6 nights of human-bait collection in Paju, the human biting rate (ma) was counted as 87.5 bites/man/night. The parity of *Anopheles sinensis* from human baiting collections fluctuated from 41% to 71% (average 48.8%) of which the rate gradually increased as time passed on: 35.2% in Jun.; 55.0% in July; 66.2% in Aug. From this proportion of parous, we could estimate the probability of daily survival rate of *An. sinensis* to be 0.79 assumed with 3 days gonotrophic cycle and the expectancy of infective life through 11 days could be defined as 0.073. Blood meal analysis was performed using ELISA to determine the blood meal source. Only 0.8% of blood meals were from human hosts. We could conclude that *An. sinensis* is highly zoophilic (cow 61.8%). Malaria is highly unstable (stability index < 0.5) in this area. From these data, vectorial capacity (VC) was determined to be 0.081. In spite of a high human biting rate (ma), malaria transmission potential is very low due to a low human blood index. Therefore, we could conclude that malaria transmission by *An. sinensis* is resulted by high population density, not by high transmission potential. For this reason, we need more effort to decrease vector population and vector-human contact to eradicate malaria in Korea.

Key words: feeding behavior, *Anopheles*, parity, longevity, human bite, mosquito, *Plasmodium vivax*

INTRODUCTION

Malaria is one of the most important vector borne diseases, transmitted by infected Anopheline mosquitoes when they feed on humans for their oviposition. In 1993, a young soldier who apparently had no history of traveling abroad was found to be infected with *P. vivax* (Chai et al., 1994). Since then, malaria has increased exponentially year after year,

totaling 13,904 cases by December 2000. Most of the malaria patients were either local residents along the border or they were once enrolled in militaries near demilitarized zone (DMZ). Furthermore, some patients had history of traveling near the border for pleasures such as fishing. There are 7 *Anopheles* species reported in Korea, and only two species, *An. sinensis* (Ree et al., 1967) and *An. yatsushiroensis* (Hong, 1977), were found to be the vector capable of transmitting *P. vivax*. *An. sinensis* was usually the dominant species, hence, recognized as primary vector.

There are two basic approaches to measure

• Received 14 March 2001, accepted after revision 11 May 2001.

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the risk of transmission of malaria in endemic areas. Transmission rates can be measured directly estimated by entomological inoculation rate (EIR) or indirectly by vectorial capacity (VC). EIR can be determined by multiplying mosquito biting-rate by the proportion of mosquitoes carrying sporozoite in their salivary glands. EIR is expressed in terms of average numbers of infective bites per person per unit time. Unlike most other parts of the world where it is difficult to determine EIR because of exceedingly low sporozoite rates, many available studies of transmission have been conducted in Africa where sporozoite rates generally range from 1% to 20%. Vectorial capacity was defined by Garret-Jones (1964) from formulae developed by Macdonald (1957). Vectorial capacity is defined as the expected number of new infections per infective case per day, assuming that all Anopheline biting that case become infected.

Determination of vectorial capacity requires measurements of man-mosquito contact, vector survivorship, and the mosquito density. Human blood index (HBI) is the proportion of human blood feed mosquito per night per female mosquito. Human blood source from the mosquito can be identified by hemoglobin crystallization method (Kim, 1994), hemoglobin precipitation test (Gad et al., 1995), enzyme linked immunosorbent assay (ELISA; Loyola et al., 1993), or DNA dot blot method (Sato et al., 1992).

Another key factor is mosquito longevity because mosquito can spread malaria parasite after imported parasite brood sporozoite. Such factor is dependent on the temperature and determines from the parity. *P. vivax* need 10.7 days at 25°C (WHO, 1975) to breed sporozoite in mosquito body, which is the average temperature during summer season in Korea. Obviously, any mosquito that lives less than this period will not be able to transmit infection through sporozoite.

The present study was conducted to evaluate the intensity and the pattern of malaria transmission in malaria endemic areas using vectorial capacity (VC) and stability index (SI). This report includes observations of the parity, human blood index, life expectancy, and human biting index of *An.*

sinensis, which is the primary malaria vector and the most abundant Anopheline in Korea. This paper describes the first study of VC and SI in Korea.

MATERIALS AND METHODS

Human baiting collection

To estimate human-biting rates, mosquitoes were fortnightly collected using volunteer human-bait catches from June to August, 2000 in Baekyeon-ri (Tongilchon), Paju-shi, Gyeonggi-do. The catchers, with the aid of torches and aspirator, collected host-seeking mosquitoes that landed on their naked legs at half-hour intervals, followed by a half-hour rest, from the sunset to the sunrise (7:00 PM to next 6:00 AM). To diminish attract and collection bias, the collectors and bait-volunteers changed their role at every 15 min intervals. Collected mosquitoes were placed in screened-topped pint cartons separating mosquitoes collected each hour. After each trap night, mosquito collections were sent to the laboratory where they were separated, counted, and identified using keys specific to Korean mosquitoes (Lee, 1998).

Parous rate (Parity)

Anopheles sinensis collected by human-bait were dissected daily to determine parity followed by WHO method (1994 a, b). *An. sinensis* were anesthetized with chloroform and dissected for ovary under a stereomicroscope. Under microscope ($\times 100$), females of which the ovaries have coiled tracheolar skeins are nulliparous and have become stretched out are parous. When some eggs were retained in the ovaries, then they were separated as parous. Less than 20 mosquitoes were dissected during a single collection time.

Human Blood Index (HBI)

Blood engorged Anopheline were collected from natural shelters (i.e. bush) and man-made structures (i.e. empty cow-shed, small warehouse, and concrete bunker), which were located in the same village as the night-biting collections using CDC backpack aspirator (John W. Hock Co., Florida, USA). All

collection sites were at least 10 m away from the blood sources (cow-shed and pigsty). Mosquito collection was carried before 8 hour in the morning.

Mosquitoes were classified as blood fed, gravid, or empty and kept separate at a time of collection. The abdomens of 20% of the blood-fed females were smeared onto Whatman No. 2 filter paper and were allowed to dry. Then they were wrapped with waxed papers and stored 4°C until they were further processed. All humans and domestic animals (cows, pigs, dogs, and chickens) were censused during each mosquito collection period in the study area.

The direct enzyme-linked immunosorbent assay (ELISA) modified by Loyola et al. (1993) was used to identify the source of the blood meal. Mosquito samples were eluted overnight in 0.2 ml of phosphate buffered saline (PBS, pH 7.4). Five μ l of each sample were placed with 50 μ l coating buffer (Sodium bicarbonate 35 mM, Sodium carbonate 15 mM, pH 9.6) in six wells of a microtiter plate (Nunk, Denmark) and incubated for 1 h at room temperature. After blocking unreacted sites with 2.5% dry skimmed milk in PBS buffer (pH 7.4), samples were treated with either rabbit anti-human, cow, dog, pig, cat, or chicken IgG (Sigma, St. Louis, USA) and incubated for 2 hr at room temperature followed by horse-radish peroxidase-conjugated anti-rabbit IgG goat serum (Sigma, USA). The color was developed using 100 μ l ABTS + H₂O₂ (1:1) solution (KPL, Maryland, USA) and read on ELISA reader (EL 340, Bio-Kinetics Instruments Co., USA) at 405 nm. The blood samples from the hosts were dried on filter paper and used as positive controls. A positive reaction was defined as an absorbance value at least 2 times above the mean absorbance of the highest cross-reacting serum among the available hosts.

Estimation of Vectorial capacity (VC)

The vectorial capacity was calculated using the Garrett-Jones and Shidrawi (1969), i.e. $VC=ma^2P^n/-\ln P$. The following variables were used to estimate the VC of *An. sinensis* to transmit malaria: ma, the human biting rate, i.e., the number of female mosquitoes per person per night; a, the human-biting

frequency per day which is indirectly estimated as the human blood index (HBI) divided by duration of gonotrophic cycle (gc) (Molineaux et al., 1988); P, the daily survival rate of the vector, was estimated from the formula $parity^{1/gc}$ (Davidson, 1954); and n, the length of the sporogonic cycle.

Stability index (SI)

The stability index was calculated using Gilles and Warrel (1993). The stability index of transmission of malaria depends on the human biting habit of the main vector (a) and its probability of survival through 1 day (P), and can be derived from the expression: $a/-\ln P$.

RESULTS

Human biting rate (ma)

The human bait collection results in Baekyeon-ri (Tongilchon), where 12 malaria cases were diagnosed until 1999 {API (malaria case per 1000 persons) = 27.2}, are shown in Table 1. *An. sinensis* was consistently the most abundant biting mosquito (89.3%) of the mosquitoes collected from 6 nights of collection from June to August, 2000. Another malaria vector, *An. yatsushiroensis*, was collected from 10 individuals (1.7%) during the entire collection period. Even though this species can mediate malaria, they do not have any epidemiological role in spreading malaria due to their low population density. Human biting rate of *An. sinensis* varied significantly from 7 to 301 bites/human/day in the study area (Table 1). In this area, the rice-planting season is from late in May to early in June, and consequently, there is enough water for mosquito to breed in the rice paddies. For this reason, human biting rate reached as high as 301 bites/human/day for *An. sinensis* in late June. An average human biting rate during survey periods was 87.5 bites/human/day.

Parity (PR)

The result of ovary dissection is shown in Table 2. In the study area, a total of 330 *An. sinensis* females from the summer season were dissected for ovaries, with 0.488 parity in average (0.352 - 0.710). In the early summer,

Table 1. Outdoor human baiting collections in Baegyeon-ri, Paju , 2000

Date	Jun. 22	Jun. 28	Jul. 12	Jul. 24	Aug. 9	Aug. 23	Total	Ratio (%)
<i>Anopheles sinensis</i>	7	301	59	78	12	68	525	89.3
<i>yatsushiroensis</i>	3	6	0	1	0	0	10	1.7
<i>Aedes vexans</i>	9	29	1	7	0	0	46	7.8
<i>Culex tritaeniorhynchus</i>	0	0	0	0	1	1	2	0.3
<i>orientalis</i>	0	1	0	0	0	0	1	0.2
<i>pipiens pallens</i>	1	0	1	0	2	0	4	0.7
Total	20	337	61	86	15	69	588	
A.s Ratio (%) ^a	35.0	89.3	96.7	90.7	80.0	98.6	89.3	

^a)A.s. Ration: *Anopheles sinensis* ratio

Table 2. Parity of *Anopheles sinensis* from human bait collection mosquitoes in Baegyeon-ri, Paju, 2000

Date	Test mosquitoes	Parous	Nulli-parous	Parity
Jun. 28	145	51	94	0.352
Subtotal Jun.	145	51	94	0.352
Jul. 12	49	31	18	0.633
Jul. 24	62	30	32	0.484
Subtotal Jul.	111	61	50	0.550
Aug. 9	12	5	7	0.417
Aug. 23	62	44	18	0.710
Subtotal Aug.	74	49	25	0.662
Total	330	161	169	0.488

the parity was low (0.352 in June 28) which suggested that the new emerging species were more abundant than the older ones. Parity reached the highest value on August 23, 2000 (0.710), one month after the raining season. After the raining season, most of the water was drained from the rice paddies. Therefore, the shortage of water caused decreased breeding places and hence increased parity.

Human blood index (HBI)

The results of blood meal identification from resting mosquito used by ELISA are shown in Table 3. In total, 340 resting *An. sinensis* were collected, of which 241 were tested for blood meal identification. Bovines (61.8%) and Pigs (21.9%) were the main blood sources for *An. sinensis* collected outdoors. Human blood was detected in 2 individuals (0.8%) of the blood-fed females. Therefore, we counted 0.008 human blood index (HBI) at the study area during the summer season of 2000. Due to a low human blood index, we were not able to detect seasonal variation of HBI.

Vectorial capacity (VC)

A summary of entomological indices necessary for calculating vectorial capacity is given in Table 4. In Paju-shi, where the mean temperature during the summer season is 25°C based on the weather station data, the sporogonic cycle for *P. vivax* was estimated to be 10.7 days. From this data, if we assume gonotrophic cycle (*gc*) to be 3 days, then VC is 0.081. However, we were not able to show the seasonal variation of malaria transmission because of very low HBI.

Stability index (SI)

The index of stability was estimated to be 0.013 in Paju-shi, based on the data for *An. sinensis* in Table 4. This data indicated that malaria in Paju-shi was highly unstable.

DISCUSSIONS

To express malaria transmission risk, vectorial capacity (VC), meaning receptivity to

Table 3. Blood-meal identification of *Anopheles sinensis* collected in Baegyeon-Ri, Paju, 2000

Date	No. of mosquitoes collected	Total tested number	No. of mosquitoes identified host animals						Mixed meals	HBI ^{a)}
			Human	Bovine	Pig	Dog	Cat	Chicken		
Jun. 29	43	14	0	10	1	1	0	0	1	0.00
Jul. 7	52	43	1	28	6	4	0	0	2	0.03
Jul. 13	84	72	1	49	23	15	4	2	18	0.01
Jul. 25	31	19	0	15	4	3	0	0	3	0.00
Aug. 10	96	58	0	42	7	5	0	0	6	0.00
Aug. 24	35	35	0	11	14	5	0	0	5	0.00
No. of Hosts		440	211	300	133 ^{b)}	ND	ND			
Total	340	241	2	155	55	33	4	2	35	0.008
Ratio(%)			0.80	61.8	21.9	13.1	1.6	0.8	13.9	

ND: No data

a)Human blood index = proportion human meals

b)Non-official data

Table 4. Vectorial capacity (VC) of *Anopheles sinensis* by malaria season in Baegyeon-ri, Paju, 2000

Date	ma ^{a)}	PR ^{b)}	HBI ^{c)}	gc ^{d)} (days)	a ^{e)} (bites/ mosq./ night)	P ^{f)} (vector survival probability /day)	n ^{g)}	Pn ^{h)}	1/-lnP ⁱ⁾	VC ^{j)}	Index of Stability
Jun.	154	0.352	0	3	0.000	0.71	11	0.023	2.920	0.000	0.000
Jul.	68.5	0.550	0.01	3	0.003	0.82	11	0.113	5.039	0.117	0.015
Aug.	40	0.662	0	3	0.000	0.87	11	0.216	7.181	0.000	0.000
Average	87.5	0.488	0.008	3	0.003	0.79	11	0.073	4.206	0.081	0.013

a)ma: Human biting rate

b)PR: parous rate

c)HBI: Human blood index

d)gc: gonotrophic cycle (Ree et al., 2001)

e)a: Human biting habit (HBI/gc)

f)P: the probability of a mosquito surviving through one day ($PR^{1/gc}$)

g)n: number of days require for completion of sporogonic cycle

h)Pn: probability of a mosquito surviving through n days (WHO, 1975)

i)1/-lnP: life expectancy of a mosquito

j)VC: Vectorial Capacity ($ma \times a \times Pn / -lnP$)

malaria from some areas, is more proper index than EIR (entomological inoculation rate). The VC of a mosquito species is not an absolute value but an index used to compare actual or potential vectorial importance of a particular species with that of another species or to evaluate vector control operations within a given area, especially in those areas where sporozoite rates are very low (Rubio-Palis et al., 1992).

The hours during which Anopheline attempt

to feed on blood may vary depending on (1) the area whether urban or rural (2) the density of the Anopheline species (3) the density of other species of Culicids (4) the time of the year (wet or dry) (5) the distance between the houses and the forest, and (6) the presence or absence of other hosts (Tadei et al., 1998). Human biting rate (ma) of *An. sinensis* at Baekyeon-ri was counted to be 154 bites/man/night (b/m/n) in June, 68.5 b/m/n in July, and 40.0 b/m/n in August,

2000. This rate was related to mosquito population density which reached its peak in late June through early July. This results are similar to other findings in high malaria risk areas (Elissa et al., 1999; Bockarie et al., 1995).

Human blood index of Anopheline mosquitoes is very important factor to indicate malaria transmission as well as its susceptibility to *Plasmodium*. *An. dirus* (anthropophilic index = 90.5) and *An. dirlingi* (Dutta et al., 1996; Tadei et al., 1998), malaria primary vector in the Indochina peninsular and Amazon region, show highly anthropophilic. However, *An. gambiae* and *An. funestus* have low human feeding habitats, with 4.5% and 9%, respectively (Fontenille et al., 1990). Human blood index of *An. sinensis* was already tested by various methods in Korea: 1.7% by serological analysis (Ree et al., 1967); 2.6-8.9% by hemoglobin crystal method (Kim, 1994). In this study, bloodmeal origin was determined in 241 samples from resting *An. sinensis* by ELISA; 0.8% (2 individuals) were human blood and 61.8% bovine blood. These data represent malaria primary vectors in Korea, *An. sinensis*, is highly zoophilic. Ree et al. (2001) presented almost same results. They tested 305 blood fed mosquitoes and found 2 positive (0.7%) for human by ELISA. For this reason, we have some problem to count seasonal variation of HBI.

Another key factor for VC is the life expectancy based on the parity. In this study, the parity of *An. sinensis* from the summer season in Paju-shi was 48.8% in average. This result is similar to Okku (52.0%), Jeollabuk-do in 1965, but significantly lower than those of Yangpyeong (76.7%), Gyeonggi-do in 1964 and Asan (81.3%), Chungcheongnam-do (Paik et al., 1965). Recently, Ree and Hwang (2000) surveyed parity in female mosquitoes resting on the walls of cowsheds in malarious and non-malarious areas and found the parities to be 64.6% and 57.8%, respectively. Anopheline mosquitoes were infected by *Plasmodium vivax* when they were fed on blood for their oviposition. So, older Anopheline have higher chance of being infected with *P. vivax*. Hence, older (parous) Anopheline are epidemiologically more important. In this study, the parity rate

gradually increased as time went on: 35.2% in June; 55.0% in July; 66.2% in August, 2000 (Table 2). Fontenille et al. (1990), found almost the same results from *An. gambiae* in Madagascar.

From this proportion of parity, we could estimate the probability of daily survival of *An. sinensis*. In the summer season, a daily survival rate is 0.79 with assumed 3 days gonotrophic cycle (Ree et al., 2001) and the expectancy of infective life through 11 days (WHO, 1975) could be defined as 0.073. This is the most important factor in the transmission of malaria, and any decrease of the expectancy of infective life may be the key to malaria control by the use of insecticides.

Given the very low anthropophagous rate (a) of the mosquitoes collected in Baekyeon-ri, the stability index is constantly <0.5 (Table 4). The value of over 2.5 indicate stability, values of 0.5-2.5 are intermediate, and the values below 0.5 indicate unstable conditions of transmission. Thus, malaria is highly unstable in this study area. Similar findings have been reported in the elevated areas of Zimbabwe (Taylor and Mutambu, 1986) and high plateau of Madagascar (Fontenille et al., 1990).

It is true that vectorial capacity is very popular index to indicate malaria transmission intensity. However, we needed to revise VC to apply our situation of very low human blood index. In spite of high human biting rate (ma), malaria transmission potential (VC) of *An. sinensis* is very low because of low human blood index (highly zoophilic), exophilic habitude, and late activity to feed. Therefore, we can conclude malaria situation in Korea is not because of high transmission potential of malaria vector, *An. sinensis*, but because of high population density.

ACKNOWLEDGEMENT

We are extremely thankful to several people for their help during this work. We greatly appreciate the assistance of Seung-Ho Kang and Yong-Bum Yi for mosquito collection. We also like to thank Kum-Nam Lee, Baekyeon-ri Branch, Paju-shi Public Health Center, Paju-shi.

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