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DAILY SURVIVAL AND HUMAN BLOOD INDEX OF *ANOPHELES SINENSIS*, THE VECTOR SPECIES OF MALARIA IN KOREA

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ABSTRACT. To evaluate the vector efficiency of *Anopheles sinensis* in transmitting vivax malaria in the northern part of Gyonggi-do, South Korea, daily survival and feeding host preferences were studied during the period of June–October 1999. Ovaries of unfed and freshly fed *An. sinensis* females were dissected and parity or nulliparity were observed. The parous rates were 75.2% in July, 56.5% in August, 78.5% in September, and 60.0% in October at Gusan-dong, Goyang-si, Gyonggi-do. The average probability of daily survival was 0.890. To determine the host feeding patterns of *An. sinensis*, outdoor-resting bloodfed mosquitoes were collected, and the sources of the blood meals were analyzed by enzyme-linked immunosorbent assay, using 6 different animal immunoglobulin G antibodies. Out of 305 blood meals tested, 0.7% were positive from humans, 89.8% from bovines, 3.3% from swine, 0.7% from dogs, 1.6% from chickens, and 0.7% from bovines and swine mixed. No blood meals were positive from mice. Though the vector efficiency of *An. sinensis* was poor because of a low human blood index and a moderate rate of daily survival, vectorial capacity would be high because of high density of the population.

KEY WORDS *Anopheles sinensis*, daily survival, human blood index, malaria vector species, Korea

INTRODUCTION

In the Republic of Korea (South Korea), malaria was eradicated in the late 1970s. One case of indigenous vivax malaria was reported in 1993. Since then the number of cases has steadily increased each year, totaling 25 cases in 1994, 107 in 1995, 356 in 1996, 1,724 in 1997, and 3,932 in 1998 (Lee et al. 1998, Chai 1999). Almost all cases were confined to the northern part of Gyonggi-do (province) along the Demilitarized Zone (DMZ) (Kho et al. 1999; Lee et al., unpublished data).

Controversy exists regarding the epidemiologic interpretation of the malaria outbreak in the northern part of Gyonggi-do. One postulation is that most of the cases are the result of infiltration (dispersal) of infected vector mosquitoes from North Korea where an epidemic has been in progress since 1993 (Ree 1998, Kho et al. 1999). Another postulation is that most of the cases are secondary cases resulting from local transmission in South Korea (Chai 1999). Parasitologic approaches cannot be used to determine the main source of infection, because the Korean strain of *Plasmodium vivax* (Grassi and Feletti) has a protracted incubation period of 5–13 months (Tiburskaja et al. 1968, Tiburskaja and Vrublevskaja 1977).

Entomological approaches such as daily survival and the degree of human feeding of vector mosquitoes are important parameters in malaria epidemiology. Daily survival studies of malaria vector mosquitoes were conducted by many workers in different countries, for example, for *Anopheles dirus* Peytonand Harrison in Thailand (Rosenberg et al. 1990), for *An. pharoensis* Theobald and *An. multicolor* Cambouliu in Egypt (Kenawy 1991), for *An. culicifacies* Giles and *An. pulcherrimus* Theobald in Iran (Zaim et al. 1993), for *An. gambiae* Giles in Sierra Leone (Bockarie et al. 1995), for *An. pseudopunctipennis* Theobald in southern Mexico (Fernandez-Salas et al. 1994), for *An. albimanus* Weid. in Mexico (Ulloa et al. 1997), and for *An. vestitipennis* Dyar and Knab in Mexico (Arredondo-Jimenez et al. 1998). Bloodfeeding behavior of anopheline mosquitoes was also studied for estimation of vectorical capacity and other epidemiologic purposes (Edrissian et al. 1985, Beier et al. 1988, Loyola et al. 1993, Mbogo et al. 1993, Rubio-Palis 1994, Bockarie et al. 1995).

This study was conducted to determine the temporal and spatial daily survival rates of vector mosquitoes and the degree of human–vector contact, as represented by the human blood index. The study was performed in the northern part of Gyonggi-do, where most of the malaria cases were reported during the period between June and October 1999.

MATERIALS AND METHODS

Study areas: Four locations in northern Gyonggi-do where most of the malaria cases occurred were selected for this study: Gusan-dong, Ilsan-gu, Goyang-si; Dongjung-ri, Wangjin-myon, Yonchon-gun; Jangpa-ri, Jindong-myon, Paju-si; and Manwu-ri, Tanhyon-myon, Paju-si (Fig. 1). In addition, a study was undertaken at Taerak-ri, Munbak-myon, Jinchon-gun, Chungchongbuk-do, which is located in a malaria-free area.

Age determination: Mosquitoes were collected by a light trap that was set up in a cowshed. Unfed and freshly fed *Anopheles sinensis* Weid. females were dissected for their ovaries, and parity or nulliparity were observed (Detinova 1962). The prob-

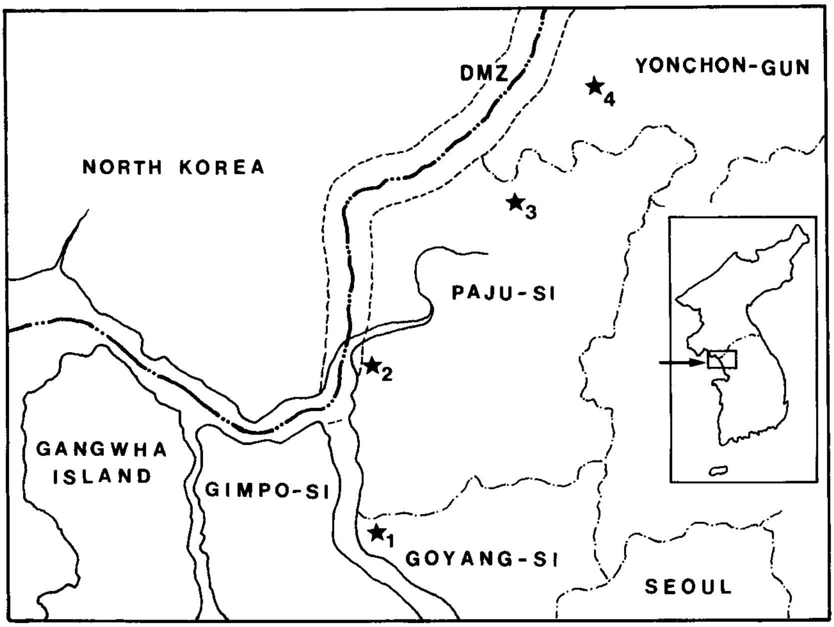

Fig. 1. Map of northern Gyonggi-do (province) showing 4 study sites. 1: Gusan-dong, Goyang-si; 2: Manwu-ri, Paju-si; 3: Jangpa-ri, Paju-si; 4: Dongjung-ri, Yonchon-gun.

ability of survival through 1 day is equivalent to the cube root of the proportion of parous females in the population sample, when the gonotrophic cycle takes place in 3 days (Gilles and Warrell 1993). To determine the gonotrophic period of *An. sinensis* at northern Gyonggi-do, fully fed females were collected on the wall of a cowshed at night. Each was put in a paper cup with sugar solution, and kept outdoors under humid conditions. Development of the ova in the follicles was observed at 12-h intervals.

Identification of blood meals: The direct enzyme-linked immunosorbent assay (ELISA) previously described by Beier et al. (1988) and modified by Loyola et al. (1990) was used to identify the source of the blood meal. Outdoor-resting mosquitoes were collected in vegetation along levees and rice fields using a backpack aspirator or by sweeping with an insect net. The collection sites were confined to places 50–100 m away from the villages. Fully fed to half-fed *An. sinensis* females were used for blood meal identification. The blood meals were taken out and resuspended with 300 μl of distilled water in eppendorf tubes using a homogenizer. The blood meal solution was aliquoted by 50 μl into 6 wells of the Costar® EIA/RIA strip ELISA plate (Corning Inc., Corning, NY). Then a 50-μl coating buffer (carbonate–bicarbonate buffer, pH 9.6) was added to each well and the plate was left overnight at 4°C in a humid box. The next morning, the plate was washed with phosphate-buffered saline (PBS)–Tween 3 times, for 3 min each time. The alkaline phosphatase–conjugated immunoglobulin Gs (IgGs) of human, bovine, dog, pig, chicken, and mouse (Sigma Co., St. Louis, MO) were tested. The dilution of the antibodies in PBS-Tween was 1:2,000, except anti-dog IgG (1:400). One hundred microliters of each antibody dilution was added to each well. The plate was incubated for 1 h in a humid box at 37°C, then removed and washed as described above. One hundred microliters of substrate solution (1 mg/ml *p*-nitrophenyl phosphate in diethanolamine buffer) was then added to each well. The reaction was stopped after half an hour by the addition of 50 μl of sodium hydroxide solution (3 mol/liter). Two wells without blood were kept blank in the same plate. The end results were read by a Vitatron spectrophotometer ELISA reader (Dynatech Lab, Inc., Chantilly, VA) at 450 nm. Background readings were subtracted from test values and final readings of ≥0.2 were taken as positive. Unfed females of *An. sinensis* were included as negative controls. The host bloods diluted to 1:1,000 were used for positive controls.

Seasonal population abundance: A black light trap (Nozawa type) was operated throughout the night (from 7:00 p.m. to 6:00 a.m.) at a half-opened cowshed with 22 cows, once a week from June to October 1999. The cowshed was located at Gusandong, Ilsan-gu, Goyang-si, Gyonggi-do. The mosquitoes collected were brought to the laboratory and identified using the key prepared by Lee (1998).

RESULTS

Proportion of daily survival

The results of ovary dissection for parity or nulliparity are shown in Table 1. The parous rate of

Table 1. Parous rates and probability of daily survival of *Anopheles sinensis* in 1999.

Locality	Month	No. dissected	Parous rate (%)	Probability of daily survival
Gusan-dong, Goyang-si	July	825	75.2	0.909
	Aug.	639	56.5	0.827
	Sept.	801	78.5	0.922
	Oct.	115	60.0	0.843
	Subtotal	2,380	70.5	0.890
Jangpa-ri, Paju-si	Aug.	288	42.0	0.749
Manwu-ri, Paju-si	Aug.	163	79.1	0.925
Dongjung-ri, Yonchon-gun	Aug.	201	56.2	0.825
Taerak-ri, Jinchon-gun	Aug.	248	47.6	0.781
Total		3,261	66.1	0.871

An. sinensis collected at Gusan-dong, Goyang-si, varied considerably by season, with the lowest rate in August (56.5%) and the highest rate in September (78.5%). The period of the gonotrophic cycle for *An. sinensis* was found to be 3 days on average (2.5–3.5 days) under the natural condition. Therefore, the proportion of daily survival was estimated as 0.890 on average during the period of July–October at Gusan-dong. The parous rate was also compared at 5 different localities during the same period (August). The results were different by location, with the lowest parous rate (42.0%) at Jangpa-ri, Paju-si, and the highest rate (79.1%) at Manwu-ri, Paju-si, given the probability of daily survival of 0.749 and 0.925, respectively.

Seasonal occurrence and parous rate

The population density of *An. sinensis* increased steadily in June, and reached its peak during the 1st week of July. Thereafter, the population kept decreasing through July–August, and very small 2nd peak appeared during mid-September. The number of *An. sinensis* mosquitoes was high throughout the 1999 season, with 71,272 females/trap/night during the peak time (1st week of July). The count was 2,057 females/trap/night during the 3rd week of August, which was the lowest count between the peaks (Fig. 2).

The parous rate of *An. sinensis* was high in early July (80.3 and 84.8% during the 1st and 2nd weeks, respectively), and continued to decrease thereafter until late August (53.5 and 54.1% during the 3rd and 4th weeks of August, respectively). In September, the parous rate was 76.3% during the 1st week, 79.3% the 2nd week, 76.1% the 3rd week, 85.6% the 4th week, and 78.7% the 5th week. The parous rate then decreased to 47.1 and 15% during the 2nd and 3rd weeks of October, respectively (Fig. 2).

Host feeding preference

In the present ELISA test, positive (homologous) absorbance values ranged from 0.281 to 0.807, and 68% of heterologous background values ranged from 0.081 to 0.197. The results of the host blood meals analyzed by ELISA are given in Table 2. At all study locations, this mosquito fed almost exclusively on bovines (89.8%). Other hosts included humans (0.7%), swine (3.3%), dogs (0.7%), and chickens (1.6%), and 3.6% of the hosts were unknown. As a result, the human blood index was 0.018 at Jangpa-ri and an average of 0.007 in all of the study locations.

DISCUSSION

In Korea, vivax malaria was prevalent throughout the country during the Korean War (1950–53), and progressively declined thereafter, particularly in the southwestern plain areas (Paik and Tsai 1963). Mass blood surveys throughout the country in 1961–65 showed that the number of malaria cases per populations of 10,000 was 0.54 in the plains areas, 10.9 in the hilly areas, and 34.6 in the mountainous areas, in spite of the fact that a much higher density of *An. sinensis* mosquitoes occurred in the plains areas (National Malaria Eradication Service 1966). In the 1960s the parous rate of *An. sinensis* was much lower in the plains areas, with 52.0% at Okku, Chollabuk-do, which is located in the plains areas of southwestern Korea, compared to those in hilly areas, with 81.3% at Asan, Chungchongnam-do, and 76.7% at Yangpyong, Gyonggi-do, both of which are located in hilly areas (Paik et al. 1965). The parous rate in our study was 66.1% on average, which is higher than that in the plains areas, but lower than that of the hilly areas, in the 1960s. The proportion of daily survival for *An. sinensis* (0.87) was estimated to be moderate, compared to those of other anopheline species. The proportion of daily survival was 0.89 for *An. pharoensis* and 0.80 for *An. multicolor* in Egypt (Kenawy 1991), 0.80 in May and 0.83 in September for *An. pulcherrimus* in Iran (Zaim et al. 1993), 0.88 for *An. pseudopunctipennis* (Fernandez-Salas et al. 1994), 0.85 for *An. gambiae* in southern Sierra Leone (Bockarie et al. 1995), 0.80–0.88 for *An. gambiae* s.l. in Sudan (Costantini et al. 1996), and 0.45–0.68 for *An. vestitipennis* in southern Mexico (Arredondo-Jimenez et al. 1998).

The seasonal occurrence pattern of *An. sinensis*

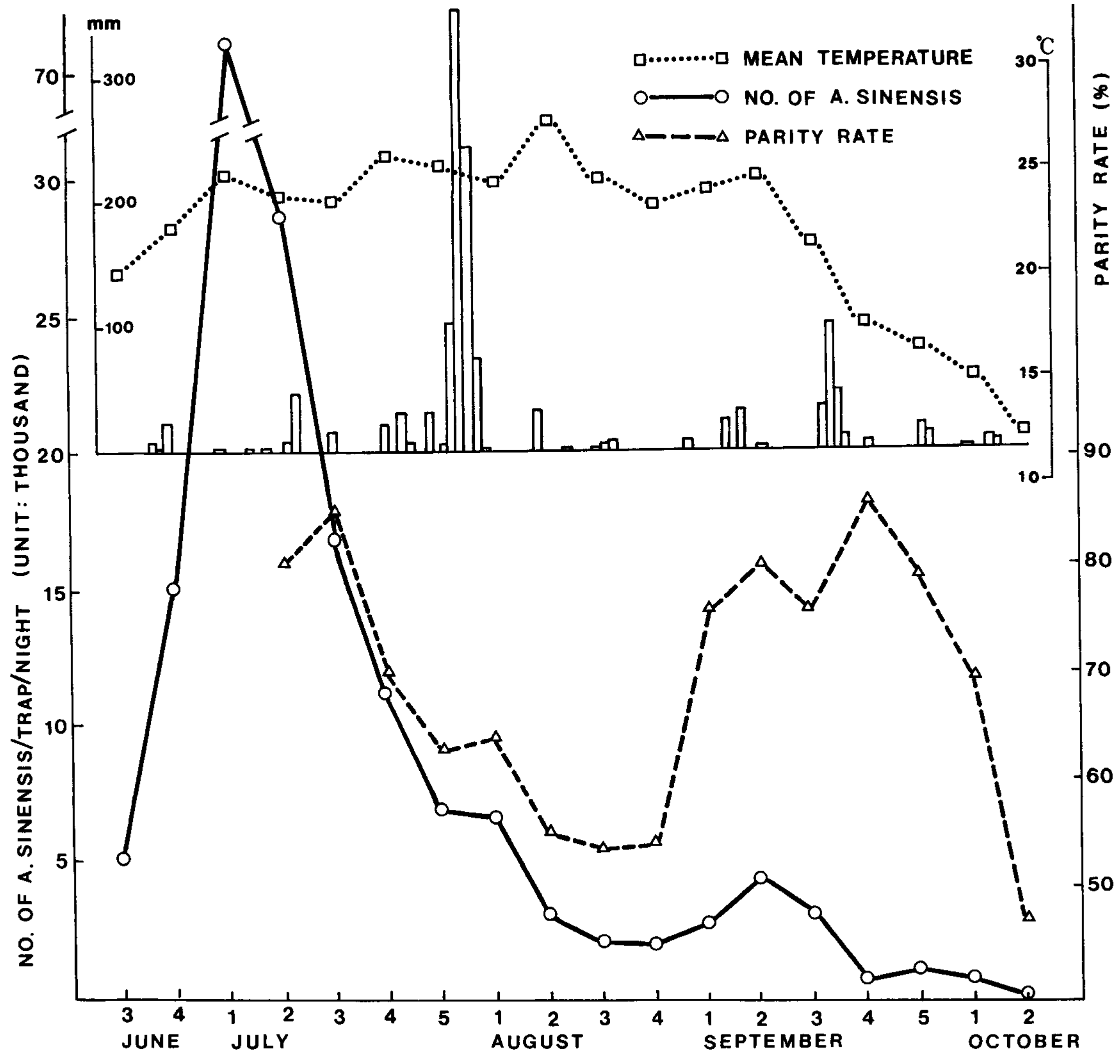

Fig. 2. Weekly occurrence of population density and parous rate of *Anopheles sinensis* at Gusan-dong, Goyang-si, Gyonggi-do, in 1999. Mean temperature by week and daily rainfall are also shown.

Table 2. Results of enzyme-linked immunosorbent assay tests for identification of blood meals from *Anopheles sinensis* collected at outdoor resting places (vegetation) in July–August 1999.

		No. (%) of host blood meals							
Locality	No. tested	Human	Bovine	Swine	Dog	Mouse	Chicken	Bo/Sw[1]	Unknown
Gusan-dong	174	0	163 (93.6)	6 (3.4)	0	0	1 (0.6)	2 (1.1)	2 (1.1)
Jangpa-ri	114	2 (1.8)	95 (83.3)	2 (1.8)	2 (1.8)	0	4 (3.5)	0	9 (7.9)
Taerak-ri	17	0	16 (94.1)	1 (5.9)	0	0	0	0	0
Total	305	2 (0.7)	274 (89.8)	10 (3.3)	2 (0.7)	0	5 (1.6)	2 (0.7)	11 (3.6)

[1] Bovine and swine mixed.

in 1999 is very similar to those found by previous workers, who reported that the peak appeared in early July (Lee and Ree 1991, Shim et al. 1997). The population density of 1999 seems to be considerably higher than that of previous years, although the data were not directly comparable. The number of *An. sinensis* per trap per night in July was 27,019 in 1999, whereas it was 8,119 in 1995 (Shim et al. 1997). Because our main study purpose was to observe temporal daily survival related to the population abundance of *An. sinensis,* collecting mosquitoes was done in only a cowshed, resulting in a rather biased sample. The parous rate declined continuously from mid-July until late August, in accordance with the decline of the population, which means that environmental factors were unfavorable both to breeding places and to the life span of adults. The conspicuous decrease of both population density and parous rate in August 1999 might be caused mainly by the weather (Fig. 2). Four typhoons hit the Korean peninsula in August 1999. Typhoon Olga (July 30—August 3) was a category 1 typhoon, and resulted in large amounts of flooding, particularly in northern Gyonggi-do. In addition, the mean temperature in August was 1.1°C lower than usual (30-year average from 1950 to 1980).

Precipitin tests of *An. sinensis* blood meals were done for the 1st time at Yongju, Gyongsangbuk-do, in 1960 (Whang 1964). Among 38 mosquitoes collected outdoors, 92% were from cows, 3% were from dogs, and 5% were from other mammals, and of 49 females collected in bedrooms, 54% were from humans, 31% were from cows, 2% were from pigs, 2% were from dogs, and 11% were from other mammals. Twenty-six blood meals of the females collected in cowsheds were 100% from cows (Whang 1964). Another serologic study on the host feeding patterns of *An. sinensis* was carried out at Yoju, Gyonggi-do, in 1965; the host blood meals of 301 females collected at outdoor resting places were 1.7% from humans, 54.8% from cows, 42.5% from pigs, 0.3% from cows and pigs mixed, and 0.7% from other mammals (Ree et al. 1967). These study results in the 1960s indicated that this mosquito fed almost exclusively on cows and pigs, with only a 0.017 human blood index. Our present study showed very similar results, showing an extremely low human blood index (0.007), although the sample size of the blood meals was rather small and the number of the study locations was limited. Obtaining bloodfed females of *An. sinensis* at outdoor resting places such as grasses, rice fields, and other vegetation was extremely difficult because they were rather evenly scattered in vast areas of such resting places.

The vector efficiency of *An. albimanus,* the vector species of malaria in Central and South America, is similar to that of *An. sinensis.* Human blood indices of *An. albimanus* collected outdoors were 0.05–0.07 (Loyola et al. 1993), and parous rates were 26.8–37.6% (Ulloa et al. 1997). Malaria transmission efficiency of this mosquito was highly dependent on overall population abundance (Bown et al. 1991). The vector efficiency of *An. sinensis* in malarial areas of northern Gyonggi-do, South Korea, in 1999 was poor because of an extremely low human blood index (0.007) and moderate daily survival (particularly in August). However, vectorial capacity, which is density dependent, would be high because of the high population density. A malaria outbreak in northern Kyonggi-do near the DMZ would result from both local transmission of secondary malaria infections and transmission by sporozoite-infected mosquitoes dispersed from North Korea across the DMZ.

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