

EPIDEMIOLOGICAL STUDIES ON MALAYAN FILARIASIS IN AN INLAND AREA IN KYUNGPOOK, KOREA. (2) THE PERIODICITY OF THE MICROFILARIAE AND THE BIONOMICS OF THE VECTOR

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ABSTRACT. The periodicity of the microfilariae of *Brugia malayi* in the peripheral blood was investigated in a high density case and a low density case. In the former, microfilariae appeared in the peripheral blood after 8 p.m., reached a peak (19 per 30 mm³) at midnight and disappeared from 9 a.m. until 3 p.m. In the low density case microfilariae occurred between 10 p.m. and 6 a.m. with the peak density at 4 a.m. (2 per

30 mm³). Therefore it can be considered that the periodicity is nocturnal.

Overnight mosquito collections using human bait produced 103 *An. sinensis*, 66 *Culex tritaeniorhynchus*, 13 *C. pipiens*, and 12 *Aedes vexans*. Five of 103 *An. sinensis* were infected with filarial larvae. *An. sinensis* appears to be an important vector of *B. malayi*.

In a previous report Kanda et al. (1975) recorded some positive cases of Malayan microfilaremia (*Brugia malayi*) found in blood surveys. Two different positive cases were selected to study the variation of the periodicity of the microfilariae in the blood stream. The biological observations relate to the epidemiological studies in which *Anopheles sinensis* was pointed out to be one of the important vectors of *B. malayi* in the inland areas of the Korean Peninsula. The present paper reports on those results.

METHODS

For the investigation of the periodicity of microfilariae, three 10 mm³ blood smears were taken every 2 to 3 hours in a 24-hour period from each of the two cases with a specially designed micro-pipette. The details of this technique were the same as previously reported (Kanda et al. 1975).

The two observed cases were as follows: the first was a male who had the highest microfilaremia (18 per 30 mm³) in his peripheral blood; the second was found as a new positive case in the second blood survey on December 29, 1973, although he

had been negative in the previous blood examination.

The biting collection on man was performed as follows: a man was allowed to lie on the floor of a room 2.5 x 2.5 m² and 2.3 m in height. An open window 1.5 x 1.0 m² permitted entry of mosquitoes. All mosquitoes biting or attempting to bite were collected between 1800 hours and 0600 hours.

The mosquitoes collected during each hour were isolated, then identified to species and dissected as soon as possible on the same day to ascertain physiological age and infection, if any, with filarial larvae. The estimate of the physiological age of the mosquitoes was determined by observing the ovarioles and also by observing the development of the filariae found in the mosquito.

Several larval surveys of mosquitoes were also carried out with a view toward identifying other possible vectors such as *Mansonia* sp. or *Aedes togoi*.

The relationship between the periodicity of the microfilariae and the mosquito biting behavior, including physiological age and stage of filarial infection, combined to further incriminate *Anopheles sinensis* as the vector.

RESULTS

INVESTIGATIONS ON THE PERIODICITY. The investigations were carried out on July 19 and 20, 1974 at Ih-do village and the re-

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sults are given in Figure 1. The data show that the peripheral blood of case "C" was positive for microfilariae after 8 p.m., with densities of 4 per 30 mm³ at 8 p.m., 7 at 9 p.m., 4 at 10 p.m., 19 at midnight, 8 at 3 a.m. and 3 at 6 a.m. No microfilariae were found in blood examinations at 9 a.m., noon or 3 p.m. The densities of microfilariae in the peripheral blood of case "L" were as follows: 1 microfilariae per 30 mm³ at midnight and at 2 a.m., and 2 microfilariae at 4 a.m. In those cases the peak times of the microfilarial density were at midnight in case "C" and 4 a.m. in case "L." Therefore it may be considered that the periodicity of the microfilariae is nocturnal.

MOSQUITO SURVEY. Observations were made of the numbers and kinds of mosquitoes which fed or attempted to feed on the human subject from 6 p.m. until 6 a.m. on July 18-19, 1974. Collected mosquitoes were grouped by hour. Table 1 shows the results of the collection. Four species, *Anopheles sinensis*, *Culex tritaeniorhynchus*, *Culex pipiens*, and *Aedes vexans*, were collected. Among these *An. sinensis*, *Culex tritaeniorhynchus* and *Aedes vexans* bit from 8 p.m. onward but *Culex pipiens* first appeared at 10 p.m. The biting cycles of these mosquitoes were all different. The peak number of *An. sinensis* was 34 during one hour between

11 p.m. and midnight, but *C. tritaeniorhynchus* showed two peaks, one between 10 p.m. and 11 p.m. and another between 4 and 5 a.m. *Aedes vexans* disappeared after 11 p.m. and *C. pipiens* appeared after 10 p.m.

All mosquitoes which came to bite the man were dissected; none except *An. sinensis* had filarial larvae. One hundred three of *An. sinensis* came to bite on the positive case during 12 hours 6 p.m. to 6 a.m., a relatively high density. Therefore the susceptibility to the filaria of this species of mosquito must be observed. To learn the physiological ages of the mosquitoes, their ovarioles were also observed in the same dissection. Five *An. sinensis* were infected with 2nd and 1st stage larvae but no 3rd stage larvae were found. Twenty-three parous mosquitoes were found among 103 females as shown in Table 2. These results show that older and also infected mosquitoes were more abundant from 8 p.m. and midnight. Table 3 shows the relation between physiological age and the larval stages of the infected *An. sinensis*. Among 5 infected mosquitoes 2 were in the 2nd ovarian age; 3 mosquitoes had 2nd stage larvae. Of the 17 filarial larvae found in these dissections, 14 were 1st stage and 3, 2nd stage.

Although those filarial larvae could not

Table 1. The results of overnight mosquito collection on human bait on July 18-19, 1974 in Ih-do, Kungpook, Korea.

Hour	Species of mosquito collected				Total
	<i>An. sinensis</i>	<i>C. tritaeniorhynchus</i>	<i>A. vexans</i>	<i>C. pipiens</i>	
6-7 p.m.					
7-8					
8-9	11	3			19
9-10	7	8			20
10-11	22	10	5		36
11-12 midnight	34	8	2	2	42
12-1 a.m.	18	5			24
1-2	7	4		1	13
2-3	3	11		2	19
3-4	1	16		5	20
4-5		1		3	1
5-6					
Total	103	66	12	13	194

Table 2. The distribution of physiological ages of *An. sinensis* and larval stages of filariae dissected from them by hours.

Hour	Mosquito no.	Physiological ages		Larval stages of filaria		
		N	1	2	I	II
6-7 p.m.		1				
7-8						
8-9	11	7	3	1		
9-10	7	6	1			
10-11	22	18	3	1	1	1
11-12 midnight	34	27	5	2	2	2
12-1 a.m.	18	14	4		2	
1-2	7	4	3			
2-3	3	3				
3-4	1	1				
4-5						
5-6						
Total	103	80	19	4	5*	3*

N = nulliparous.

* = number of larvae.

18th, 19th and 20th of July, 1974 and also again from the 6th to the 15th of August of the same year. In spite of these and several other larval surveys, we were unable to find any larvae of other possible vectors. Neither did we find any other species infected except *An. sinensis*.

DISCUSSION

There were not as many high density positive cases found in our blood surveys as in the surveys performed by Seo and Kang (1974) in Cheju Island, or by Hayashi (1962) in Hachijo-Koshima. Therefore the case "C" with 18 microfilariae per 30 mm³ was chosen for observation. The other case was also chosen to ascertain how

variable was the periodicity between individuals with low and high densities. Seo and Kang (1974) pointed out that the periodicity of this kind of microfilariae at a location close to our research site was of the nocturnal periodic type of which the peak point was at midnight or at 2 a.m. However, from the present results it also may be considered that there is possibly some variation in the peak period of microfilarial density as the new positive case showed. Other factors such as physiological variation of the host and technical problems in taking blood from the hosts might have influenced the results. It is more variable especially in the case of low density, as shown in case "L" in Fig. 1. In an event from our data the periodicity may be confirmed to be of the nocturnal periodic type in this area.

The previous paper reported the anthropophilic character, the high infectivity (susceptibility) to Malayan filariasis and the longevity of *An. sinensis*. The present investigation shows similar results; furthermore the data on the bionomics of *An. sinensis* as shown in Fig. 1 and Tables 1 and 2 clearly correlate with the periodicity of the microfilariae in the positive cases. Therefore it is clear that *An. sinensis* in this area has sufficient opportunity to be one of the important vectors.

Table 3. The relationship between physiological ages of mosquitoes and the larval stages of the filaria in each mosquito.

Mosquito no.	Physiological ages	Larval stages	
		I	II
1	2	3	1
2	1	4	1
3	1	3	1
4	2	2	
5	1	2	
		14	3

The present and previous data give some possible epidemiological important suggestions on filarial infection by *An. sinensis*. Although there are some possible variations on the age composition of the mosquitoes by year or other conditions, our data suggest that older mosquitoes are relatively abundant during autumn. It can be considered that those characters such as anthropophilic behavior, longevity in the life span of the mosquitoes, susceptibility of its natural infection, seasonal condition and also custom of life of the people, etc., are related to the vector capacity.

The present authors have information that *An. sinensis* from Japan was not a suitable intermediate host for a strain of *Brugia malayi* which originated in Malaysia. Third stage filarial larvae which had degenerated were found in experimental infections when a Japanese strain of *An. sinensis* and a Malaysian strain of *B. malayi* were used. This would suggest differences in the geographical strains of the mosquito, or the parasite, or both.

With respect to the vector capacity of *An. sinensis* for *B. malayi*, there are several papers which indicate the incrimination in China (Hu et al. 1937, Feng and Ma 1957, Feng et al. 1958, and Harrison 1973). Harrison (1973) considered that the strain of *An. sinensis* with a narrow decked egg is one of the vectors of Malayan filariasis in the inland area of China because it is more anthropophilic than the wide decked egg strain. Narrow decked eggs are always seen in *lesteri*, *yatsushiroensis* and *sineroides* in Japan. The mosquito which has a narrow decked egg can be considered to be *An. lesteri*. Very few *koreicus* were collected in the present surveys in Korea, but they had no filarial larva; and no other species of mosquitoes were collected. The present authors observed that the eggs which were the progeny of the adults collected on human bait were all wide decked. Kanda and Oguma (1974) reported that the progeny of any cross among species of the *hyrcanus* species group always shows maternal inheritance of egg characters. The cytogenetic char-

acters of the Japanese type and the Korean type of *sinensis* were also observed. Those two types were only identified in the progeny of the cross between *lesteri* and these two types of *sinensis* by the cytogenetic characters which show different synapsis. *Anopheles sinensis* of both types thus shows differences in biological as well as epidemiological parameters.

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