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STUDIES ON MALAYAN FILARIASIS IN CHE-JU IS., KOREA

4 Experimental transmission of *Brugia malayi* (Che-ju strain) to domestic cats

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Abstract: Che-ju strain of *B. malayi* was successfully transmitted to three domestic cats by subcutaneous injection of infective-stage larvae obtained from naturally-infected *Ae. togoi*. The second generation of the strain of *B. malayi* was established in nine cats which had been inoculated subcutaneously with the infective larvae developed in laboratory-bred Nagasaki strain of *Ae. togoi*. The third generation was built up by subcutaneous inoculation in two cats through *Ae. togoi* and four cats through Liverpool strain of *Ae. aegypti*. The prepatent periods were 91–131 days. The microfilaria counts in the peripheral blood gradually increased in one third to two thirds of successfully transmitted cats. The microfilariae of Che-ju strain exhibited a sub-periodic tendency in the cat. The average infection rate of laboratory-bred Nagasaki strain of *Ae. togoi* was 69.3% with infective larvae of *B. malayi*. The mean number of larvae per mosquito was 5.8 with the infective larvae. Nagasaki strain of *Ar. subalbatus* was not susceptible to *B. malayi* infection. *B. pahangi* developed to the infective form in both *Ae. togoi* and *Ar. subalbatus*.

As to *Brugia malayi*, only the periodic and sub-periodic forms from Malaya were reported to have been successfully transmitted to domestic cats through the insect vectors, *Mansonia* mosquitoes (Edeson and Wharton, 1957, 1958; Wharton *et al.*, 1958), *Aedes aegypti* (Ramachandran *et al.*, 1960), and also *Anopheles barbirostris* (Laing *et al.*, 1961). It was described that the periodic form does not develop well in the cat with a long prepatent period and low microfilaremia, although marked nocturnal periodicity is maintained in the cat after the successful transmission (Laing *et al.*, 1961). In Che-ju Island, Korea, only the periodic form of *B. malayi* is known to cause filariasis in man; the main vector is *Ae. togoi* (Wada *et al.*, 1973).

The present paper gives the results of transmission experiments made on the domestic cats through *Ae. togoi* as well as *Ae. aegypti* from 1972 to 1976, and the results of susceptibility studies of two species of laboratory-bred mosquitoes to *Brugia* infection.

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Materials and Methods

I. Experimental transmission of infective larvae to cats.

Wild *Aedes togoi* were collected early morning from the inside of the houses which *B. malayi* microfilaria carriers occupied in the endemic areas of Che-ju Island, Korea. The mosquitoes were kept in the laboratory for 8–10 days and then dissected to obtain infective-stage larvae (Table 1). Three domestic cats carried from Japan were injected subcutaneously into the inguinal regions with 114–235 infective-stage larvae divided into two to four inoculations (Table 2). Stained thick smears of 60 cmm

Table 1 Stage III larvae of *B. malayi* in *Ae. togoi* kept in laboratory after collection at microfilaria carriers' houses

Days after collection	No. of mosquitoes dissected	No. of mosquitoes infected	Stage III larvae			
			Site of occurrence			Total no.
			Head	Thorax	Abdomen	
8	139	37 (26.6)	56 (29.0)	110 (57.0)	27 (14.0)	193
9	454	105 (23.1)	179 (55.6)	73 (22.7)	70 (21.7)	322
10	71	14 (19.7)	48 (63.2)	19 (0.25)	9 (11.8)	76
Total	664	156 (23.5)	283 (47.9)	202 (34.2)	106 (17.9)	591

( ): %

Table 2 Inoculation of stage III larvae obtained from naturally-infected mosquitoes to domestic cats

Date of inoculation	No. of larvae inoculated		
	Cat 1	Cat 2	Cat 3
31 Aug. '72		79	
2 Sept. '72	74		
3 Sept. '72		42	
5 Sept. '72			51
6 Sept. '72	40		
7 Sept. '72		42	48
8 Sept. '72		72	
10 Sept. '72			50
Total no. of larvae inoculated	114	235	149

peripheral blood, exclusive of occasional 30 cmm blood instead, were examined for microfilaria weekly after the inoculation (Tables 3, 4).

Furthermore, laboratory-bred *Ae. togoi* (Nagasaki strain) were fed on Cat 3

with moderate microfilaremia (Fig. 1). The infective-stage larvae obtained from the same mosquitoes were injected into the inguinal regions of 29 cats to establish the second generation in the cat (Table 5). Each of them was given 52–356 larvae as a single inoculation or divided into two. Also laboratory-bred *Ae. aegypti* (Liverpool strain) were fed on Cat 3 and kept in the laboratory at 25 C. Cat 30 received 200 infective-stage larvae developed in *Ae. aegypti*. During the prepatent period 17 cats (including Cat 30) out of 30 died, most frequently from panleukopenia.

Cat 31 was chosen for the source of microfilariae to build up the third generation in the cat (Table 7). The infective-stage larvae from *Ae. togoi* were introduced subcutaneously into the inguinal regions of Cat 34 and 35, which received 125 and 104 larvae, respectively. In addition to that, 8 cats were given 230–350 infective-stage larvae developed in *Ae. aegypti* fed on Cat 31.

The cats with microfilaremia were usually examined in circadian fluctuations in appearance of microfilariae into the peripheral blood every month or two. Blood films of 60 cmm were taken every 2 hours for 24 hours from the pinnae of the ears of cats. Periodicity studies were also made on Cat 2 before and after transportation to Los Angeles, California, by air (Table 8).

II. Susceptibility of two species of mosquitoes to *B. malayi* and *B. pahangi* infections.

Laboratory-bred Nagasaki strains of *Aedes togoi* and *Armigeres subalbatus* were fed on a cat with *B. malayi* microfilaremia under Nembutal anesthesia. The expected numbers of microfilariae taken up by mosquitoes were estimated from the quantities of ingested blood and the microfilaria counts in the peripheral blood of the cat at the time of feeding. The estimates were 1.7–4.5 for *Ae. togoi* and 2.0–7.5 for *Ar. subalbatus* (Table 11). The mosquitoes were kept at 25 C and dissected daily 1–13 days after feeding. The total number of examined *Ae. togoi* was 713 (Table 12), and that of *Ar. subalbatus* was 279 (Table 13).

Also the two species of laboratory-bred mosquitoes were fed on a dog with *B. pahangi* microfilaremia under Nembutal anesthesia, kept at 25 C and examined for the development of microfilariae to the infective larvae. The dissected number of *Ae. togoi* was 107, and that of *Ar. subalbatus* was 215 (Table 15).

III. The periodicity of *B. malayi* microfilariae in human carriers.

Blood films of 60 cmm were taken every 2 hours throughout the 24 hours from the ear lobes of 10 microfilaria carriers in the endemic areas. The blood was taken up into graduated capillary tubes. Thick films of parallel lines were made on clean glass slides, dried for about 8 hours, hemolysed in distilled water, and stained in buffered Giemsa solution.

The average counts of samples were calculated from the microfilaria counts of every individual to give the secondary histogram indicating the percentage rate of each area between two adjoining vertical lines at 2-hour interval to the total areas in the primary histogram (Era, 1959; Katamine, 1972).

Results

As indicated in Table 1 the total of 591 infective (stage III) larvae were obtained from 156 mosquitoes out of 665 dissected ones which had been collected from the microfilaria-carriers' houses and kept in the laboratory for 8 to 10 days. The infection rate of the mosquitoes corresponded to 23.2 per cent. Most of the stage III larvae were harbored in the head and thorax; less than 22% in the abdomen. The microfilariae were first found in the peripheral blood 91, 99 and 104 days after inoculation in three domestic cats which had been given the infective larvae from the naturally-infected mosquitoes (Tables 3, 4). In Cats 2 and 3 microfilaria levels

Table 3 Experimental transmission of *B. malayi* from naturally-infected mosquitoes to cats

Cat no.	Sex	Weight in kg	No. of larvae inoculated	No. of days observed after inoculation	No. of days from inoculation to mf. detection in blood
Cat 1	F	2.2	114	993	91
Cat 2	M	2.8	235	330*	104
Cat 3	M	3.2	149	1,095**	99

* Then transferred to Los Angeles

** Adult *B. malayi* recovered at necropsy

Table 4 Prepatent periods and changes of microfilaremia in cats inoculated with larvae from naturally-infected *Ae. togoi*

Cat no.	Cat 1	Cat 2	Cat 3
No. of larvae inoculated	74, 40	79, 42, 42, 72	51, 48, 50
Date of 1st inoculation	2 Sept. '72	31 Aug. '72	5 Sept. '72
Prepatent period in days	91	104	99
Date (hours on 24-hour clock)	Mf. count per 60 cmm blood		
7 Nov. (23: 00)	0*	0*	0*
2 Dec. (23: 00)	1*	0*	0*
13 Dec. (23: 00)	0	1*	1*
24 Dec. (23: 00)	0	3	2*
8 Jan. (11: 00)	1	8	14
(23: 00)	0	9	2
22 Jan. (11: 00)	0	20	16
(23: 00)	0	16	22

* per 30 cmm blood

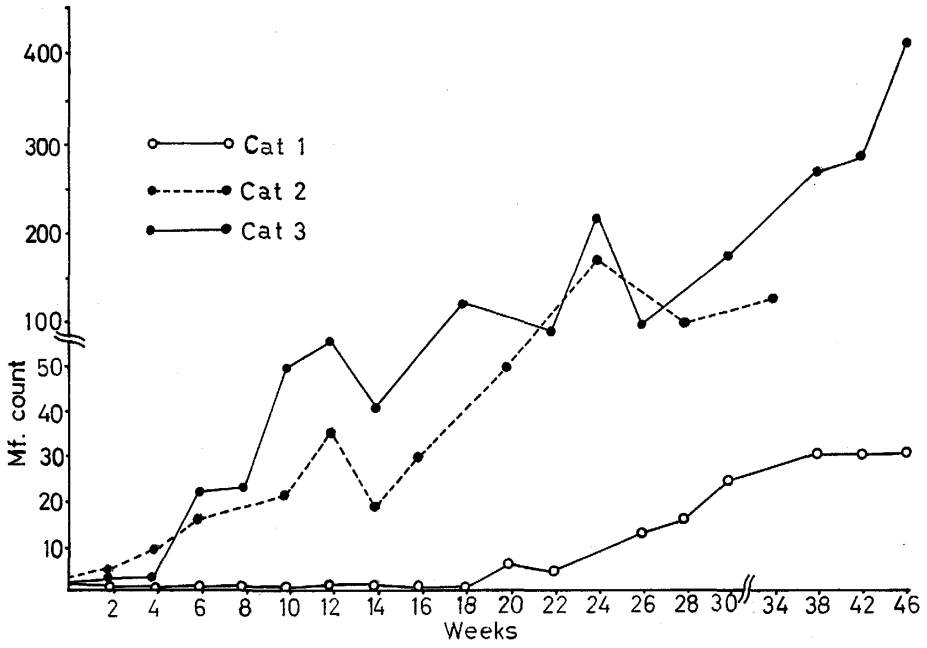

Fig. 1 Increase of microfilaria counts per 60 cmm peripheral blood after the first detection of microfilaremia in cats inoculated with larvae from naturally-infected *Ae. togoi*.

gradually rose to 174 and 417 microfilariae per 60 cmm blood film, respectively, during one year observation after the first detection in the peripheral blood, whereas in Cat 1 microfilaria levels were low, varying from 0 to 31 per 60 cmm film throughout the period of observation (Fig. 1). The microfilariae detected in the cats were identified as *B. malayi* microfilariae because their morphological characteristics were exactly the same as those obtained from the human carriers in Che-ju Island (Aoki *et al.*, 1976). The microfilariae were first detected 102–131 days after inoculation in the peripheral blood of 8 cats of the surviving 13 which had been inoculated to establish the second generation in the cat. Although the peripheral blood of Cat 23 remained negative during the period of 138 days under observation, the necropsy revealed the microfilariae in the pulmonary blood vessels. Thus, the second generation of *B. malayi* was established in 9 cats which had received the infective larvae developed in *Ae. togoi* (Table 5). The microfilariae in the peripheral blood increased gradually in 6 cats, while Cats 16 and 17 produced low counts of 0 to 4 per 60 cmm blood throughout the period of observation (Fig. 2, Table 6).

The third generation was built up in 6 cats out of 10 cats inoculated with the infective larvae from the mosquitoes fed on Cat 31. Two of them had received the larvae developed in *Ae. togoi* and four those developed in *Ae. aegypti*. The prepatent periods were 95–114 days (Table 7). The counts rose to 52 and 183 microfilariae per 60 cmm peripheral blood in Cats 34 and 40, respectively, 10 weeks after the first detection of microfilariae, and to 285 in another 10 weeks in Cat 40 which had been given the infective larvae developed in *Ae. aegypti* (Fig. 3). In the others microfilaria levels remained rather low.

TABLE 5 Experimental transmission of *B. malayi* from Cat 3 to Cats 4–33 to establish the second generation in the cat

Cat no.*	Sex	Weight in kg	No. of larvae inoculated	No. of days observed after inoculation	No. of days from inoculation to mf. detection in blood
Cat 4	F	2.0	130 60	314	115
Cat 5	F	1.5	112	311	102
Cat 9	F	1.7	217	159	110
Cat 14	F	2.5	200 155	441	
Cat 16**	M	2.2	52	392	131
Cat 17	F	2.4	159	743	117
Cat 18	M	1.9	92	159	
Cat 21	F	2.6	186	205	
Cat 23***	F	2.5	200	135	
Cat 24	F	2.4	200	395	
Cat 31	M	4.5	200	582	117
Cat 32	F	3.3	178	461	117
Cat 33	F	2.7	200	434	103

* The cats died shortly after inoculation were not included in the table
** Adult *B. malayi* recovered at necropsy
*** Necropsy revealed microfilariae in the pulmonary blood vessels

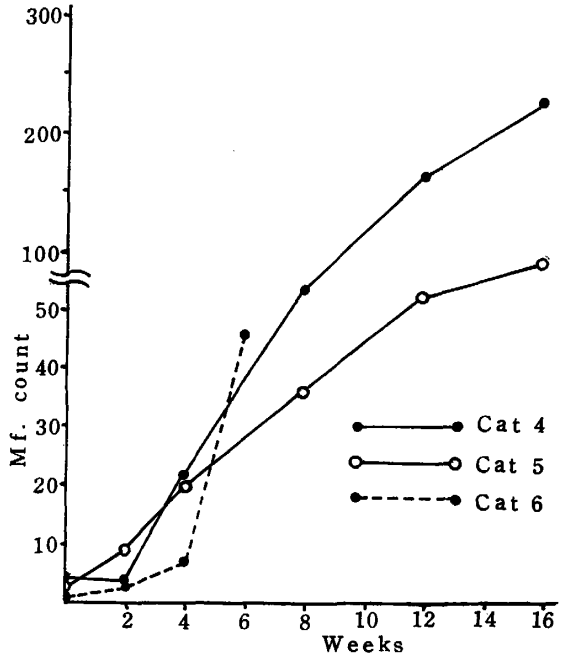

Fig. 2 Increase of microfilaria counts per 60 cmm peripheral blood after the first detection of microfilaremia in Cats 4, 5, 9 inoculated with infective larvae of *B. malayi* from Cat 3.

TABLE 6 Increase of microfilaria counts per 60 cmm peripheral blood in Cats 16–33 after the first detection of microfilaremia

Cat no.	Mf. count at weeks after the 1st detection of microfilaremia																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	20	24
Cat 16	1	0	1	1	0	1	3	4	1			0		3			
Cat 17	1	2	0	0	1	2	0	0	2	1			0		2		
Cat 31	5		118		213		317				368						867
Cat 32	2		9					67									102
Cat 33	16		12			150										174	

TABLE 7 Experimental transmission of *B. malayi* from Cat 31 to Cats 34–43 to establish the third generation in the cat

Cat no.	Sex	Weight in kg	No. of larvae inoculated	Vector	No. of days observed after inoculation	No. of days from inoculation to mf. detection in blood
Cat 34	M	4.1	125	*Ae. togoi*	265	104
Cat 35	F	3.7	104	*Ae. togoi*	232	95
Cat 36	F	3.3	300	*Ae. aegypti*	170	
Cat 37	M	3.7	300	*Ae. aegypti*	128	102
Cat 38	F	2.8	230	*Ae. aegypti*	170	
Cat 39	M	3.0	350	*Ae. aegypti*	165	
Cat 40	M	4.2	350	*Ae. aegypti*	265	97
Cat 41	M	3.2	300	*Ae. aegypti*	282	
Cat 42	M	3.7	300	*Ae. aegypti*	282	114
Cat 43	M	1.9	280	*Ae. aegypti*	282	114

In contrast to the marked nocturnal periodicity in the human carriers from Che-ju Island, moderate numbers of microfilariae always appeared in the peripheral blood of the cats in the daytime and the nocturnal rise in the microfilaria counts was less obvious (Figs. 4, 5, Tables 8, 9, 10). Usually the peak counts were only two to three times greater than the lowest counts in the cats. The pattern in the cat exhibited a sub-periodic tendency of the microfilariae.

As shown in Table 8 the highest microfilaria count was recorded at 10 p.m. and the lowest at noon in Japanese standard time in Cat 2 shortly after the transportation to Los Angeles, California, by air. Although the periodic changes in numbers of microfilariae at 2-hour intervals were not marked, the highest count was found at noon and the lowest at midnight in Japanese standard time 18 weeks afterwards.

Adult worms were recovered only from Cat 3 and 16. The worms which harbored in the perirenal adipose tissue were identified as *B. malayi* from their morphological characteristics (unpublished data).

In *Ae. togoi* most of ingested *B. malayi* microfilariae exsheathed in the stomach,

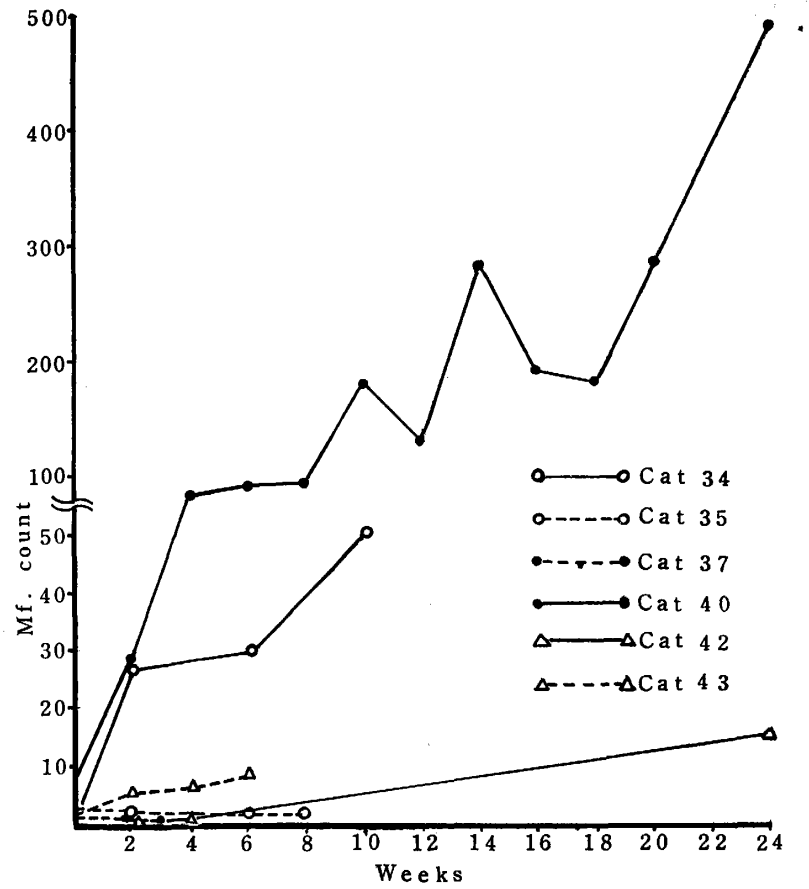

Fig. 3 Increase of microfilaria counts per 60 cmm peripheral blood after the first detection of microfilaremia in Cats 34–43 inoculated with infective larvae of *B. malayi* from Cat 31.

TABLE 8 Microfilaria counts per 60 cmm blood in Cat 2 at 2-hour intervals before and after transportation to Los Angeles. The cat was sent by air late in July, 1973

Date of examination	Mf. count at Japanese standard time on 24-hour clock												
	12: 00	14: 00	16: 00	18: 00	20: 00	22: 00	24: 00	02: 00	04: 00	06: 00	08: 00	10: 00	12: 00
14 Jul. '73	53	47	51	55	103	123	88	109	46	118	130	88	55
22 Aug. '73	31	54	69	64	72	106	89	88	92	88	53	40	51
8 Jan. '74	242	206	208	159	212	223	142	164	172	228	190	189	201

penetrated the stomach wall early, migrated into the thorax and began to grow in the muscles. At 25 C the larvae developed to stage II in 6 days and to stage III in 9 days after intake. The infective larvae finally tended toward the proboscis. The average infection rates were as high as 75.0% with stage I larvae, 69.0% with

TABLE 9 Microfilaria counts per 60 cmm peripheral blood at 2-hour intervals in Cat 31 at the 66th week of microfilaremia and in Cats 40 and 42 at the 24th week

Cat no.	Mf. count at hours on 24-hour clock												
	12: 00	14: 00	16: 00	18: 00	20: 00	22: 00	24: 00	02: 00	04: 00	06: 00	08: 00	10: 00	12: 00
Cat 31	393	410	208	263	298	223	355	224	366	215	289	171	361
Cat 40	506	647	393	500	485	331	507	430	516	464	590	533	590
Cat 42	1	6	12	17	25	15	18	9	13	7	8	10	11

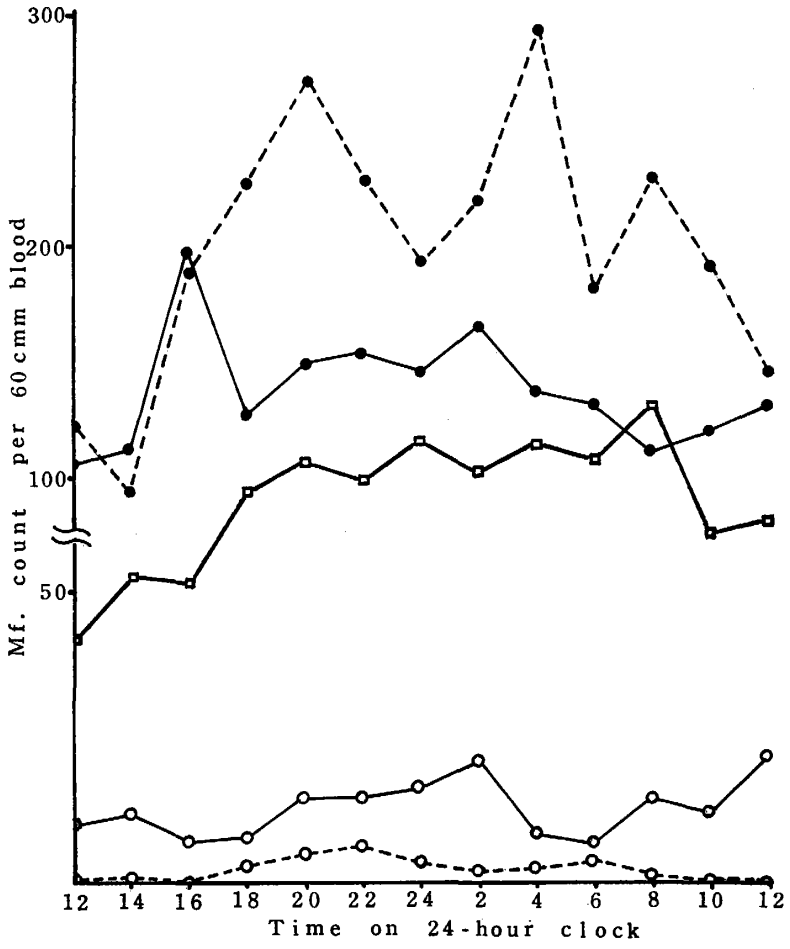

Fig. 4 Periodicity of the microfilariae of *B. malayi* in Cats 1–3 at the 27th week of microfilaremia and at the 86th week.
Open circles connected by solid line, Cat 1 at the 27th week; open circles connected by broken line, Cat 1 at the 86th week; open squares connected by solid line, Cat 2 at the 27th week; solid circles connected by solid line, Cat 3 at the 27th week; solid circles connected by broken line, Cat 3 at the 86th week.

TABLE 10 Microfilaria counts at 2-hour intervals in human carriers of *B. malayi* from Che-ju Is.

Carrier no.	Mf. count per 60 cmm blood at hours on 24-hour clock												
	12:00	14:00	16:00	18:00	20:00	22:00	24:00	02:00	04:00	06:00	08:00	10:00	12:00
1	150	182	445	829	1,019	967	917	997	763	751	303	169	65
2	119	57	115	260	895	972	1,206	1,436	1,306	968	492	150	128
3	73	43	134	315	526	439	460	604	469	507	165	67	33
4	49	30	44	83	269	215	208	272	324	185	62	60	33
5	140	153	285	599	885	1,002	1,059	1,053	819	730	539	193	152
6	72	47	176	513	741	772	808	662	807	610	210	44	51
7	7	3	10	42	111	113	80	90	93	116	37	23	2
8	20	4	12	7	67	246	164	147	109	111	29	21	10
9	61	56	68	82	263	499	418	572	376	373	219	116	82
10	0	1	0	0	8	9	17	21	19	16	20	6	4

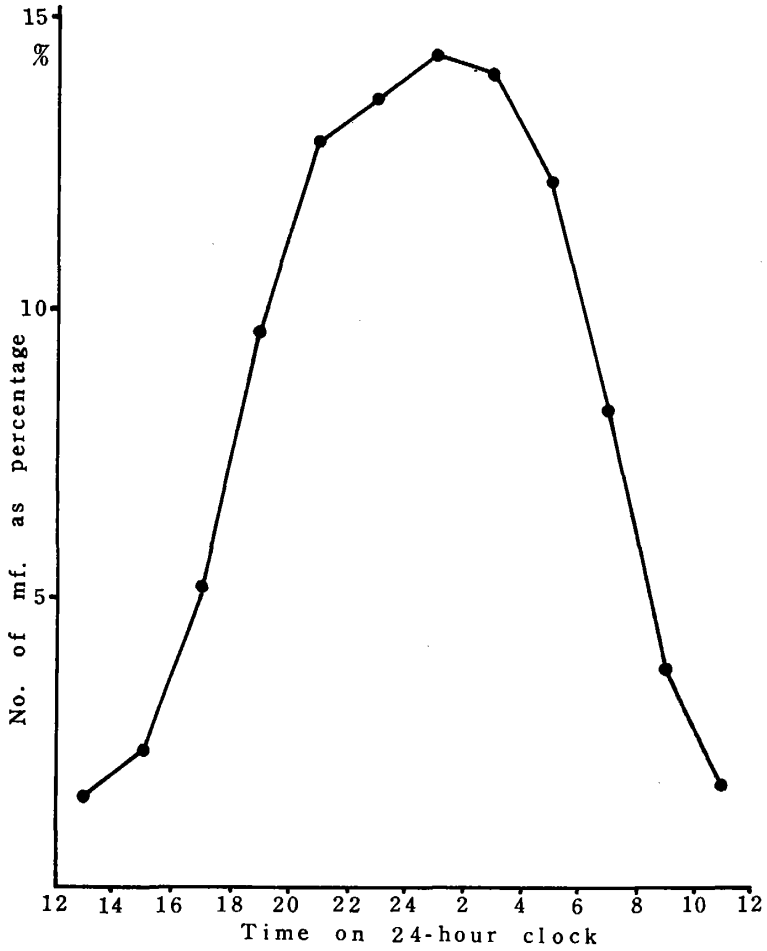

Fig. 5 Secondary histogram drawn from average counts of 10 human carriers. The number of microfilaria as percentage indicates the rate of an area between two adjoining vertical lines to the total areas in the primary histogram.

stage II and 69.3% with stage III (Table 14). The individual counts of larvae varied considerably among the dissected mosquitoes. A mosquito, for instance, harbored more than 40 infective larvae. The mean number of larvae per mosquito (*e.g.* 5.8 with stage III larvae) was greater than the expected number of microfilarial intake (*i.e.* 1.7–4.5 per mosquito) which was estimated from the concentration of microfilariae in the cat and the quantity of blood ingested by *Ae. togoi* (Tables 11, 12).

TABLE 11 Expected numbers of *B. malayi* microfilariae taken up by *Ae. togoi* and *Ar. subalbatus*

Lot no.	Species	Mf. count per 60 cmm blood	Weight of blood-meal (mg/mosquito)	Expected no. of mf. per mosquito
1	*Ae. togoi*	52	2	1.7
2	*Ae. togoi*	52	2	1.7
5	*Ae. togoi*	90	3	4.5
7	*Ae. togoi*	94	2.5	3.9
3	*Ar. subalbatus*	40	3	2.0
4	*Ar. subalbatus*	40	3	2.0
6	*Ar. subalbatus*	150	3	7.5

TABLE 12 Susceptibility of laboratory-bred *Aedes togoi* to *B. malayi* infection

Lot no.	Stage I larvae			Stage II larvae			Stage III larvae		
	No. of mosquitoes		Mean no. of larvae per mosquito	No. of mosquitoes		Mean no. of larvae per mosquito	No. of mosquitoes		Mean no. of larvae per mosquito
	dissected	infected (%)		dissected	infected (%)		dissected	infected (%)	
1				4	4 (100.0)	1.5	94	66 (70.2)	3.7
2				5	2 (40.0)	1.5	144	84 (58.3)	3.4
5	6	4 (66.7)	5.3	2	1 (50.0)	2.0	148	117 (79.0)	6.5
7	34	26 (76.5)	11.2	76	53 (69.7)	8.0	200	139 (69.5)	7.6
Total	40	30 (75.0)	10.4	87	60 (69.0)	7.3	586	406 (69.3)	5.8

In *Ar. subalbatus* most of *B. malayi* microfilariae died in the stomach cavity without penetrating the stomach wall. A few found in the thoracic muscles did not develop and died 2 or 3 days later. Consequently, the larvae were not detected in *Ar. subalbatus* dissected after the 5th day of intake (Tables 13, 14).

As shown in Tables 14 and 15, *Ar. subalbatus* was much more susceptible than *Ae. togoi* to *B. pahangi* infection in contrast to *B. malayi* infection. Although *B. pahangi* microfilariae developed to stage III at 25 C 11 days after intake in both species of mosquitoes, the mean number of infective larvae per mosquito was 5.4 in *Ae. togoi* and 22.9 in *Ar. subalbatus*.

TABLE 13 Susceptibility of laboratory-bred *Armigeres subalbatus* to *B. malayi* infection

Lot no.	Stage I larvae			Stage II larvae			Stage III larvae		
	No. of mosquitoes		Mean no. of larvae per mosquito	No. of mosquitoes		Mean no. of larvae per mosquito	No. of mosquitoes		Mean no. of larvae per mosquito
	dissected	infected (%)		dissected	infected (%)		dissected	infected (%)	
3	46	14 (30.4)	1.8	19	0 (0)	0	20	0 (0)	0
4	63	26 (41.3)	1.6	12	0 (0)	0	8	0 (0)	0
6	50	40 (80.0)	3.4	11	0 (0)	0	50	0 (0)	0
Total	159	80 (50.3)	2.5	42	0 (0)	0	78	0 (0)	0

TABLE 14 Development of Che-ju strain of *B. malayi* in laboratory-bred Nagasaki strains of *Ae. togoi* and *Ar. subalbatus*

Stage of larvae	Days after feeding	*Ae. togoi* No. of mosquitoes		*Ar. subalbatus* No. of mosquitoes	
		dissected	infected (%)	dissected	infected (%)
I	1– 5	40	30 (75.0)	159	80 (50.3)
II	6– 8	87	60 (69.0)	42	0 (0)
III	9–13	586	406 (69.3)	78	0 (0)

TABLE 15 Development of *Brugia pahangi* in laboratory-bred Nagasaki strains of *Ae. togoi* and *Ar. subalbatus*

Stage of larvae	Days after feeding	*Ae. togoi* No. of mosquitoes		*Ar. subalbatus* No. of mosquitoes	
		dissected	infected (%)	dissected	infected (%)
I	1– 5			42	42 (100.0)
II	7– 9			14	14 (100.0)
III	11–13	107	63 (58.9)	159	152 (95.6)

DISCUSSION

It is generally accepted that *Ae. togoi* breeding in rock pools on the seacoast is the main vector of *B. malayi* in the endemic areas of Che-ju Island (Katamine *et. al,* 1973; Wada *et. al,* 1973), whereas *Anopheles sinensis* is suspected to be a probable vector in the inland Korea (Kim *et al.,* 1973). Our present results indicate that the laboratory-bred Nagasaki strain of *Ae. togoi* is competent to develop Che-ju strain of *B. malayi* microfilaria to the infective form and useful as a laboratory vector. It is interesting that *Ar. subalbatus* (Nagasaki strain) is not susceptible to *B. malayi* infection, though the closely related species, *B. pahangi* develops to the infective larva in this mosquito. It was reported that Liverpool strain of *Ae. aegypti* is more susceptible to both periodic and sub-periodic forms of *B. malayi* from Malaya than two other strains of *Ae. aegypti* (Ramachandran *et al.,* 1960). In our studies Liver-

pool strain of *Ae. aegypti* also turned out to be useful as a laboratory vector of Che-ju strain of *B. malayi* (unpublished data).

It is indicated that *Ae. togoi* takes up more microfilariae than would be expected by the present findings that the mean number of larvae per mosquito is greater than the expected number of microfilarial intake estimated from the quantity of ingested blood and the concentration of microfilariae at the time of feeding. Similar observations were reported by Wharton (1957) in the microfilarial intake of *Mansonia (Mansonioides) longipalpis*. The difference in the numbers seems to stem partly from possible discharge of microfilaria-free or microfilaria-scanty droplets during feeding of *Ae. togoi*, although it is unknown to what extent the discharge of droplets is implicated in the underestimation of the quantity of ingested blood.

Malayan strains of periodic and sub-periodic forms of *B. malayi* were transmitted experimentally from man to domestic cats (Edeson and Wharton, 1957; Wharton *et al.*, 1958), and from experimentally infected cats to cats (Edeson and Wharton, 1958; Wharton *et al.*, 1958; Ramachandran *et al.*, 1960; Laing *et al.*, 1961). The periodic form from Malaya was reported not to develop well in cats with long prepatent periods (95–175 days) and low microfilaria counts in the peripheral blood, in contrast to the sub-periodic form from Malaya which was readily transmitted with short prepatent periods and high microfilaremia (Wilson *et al.*, 1958; Laing *et al.*, 1961). The prepatent periods of Che-ju strain are 91–131 days, almost equal to those of the periodic form from Malaya, in the experimental transmission to domestic cats. The microfilaria counts in the peripheral blood, however, gradually increase in one third to two thirds of cats successfully infected with Che-ju strain. Although the inoculated numbers of infective larvae are larger in our experiments than in the previous authors', the high microfilaremia indicates that domestic cats are relatively "good" hosts for Che-ju strain.

As to the periodic form from Malaya, it was reported that the marked nocturnal periodicity was maintained in cats after transmission (Edeson, 1959; Laing *et al.*, 1961). In contrast to those of Malayan strain, the microfilariae of Che-ju strain exhibit a sub-periodic tendency in the cat, although Che-ju strain is characterized by a marked nocturnal periodicity of microfilariae in human carriers. The sub-periodic tendency might be caused by the physiology of cats, which are mainly nocturnal. To confirm it, "the apparently sub-periodic Che-ju strain in cats" would have to be transmitted to the monkeys with a strictly diurnal activity-cycle.

It is interesting to note that the microfilaria counts of Cat 2 are higher at night and lower in the daytime in Japanese standard time shortly after the transportation to California by air, and that the higher counts are found at night and the lower in the morning in U.S. Pacific standard time 18 weeks afterwards. It may indicate that some little or a certain fixed time must elapse before *B. malayi* and/or the cat become adapted to the local time.

Developing or adult worms were found in the cats inoculated with Malayan strains in the high percentage of 72, although mature worms were recovered small in numbers (Edeson and Buckley, 1959). In the present studies on Che-ju strain adult worms have been recovered from only 2 cats out of 8 in which microfilariae were detected earlier or at necropsy. The reasons why the rate of adult worm

detection is low remain to be explained. The localization of recovered adult worms in the perirenal adipose tissue may suggest the migration of the larvae to the internal lymph vessels, which put a difficulty in detecting the worms at necropsy.

The presence of natural infection of *B. malayi* in forest animals and the domestic cat was reported in Malaya, where the dusky leaf-monkey was supposed to be an important reservoir of the sub-periodic form (Edeson, 1959; Laing, 1959; Laing *et al.*, 1960; Wilson, 1961). Also a zoonosis of the sub-periodic form was reported in the Phillippines (Roseboom and Cabrera, 1965). However, the periodic form is thought to be more specialized and more host-specific (*i.e.* rare in vertebrate hosts other than man) than the sub-periodic form, whose wide range of hosts indicates a less-developed, younger stage in the evolution of parasites (Laing *et al.*, 1960). In Che-ju Island the domestic cats, which are few because of the folk custom of not keeping cat, are negative for microfilariae: The natural infection of *B. malayi* is not detected in the dogs either (unpublished data). Very few monkeys, if any, are kept by the islanders. Thus, there seems to be no substantial evidence to suggest that any mammal other than man may form a reservoir of the periodic form infection in the island. Further studies on wild animals are necessary to conclude that only man is the definitive host of the Che-ju strain of *B. malayi*.

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韓国済州島のマレー糸状虫に関する研究

4 *Brugia malayi* (Che-ju strain) のネコへの感染実験

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済州島糸状虫浸淫地のミクロフィラリア (mf.) 陽性者の住居で採集した，自然感染 *Aedes togoi* より得た感染幼虫を，鼠径部皮下に注射して，3頭のイエネコに Che-ju strain の *B. malayi* を感染させることに成功した。その感染ネコを，恒温室内で累代飼育中の *Ae. togoi* (Nagasaki strain) に吸血させて得た感染幼虫を接種し，9頭のネコに第2代の感染を成立させた。*Ae. togoi* を用い2頭の，*Ae. aegypti* (Liverpool strain) を用い4頭のネコに，皮下接種により第3代の感染を成立させた。接種後 mf. を末梢血に検出するまでに91～131日を要した。末梢血中の mf. 数は感染の成立したネコの1/3～2/3では，次第に増加した。従ってネコは Che-ju strainの *B. malayi* の比較的好適な宿主と考えられる。Che-ju strain の mf. はヒトでは periodic type であるが，ネコでは夜間出現性が顕著でなく，昼間にもかなり末梢血中に認められ，sub-periodic の傾向を示した。生前或は剖検時 mf. が検出された8頭のうち，2頭の腎周囲脂肪組織より成虫が回収された。成虫の局在部位は，接種された虫体の体内部リンパ管への移動を示唆し，その為，剖検時成虫の検出が困難となると思われる。累代飼育中の *Ae. togoi* の *B. malayi* 感染幼虫による感染率は69.3％で，感染蚊1個当たりの平均保有感染幼虫数は，5.8隻であった。従って *Ae. togoi* は *B. malayi* の laboratory vector として用い得る。*Armigeres subalbatus* は *B. malayi* に感受性を示さなかったが，近似種の *B. pahangi* は両種の蚊にて感染幼虫まで発育した。

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