

Filariasis transmission in Samoa

I. Relation between density of microfilariae and larval density in laboratory-bred and wild-caught *Aedes (Stegomyia) polynesiensis* (Marks) and wild-caught *Aedes (Finlaya) samoanus* (Gruenberg)

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Transmission experiments of laboratory-bred and wild-caught *Aedes polynesiensis* and wild-caught *Ae. samoanus* on carriers with different levels of microfilaraemia showed that the percentage of mosquitoes infected, the average number and range of larvae found in each infected mosquito were directly proportional to the microfilarial densities in the carrier at the time of feeding. There was no difference between the results for laboratory-bred and wild-caught mosquitoes. *Aedes polynesiensis* fed on low carriers (around 20 Mf ml⁻¹ and less) gave an average infection rate of 4·9%. Each infected mosquito harboured only one larva.

A plot of the probit of percentage *Ae. polynesiensis* positive against the logarithm of microfilarial density showed a linear relationship. Using the regression line, at 95% confidence interval, a microfilarial density of 1 ml⁻¹ would give an infection rate of 0·22–2·51%, a density of 0·1 Mf ml⁻¹ an infection rate of 0·01–1·0%.

The concentrating capacity of *Ae. polynesiensis* ranged from 0·70 to 4·74. As microfilaria densities decreased, concentration increased. The microfilarial intake, the subsequent worm burden and concentrating capacity were less in *Ae. samoanus* than *Ae. polynesiensis*. There was no evidence of any association between microfilarial density and concentration in *Ae. samoanus*.

Filariasis due to sub-periodic *Wuchereria bancrofti* is an important public health problem in the South Pacific. In Samoa the disease has been endemic for a long time and is transmitted by two species of mosquito, the day-biting *Aedes (Stegomyia) polynesiensis* (Marks) and night-biting *Aedes (Finlaya) samoanus* (Gruenberg).

The first blood surveys in the country, conducted in 1965 in 21 villages, showed a microfilarial rate of 19·1% and a median value (MfD_{50}) of microfilarial density of 18·4 (Maung and Penaia, unpublished report). A mass drug administration programme for the entire population above the age of two years was carried out in 1965–1966, using diethylcarbamazine (DEC) citrate tablets orally at a dosage of 5 mg kg⁻¹ body weight once a week for six weeks followed by once a month for 12 months. A subsequent blood survey in 115 sample villages showed a drastic reduction in the microfilarial rate to 1·6% and MfD_{50} to 2·8. The infective rates in *Ae. polynesiensis* fell from 2·95 to 0·082% after drug treatment.

A repeat survey in 1969 showed a rise in microfilarial rate to 2·26%. Thus, in order to reduce further the chances of transmission, a second mass drug administration was carried out in 1971 using a modified monthly dosage schedule of 6 mg kg⁻¹ body weight for 12 months. The complete 12 doses were taken by 51% of the population, coverage being lowest in young adult males. Following this treatment, the results of blood surveys by Maung and Penaia showed microfilarial rates of 0·19% in 1972 and 0·14% in 1973. In 1975 the rate was

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2·12% but in some villages it was as high as 5·7%. All the above mentioned surveys have been carried out using the relatively insensitive technique of examining stained blood films of 20–60 mm³ of capillary blood.

Recently more sensitive methods for the detection of microfilariae by membrane filtration of 1 ml of venous blood have been tested in the South Pacific areas (Desowitz and Southgate, 1973; Desowitz *et al.*, 1973; Desowitz and Hitchcock, 1974; Southgate, 1974); the results show that large numbers of people have very low densities, many with less than ten microfilariae in 1 ml of venous blood. The finding of these ultra-low density carriers introduces new problems in planning control programmes. It is important to determine the capacity of these carriers to act as a source of infection for vectors, particularly when microfilaraemia persists after treatment.

In 1976, the Government of Samoa and the World Health Organization agreed to co-operate in a filariasis research project in Samoa to study further the epidemiology of filariasis with emphasis on the dynamics of transmission at the low level of microfilaraemia; to study the ecology and bionomics of vectors; and to evaluate methods of control which could be applied to all countries of the South Pacific.

Preliminary studies by Bryan and Southgate (1976) in Samoa showed that a low-level microfilaraemia can readily infect a significant proportion of *Ae. polynesiensis*, hence low density carriers could be positive reservoirs if vector mosquitoes were not controlled. The present study describes feeding experiments on carriers with different levels of *W. bancrofti* microfilaraemia by *Ae. polynesiensis* and *Ae. samoanus*.

MATERIALS AND METHODS

Experiments were carried out with laboratory-bred and wild-caught *Ae. polynesiensis* and wild-caught *Ae. samoanus*. *Aedes polynesiensis* caught on human bait in Apia were fed on human blood and maintained in cages to obtain eggs for laboratory culture. Details of culture and biology are reported separately. In the first series of experiments laboratory-bred *Ae. polynesiensis* were fed on carriers with different levels of microfilaraemia. The susceptibility level of *W. bancrofti* in laboratory-bred *Ae. polynesiensis* was then compared with that in wild *Ae. polynesiensis*. In a third series, batches of *Ae. samoanus* taken in a village near Apia were fed on carriers with different levels of microfilaraemia. Finally, wild-caught *Ae. polynesiensis* were tested with wild-caught *Ae. samoanus* on the same volunteer.

The Human Volunteer

Volunteers for feeding experiments were selected from among the carriers identified in the pilot survey of the microfilaria prevalence study (Shibuya *et al.*, 1979). In this study the nucleopore filter technique on 1 ml of venous blood was adopted. Sixteen carriers were used, ranging from the highest density recorded to the lowest (see Table 1). Most volunteers were used in more than one experiment. Before each experiment, 1 ml of venous blood was drawn from the volunteer and processed for nucleopore filtration. After the feeding, 60 mm³ of blood was taken by finger-prick on to a slide for staining and examination. The microfilarial density of a volunteer recorded in Table 1 was the count taken at the first experiment. Each volunteer was given a serial number (Table 1) and counts from the same volunteer shown in subsequent experiments are given in later tables using the same serial number.

Feeding Experiments on the Carrier

A batch of 100–400 mosquitoes for a feeding experiment on a volunteer was held in a cage of 20 × 20 × 30 cm. The sugar meal inside the cage was removed 12 hours before blood-feed. To feed the mosquitoes the arm of the volunteer was introduced into the cage through the sleeve.

TABLE I

Age, sex, microfilarial densities at initial test and history of diethylcarbamazine treatment of carriers used in the feeding experiments

Serial number	Name	Age	Sex	Microfilarial density		History of prior treatment with diethylcarbamazine
				Nuclepore (1 ml)	Fingerprick (60 mm ³)	
1	Fa'asina	49	Male	2616	175	No treatment
2	Liasi	18	Female	583	48	No treatment
3	Litara	33	Female	0*	0	No treatment
4	Ifi	59	Male	37	2	No treatment
5	Niupopo	50	Male	5290	435	No treatment
6	Talita	13	Male	7	0	No treatment
7	Eti	28	Male	1160	56	No treatment
8	Fa'amana	14	Male	581	51	No treatment
9	Tu'utele	49	Male	32	2	Ten monthly doses 1971
10	Meafou	60	Female	1249	80	No treatment
11	Neva	45	Female	460	44	Five monthly doses 1971
12	Kapisi	14	Male	397	46	No treatment
13	Pisila	14	Female	328	34	No treatment
14	I'umai	55	Male	586	44	No treatment
15	Taimi	30	Female	789	60	No treatment
16	Alaesc	30	Male	1	0	No treatment

*This carrier showed a count of 3 Mf ml^{-1} of venous blood at the pilot survey of Shibuya et al. (1979).

Unless otherwise stated, *Ae. polynesiensis* was fed between 1500 and 1700 hours and *Ae. samoanus* between 1900 and 2000 hours. The starved *Ae. samoanus* quickly settled on the arm and most females were replete in ten minutes. *Aedes polynesiensis* were noticeably slower, but most of them had fed by 30–45 minutes. Soon after feeding, five mosquitoes were killed for immediate dissection.

Wild-caught Mosquitoes for Feeding Experiments

As wild-caught mosquitoes were used in nearly all experiments with *Ae. samoanus* it was important to ensure that the mosquitoes used were not infected with *W. bancrofti*. *Aedes samoanus* for the experiments were captured from areas far away from human habitation and the several hundred specimens dissected gave negative results. In all the experiments with *Ae. samoanus* fed on different carriers, no infection older than the expected stage of development was found. The same was true for *Ae. polynesiensis*. *Aedes polynesiensis* for feeding experiments were captured at Mulinuu by the sea coast away from human habitation.

Maintenance of Fed Mosquitoes

Fed females were transferred to a clean cage and humidity provided immediately with wet lint draped on the top and on the sides of the cage. It was very difficult to keep the infected mosquitoes alive for 12–14 days at the prevailing room temperature. There were many deaths during the first 24 hours after the infecting blood-meal. To prevent this in later experiments, the mosquitoes were transferred to a clean cage 12 hours after the feeding. Nevertheless, there was high mortality in some experiments because of fluctuations in temperature. From the third day after feeding, wet paper towelling was provided for egg laying on the floor of the cage, lined inside a 100 ml beaker and above the mesh on the top

side. Each morning the paper towels were replaced with fresh ones. Blood meals were offered to the infected females daily from the third day to the tenth day of incubation. It was found that the mosquitoes survived better when they were allowed to ovipost and feed in the cage. Each day all dead mosquitoes were collected from the bottom of the cage and dissected for any developing larvae. At the end of the incubation period all surviving mosquitoes were anaesthetized and dissected.

Dissection of Mosquitoes

Dead mosquitoes were dissected each day. In mosquitoes which died up to the tenth day after feeding only the thorax was dissected and the muscles teased out to observe the developing stages. Subsequently individual mosquitoes were separated into head, thorax and abdomen in three drops of saline and each part dissected separately. The infections were noted under the stereoscopic microscope and subsequently identified under the compound microscope. The larvae in each infected mosquito were counted and measured.

Blood-meal Size of Mosquitoes

The number of microfilariae that a batch of test mosquitoes would be expected to ingest from a given carrier was calculated by determining the number of specimens that fed on 1 ml of blood using the method of Bryan and Southgate (1976). Eight weighing experiments using an electrically-operated analytical balance were carried out on laboratory-bred *Ae. polynesiensis*, 13 on wild-caught *Ae. polynesiensis* and 13 on wild-caught *Ae. samoanus* totalling 346, 653 and 827 mosquitoes in each respective category. The average number of mosquitoes which ingested 1 ml of blood was determined as 584 for laboratory-bred *Ae. polynesiensis*, 611 for wild-caught *Ae. polynesiensis* and 582 for wild-caught *Ae. samoanus*. These values have been used in calculating the expected number of microfilariae ingested by a batch of mosquitoes. During these experiments neither *Ae. polynesiensis* nor *Ae. samoanus* expelled any blood while feeding.

RESULTS

Relation between Density of Microfilariae and Larval Density in Laboratory-bred *Ae. polynesiensis*

The results of 32 experiments (Table 2) show that the percentage of mosquitoes which became infected, the average number of larvae found in *Ae. polynesiensis* dissected, the average and range of larvae found in infected specimens were directly proportional to the microfilarial densities in the carrier at the time of feeding. In each experiment the larval count comprised developing larvae from dead mosquitoes dissected daily and developing and infective larvae from specimens killed at the end of the incubation period. With only one exception at least half of the mosquitoes became infected when fed on carriers with densities over about 500 Mf ml^{-1} or $40 \text{ Mf } 60 \text{ mm}^3$ (range 30–50) of fingerprick blood. Less than 10% of mosquitoes were infected using microfilaria levels of up to 25 Mf ml^{-1} .

Since the interest lies in low and ultra-low levels of infection, several experiments were carried out with carriers 4, 9 and 6 (Table 2). In four experiments using carrier 4 when his count was less than 40 Mf ml^{-1} , 278 mosquitoes were dissected of which 27 were infected; 22 of them harboured a single larva each and five had two larvae each. In five experiments with carrier 9335 mosquitoes were dissected and 21 were infected. Here 16 mosquitoes harboured a single larva each and five had two larvae each. In six experiments with the ultra-low carrier 6576 mosquitoes were dissected and 30 were infected, each with only a single larva.

A plot of the probit of percentage positive against the logarithm of microfilarial density showed a generally linear relationship but with some dispersion. Because the interest lies in

TABLE 2

Laboratory-bred *Aedes polynesiensis* fed on carriers with different densities of microfilariae*

No. of adult mosq. carriers	Mf density no. of microfilariae/ page/pinch	No. of mosquitoes dissected	Percentage injected	Average no. of larvae per mosquito	Range of larvae per infected mosquito	No. of mosquito with indicated no. of larvae										Total no. of larvae observed (E)	Total no. of larvae expected (E)					
						No. of mosquito with indicated no. of larvae																
						0	1	2	3	4	5	6	7	8	9	10	11	12	13			
5	5290 (435)	60	95.0	1.98	12.61	1-57	3	2	2	2	1	5	2	6	3	13	10	7	2	1	719	543.5
1	2616 (175)	63	76.2	4.78	6.27	1-25	15	11	3	5	6	2	2	6	1	1	6	2	2	1	301	282.2
8	1390 (89)	52	57.7	3.33	5.77	1-15	22	8	2	1	3	2	3	2	1	2	4	-	-	-	173	123.8
10	1249 (80)	79	46.8	1.09	2.32	1-10	42	12	13	3	5	2	-	-	-	-	-	-	-	-	86	78.6
7	1160 (56)	29	54.5	2.95	5.42	1-12	10	1	2	1	1	1	1	1	1	1	1	1	1	1	65	47.1
2	533 (46)	15	60.0	3.07	6.20	1-18	9	2	4	2	1	3	2	1	1	1	1	1	1	1	124	57.6
11	460 (44)	94	43.6	1.39	3.20	1-10	53	13	8	4	3	5	1	0	1	-	-	-	-	-	46	15.0
12	357 (36)	46	30.4	0.48	1.57	1-5	32	9	3	1	-	-	-	-	-	-	-	-	-	-	22	31.3
13	326 (33)	34	29.4	0.56	1.90	1-3	24	4	3	-	-	-	-	-	-	-	-	-	-	-	19	19.1
4	101 (8)	53	13.2	0.15	1.14	1-2	46	6	1	-	-	-	-	-	-	-	-	-	-	-	8	9.2
72	52 (5)	27	11.1	0.19	1.67	1-3	24	0	1	-	-	-	-	-	-	-	-	-	-	-	5	3.5
67	4 (4)	57	15.8	0.18	1.11	1-2	48	8	1	-	-	-	-	-	-	-	-	-	-	-	10	6.5
37	(2)	30	20.0	0.30	1.50	1-2	24	3	-	-	-	-	-	-	-	-	-	-	-	-	9	1.9
29	1 (1)	52	9.6	0.10	1-0	1-1	47	5	-	-	-	-	-	-	-	-	-	-	-	-	5	2.6
29	(2)	84	11.9	0.13	1-10	1-2	74	9	1	-	-	-	-	-	-	-	-	-	-	-	11	4.2
28	(0)	112	5.4	0.06	1.17	1-2	106	5	1	-	-	-	-	-	-	-	-	-	-	-	7	5.4
9	32 (2)	90	8.9	0.11	1.25	1-2	82	6	2	-	-	-	-	-	-	-	-	-	-	-	10	4.9
20	1 (1)	63	6.4	0.08	1.25	1-2	59	3	1	-	-	-	-	-	-	-	-	-	-	-	5	2.8
23	(1)	57	7.0	0.09	1.25	1-2	53	3	1	-	-	-	-	-	-	-	-	-	-	-	5	2.2
16	1 (1)	42	7.4	0.07	1-0	1-1	39	3	1	-	-	-	-	-	-	-	-	-	-	-	3	1.2
12	(0)	53	2.4	0.04	1.50	1-2	81	1	-	-	-	-	-	-	-	-	-	-	-	-	3	1.7
12	10 (0)	83	2.4	0.06	1-0	1-1	167	11	-	-	-	-	-	-	-	-	-	-	-	-	11	7.0
6	23 (1)	178	6.2	0.03	1.00	1-0	1-1	71	2	-	-	-	-	-	-	-	-	-	-	-	2	1.8
13	13 (0)	73	2.7	0.03	1.00	1-0	1-1	74	4	-	-	-	-	-	-	-	-	-	-	-	0	1.1
12	12 (0)	91	6.5	0.07	1-0	1-1	85	6	-	-	-	-	-	-	-	-	-	-	-	-	6	1.9
10	10 (0)	53	5.4	0.05	1.00	1-1	52	3	-	-	-	-	-	-	-	-	-	-	-	-	3	3.3
7	7 (0)	101	3.9	0.04	1-0	1-1	97	4	-	-	-	-	-	-	-	-	-	-	-	-	4	1.2
7	7 (0)	78	5.4	0.05	1-0	1-1	74	4	-	-	-	-	-	-	-	-	-	-	-	-	4	0.9
16	1 (0)	271	0.0	0.00	0.00	0	271	0	-	-	-	-	-	-	-	-	-	-	-	-	0	0.5
3	1 (0)	96	0.0	0.00	0.00	0	96	0	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0
3	0	35	0.0	0.00	0.00	0	35	0	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0
3	0	63	0.0	0.00	0.00	0	65	0	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0
9	0	97	0.0	0.00	0.00	0	97	0	-	-	-	-	-	-	-	-	-	-	-	-	0	0.0

* Experiments with carriers subsequently treated with diethylcarbamazine tablets are not included in this table.

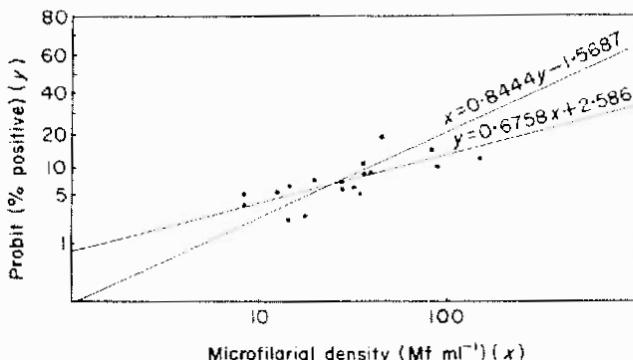


Fig. Regression of probit of percentage positive against logarithm of microfilarial density.

the lower Mf range a more complete analysis using data from carriers 4, 9 and 6 was carried out. The plot for 18 experiments shows a good degree of association between variables ($r=0.755$, $16\ df$, $P<0.001$) and shown in the Figure.

A regression analysis of the y (probit percentage positive) and x (logarithm of Mf) gives the regression lines $y=0.6758x+2.5860$ and $x=0.8445y-1.5687$. Using the line y on x for $Mf=1$ gives a 95% confidence interval for percentage positive of 0.22–2.51 and for $Mf=0.1$ the 95% confidence interval for percentage positive of 0.01–1.0.

Using the data on the total number of larvae observed in a batch of mosquitoes and the calculated expected number of microfilariae ingested by the same batch, it is possible to determine whether *Ae. polynesiensis* took up and developed more microfilariae than would be expected from their density in the carrier. This capacity for concentrating microfilariae, shown in Table 2, ranged from 0.70 to 4.74. Regression analysis of y (O/E) and x (logarithm of Mf) gave the equation $y=0.59x+3.13$. The regression coefficient (-0.59) differs significantly from zero ($t=2.97$, $26\ df$, $0.005 < P < 0.01$). Thus, as microfilarial densities decreased, concentration increased. The estimate of microfilaraemia at which the observed number of larvae is equivalent to the expected value is at the density of $1848\ Mf\ ml^{-1}$ in the carrier.

Susceptibility of Laboratory-bred and Wild-caught *Ae. polynesiensis* to *W. bancrofti*

In order to show if there was any difference between the susceptibility to *W. bancrofti* of laboratory-bred and wild-caught *Ae. polynesiensis*, ten experiments were carried out using carriers with different levels of microfilaraemia. Wild *Ae. polynesiensis* were caught on the morning of the experiment and kept starved until their blood-feed. Laboratory-bred and wild-caught *Ae. polynesiensis* held in separate cages were fed simultaneously on the two arms of the carrier.

The results given in Table 3 show that, except for the experiment using carrier 11, the difference between the percentage mosquitoes infected in the two batches is not statistically significant ($\chi^2=14.88$, $10\ df$, $0.1 < P < 0.2$), neither is there any difference between the values of O/E in the two batches of mosquitoes.

Relation between Density of Microfilariae and Larval Density in Wild-caught *Ae. samoanus*

In 18 experiments, batches of wild-caught *Ae. samoanus* were fed on carriers with different levels of microfilaraemia. The results (Table 4) show that, as for *Ae. polynesiensis*, the percentage of *Ae. samoanus* which became infected, the average number of larvae found in the

TABLE 3

Comparison of results of susceptibility of laboratory-bred and wild-caught Aedes polynesiensis to Wuchereria bancrofti in carriers with different levels of microfilariae

Serial no. of carrier	Mf density (nucleopore 1 ml)	Mosquito batch	No. of mosquitoes dissected	Percentage infected	Average no. of larvae per mosquito	Average no. of larvae per infected mosquito	Total no. of larvae observed (O)	Total no. of larvae expected (E)	O/E
8	1390	L	52	57.69	3.33	3.77	173	123.8	1.40
		W	121	59.50	3.64	6.11	440	275.3	1.60
10	1249	L	22	54.54	2.95	5.42	65	47.1	1.38
		W	25	60.00	4.80	8.00	120	51.1	2.35
11	460	L	94	43.62	1.39	3.20	131	74.0	1.77
		W	38	15.79	0.40	2.50	15	28.6	0.52
12	397	L	46	30.43	0.48	1.57	22	31.3	0.70
		W	43	51.16	1.14	2.23	49	27.9	1.76
13	328	L	34	29.41	0.56	1.90	19	19.1	0.99
		W	33	33.30	1.21	3.63	40	17.7	2.25
6	120	L	112	16.07	0.20	1.22	22	23.0	0.95
		W	118	27.12	0.46	1.69	54	23.2	2.33
4	72	L	27	11.10	0.19	1.67	5	3.3	1.52
		W	54	13.00	0.30	2.29	16	6.4	2.50
9	16	L	42	7.14	0.07	1.00	3	1.2	2.50
		W	31	6.45	0.06	1.00	2	0.8	2.50
9	12	L	83	2.41	0.04	1.50	3	1.7	1.76
		W	64	3.13	0.03	1.00	2	1.3	1.54
1	9	L	53	3.80	0.02	1.00	2	0.8	2.50
		W	99	3.03	0.03	1.00	3	1.5	2.00

Abbreviations: L, laboratory-bred; and W, wild-caught.

mosquitoes, and the average and range of larvae found in the infected specimens were directly proportional to the microfilarial densities in the carrier at the time of feeding.

The results suggest that fewer than 10% of *Ae. samoanus* became infected when fed on densities as high as 50 Mf ml⁻¹, a microfilarial level higher than that required to infect 10% of *Ae. polynesiensis*. It is also possible to compare the results of *Ae. samoanus* and *Ae. polynesiensis* fed on low carriers 4 and 9 because of the similar range of density in the carriers when fed by the two species.

Thus with carrier 4, two experiments with *Ae. samoanus* fed on densities 55 and 87 Mf ml⁻¹ gave an infected proportion of 12 out of 158 or 7.6%, while in six experiments with *Ae. polynesiensis* fed on the same carrier at an Mf range of 28–72 (see Table 2), a higher proportion of mosquitoes, 39 (10.8%) out of 362 were infected.

Carrier 9 had a microfilarial density range of 12–34 during the experiments on both *Ae. polynesiensis* and *Ae. samoanus* conducted some seven to eight months apart. In three experiments on *Ae. samoanus*, 11 out of 233 or 4.7% were infected while in five experiments on *Ae. polynesiensis* there was a higher infection rate of 6.3%. In all experiments under a density of 55 Mf ml⁻¹ each infected mosquito harboured only one larva.

A plot of the probit of percentage positive against the logarithm of number of microfilariae shows a linear relationship in this series also. Concentrating capacity is lower than for *Ae. polynesiensis* and there is no evidence of any association between microfilarial density and concentration.

TABLE 4

Wild-caught *Aedes samoaicus* fed on carriers with different densities of microfilariae

Serial no. of carriers	Off density at capture (stage/patch)	No. of mosquitoes dissected	Percentage infected	Average no. of larvae per mosquito	Range of no. of larvae per infected mosquito	Average no. of larvae per mosquito	Range of no. of larvae per infected mosquito	Total								Total											
								No. of mosquitoes with indicated no. of larvae	0	1	2	3	4	5	6	7	8	9	10-14	15-19	20-24	25-29	30-60	60+	no. of larvae observed	no. of larvae expected	O
10	1.275 (168)	95	69.5	4.28	6-17	1-31	29	7	10	8	5	8	2	6	2	7	1	1	1	1	1	1	1	407	208.1	1.96	
	1.275 (168)	103	72.8	2.29	3-15	1-32	28	8	15	8	10	5	8	4	4	5	6	1	1	1	1	1	1	393	225.6	1.74	
8	1.449 (102)	43	67.4	2.42	3-18	1-15	15	6	6	7	2	1	1	1	3	1	1	1	1	1	1	1	1	104	107.0	0.97	
	1.412 (82)	89	42.7	1.28	3.00	1-11	51	16	6	5	1	3	3	2	0	1	1	1	1	1	1	1	1	114	139.5	0.82	
11	3.97 (66)	62	54.8	1.94	3.53	1-13	28	8	9	4	3	4	3	0	1	1	1	1	1	1	1	1	1	1	120	95.6	1.26
	3.95 (33)	52	51.9	1.12	2.15	1-6	25	10	9	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	58	26.4	2.20
12	3.65 (21)	93	32.3	0.47	1.47	1-3	63	19	8	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	44	42.3	1.04
	3.81 (35)	66	33.3	0.61	1.82	1-6	44	16	3	2	3	0	1	0	0	1	1	1	1	1	1	1	1	1	40	31.9	1.25
13	3.30 (28)	119	35.3	0.85	2.41	1-8	77	17	9	7	5	2	0	1	1	1	1	1	1	1	1	1	1	101	47.0	2.15	
	3.87 (8)	73	11.0	0.16	1.50	1-3	63	5	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	12	10.9	1.10	
4	5.5 (2)	85	4.7	0.05	1.00	1-1	81	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	8.0	0.50	
	3.34 (4)	57	7.0	0.09	1.25	1-2	53	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	3.3	1.52	
9	19.1	50	0.0	0.00	0.00	—	50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	1.6	—	—	
	18.1	126	4.8	0.05	1.00	1-1	120	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	120	6	3.9	
1	24.2	37	5.4	0.05	1.00	1-1	35	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1.5	
	10.0	120	3.3	0.03	1.00	1-1	116	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	2.1	1.90	
16	1.0	54	0.0	0.00	0.00	—	54	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0.1	—		
	1.0	56	0.0	0.00	0.00	—	56	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0.1	—		

TABLE 5

Intake of microfilariae in wild-caught Aedes polynesiensis and Aedes samoanus when fed on the same carrier

Serial no. of carrier	Mf density (nucleopore 1 ml)	Mosquito batch	No. of mosquitoes dissected	Percentage infected	Average no. of larvae per mosquito	Average no. of larvae per infected mosquito	Total no. of larvae observed (O)	Total no. of larvae expected (E)	O/E
8	1464 1542	P S	24 33	95.8 72.7	5.92 2.18	6.17 3.00	142 72	57.5 87.4	2.47 0.82
15	771 789	P S	32 40	68.8 67.5	3.94 2.73	5.73 4.04	126 109	40.4 54.2	3.12 2.01
14	687 586	P S	50 50	78.0 26.0	3.62 0.62	4.64 2.38	181 31	56.2 50.3	3.22 0.62
14	549 618	P S	42 40	61.9 52.5	1.79 1.00	2.88 1.90	75 40	37.7 42.5	1.99 0.94
4	79 104	P S	42 60	39.9 6.7	0.50 0.07	1.62 1.00	21 4	5.4 10.7	3.89 0.37
1	28 23	P S	47 60	10.6 6.7	0.15 0.07	1.40 1.00	7 4	2.2 2.4	3.18 1.67
6	11 11	P S	70 70	5.7 2.9	0.06 0.03	1.00 1.00	4 2	1.3 1.3	3.08 1.54
9	5 5	P S	48 82	2.1 1.2	0.02 0.01	1.00 1.00	1 1	0.4 0.7	2.50 1.43

Ae. polynesiensis fed at 1600–1700 hours and *Ae. samoanus* at 1900–2000 hours.

Abbreviations: P, *Ae. polynesiensis*; S, *Ae. samoanus*.

Comparison of the Intake of Microfilariae by *Ae. polynesiensis* and *Ae. samoanus*

The experiments reported above suggested that the microfilarial intake in *Ae. samoanus* was less than in *Ae. polynesiensis* when fed on blood with comparable microfilarial densities. In order to investigate this further, batches of wild-caught *Ae. polynesiensis* and wild-caught *Ae. samoanus* were fed on a carrier on the same day but at different times, *Ae. polynesiensis* at 1600–1700 hours and *Ae. samoanus* at 1900–2000 hours. Blood for microfilarial counts was taken from the carrier each time before the mosquito feeding. In this series of experiments mosquitoes were killed immediately after feeding, stored in the deep freeze at –25°C and dissected later. In the dissection the blood mass of each mosquito was thinly smeared in a drop of distilled water and examined under a coverslip for microfilariae.

Results of eight experiments with different densities of microfilariae are given in Table 5. The proportion of mosquitoes infected, the average number of microfilariae per infected mosquito and concentrating capacity were all higher in *Ae. polynesiensis* despite the very similar microfilariae densities in the carriers at the times of mosquito feeding.

DISCUSSION

The direct relationship shown in our studies between the percentage of *Ae. polynesiensis* infected and the microfilarial density of the carrier at the time of feeding agrees with the findings of Rosen (1955) for the same combination of parasite and vector in French Oceania. A similar relationship has been reported for *Brugia malayi* and *Mansonia* (Wharton, 1957) and for *B. malayi* and *Ae. togoi* (Ramachandran and Zaini, 1968).

Rosen (1955) reported experimental transmission studies on 23 batches of *Ae. polynesiensis* fed on carriers of microfilarial counts ranging from 0·4 to 555 in 20 mm³ of blood in Tahiti. The lowest counts used by him, 0·4 and 0·6 microfilariae 20 mm⁻³ of blood (equivalent to 20 and 30 microfilariae 1 ml⁻¹, respectively) gave 5·3 and 11·6% infections. Rosen concluded that any density of microfilariae that could be detected in 20 mm³ of blood can be infective to mosquitoes. He did not directly investigate the claim that mosquitoes ingested more microfilariae than could be found in an equal amount of blood obtained by other means. He stated that there was nothing to suggest that such a phenomenon occurred to a significant extent in *Ae. polynesiensis*. Calculation of the expected intake of microfilariae by *Ae. polynesiensis* to Rosen's data shows a range of concentration of 0·5–6·4 times (Zahar *et al.*, 1980). Pichon (1974) examined the data of Rosen mathematically and found a more or less similar degree of concentration.

Symes (1960) indicated that *Ae. pseudoscutellaris*, *Ae. fijiensis* and *Culex quinquefasciatus* became infective after feeding on blood counts as low as 0·04, 0·03 and 0·11–0·13 mm⁻³ of blood, respectively and concluded that these mosquitoes would become infective after feeding on a carrier with a microfilaria density which cannot be detected in a 20 mm³ sample. He also reported first stage developing forms in one of 49 and two of 112 specimens of *Ae. pseudoscutellaris* fed on a carrier whose count in 1 ml had been reduced from 4050 to zero by treatment with DEC.

Bryan and Southgate (1976) reported surprisingly high infection rates of 11·8, 7·2, 9·6 and 9·3% in wild-caught *Ae. polynesiensis* fed on a carrier with low counts of eight, six, five and four microfilariae in 1 ml of blood, respectively. They also found that wild-caught *Ae. polynesiensis* took up and sustained the development of microfilariae in 12 times the number expected to be present in a similar volume of peripheral blood. Their data showed that the actual intake averaged 17 times the expected intake. Thus, a rigorous re-evaluation of the question of infectivity of ultra-low microfilaraemia to different species of mosquito vectors has become necessary.

The present studies involving several experiments on both laboratory-bred and wild-caught *Ae. polynesiensis* fed on subjects with a low microfilaraemia demonstrate infection rates and a degree of concentration much lower than the results reported by Bryan and Southgate. There is also apparently no difference between the results for laboratory-bred and wild-caught *Ae. polynesiensis*. Our data are, in fact, more consistent with those of Rosen. Regression analysis of our results suggests that there can be no truly non-infective microfilarial level but zero. Carrier 3, who showed a microfilaraemia of 3 ml⁻¹ in the pilot survey, was negative on three subsequent occasions and each batch of *Ae. polynesiensis* fed on her produced no infected mosquitoes (Table 2).

Bryan and Southgate (1976) reported that many of their *Ae. polynesiensis* harboured more than one parasite. In one experiment involving microfilaraemia of 8 Mf ml⁻¹ they found two specimens with five sausage stages and one with three sausage stages. In our experiments a microfilaraemia of less than about 20 ml⁻¹ of blood produced one larva in each infected mosquito while a microfilaraemia of less than 60 ml⁻¹ gave infected mosquitoes which had one larva each and some with two larvae each. Again, these results are consistent with those of Rosen (1955).

In discussing the inefficiency of transmission of *W. bancrofti* from *Cx. quinquefasciatus* to the human host based on observations in Rangoon, Hairston and De Meillon (1968) implied that it was unlikely that infective mosquitoes harbouring one, two or three larvae would produce patent infections in the human host. Plainly, for the human host to produce a microfilaraemia both male and female parasites must be present and mate, so that the bite of a mosquito carrying a single infective larva cannot cause microfilaraemia. On the assumption of an equal sex ratio and independent distribution of the two sexes among mosquitoes, half the mosquitoes carrying two larvae and one-quarter of those carrying three larvae cannot

cause microfilaraemia. The studies of De Meillon *et al.* (1967) suggest that only a proportion of the infective larvae leave the mosquito host at one feeding. Thus, as suggested by Gubler and Bhattacharya (1974), the human population will receive a single larva or none at all from the bites of these infective mosquitoes. Patent infections would therefore rarely occur from a single bite. The people would be repeatedly infected with low or sub-minimal doses of infective larvae. Gubler and Bhattacharya (1974) suggested that instead of producing patent infection such exposure would be more likely to induce an immune response by the host. This trend of argument based on the data presented suggests that ultra-low density carriers do not play an important role as a potential source of infection in Samoa.

The present work forms the first major experimental transmission study of *Ae. samoanus* reported in Samoa. In the only other study Ramalingam (1968) fed wild *Ae. samoanus* on two carriers who had seven and 11 microfilariae in 20 mm^3 of blood. The ten surviving mosquitoes dissected 11 days after feeding contained infective larvae. From this data and the examination of 751 wild females Ramalingam (1968) established for the first time the efficiency of *Ae. samoanus* as a vector of subperiodic *W. bancrofti* in Samoa. The taxonomic status of *Ae. samoanus* has been established only recently by Belkin (1962), who distinguished a pale form as opposed to the dark form described as a new species *Aedes (Finlaya) oceanicus* (Belkin). He suspected that in the past *Ae. samoanus* and *Ae. oceanicus* were probably confused in epidemiological studies.

Our studies confirm the efficient vector potential of *Ae. samoanus*, although there is a clear difference between the vector potentials of *Ae. polynesiensis* and *Ae. samoanus*. The results of the two series (Tables 2 and 4) show that the worm burden in *Ae. samoanus* was less than in *Ae. polynesiensis* when both species were fed on carriers with similar microfilaraemias. The capacity to concentrate microfilariae shown by the two species was further evidence of the lower intake of microfilariae by *Ae. samoanus* compared with *Ae. polynesiensis*. Final confirmation of the difference in the microfilariae intake by the two species was borne out in the comparative experiments (Table 5).

Although the time taken to feed by individual specimens was not noted, the wide difference in this period for the two species shows that individual *Ae. samoanus* take a full blood-meal more quickly than *Ae. polynesiensis*. Thus it is likely that fewer microfilariae succeed in entering the stomach of *Ae. samoanus* during the time taken for ingestion while the slower rate of ingestion of blood in *Ae. polynesiensis* allows more microfilariae to be drawn in. Further, the mean numbers of *Ae. polynesiensis* and *Ae. samoanus* which theoretically ingest 1 ml of blood are similar. Hence, a possible explanation for the lower intake of microfilariae by *Ae. samoanus* could be its more rapid ingestion of blood. The difference between worm burdens in *Ae. polynesiensis* and *Ae. samoanus* observed here is consistent with the worm burdens in infected and infective specimens of the two species from wild-caught populations dissected throughout the year.

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