Annals of Tropical Medicine and Parasitology, Vol. 79, No. 1, 101–107

Filariasis transmission in Samoa

II. Some factors related to the development of microfilariae in the intermediate host

By W. A. SAMARAWICKREMA*, G. F. S. SPEARS†, FOLA SONE, KAZUYO ICHIMORI AND R. F. CUMMINGS

WHO Samoa Filariasis Research Project, P.O. Box 77, Apia, Samoa

Received 20 July 1983

The developmental period of microfilariae of sub-periodic *Wuchereria bancrofti* in laboratory-bred *Aedes polynesiensis* and *Ae. samoanus* was shorter in the warm season (December to May) than in the cool season (June to November). In the warm season the microfilariae reached the 'sausage' stage in three days, cylindrical second stage in seven days and the infective stage in 12 days after the infecting meal. During the cool season the incubation period was extended to 14 days. Microfilariae persisting in a carrier recently treated with diethylcarbamazine citrate readily infected *Ae. polynesiensis* and *Ae. samoanus* and developed into the infective stage, but the proportion which became infected was significantly less than the proportion infected on an untreated carrier having comparable microfilarial density. Under the conditions of the experiments the survival of infected *Ae. polynesiensis* and *Ae. samoanus* was dependent on physical conditions. The average level of infective worm burden did not appear to affect the mortality of the vectors.

Transmission studies of *Aedes (Stegomyia) polynesiensis* (Marks) and *Ae. (Finlaya) samoanus* (Gruenberg) on carriers with different levels of microfilaraemia of sub-periodic *Wuchereria bancrofti*, with special reference to low density microfilaraemia, were carried out in Samoa in 1978. Most of the results have been published (Samarawickrema *et al.*, 1985). The present paper describes the effect of climate and the effect of diethylcarbamazine citrate (DEC-C) treatment on the development of microfilariae. The survival of mosquitoes infected with different densities of microfilaraemia is also discussed.

MATERIALS AND METHODS

The general background of the investigation and the methods used are described in the previous paper (Samarawickrema *et al.*, 1985). Only the relevant details not mentioned previously will be described here. At first it was thought essential to establish, for the local conditions, the time taken for microfilariae to reach the recognized stages of development in the mosquito. A batch of laboratory-bred *Ae. polynesiensis* was fed on a carrier with a moderate microfilaraemia and maintained in a cage. Four mosquitoes were killed each day and dissected to determine the stage of development of larvae. The length and width of the

*Present address: WHO Entomologist, Malaria Control Project, P.O. Box 22, Honiara, Solomon Islands.

†Senior Lecturer in Statistics, Department of Preventive and Social Medicine, Medical School, University of Otago, Dunedin, New Zealand.

0003–4983/85/010101+07 $03.00/0

TABLE 1

Growth of Wuchereria bancrofti *larvae in laboratory-bred* Aedes polynesiensis *in warm season, January 1978, fed on a carrier with count of 2616 Mf ml^{-1} and in cool season, August 1978, fed on carrier with count of 1616 Mf ml^{-1}*

	January 1978—warm season							*August 1978—cool season*						
Period in mosquito in days	*Mean no. larvae/ mosquito*	*No. of larvae*			*Mean length / Mean width*			*Mean no. larvae/ mosquito*	*No. of larvae*			*Mean length / Mean width*		
		I	*II*	*III*	*I*	*II*	*III*		*I*	*II*	*III*	*I*	*II*	*III*
1	1·4	7			243/7				10			250/67		
2	8·7	26			227/9				7			227/9		
3	5·0	15			190/20				8			188/23		
4	4·2	25			162/22				12			191/23		
5	4·2	46			235/28			5·0	25			192/20		
6	3·1	22	3		240/30	297/32		6·4	37			195/29		
7	5·0	4	12		246/31	407/32		6·7	20			250/36		
8	4·3	2	24		253/30	574/33		2·8	3	22		260/35	400/32	
9	5·1		41			850/33		5·0	1	14			625/32	
10	2·3		7	1		870/34	1562/21	8·0		24			813/35	
11	6·8		23	4		1010/33	1596/22							
12	11·5		8	38		1058/30	1618/21	10·7		32			1205/34	
13								9·0		20	2		1305/33	1712/23
14								9·6		2	18			1650/22
15								10·5			21			1680/22

larvae were recorded daily. The experiment was repeated from time to time to observe any changes in the duration of the incubation period.

The two carriers with the highest densities, after initial feeding experiments on laboratory-bred *Ae. polynesiensis*, were treated with DEC-C on a dosage schedule of 6 mg kg^{-1} body weight daily for 14 days. At the end of the course of treatment feeding experiments on the carriers were repeated with *Ae. polynesiensis* at different intervals.

In the experiments on survival of infected mosquitoes, batches of *Ae. polynesiensis* and *Ae. samoanus* fed on carriers with different levels of microfilaraemia were maintained in the laboratory till the end of the incubation period. The specimens were then dissected and the worm burden in each mosquito recorded.

RESULTS

Development of *W. bancrofti* in *Ae. polynesiensis* and *Ae. samoanus*

The results of two experiments, one in the warm season (January 1978) and the other in the cool season (August 1978) are given in Table 1. In the warm season the 'sausage' stage was reached in three days, cylindrical second stage in six to seven days and the infective stage in ten to 12 days. These results agreed very well with the findings for the nocturnally periodic *W. bancrofti* in *Culex quinquefasciatus*. The mean length and width of the larvae were in good agreement with those for the periodic form.

From June to November the climate in Samoa was milder. The temperatures were the same during the day as in the warm season from December to May but dropped below 23°C on several nights each month (Table 2). The 'sausage' stage was reached in three days during the cool season. The cylindrical second stage appeared on the eighth day and the infective larvae on the fourteenth day. Four experiments, two with laboratory-bred and two with wild-caught *Ae. samoanus* produced identical results.

The average counts of larvae per mosquito were made each day for both species and although there were variations from day to day, no dead or malformed larvae were observed at any time. Thus once they invaded the thoracic muscles shortly after ingestion the microfilariae proceeded to develop to the infective stage. The larvae on maturation became very active both in *Ae. polynesiensis* and *Ae. samoanus* and migrated to all parts of the body. Sometimes they were found in the legs, base of wing, base of antennae and in the abdomen among the tracheal trunks of the ovaries.

Effect of DEC-C Treatment on Development of Microfilariae

Results of initial experiments with *Ae. polynesiensis* on carriers with densities of 5290 and 2616 Mf ml^{-1} and subsequent experiments after DEC-C treatment are given in Table 3. Carrier 5 was tested three days, three weeks and four weeks after treatment when his counts were 8, 20 and 22 Mf ml^{-1} respectively. Carrier 1 was tested 14, 16 and 18 weeks after treatment when his counts were 7, 9 and 3 Mf ml^{-1} respectively. The microfilariae persisting in a carrier recently treated with DEC-C readily infected laboratory-bred *Ae. polynesiensis* and developed into the infective stage.

TABLE 2

Maximum and minimum temperatures during warm and cool seasons for 1978 recorded at Apia Observatory

Warm season				*Cool season*			
	Temperature				*Temperature*		
Month	*Maximum*	*Minimum*	*Nights with temperature below 23°C*	*Month*	*Maximum*	*Minimum*	*Nights with temperature below 23°C*
January	30·9	22·6	2	June	31·0	20·7	16
February	33·4	21·4	3	July	31·2	19·2	21
March	32·1	21·5	5	August	31·5	20·7	15
April	31·8	22·3	6	September	31·4	19·2	15
May	31·9	21·6	13	October	31·6	21·5	8
December	31·8	22·0	8	November	31·5	19·2	13
Total			37 nights Mean = 6 nights per month	Total			88 nights Mean = 15 nights per month

TABLE 3

Effect of DEC-C on development of microfilariae in laboratory-bred Aedes polynesiensis

Serial no. of carrier	*Date of experiment*	*Mf density (nucleopore 1 ml)*	*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*
5	21.2.1978	5290	60	95·0	11·98	12·61	719	543·5	1·32
	17.3.1978	8	98	1·0	0·01	1·00	1	1·3	0·77
	31.3.1978	20	70	4·3	0·04	1·00	3	2·4	1·25
	7.4.1978	22	91	2·2	0·02	1·00	2	3·4	0·59
1	25.1.1978	2616	63	76·2	4·78	6·27	301	282·2	1·07
	5.5.1978	7	64	4·7	0·05	1·00	3	0·8	3·75
	19.5.1978	9	53	3·8	0·02	1·00	2	0·8	2·50
	30.5.1978	3	65	0·0	0·00	0·00	0	0·0	0·00

Results of one experiment on wild-caught *Ae. polynesiensis* fed on carrier 1 18 weeks after DEC-C treatment and two experiments on wild-caught *Ae. samoanus* on the same carrier 29 weeks after DEC treatment showed that persisting microfilariae developed into the infective stage in these mosquitoes.

Survival of *Ae. polynesiensis* and *Ae. samoanus*

Tables 4 and 5 show that the percentage of mosquitoes surviving the incubation period varied from 4·2 to 41·5 for laboratory-bred *Ae. polynesiensis* and from 1·7 to 43·6 for wild-caught *Ae. samoanus*. Such a wide variation in survival was due to fluctuation in temperature in the absence of controlled conditions in the laboratory. In the results of experiments in Tables 4 and 5 for different levels of microfilaraemia, the highest infective worm burden was 16 for *Ae. polynesiensis* and 17 for *Ae. samoanus*. In a single experiment with *Ae. polynesiensis* on a carrier having a density of 5290 Mf ml^{-1}, of the 25 specimens dying between the seventh and the tenth day of incubation 15 had worm burdens ranging from eight to 16 and ten had a range between 21 and 57. Thus in these experiments, except for the high infections of between 21 and 57 in *Ae. polynesiensis* which may have killed the mosquitoes, the average level of infective worm burden did not appear to affect the mortality of the vectors.

DISCUSSION

Different workers have found varying developmental periods of sub-periodic *W. bancrofti* in *Ae. polynesiensis* in different countries of the South Pacific region. Thus, Byrd and St. Amant (1959) found the average time for completion of development to be 11–17 days; Rosen (1955) in Tahiti found the period to be 12–13 days at 24–28°C and Rageau and Estienne (1959) reported that the development was completed in 13–16 days. Recently Bryan and Southgate (1976) in Samoa determined the period of development to be 12 days. The results of the last named workers agree with our data for the warm season. Symes (1960) found seasonal fluctuation of the developmental period in *Ae. pseudoscutellaris*, *Ae. fijiensis* and *Cx. quinquefasciatus*. The accurate determination of the period required for microfilariae to reach 'sausage' stage, the cylindrical second stage and the infective stage during the warm and cool seasons of the year is important in calculating the probability of survival of an infected vector population in the field using the method of Laurence (1963).

TABLE 4

Percentage of Ae. polynesiensis *fed on different levels of microfilaraemia surviving the incubation period*

Serial no. of carrier	*Mf density (nuclepore 1 ml)*	*No. of mosquitoes fed*	*Number surviving incubation period (%)*	*No. of larvae per infective mosquito*	*Range per infective mosquito*
5	5290	220	25 (11·4)	10·8	1–16
8	1390	172	34 (19·8)	6·8	1–15
	581	230	79 (34·4)	2·3	1–10
10	1249	172	9 (5·2)	4·3	1–6
7	1160	200	12 (6·0)	4·8	1–13
2	583	59	10 (17·0)	3·2	1–16
11	460	223	61 (27·4)	3·2	1–10
12	397	90	21 (23·3)	1·7	1–4
13	328	120	5 (4·2)	2·0	1–3
4	101	120	14 (11·7)	1·0	1–1
	72	165	12 (7·3)	1·7	1–3
	67	178	57 (32·0)	1·1	1–2
	37	200	20 (10·0)	1·4	1–2
	29	175	19 (10·9)	1·0	1–1
	29	175	40 (22·9)	1·2	1–2
	28	435	30 (6·9)	1·0	1–1
9	32	220	90 (40·9)	1·2	1–2
	26	280	63 (22·5)	1·2	1–2
	23	211	57 (27·0)	1·2	1–2
	16	140	42 (30·0)	1·0	1–1
	12	200	83 (41·5)	1·5	1–2
6	23	320	108 (33·8)	1·0	1–1
	14	205	46 (22·4)	1·0	1–1
	12	213	52 (24·4)	1·0	1–1
	10	212	19 (8·9)	1·0	1–1
	7	282	43 (15·3)	1·0	1–1
	7	400	0	—	—
16	1	490	146 (29·8)	—	—
	1	184	45 (24·5)	—	—

Laigret *et al.* (1965), analyzing the data during 11 years of chemotherapy by DEC-C in French Polynesia, assumed that the decrease in both the proportion of infective mosquitoes and infective worm burden could have been due to the effect of DEC-C in reducing the chances for the larvae to develop to the mature stage and suggested further investigation. Microfilariae of *W. bancrofti* which persist in the blood after DEC-C treatment will readily develop into the infective stage in *Ae. polynesiensis* (Rosen, 1955; Bryan and Southgate, 1976), in *Ae. pseudoscutellaris* (Symes, 1960) and in *Cx. quinquefasciatus* (Jordan, 1967; Kanda *et al.*, 1967).

Chen and Fan (1977) made similar observations in *Cx. quinquefasciatus* fed on a carrier with microfilariae which survived three courses of treatment. They also found that the number of larvae per infective mosquito fed on a DEC-C treated carrier was much lower than the number of larvae per infective mosquito fed on an untreated carrier with a similar level of microfilaraemia. Our results confirm the observations of all the above workers with regard to the development of microfilariae surviving the DEC-C treatment in *Ae. polynesiensis*

TABLE 5

Percentage of Aedes samoanus *fed on different levels of microfilaraemia surviving the incubation period*

Serial no. of carrier	*Mf density (nuclepore 1 ml)*	*No. of mosquitoes fed*	*Number surviving incubation period (%)*	*No. of larvae per infective mosquito*	*Range per infective mosquito*
10	1275	220	4 (1·8)	6·3	1–14
	1275	208	61 (29·3)	4·9	1–17
8	1449	184	33 (17·9)	3·2	1–13
	912	223	14 (6·3)	2·7	1–7
11	897	290	16 (5·5)	3·5	1–13
12	295	190	44 (23·2)	2·2	1–6
	265	188	66 (35·1)	1·5	1–3
13	281	187	39 (20·9)	1·9	1–6
	230	225	84 (37·3)	2·4	1–8
4	87	296	19 (6·4)	1·5	1–2
	55	156	42 (26·9)	1·0	1–1
9	34	175	34 (19·4)	1·2	1–2
	19	93	18	—	—
	18	211	92 (43·6)	1·0	1–1
1	24	171	22 (12·9)	1·0	1–1
	10	236	76 (30·5)	1·0	1–1
16	1	173	3 (1·7)	—	—
	1	159	12 (7·5)	—	—

and *Ae. samoanus*. Further, a summary of the results of the experiments in Table 3 shows that 11 of the 441 *Ae. polynesiensis* dissected were infective. These results may be compared with those on carrier 6 in Table 4 of Samarawickrema *et al.* (1985), who was untreated and had a naturally low microfilaraemia of the same intensity. In six experiments on carrier 6, 18 of 268 *Ae. polynesiensis* dissected were infective. The mean microfilarial counts in the two treated carriers and the untreated carrier were not significantly different ($t=0{\cdot}11$, 10 *df*, $0{\cdot}9<P<0{\cdot}95$) but the proportion infected in the treated series was significantly less than the proportion infected in the untreated series ($\chi^2=4{\cdot}08$, 1 *df*, $0{\cdot}025<P<0{\cdot}05$). The observations confirm the findings of Chen and Fan (1977).

Rosen (1955) observed high mortalities in *Ae. polynesiensis* when fed on microfilarial densities over 9000 ml^{-1}. There was no recognizable effect of the heavy infection on the longevity of the mosquito during the first week after the infecting feed. The effect of the parasite load was observed near the end of the period of development to infective stage. Rosen concluded that more heavily infected mosquitoes died before those with lighter infections. Hairston and Jachowski (1968) re-examined the data of Rosen and reported that the average success of the larvae through the mosquito phase of the life cycle was much higher for intermediate densities than for higher densities. Symes (1960) suggested that high mortality became apparent when feeding was on carriers with microfilariae counts exceeding 3500 ml^{-1}. Conditions under which our studies were carried out were not suitable for observations of survival of infected *Ae. polynesiensis* and *Ae. samoanus*. However, on the basis of the above observations the range of microfilariae counts of carriers used in our experiments, except for the count of 5290 Mf ml^{-1}, fell within the limits of intermediate densities and therefore had no obvious effect on the longevity of the vectors.

REFERENCES

BRYAN, J. H. & SOUTHGATE, B. A. (1976). Some observations in filariasis in Western Samoa after mass administration of diethylcarbamazine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70,** 39–48.

BYRD, E. E. & ST. AMANT, L. S. (1959). Studies on the epidemiology of filariasis in Central and South Pacific. *South Pacific Commission, Technical Paper No. 125.*

CHEN, C. C. & FAN, P. C. (1977). The effect of diethylcarbamazine treatment on the development of bancroftian microfilaraemia in *Culex fatigans*. *Southeast Asian Journal of Tropical Medicine and Public Health*, **8,** 53–55.

HAIRSTON, N. G. & JACHOWSKI, L. A. (1968). Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization*, **38,** 29–59.

JORDAN, P. (1967). The possible role of low density microfilaraemia in the spread of *Wuchereria bancrofti* by *Culex fatigans* in East Africa. *Annals of Tropical Medicine and Parasitology*, **53,** 42–46.

KANDA, T., TANAKA, S. & SASA, M. (1967). The effects of diethylcarbamazine treatment on the viability of microfilariae ingested by intermediate hosts. *Japanese Journal of Experimental Medicine*, **37,** 149–155.

LAIGRET, J., KESSEL, J. F., MALARDE, L., BAMBRIDGE, B. & ADAMS, H. (1965). La lutte contre la filariose lymphatique apériodique en Polynésie française. *Bulletin de la Société de Pathologie Exotique*, **58,** 895–916.

LAURENCE, B. R. (1963). Natural mortality in two filarial vectors. *Bulletin of the World Health Organization*, **28,** 229–234.

RAGEANU, J. & ESTIENNE, J. (1959). *Enquête sur la filariose à Wallis*. Mimeographed document, 37 pp. Institut Français, Noumea.

ROSEN, L. (1955). Observations on the epidemiology of human filariasis in French Oceania. *American Journal of Hygiene*, **61,** 219–284.

SAMARAWICKREMA, W. A., SPEARS, G. F. S., SONE, F., ICHIMORI, K. & CUMMINGS, R. F. (1985). 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). *Annals of Tropical Medicine and Parasitology*, **79,** 89–100.

SYMES, C. B. (1960). Observations on the epidemiology of filariasis in Fiji. Part II. Laboratory studies and human infections. *Journal of Tropical Medicine and Hygiene*, **63,** 31–44.