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Filarial vector studies in a diethylcarbamazine-treated and in untreated villages in Papua New Guinea

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SUMMARY

Entomological studies were undertaken in three villages in the East Sepik Province of Papua New Guinea. The inhabitants of one village, Nanaha, had been treated with diethylcarbamazine (DEC) to reduce the prevalence and density of microfilaraemia of *Wuchereria bancrofti*. No intervention was undertaken in the other two villages, Yauatong and Musenau, in which bancroftian filariasis was present but with markedly different human prevalence rates and mean parasite densities. In Yauatong, infection rates in anopheline vectors (*Anopheles punctulatus* and *An. koliensis*) varied from 20.5 to 46.6% with infectivity rates of 0–1.4% while these rates were 10.9–14.3% and 0–1.1% respectively in *Culex quinquefasciatus*. In Nanaha after DEC treatment, infection rates were as high as 16.3% in *An . koliensis* and infectivity rates reached 7.0% for *An. punctulatus* despite a 45% reduction in the number of people with detectable microfilariae (mf) and a 94% reduction in mf density in those who remained positive.

Keywords: filariasis, *Wuchereria bancrofti, Anopheles koliensis, Anopheles punctulatus, Culex quinquefasciatus*, diethylcarbamazine, Papua New Guinea

INTRODUCTION

A multidisciplinary study of filariasis was undertaken in the East Sepik Province of Papua New Guinea (PNG); the area and disease patterns were described by Kazura *et al.* (1984) and worm burdens were quantified by blood examination and by circulating antigens (Forsyth *et al.* 1985; Day *et al.* 1991a). *Wuchereria bancrofti* was the only filarial parasite of humans detected; infective and preinfective larvae were found in *Anopheles punctulatus* and pre-infective stages only in *An. koliensis* and *Culex quinquefasciatus* (Bryan 1986).

The present investigations were carried out in one village subjected to mass drug administration (MDA) with diethylcarbamazine citrate (DEC) and two untreated villages with the following objectives:

(a) to relate vector findings to parasitological data from humans;
(b) to seek evidence for the presence or absence of facilitation in anopheline vectors after MDA with DEC.

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MATERIALS AND METHODS

The study area

The study villages were Nanaha, Musenau and Yauatong which are within 20 km of the Government station at Dreikikier along the Wewak–Lumi road; they are similar in house construction and animal husbandry

Table 1. Prevalence rates and intensities of microfilaraemia in three villages of East Sepik Province, Papua New Guinea

Village	Prevalence rate of mf (%)	Arithmetic mean intensity mf ml^{-1} venous blood ± s.e.m.
Baseline data		
Nanaha		
(Day *et al.* 1991b)	66	112.7 ± 1.4
Musenau		
(Day pers. comm.)	20	64.3
Yauatong		
(including Albalum)		
Kazura *et al.* 1984)	68	3198.0
One year after MDA with DEC in DEC recipients		
Nanaha		
(Day *et al.* 1991b)	38	6.5 ± 1·3

practices. Musenau and Nanaha are situated along ridges whereas Yauatong consists of small clusters of houses some of which border on a stream.

Originally MDA and DEC at a dosage of 6 mg kg^{-1} daily for 12 consecutive days was planned for Yauatong with Nanaha and Musenau as non-intervention villages. For operational reasons, Nanaha was substituted for Yauatong, and MDA was carried out in Nanaha in June 1985 without collection of preintervention entomological data. Baseline prevalence rates and densities of microfilariae (mf) in all three villages and post-intervention data for those who completed MDA and DEC in Nanaha are summarized in Table 1. One year after MDA of DEC, mf prevalence in DEC recipients in Nanaha had fallen by 42% and the arithmetic mean mf density by 94%.

Entomological studies

Mosquitoes were collected between November 1984 and January 1987 at Yauatong and Musenau and between April 1986 and April 1987 at Nanaha. To obtain data on vector densities, four whole-night landing collections (1800–0600 h) on humans were made both inside and outside houses in each village. Additional mosquitoes were obtained from daytime indoor resting collections and by part-night landing catches. Monthly collections were planned but were not always possible.

Mosquitoes, identified on morphological characters, were mostly members of the *An. punctulatus* complex (Belkin 1962). Morphological identification within this complex is not always reliable (Foley *et al.* 1993), but allozyme analysis of some specimens (Foley & Bryan 1993) confirmed the morphological identifications. *Culex quinquefasciatus* was collected only in Musenau and identified by the key of Belkin (1962).

Mosquitoes were preserved and stained in Mayer's acid haemalum (Nelson 1959) before dissection and examination for filariae. Where possible at least 40 specimens were examined from each set of collections for each village.

Statistical methods

Data from whole-night landing catches were subjected to two-way analysis of variance of the natural logarithm of (number of mosquitoes per night +1)/(number of collectors) to test for the significance of landing rate differences between villages and seasons. Infection rates in mosquitoes were compared using χ^2-tests; mean densities of filarial larvae at successive instars were compared using Student's *t*-test.

RESULTS

Mosquitoes obtained from night landing collections are shown in Table 2; all those infected were members of

Table 2. Mosquito landing rates/person/night in four whole-night landing catches in three villages, East Sepik Privince, Papua New Guinea

	Indoors			Outdoors		
	No. bait	No. *An. kol.*	No. *An. pun.*	No. bait	No. *An. kol.*	No. *An. pun.*
Yauatong						
April 86	3	3	25.3	3	2.7	25
June 86	3	3.3	173.3	3	0	118.3
Sept 86	3	5.3	58.3	3	4.7	94
Jan 87	4	1.8	29.3	4	0.8	20.3
Mean mosquito/person/night		71.5			62.9	
Nanaha						
April 86	2	15	1	2	6.5	0
June 86	3	28	5.7	3	13.7	6
Sept 86	3	2.3	9	5	3.0	26.4
Jan 87	4	9.8	1.8	4	2.5	0.8
Mean mosquito/person/night		17.8			16.6	
Musenau						
April 86	3	0	0	3	0	0
June 86	4	0.3	1.5	4	1	7.3
Sept 86	3	1	19	3	1.7	20.7
Jan 87	6	0.2	3.2	6	0	2.7
Mean mosquito/person/night		5.4			7.3	

An. kol., *An. koliensis*.
An. pun., *An. punctulatus*.

the *An. punctulatus* complex. Villages differed in their vector composition with *An. punctulatus* comprising 96.2% of the catches at Yauatong and 93.1% at Musenau but only 46.3% at Nanaha, where *An. koliensis* was relatively more abundant. At Yauatong and Musenau the results for *An. koliensis* and *An. punctulatus* have been pooled when analysing village and seasonal differences in landing densities.

Pooled data (whole-night and part-night landing catches and indoor resting catches) on infection rates and densities of *W. bancrofti* larvae in infected mosquitoes are recorded in Tables 3 and 4. In Nanaha, filarial infection rates in *An. koliensis* and *An. punctulatus* did not differ significantly either in 1986 ($\chi^2_{(1)}=0.30$, $P=0.58$) or in 1987 ($\chi^2_{(1)}=2.55$, $P=0.11$), so data from these two species were pooled for comparisons with data from Yauatong and Musenau. Generally, the proportion of mosquitoes infected declined as infections aged (Table 4). In Yauatong, the mean density of filariae in infected mosquitoes decreased from the first to the second stage and from the second to the third stage; these decreases were significant in five of eight cases. No worms which had died in a living mosquito were detected. A significant reduction in worm burden with increasing age of infection also occurred in *Cx. quinquefasciatus* in Musenau in 1984.

DISCUSSION

Entomological studies were undertaken in Nanaha over 13 months and in the non-intervention villages (Musenau and Yauatong) over 27 months. In spite of the lack of preintervention data at Nanaha, the study was continued because of the total absence of field data following MDA in areas of anopheline transmission of *W. bancrofti*.

Whole-night landing catches, very surprisingly, showed few significant differences in seasonal rates. Of the single species/single site of collection pairs only outdoor *An. punctulatus* yielded a significant result ($P=0.004$). When data on *An. punctulatus* indoors and outdoors are combined a significant seasonal difference is found ($P=0.018$) due to the outdoor data; similarly,

Table 3. Percentage of mosquitoes caught in part- and whole-night landing catches and resting indoors in East Sepik Province, Papua New Guinea, 1984–1987 infected with *Wuchereria bancrofti*

Year	No. dissected	With larvae (all stages) No.	(%)	With L1 (%)	With L2 (%)	With L3 (%)
Yauatong: *An. punctulatus* and *An. koliensis*						
1984	482	100	20.7	17.4	5.8	0.6
1985	1129	453	40.1	31.0	12.8	4.1
1986	625	166	26.6	21.0	8.8	2.6
1987	191	89	46.6	29.3	22.5	6.3
Nanaha: *An. punculatus*						
1986	276	42	15.2	9.4	7.0	2.2
1987	43	7	16.3	7.0	7.0	7.0
Nanaha: *An. koliensis*						
1986	720	98	13.6	7.8	6.5	1.5
1987	290	22	7.6	4.1	4.8	0
Nanaha: *An punctulatus* and *An. koliensis*						
1986	996	140	14.1	8.2	6.7	1.7
1987	333	29	8.7	4.5	5.1	0.9
Musenau: *An punctulatus* and *An. koliensis*						
1984	326	21	6.4	3.4	2.8	0.9
1985	54	8	14.8	5.6	9.3	0
1986	296	37	12.5	8.1	4.4	1.4
1987	89	10	11.2	4.5	6.7	1.1
Musenau: *Cx. quinquefasciatus*						
1984	320	35	10.9	9.7	2.2	0
1985	182	26	14.3	12.6	1.7	1.1

L1, First instar larva; L2, second instar larva; L3, third instar larva.

pooling outdoor catching results for *An. punctulatus* and *An. koliensis* gives a significant seasonal difference (P=0.024) due to *An. punctulatus*. All species/site collection pairs showed significant differences between villages (P=0.002 to 0.019) in whole-night landing rates with the exception of *An. koliensis* caught outdoors (P=0.052), where the conventional P=0.05 was not quite attained. Variations in relative abundance and absolute densities of species within the *An. punctulatus* complex in villages in close proximity are common in PNG and have been described before (Burkot *et al.* 1988).

Table 3 shows considerable yearly variations in overall filarial infection rates in vectors and consistent differences between villages, with untreated Yauatong having higher values than untreated Musenau and treated Nanaha. The original prevalence of mf at Nanaha was 66%; 80% of the population received DEC, and at the start of these studies, 38% of these persons were microfilaraemic, with an arithmetic mean mf ml^{-1} of 6.5 (Table 1). Pooled mosquito infection rates at Nanaha (14.1 and 8.7%, Table 3) are inexplicably high given the mf rate and density in those who completed a course of DEC. Many of the untreated persons would be amicrofilaraemic as this group included all children under 5 years. Infective larvae at Nanaha occurred in 1.7% of vectors in 1986 and in 0.9% in 1987, rates which are very similar to those in Musenau. All-stage infection rates and to a greater extent all-stage filarial densities in anophelines in treated Nanaha are remarkably high compared to untreated Musenau; figures for Yauatong are higher, but by a lesser order of magnitude than that expected from the human mf data. No plausible hypothesis explains these findings.

Because of higher vector densities, the 'force of infection' in Nanaha is higher than in Musenau. At Musenau, 4% of the population had obstructive disease

Table 4. Arithmetic mean number of *Wuchereria bancrofti* larvae in infected mosquitoes caught in part-night and whole-night landing catches and resting indoors in East Sepik Province, Papua New Guinea, 1984–1987 including statistical analysis of change of mean number of larvae at successive instars

Year	mean L1	mean L2	mean L3	L1 to L2 *t*-value	L2 to L3 *t*-value
Yauatong: *An. punctulatus* and *An. koliensis*					
1984	11.0	5.9	2.0	2.712*	2.56 NS
1985	12.3	6.5	4.6	4.38***	3.38***
1986	12.5	6.4	3.4	2.48*	1.67 NS
1987	12.9	8.3	2.8	1.104 NS	2.69*
Nanaha: *An. koliensis*					
1986	6.8	4.2	3.8	0.88 NS	0.24 NS
1987	10.3	4.3	2.0	0.98 NS	1.75 NS
Nanaha: *An. koliensis*					
1986	12.0	7.5	3.2	1.62 NS	1.99 NS
1987	9.8	4.8	0	0.75 NS	not done
Musenau: *An. punctulatus* and *An. koliensis*					
1984	2.7	3.1	6.7	− 0.33 NS	− 0.62 NS
1985	6.0	9.2	0	− 0.50 NS	not done
1986	8.6	6.8	1.0	1.01 NS	1.45 NS
1987	1.3	6.0	1.0	1.71 NS	not done
Musenau: *Cx. quinquefasciatus*					
1984	4.9	1.4	0	2.67*	not done
1985	3.3	1.0	2.0	2.62 NS	not done

*$P<0.05$; **$P<0.01$, ***$P<0.005$.
NS, Not significant.

indicating that additional control measures will be required at Nanaha to prevent new episodes and new cases of filarial disease.

The significant decreases in mean densities of larvae as infections aged in anophelines at Yauatong (Table 4) could have arisen from death of larvae in the vectors or from a higher mortality rate in the more heavily infected vectors. We reject the first hypothesis as filariae which had died in the vectors were not seen; although young dead larvae may be missed, older dead worms are readily detectable. Death of heavily infected mosquitoes in nature is expected from laboratory studies (Townson 1971) and has been recorded in *Cx. quinquefasciatus* naturally infected with *W. bancrofti* in Sri Lanka where the median density of mf ingested was 10.3 (Samarawickrema & Laurence 1978). Significant declines in parasite load with age were not detected in anophelines in Musenau and Nanaha, but the mean larval density was usually lower in these villages and may not have been sufficient to increase mortality. Similarly, density of infection with *W. bancrofti* did not influence survival of *An. funestus* in Tanzania where larval densities were low with over 90% of infected mosquitoes containing one to four worms; the highest number of larvae in one vector was twelve (Krafsur & Garrett-Jones 1977).

A significant reduction in worm burden with age occurred in *Cx. quinquefasciatus* in Musenau in 1984 although the mean number of first stage larvae was only five, suggesting that *Cx. quinquefasciatus* is more readily killed by filariae than anophelines. This is the first published record of infective larvae in wild-caught *Cx. quinquefasciatus* from PNG.

The relationship of mf density in blood, number of mf ingested by vectors and number of infective larvae which subsequently develop has been studied for several *W. bancrofti* vector pairs. The host/parasite relationship between *W. bancrofti* and some anopheline vectors in Africa has been investigated observationally, experimentally and mathematically (see review in Southgate & Bryan 1992). An important finding was that as the number of ingested mf increased, the parasite yield also increased, and Bain (1971) termed this relationship 'facilitation'. The epidemiological significance of facilitation and its implications for control programmes were discussed recently by Webber

(1991), Southgate (1992, a,b), Southgate & Bryan (1992) and Dye (1990, 1992). Southgate (1992b) argued that where control measures have '... produced low mf prevalence rates combined with low mf densities ... predictions of parasite extinction or parasite resurgence can be made with confidence, based on the local vector/filaria relationships ...'. The eradication of *W. bancrofti* from Solomon Islands through malaria vector control activities (Webber 1979; Webber & Southgate 1981) is suggestive of facilitation; the vectors in that area are members of the *An. punctulatus* complex, as in PNG. A similar mechanism seems to have operated with *Brugia malayi* transmitted by *An. sinensis* from China after MDA of DEC (Zhang *et al.* 1991). Therefore control of transmission was anticipated in PNG by MDA with DEC, which while not clearing mf from all infected persons, was expected to reduce prevalence and intensity of microfilaraemia to a level where facilitation might operate and parasite extinction might be possible.

An original objective of the present study was to investigate the possibility of facilitation in PNG following MDA with DEC. Unfortunately the condition of '... low mf prevalence rates combined with low mf densities ...' quoted above from Southgate (1992b) was not achieved in Nanaha for reasons which are quite unclear. The conditions under which facilitation might be expected to operate did not occur and the topic must remain a priority for future research in PNG.

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