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## Studies on the epidemiology of subperiodic *Brugia malayi* in Malaysia: problems in its control

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### Summary

The dynamics of the transmission of subperiodic *Brugia malayi* in a typical endemic area in Malaysia was studied over a period of 4 years. Mass chemotherapeutic control with diethylcarbamazine citrate was found to be inefficient, new cases being detected even after the fifth treatment cycle of 6 mg/kg  $\times$  6 days per cycle. This is in marked contrast to the situation in periodic *B. malayi* areas where mass treatment efficiently controlled the infection. The disparity in results in these two areas is attributed to zoonotic transmission of subperiodic *B. malayi* from non-human primates where a mean infection rate of 76.3% was found.

**Key words:** filariasis; subperiodic *Brugia malayi*; mass treatment; monkey reservoirs.

### Introduction

*Brugia malayi* and *Wuchereria bancrofti* are endemic in Malaysia, the former predominating. Periodic *B. malayi* occurs mainly in humans in coastal ricefield regions whilst the subperiodic form affects both man and animals in swamp forests. Since the discovery of *B. malayi*-like microfilariae in macaque monkeys and other animals (Poynton and Hodgkin, 1939; Edeson et al., 1955) and the experimental transmission of the parasite from man to animals and vice versa (Edeson and Wharton, 1958; Dondero et al., 1972) the possible occurrence of zoonotic transmission in nature has been postulated. However, no study has been carried out to assess the impact of zoonotic transmission on control programmes in endemic areas. In an attempt to understand the transmission dynamics of subperiodic *B. malayi* and the impact of zoonotic transmis-

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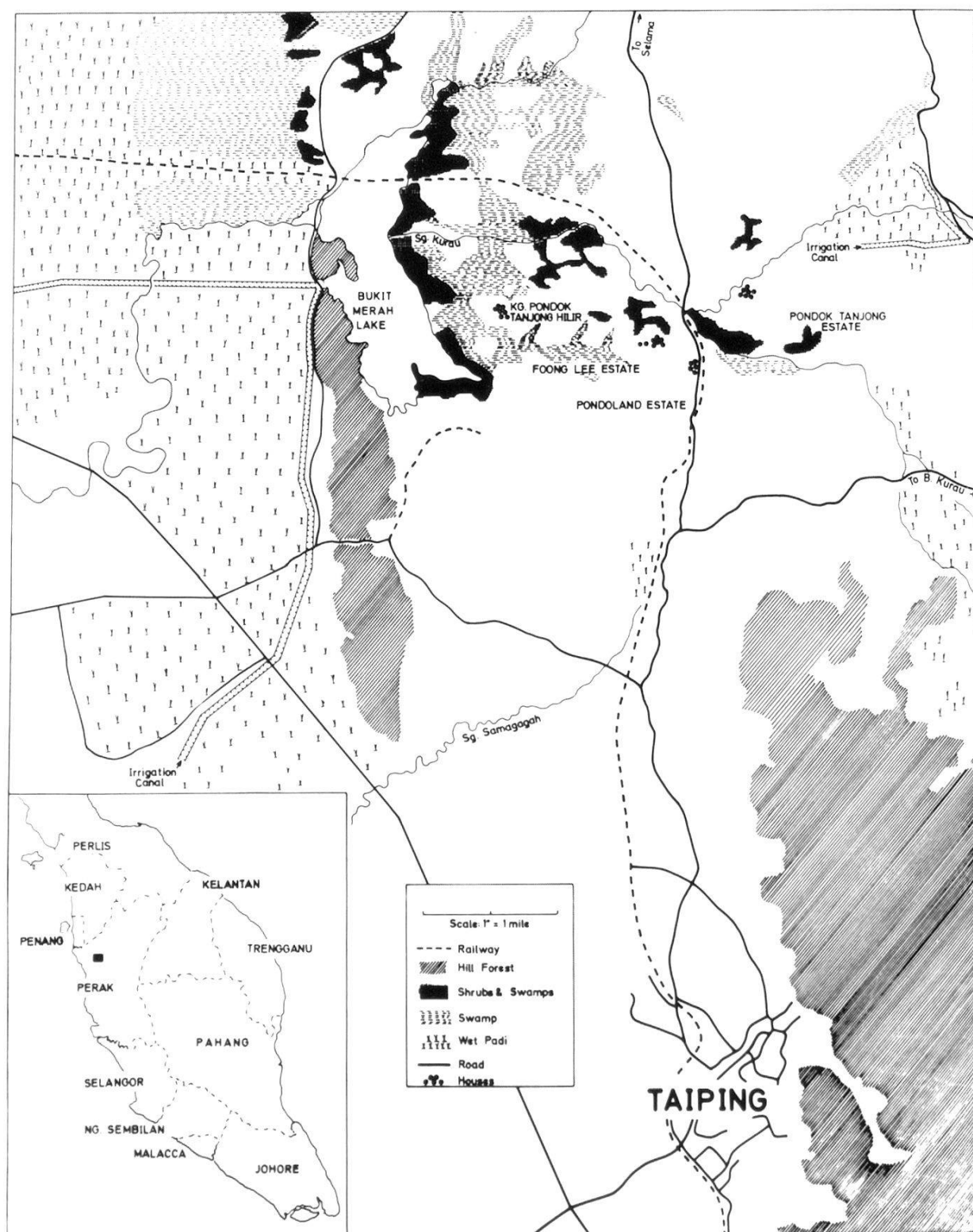


Fig. 1. Filariasis study area near Taiping, Perak.

sion on the mass chemotherapeutic control programme with diethylcarbamazine citrate (DEC) in an endemic area, a longitudinal study was undertaken in Pondok Tanjong, Taiping. The effectiveness of the control measures taken will be compared with those obtained in periodic and subperiodic *B. malayi* endemic areas under mass chemotherapeutic control with DEC.

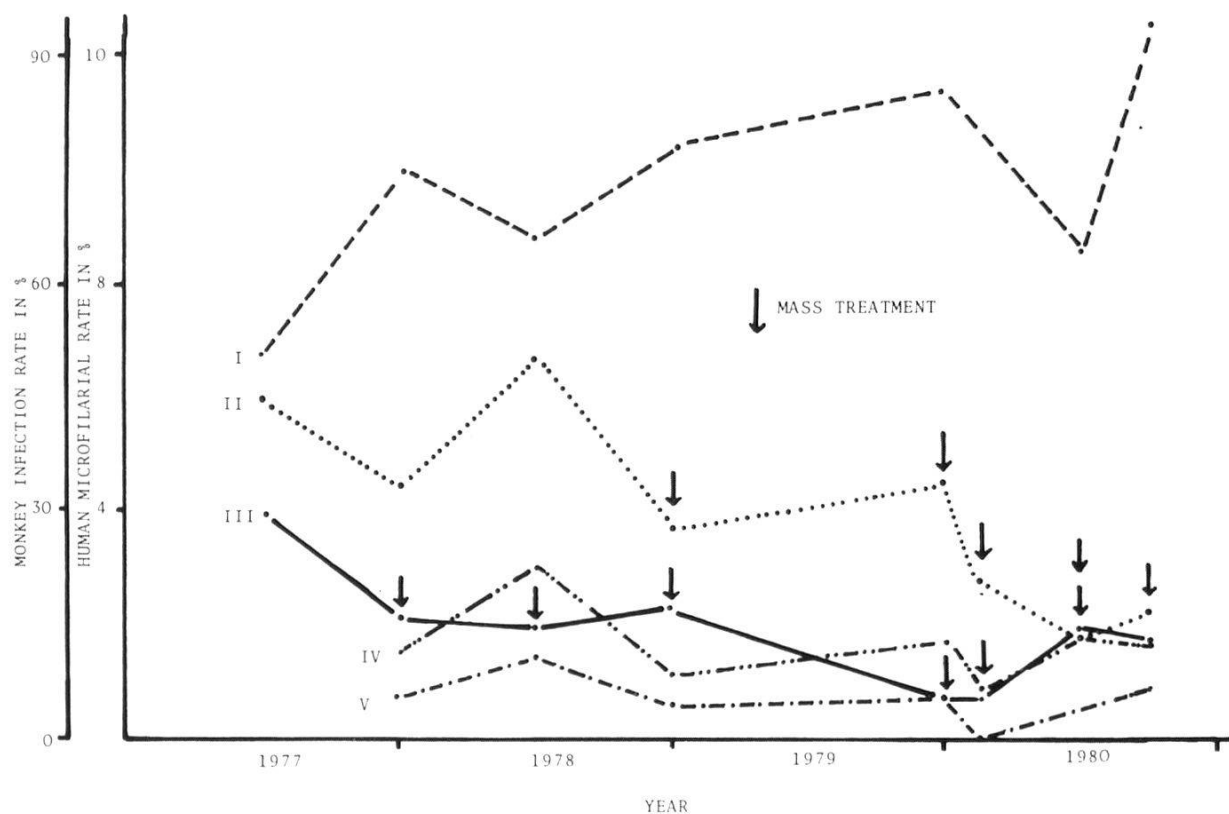


Fig. 2. Effect of diethylcarbamazine citrate 6 mg/kg body weight daily  $\times$  6 days on monkey infection (I) and human microfilarial (II – Pondoland and Foong Lee Estates; III – Pondok Tanjong Estate; IV – new cases in Pondoland and Foong Lee Estates; V – new cases in Pondok Tanjong Estate) rates.

## Materials and Methods

**Study area.** The study area, situated about 15 miles from Taiping town consists of 3 mature rubber estates (Pondok Tanjong, Pondoland and Foong Lee Estates) situated on both sides of the main road (Fig. 1). The total population is about 1000 (Pondok Tanjong Estate 500, Pondoland Estate 300 and Foong Lee Estate 200), the majority being Indians. The people are estate employees or their dependants. The majority of the residents were born in the estates except for a few older members who had migrated from India. The three estates are situated in similar surroundings, these being swamp forests inhabited by numerous monkeys and other wild animals. The estates are predominantly under rubber cultivation although some oil palms are being planted under the agricultural diversification scheme. The workers are mostly rubber tappers, labourers, weeders and some latex factory hands. Normally all adult members of the households are involved in estate employment.

**Clinical and parasitological examinations.** During surveys, a clinical history was taken and physical examination for signs of filariasis carried out. 60  $\mu$ l thick blood smears were taken from the inhabitants from 20.00 h onwards. Blood smears were dried overnight and stained with diluted Giemsa (1.5 ml in 100 ml buffered water at pH 7.2). Surveys were carried out at 6-monthly intervals for the first 18 months, then a year later and subsequently at 3-monthly intervals.

**Mass treatment.** The populations from the three estates were divided into two groups. Group 1 consisting of the Pondok Tanjong Estate population was given mass treatment with DEC at a dosage of 6 mg/kg body weight daily  $\times$  6 days at 6-monthly intervals during the first 18 months, then a year later and subsequently at alternate 3-monthly intervals (Fig. 2). Group 2 consisting of populations from Pondoland and Foong Lee Estates were left untreated until the third survey, i.e. a year after the initial survey. Subsequent mass treatments were given after 6 months, one year and then 3-

monthly. The drug was given under supervision and reactions to it looked for. Records of sickness due to filarial disease were monitored throughout the study period.

*Entomological studies.* Longitudinal studies were carried out to determine the types, abundance and filarial infection rates of mosquitoes in the study area. Bionomics of vector mosquitoes were also studied. These studies will be the subject of another paper.

*Animal studies.* Domestic cats and dogs were examined for microfilaraemia using Giemsa stained thick blood films taken from ear pricks. In addition, about 20 monkeys of various species were shot at each survey and complete autopsies carried out. Adult worms were recovered from lymph glands by the method of Buckley and Edeson (1956). Worms recovered were fixed in hot 70% alcohol and then preserved in a mixture of 5% glycerine in 70% alcohol for further study.

## Results

### *Parasitological findings*

In the preliminary survey 19 out of 448 (4.0%) and 26 out of 432 (6.0%) in Groups 1 and 2 respectively had microfilaraemia giving a combined rate of 4.6%. All infections were due to subperiodic *B. malayi*. The median microfilaria counts (MfD<sub>50</sub>) were 2.9 and 10.0/60  $\mu$ l in Groups 1 and 2 respectively, giving a combined count of 6.2/60  $\mu$ l. After the institution of mass chemotherapy in Group 1, there was a decline in microfilarial rate (Fig. 2). However, the microfilarial rates were not significantly different from that of the pre-treatment until after the fourth course of treatment (November 1979) when the rate was 0.7% (Chi-square = 4.515, 0.05 > P > 0.02). Only 2 out of 294 people examined were positive, both of them being new cases. No new case was detected during the next 3 months, and the rate remained at 0.7%. However, this subsequently increased again to 2.0% and 1.8% during the next 3-monthly surveys. The MfD<sub>50</sub> did not change much until November 1979 and February 1980 when only 2 people each were positive. However, this increased to 14.0/60  $\mu$ l and 6.0/60  $\mu$ l in June and September 1980 respectively.

In Group 2, there was a progressive drop in the microfilaria rate after the initiation of mass chemotherapy with DEC in June 1978. By June 1980, i.e. after the 5th course of DEC, it had dropped to 1.8% (4 out of 221 compared to the initial rate of 6.0% (26 out of 432). This lower rate was statistically significant (Chi-square = 4.9869, 0.05 > P > 0.02). However, this subsequently increased to 2.3% (4 out of 173) at the last survey. The initial MfD<sub>50</sub> before treatment was 10.0/60  $\mu$ l. This fluctuated at subsequent surveys and after the 5th and 6th courses of DEC were 7.0 and 5.4/60  $\mu$ l respectively.

### *Clinical findings*

A combined total of 63 out of 880 (7.2%) in the study area gave histories consistent with filarial disease, these being recurrent fever associated with lymphadenitis, retrograde lymphangitis and lymphoedema. Of these 30 out of 448 (6.7%) and 32 out of 432 (7.4%) from Groups 1 and 2 respectively gave positive histories. Only 7 out of the 63 (11.1%) who gave positive clinical histories were microfilaraemic. Clinical signs consistent with filarial diseases were

Table 1. Filarial infections in monkeys from Pondok Tanjung, Taiping, May 1977 to September 1980

Monkey species	No. (%) positive for			Negative (%)	Total examined
	<i>Brugia malayi</i>	<i>Dirofilaria magnilarvatum</i>	<i>B. malayi</i> and <i>D. magnilarvatum</i>		
<i>Presbytis cristata</i> . . . . .	5 (62.5)	—	—	3 (37.5)	8
<i>P. melalophos</i> . . . . .	40 (59.7)	2 (3.0)	9 (13.4)	16 (23.9)	67
<i>P. obscura</i> . . . . .	43 (52.4)	—	25 (30.5)	14 (17.1)	82
<i>Macaca fascicularis</i> . . . . .	—	2 (66.7)	—	1 (33.3)	3
Total	88 (55.0)	4 (2.5)	34 (21.3)	34 (21.3)	160

seen in 47 out of 448 (10.5%) and 38 out of 432 (8.8%) in Groups 1 and 2 respectively giving a combined rate of 9.2%. Only 4 out of the 889 (0.5%) were microfilaraemic. Lymphadenitis (greater than 1.5 cm in diameter), usually bilateral inguino-femoral, was the most common sign (6.7%), followed by lymphoedema/elephantiasis usually of both the lower limbs below the knees (5.1%). Abscess scars along the course of the lymphatics was only seen in 1 patient.

There was an appreciable drop in attacks of filarial symptoms after the institution of chemotherapy. Thus of the 294 examined in Group 1 after the 4th course of DEC, only 5 (1.7%) had positive symptoms and 18 (6.1%) positive signs of disease. In Group 2, after the 2nd course of DEC, of 292 examined 2 (0.7%) had positive symptoms and 8 (2.7%) had positive clinical signs.

#### Animal surveys

Approximately 20 monkeys of various species, especially *Presbytis* spp., were shot and autopsied during each survey. A total of 160 monkeys were examined (Table 1, Fig. 2). Infection rates with subperiodic *B. malayi* in monkeys ranged from 50–95% at each survey with a mean of 76.3% (122 out of 160). Of the 4 species of monkeys examined the highest rate of *B. malayi* infection was seen in *P. obscura*, this being 82.9% (68 out of 82). 73.1% (49 out of 67) *P. Melalophos* and 62.5% (5 out of 8) *P. cristata* were also infected. Monkeys were also found to be commonly infected with *Dirofilaria magnilarvatum*.

At the initial survey, 12 out of 14 (85.7%) dogs were found infected with *Dirofilaria immitis* and one each of 30 (3.3%) cats infected with subperiodic *B. malayi* and *D. repens*. All 3 goats examined were negative.

#### Discussion

Since Wilson (1950) showed the effectiveness of DEC against *B. malayi* infection and Turner and Sodhy (1959) the possibility of its mass administra-



tion, the drug has been used in the mass chemotherapeutic control of Brugian filariasis in Kedah, Malaysia. Results indicated that this control method was extremely effective in periodic *B. malayi* areas (Yusoff, 1959). Similar control campaigns like that in Malaysia are being carried out in countries endemic for *B. malayi* in Southeast Asia. In areas where both the periodic and subperiodic forms of *B. malayi* exists, the same control strategy has been applied to both forms. Yet the epidemiology of the two forms differ in many ways. Whereas periodic *B. malayi* is principally transmitted by *A. campestris*, *Mansoni indiana*, *M. uniformis* and *M. annulifera* (Reid et al., 1962), that of subperiodic *B. malayi* is mainly by *Mansonia* spp. these being *M. bonneae*, *M. dives*, *M. annulata* and *M. uniformis* (Wharton, 1960). Furthermore, animal reservoirs like leaf-monkeys are important in subperiodic *B. malayi* but is rare in the periodic form (Laing et al., 1960).

It has been shown that *A. campestris*, an endophilic species, rapidly disappeared from areas under DDT residual spraying (Moorhouse and Chooi, 1964). In areas under the Malaria Control Programme, the regular spraying of residual insecticides markedly reduced *A. campestris*, producing a beneficial effect on the control of periodic *B. malayi* (Mak et al., 1977). Although Iyengar (1953) found residual spraying with DDT effectively reduced vector *Mansonia* and *Anopheles* mosquitoes in Southern Thailand and Harinasuta et al. (1970) found a similar effect on *Mansonia* mosquitoes, disappointing results were obtained in Malaysia, there being a lack of observable effect on the intensity of transmission as measured by the biting and infection rates of *M. dives/bonneae* (Wharton et al., 1958). One of the reasons attributed to this was the presence of animal reservoirs contributing to the source of infective mosquitoes. This was in spite of the finding that even 2 months after residual spraying, more than 80% of blood-fed *Mansonia* mosquitoes caught resting in sprayed houses, died within 24 h of capture, thus effectively reducing the number of infective mosquitoes infected from human carriers.

Thus it is seen that the control of subperiodic *B. malayi* is much more complicated than that of the periodic form and other control strategies are needed. Wilson (1969) in a report on the Filariasis Control Programme in Malaysia, also noted a very great reduction in microfilarial rates and transmission even more than 10 years after mass chemotherapy in periodic *B. malayi* endemic areas. Results from subperiodic *B. malayi* areas were not as good. In the present study, mass chemotherapy decreased slightly the microfilarial rates as can be seen when the survey results for June 1978 are compared between Group I (treated) and Group 2 (untreated). This was, however, not reflected in the change in the mean microfilarial densities, these being 8.1/60  $\mu$ l compared to the initial 8.6/60  $\mu$ l in Group 1 and 10.5/60  $\mu$ l compared to the initial 24.4/60  $\mu$ l in Group 2. Neither was there any significant changes in the MfD<sub>50</sub>. There was only a transient significant decrease in the microfilarial rate after the 5th course of DEC in Group 1. Although there was a marked decrease in those having

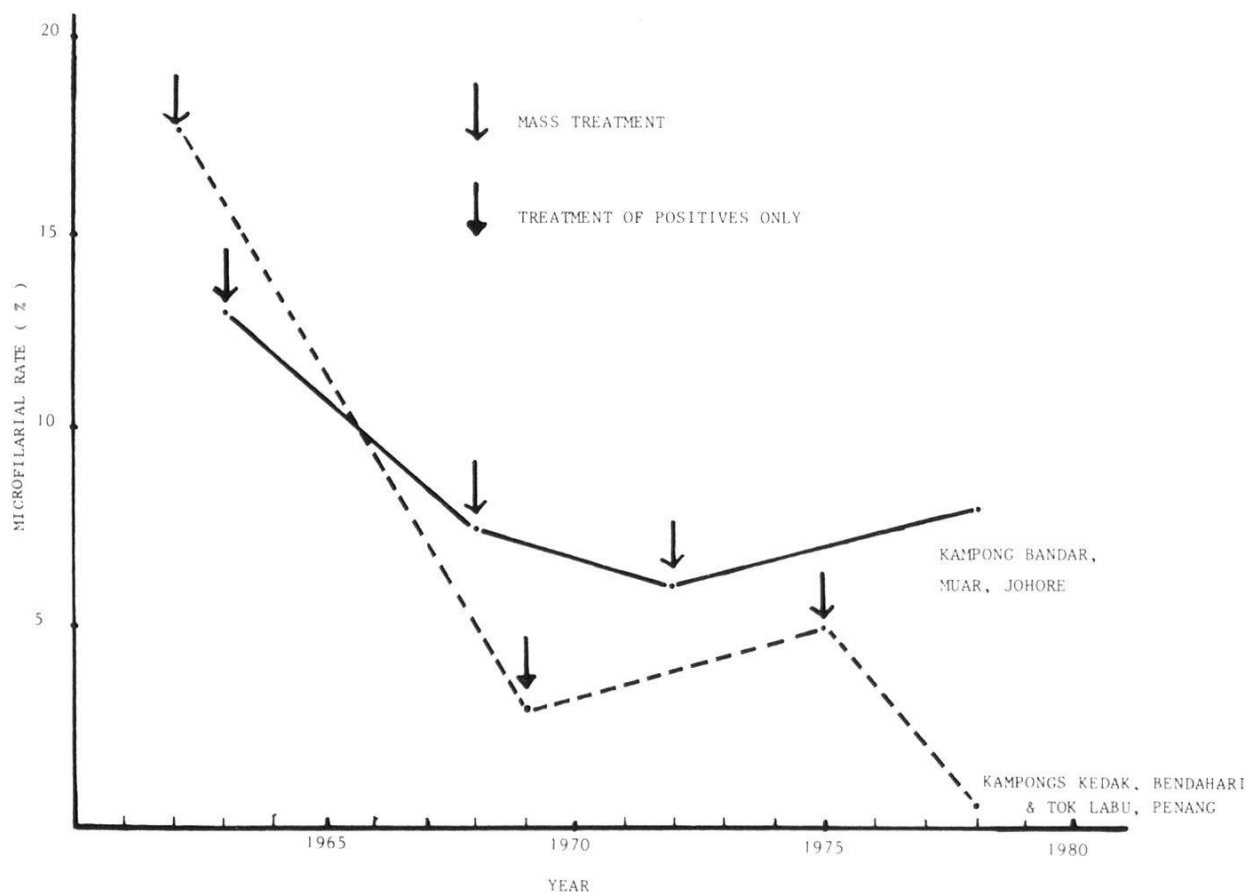


Fig. 3. Effect of mass treatment or treatment of positives only with diethylcarbamazine citrate at 6 mg/kg body weight weekly  $\times$  6 weeks on microfilarial rates in subperiodic (Kampong Bandar) and periodic (Kampongs Kedak, Bendahari and Tok Labu) *Brugia malayi* endemic areas.

attacks of symptoms due to filarial infection, new cases continued to be present in both areas, thus showing that active transmission still occurred. It would therefore mean that in the presence of zoonotic transmission from leaf-monkeys, mass chemotherapeutic control of subperiodic *B. malayi* with DEC is not very effective. Analysis of results from the National Filariasis Control Programme showed a similar picture. Thus the mass chemotherapeutic control with DEC at a dose of 6 mg/kg body weight weekly  $\times$  6 weeks in a subperiodic *B. malayi* endemic village in Kampong Bandar, Muar, Johore, did not reduce the microfilarial rate significantly even after 3 courses (Fig. 3).

The initial microfilarial rate of 13.2% (40 out of 302) in 1963 was 8.2% (25 out of 306) in 1968, 6.2% (17 out of 276) in 1972 and 8.4% (21 out of 251) in 1978 in spite of mass treatment with DEC at the above dosage regime during those years. In contrast, in a periodic *B. malayi* area in Penang, an initial mass treatment in 1962 followed by selective treatment only of positive patients with the above dosage regime decreased the microfilarial rate from 17.7% (161 out of 910) in 1962 to 3.0% (27 out of 889) in 1969, 5.2% (48 out of 920) in 1974 and 0.4% (4 out of 950) in 1978. As in the present study, disease rates declined in all these areas. In the study area there was an appreciable drop in positive clinical signs after the institution of chemotherapy.



The above study demonstrates that there is intense subperiodic *B. malayi* infection among the non-human primates with as high as 82.9% of *P. obscura* being infected. As *Mansonia* spp. are catholic in their feeding habits (Wharton, 1962) intense transmission from animals to man is probably going on. All 3 species of Malaysian leaf monkeys are found in the study area. Troop sizes of these animals range from 20–50 individuals and they are found in inland and coastal swamp forests (Lim and Mak, 1978). Nothing much is known about the habits and ecology of these animals but *P. cristata* is known to move 2–3 kilometers in distance each day and in the study area all 3 species of leaf-monkeys are seen in the rubber estates near human habitation. It is believed that the population of these monkeys in the study areas must be considerable. Further, as the infection rates in these monkeys have continued to increase during the period of observation, mass chemotherapy in the human population has practically no effect on the transmission of filariasis to and among them.

In the light of the above findings, it is evident that other control strategies must be found to complement mass chemotherapy as a control measure in subperiodic *B. malayi* endemic areas where zoonotic transmission is present. Antilarval measures in the control of *Mansonia* vectors are difficult due to the nature of the biology of these mosquitoes. As shown by Wharton et al. (1958), residual spraying of insecticides would not reduce the number of infective mosquitoes infected from animal reservoir sources. Furthermore *Mansonia* spp. bite both inside as well as outside houses (Wharton, 1962), thus reducing the effectiveness of residual insecticide spraying. It would be ideal if the animal reservoirs of subperiodic *B. malayi* could be eliminated. It will probably be extremely difficult to treat them but if available, a long acting depot antifilarial drug would help. To kill off these monkeys would be unacceptable to conservationists. As the production of an effective and safe vaccine is not feasible in the near future, the use of a prophylactic drug must be considered. Although DEC has been shown to kill infective larvae, fourth stage and juvenile adults of *B. malayi* in cats (Ewert and Emerson, 1975, 1979), no proper assessment has been made in its use as a chemoprophylactic in Bancroftian and Brugian filariasis. Duke (1961, 1963) has shown it to be useful as a prophylactic agent against *Loa loa* infection. Studies are being undertaken to assess its use as a prophylactic drug in Brugian filariasis.

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