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Transmission Intensity and Its Relationship to Infection and Disease Due to *Wuchereria bancrofti* in Papua New Guinea

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This study describes the relationship between transmission intensity and infection and disease due to *Wuchereria bancrofti* in an endemic area of Papua New Guinea. The prevalence of microfilaremia in the entire study population was 66%. Of 1892 persons examined, 6.2% and 12.3% had lymphedema of the legs and hydroceles, respectively. The prevalences of microfilaremia and clinical morbidity were lowest in persons <20 years old and increased progressively with age. Annual transmission potential and annual infective biting were monitored in five villages where *Anopheles punctulatus* and *Anopheles koliensis* are the only vectors of *W. bancrofti*. Both measures of the entomologic inoculation rate were positively associated with the village-specific microfilarial rate, mean intensity of microfilaremia, and prevalence of leg edema. These data indicate that transmission intensity is a major determinant of patent infection and morbidity rates in bancroftian filariasis.

Lymphatic filariae infect >120 million persons living in tropical areas of Latin America, Africa, Asia, and the Pacific. It is estimated that ~40 million of these individuals suffer from the pathologic sequelae of chronic infection, most commonly lymphedema of the lower extremities (elephantiasis) and hydroceles [1]. Studies with noninvasive imaging techniques indicate that many clinically asymptomatic persons also have significant lymphatic dysfunction [2, 3]. The need for a comprehensive understanding of the individual, epidemiologic, and ecologic risk factors for infection and disease in endemic populations has become more urgent, since it has been recognized that the cost-effective public health measures, such as mass chemotherapy, decrease transmission or may even eradicate lymphatic filariasis [4–6].

Several factors may predispose to lymphedema of the legs and hydroceles, the two most common and debilitating manifestations of Bancroftian filariasis. First, some studies suggest that lack of patent infection (i.e., amicrofilaremia) is associated with elephantiasis, whereas microfilaremia is more common among clinically asymptomatic persons. Because these clinical manifestations correlate with differences in the pattern of filarial-specific T cell responses, it is possible that the qualitative nature of the host immunity affects the pathologic outcome of infection [7–9]. Second, epidemiologic, immunologic, and parasitologic observations have been interpreted to indicate that prenatal sensitization and the consequent immunologic tolerance of filarial antigens influence clinical morbidity and the propensity to develop microfilaremia [10–13]. Third, on the basis of observations of experimentally infected immunodeficient mice, it is possible that parasite-derived molecules directly impair lymphatic function [14]. Finally, correlations between various entomologic indices of transmission and crude patent infection and disease rates suggest that the degree of exposure to mosquitoes harboring infective larvae determines the frequency of patent infection and possibly clinical morbidity [15].

Indeed, the contribution of these and other host immune or genetic factors to infection and disease from lymphatic filariae are not mutually exclusive and should be considered to interact with broader ecologic and epidemiologic features in areas with endemic filariasis. A quantitative appreciation of the importance of vector-host interactions is dependent on detailed monitoring of entomologic inoculation rates coincidental with sensitive and standardized indicators of infection and disease. Ideally, these relationships should be assessed in *Wuchereria bancrofti*–endemic areas where transmission has been stable and not altered by previous chemotherapy- or vector-based interventions. The current report describes the results of such a study conducted in East Sepik Province, Papua New Guinea.

Materials and Methods

Study population. The study population included 1892 persons ≥5 years old living in 12 villages located within 20 km of the Dreikikir government station in East Sepik Province, Papua New Guinea. In total, 1666 persons (88% of eligible individuals) gave blood samples for determination of microfilaremia. Earlier limited

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Informed consent was obtained from all adults and parents of children. The studies were approved by the appropriate authorities of the Government of Papua New Guinea and the Human Studies Committee of University Hospitals of Cleveland, Case Western Reserve University, Cleveland.

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surveys in this area have demonstrated microfilaria (mf) carrier rates of 68% and 30% prevalence of lymphatic disease of the legs [16]. Systematic mosquito control measures and individual or population-based chemotherapy have not been used in any of the study villages.

Clinical and parasitologic examinations. Physical examination for hydrocele and lymphedema of the lower extremities was performed according to protocols described by the World Health Organization [17]. The current study describes only grade II or III lymphedema of the lower extremities (defined, respectively, as nonpitting lymphedema not reversible upon elevation of the leg and gross increase in leg volume with dermatosclerosis and papillomatous changes). Lymphedema of the upper extremities was observed in 1.1% of the population.

Microfilaremia was quantified from a 1-mL blood sample obtained between 2200 and 0200 h. Filtration (Nuclepore, Pleasanton, CA) and counting of mf were done as described [18] and results were expressed as mf per milliliter of blood. Mf counts >999/mL were assigned values of either 1000 or 10,000, depending on the estimated abundance of parasites. Mf counts were log-transformed, and geometric mean intensities were calculated from the absolute value + 1.

Entomologic monitoring. Detailed entomologic studies were done in five villages (each of which constituted a transmission zone)—Yauatong, Albulum, Peneng, Nanaha, and Ngahmbule. Human bait and the landing catch method were used to capture mosquitoes between 1800 and 0600 h during 4 days per month from April 1993 to March 1994. Mosquitoes were dissected and *W. bancrofti* third-stage larvae (L3) were identified as described [19–21]. Annual transmission potential (ATP, the total number of L3 to which an individual is exposed per year) and annual infective biting rate (AIBR, the number of bites per year by mosquitoes containing at least one L3) were calculated using formulas described by Walsh et al. [22]. *Anopheles punctulatus* and *Anopheles koliensis* are the only two vectors of *W. bancrofti* in this area, although the majority of mosquitoes caught are *Culex* species [23]. An earlier report from a single site in East Sepik Province also showed that anopheline mosquitoes contain *W. bancrofti* L3 [24].

Because the use of bed nets may affect mosquito infection rates [25], we ascertained whether each study subject had slept under a bed net the night before venipuncture. In Yauatong, Albulum, Peneng, Nanaha, and Ngahmbule, respectively, 36.3%, 12.4%, 11.8%, 32.4%, and 8.1% of individuals ≥5 years old used a bed net. Insecticide-impregnated bed nets were not used.

Statistics. The significance of associations between microfilaremia and clinical disease status was calculated using the Mantel-Haenszel (χ^2) test. Correlation coefficients were used to determine the relationship between entomologic indices of transmission and intensities of microfilaremia, numbers of mf carriers, and disease rates in individual villages [26]. $P < .05$ was considered significant.

Results

Microfilarial status and disease profile of the entire study population. Age-specific mf carrier rates and geometric mean intensities of microfilaremia are described in table 1. The lowest prevalence of microfilaremia (38%) was in the group 5–9 years old. This indicator of patent infection increased in the second decade of life and reached a plateau of 70%–80% for all age groups >20 years. There was a similar age-related increase in the geometric mean intensity of microfilaremia, from a low of 4.2 mf/mL in those 5–9 years old to ~100 mf/mL in persons >40 years old. There were no sex-associated differences in age-specific or aggregate mf carrier rates or intensities of parasitemia (e.g., aggregate mf carrier rates were 65.8% and 66.1% for female and male subjects, respectively).

Table 1. Age-specific prevalence of microfilarial (mf) status and clinical morbidity.

Age group (years)	Mf carrier rate (% positive)	Mean mf level (mf + 1/ml)*	Leg edema (% positive)†	Hydrocele (% positive)‡
5–9	38.3	4.2	1.2	0
	(222)		(332)	(181)
10–19	59.7	11.8	3.5	3.3
	(437)		(483)	(239)
20–29	70.7	29.5	7.8	13.3
	(372)		(398)	(165)
30–39	72.0	48.6	8.0	12.4
	(236)		(249)	(113)
40–49	80.9	104.7	10.8	34.9
	(173)		(186)	(86)
≥50	79.7	93.3	10.2	30.9
	(226)		(244)	(123)
Total	66.0	25.6	6.2	12.3
	(1666)		(1892)	(907)

NOTE. Nos. in parentheses are no. of persons examined.

* Geometric mean mf count + 1/mL of blood for all samples, including those with no mf.

† No. of individuals examined for grade II or III lymphedema of legs and hydroceles was greater than no. for whom mf values were obtained because not all persons donated blood.

‡ Total includes only males.

The age-related profile of leg lymphedema and hydroceles also indicated that individuals ≥20 years old were more likely than younger persons to have clinical morbidity. Leg edema was observed in only 1.2% of those 5–9 years old, with an increase to a maximum of 10% in the fifth and sixth decades of life (table 1). Female subjects were about two times more likely than male subjects to have lymphedema of the lower extremity (prevalences of 7.9% and 4.3%, respectively, $P = .001$). Cases of hydroceles also increased with age, except that the frequency of this disease manifestation was higher than lymphedema of the legs in men ≥20 years old. For example, the prevalences of hydrocele and leg edema were 31% and 10%, respectively, in males >50 years old (table 1).

To determine whether leg lymphedema predisposed to hydroceles (or vice versa), the prevalences of lymphedema in male subjects with hydroceles was compared with that of male subjects without hydroceles. Sixteen (14.3%) of 112 males with hydrocele had leg lymphedema, whereas 23 (2.9%) of 795 without hydrocele had lymphedema of the lower extremity

Table 2. Relationship between microfilarial (mf) status and clinical morbidity in persons ≥20 years old.

	Leg edema			Hydrocele		
	Yes	No	Total	Yes	No	Total
Mf^+	64 (8.5)	688	752	78 (22)	269	347
Mf^-	30 (12)*	223	253	21 (20)†	83	104

NOTE. Nos. in parentheses are % of total in given category.
* Mantel-Haenszel: $\chi^2 = 2.5$, $P = .11$.
† Mantel-Haenszel: $\chi^2 = 0.24$, $P = .62$.

($P < .0001$). The association was also significant ($P < .0001$) when only men ≥20 years old were included in the analysis.

Correlation between microfilaremia and lymphatic disease. It has been suggested that persons with chronic disease manifestations of lymphatic filariasis, particularly elephantiasis of the lower extremities, have a propensity to be amicrofilaremic, whereas clinically asymptomatic adults are microfilaremic [7–9]. Therefore, this relationship was examined in persons ≥20 years old, the age group at highest risk for lymphatic disease. Mf status did not correlate with the presence of leg lymphedema for both sexes combined ($P = .11$) or with hydroceles in men ≥20 years old (table 2).

Interrelationships between entomologic inoculation rate, microfilarial status, and clinical morbidity. To assess the relation of village-specific mf carrier rates and levels of parasitemia to transmission intensity, microfilaremia was quantified in 695 of 810 persons ≥5 years old residing in the five villages undergoing concurrent monthly entomologic monitoring (the age distribution was similar for residents of each of the villages). ATPs were normally distributed and were 2344, 1338, 179, 279, and 31 L3/year in Yauatong, Albulum, Peneng, Nanaha, and Ngahmbule, respectively. The corresponding mf carrier rates were 93.8%, 81.5%, 65.6%, 55.5%, and 51.7%. There was a significant positive association between the log of the ATP and the village-specific mf carrier rate ($r = .91$, $P = .034$) and the geometric mean intensities of microfilaremia ($r = .93$, $P = .024$; figure 1). A similar positive association existed between the log AIBR and the mf carrier rate ($r = .97$, $P < .01$).

To evaluate the relationship between transmission intensity and clinical morbidity, the correlation coefficient between the log ATP and the prevalence of lymphatic disease of the legs and male genitalia was determined for persons ≥20 years old. This analysis was performed for older individuals, not children or adolescents, because the older persons are most prone to leg edema and hydroceles (table 1). The prevalence of leg edema was positively related to the log ATP ($r = .89$, $P = .04$). There was not, however, a statistically significant correlation between the log ATP and the prevalence of hydroceles ($r = .80$, $P = .11$; figure 2).

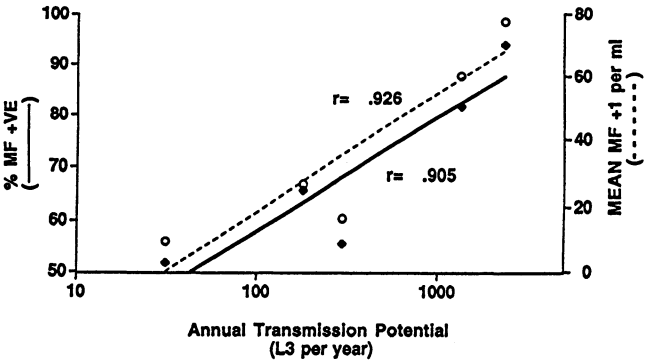

Figure 1. Relationship between log of annual transmission potential and village-specific microfilaria (mf) carrier rate and geometric mean level of microfilaremia. ♦, mf carrier rates for each village; ○, geometric mean intensity of microfilaremia. $P < .05$ for each linear regression plot. +VE = positive.

Discussion

Mathematical models of infectious diseases ideally describe the quantitative interrelationships among transmission, infection, and disease [27]. Generation of appropriate data sets to examine these complex interactions in human lymphatic filariasis has been difficult for several reasons. With respect to the vector, the abundance of mosquito populations, their infection rates, and the competence of various mosquito species to transmit L3 may be highly variable, even within relatively small geographic areas [28]. Accurate assessment of transmission intensity is also often difficult logistically, since there may be seasonal variations of entomologic inoculation rates that require repeated observations over long periods of time to appreciate.

With regard to the human population, uniform and sensitive indicators of infection and disease should be used to evaluate large numbers of persons in all age groups, ideally at the same

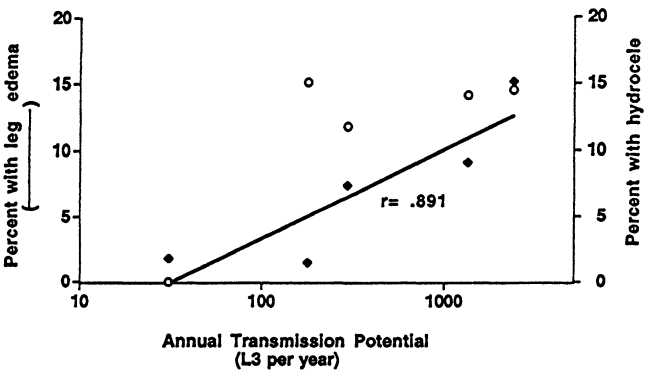

Figure 2. Relationship between annual transmission potential and village-specific prevalence of leg edema and hydrocele. ♦, Prevalence of leg edema in each village; ○, hydrocele prevalence. $P < .05$ for linear regression plot of leg edema vs. annual transmission potential.

time that entomologic monitoring is conducted. Reliable determinations of human infection and disease rates in mf-endemic areas may also be complicated by the fact that diethylcarbamazine, a drug that decreases the level of microfilaremia and kills adult *W. bancrofti,* is readily available in many areas of the world. In the current study, many of these impediments were overcome by conducting entomologic studies coincidental with determinations of human infection and disease rates in nearly 1900 residents of a rural area of Papua New Guinea, where systematic control measures have not yet been instituted.

Several remarkable features of the relationship between the entomologic inoculation rate and the patterns of patent infection and lymphatic disease were observed. First, transmission intensity, measured by the log ATP and AIBR, was positively associated with village-specific mf carrier rates and mean intensities of microfilaremia. (A similar positive association was noted for the untransformed value of ATP.) Taken together with the findings that (1) the aggregate mf carrier rate and mean intensity of parasitemia increase with age and (2) mf carrier rates in children 5–9 years in villages with the highest transmission intensities (Yauatong and Albulum) were greater than those of age-matched residents of the villages with lower transmission intensities ($\geq$84% vs. $\leq$24%), these data suggest that the cumulative number of L3 to which residents of relatively small geographic foci of transmission are exposed is a critical determinant of the propensity to develop patent infection.

Second, results of the current study indicate that transmission intensity correlates in a positive manner with the prevalence of lymphedema of the legs. In contrast, entomologic inoculation rates are not directly proportional to the frequency of hydroceles. Rather, the prevalence of hydroceles was similar for men living in villages where ATPs ranged from 300 to ~2,300 L3/year. Along with the fact that the frequencies of both forms of clinical morbidity increased with age, these results suggest that there are differences in the cumulative levels of exposure to L3 that predispose to elephantiasis and hydroceles, that is, the risk for lymphedema of the legs increases continuously with transmission intensity, whereas a lower or critical threshold value predisposes to hydroceles. By extension, the findings are consistent with the notion that lower adult worm burdens predispose to hydroceles relative to lymphedema of the lower extremities. Comparisons of the level of circulating parasite antigens—an indirect measure of adult worm burden [29, 30]—among groups of infected asymptomatic men and those with elephantiasis or hydrocele alone should provide some insight into this issue. In any event, in the framework of a population-based control program, the current data demonstrate that the pattern of disease within a given geographic region is heterogeneous and that reduction in transmission intensity may have different effects on the rates of leg lymphedema and hydroceles.

Finally, in view of the finding that the mf carrier rates were high, even in villages where transmission intensity is relatively low (e.g., the mf carrier rate is >50% in Ngahmbule, where the ATP is 31 L3/year), the current report confirms previous work that suggested that *Anopheles* mosquitoes are extremely efficient vectors of *W. bancrofti* [31–33].

Examination of the relationship between patent infection and disease indicates that neither lymphedema of the lower extremities nor hydroceles correlated with mf status. This finding is similar to the conclusions derived from a recent metanalysis of data from other mf-endemic areas [34], which showed that lymphatic pathology was either positively or not correlated with microfilaremia. Although analysis of the relationship between infection and disease in other areas of the world has led to the conclusion that microfilaremia decreases the risk of lymphatic disease of the legs and, conversely, amicrofilaremia predisposes to elephantiasis [7], these observations may have been biased by selection of subjects from a hospital or clinic setting and the availability of diethylcarbamazine. It is also possible that the relationship between patent infection and clinical morbidity is different in mf-endemic areas where transmission intensity is lower than in East Sepik Province.

Some studies have suggested that there are sex differences in lymphatic filariasis such that females are less prone to lymphatic pathology and have lower levels of microfilaremia than males [35, 36]. Our current data indicate this is not the case, at least in this area of Papua New Guinea. Mf carrier rates and levels of parasitemia were similar for both sexes, and the frequency of leg edema was twice as common among women as men. We speculate that the impression of there being a greater prevalence of disease among men may be due to selection bias for men with hydroceles. In any case, our data underscore the need for studies to determine whether pregnancy increases the risk for lymphatic pathology of the lower extremities and the importance of lymphatic filariasis as a public health problem of women.

Data presented here and observations made in other mf-endemic areas [37–40] should facilitate the creation of biologically relevant mathematical models that describe the complex interactions among transmission intensity, infection, and disease in lymphatic filariasis. Such tools have been used to evaluate the success of control programs for other arthropodborne parasitic diseases, such as onchocerciasis [41]. For lymphatic filariasis, we hope these models can be used to predict whether control schemes using mass chemotherapy or vector-based interventions are theoretically sound and feasible.

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