

TRANSMISSION DYNAMICS OF *WUCHERERIA BANCROFTI* IN EAST SEPIK PROVINCE, PAPUA NEW GUINEA

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Abstract. Bancroftian filariasis is endemic in many areas of Papua New Guinea. This study describes the entomologic indices of transmission near Dreikikir in East Sepik Province, Papua New Guinea. A total of 1,735 culicine mosquitoes, including *Culex* and *Mansonia* species, were dissected, but none were infected with filarial larvae. In contrast, *Anopheles punctulatus* and *An. koliensis* were found to be potential vectors: 7.3% of *Anopheles* were infected and the mean number of first- to third-stage larvae per infected mosquito was 2.7. Transmission indices varied significantly in five villages located within a 50-km radius of each other. Annual biting rates ranged from 4,789 to 48,020 bites/person/year; annual infective biting rates from 15 to 836/person/year; and annual transmission potential from 31 to 2,340 third-stage larvae/person/year. Monthly transmission potential and monthly infective biting rate varied significantly in each village, with the highest indices of transmission observed in villages nearest sites where puddles formed in river beds during the dry season. These data indicate that there is small area variation in the intensity and temporal pattern of filariasis transmission and that culicine mosquitoes are not important vectors of *W. bancrofti* in this area.

An appreciation of the transmission dynamics of lymphatic filariasis is important in advancing knowledge of how vector competence, behavior, and abundance influence the level of infection and disease in susceptible human populations. From a practical perspective, quantitative analyses of potential vectors and their level of infection may also provide cost-effective and noninvasive means for rapid assessment of the need for and success of various control measures.¹

Wuchereria bancrofti infection has been reported to be endemic in all lowland areas of Papua New Guinea so far investigated, and in the Ok Tedi and other fringe highland regions.²⁻⁷ Although entomologic studies in several areas of the country have shown the *Anopheles punctulatus* group are the principal vectors,⁸⁻¹⁰ only the work of Burkot and others⁹ conducted in Madang Province involved monthly collections of mosquitoes for a period exceeding one year. In April 1993, detailed monthly entomologic surveys were initiated in five inland villages in East Sepik Province as part of a multidisciplinary study investigating the epidemiology of bancroftian filariasis and the efficacy of mass chemotherapy as a control measure. Quantitative indices that have been shown to be useful in evaluating transmission of the related arthropod-borne filaria *Onchocerca volvulus*, the annual biting rate (ABR), annual infective biting rate (AIBR), and annual transmission potential (ATP), were determined along with the relationship between rainfall and mosquito infection and infective rates.¹¹ The current report describes the results of studies conducted in the year prior to administration of mass chemotherapy.

MATERIALS AND METHODS

Study area. Surveys were conducted in five villages in Dreikikir District in the East Sepik Province of Papua New Guinea. These villages, Albulum, Ngahmbule, Peneng, Yauatong, and Nanaha, are located in the southern foothills of the Torricelli Mountains. The natural vegetation is rain forest that has been cleared in many areas to make gardens.

Total rainfall between April 1993 and March 1994 was 1,499 mm. Most rainfall occurred from April to June and from December to March. The period from July to November was considered the dry season. Rainfall was measured at a station located 50 km away in the town of Maprik. Inhabitants of this area of East Sepik Province are predominantly subsistence farmers living in thatched huts.

Collection and dissection of mosquitoes. The all-night landing catch method was used to collect mosquitoes biting indoors and outdoors. Mosquitoes landing on humans from 6:00 PM to 6:00 AM were collected by resident adult volunteers with feet and legs bared to the knee. Using aspirators and aided by light from torches, the collectors captured mosquitoes that landed on them in search of a blood meal. Collectors worked in pairs at 6-hr intervals. One pair began at 6:00 PM and the other at midnight. One individual of a pair worked inside a house, and the other outside. Depending on geographic size, each village was divided into two or four sections; collectors rotated through the sections on different nights. Landing collections were performed regularly for four nights every month from April 1993 to March 1994, with the exception of October when studies were not performed because of lack of funding.

Female mosquitoes were sorted according to species and stored individually in 70% ethanol for transport to the laboratory. Species identification was based on keys by Belkin.¹² Mosquitoes were stained for filarial parasites using Mayer's acid haemalum and individually dissected with the head, thorax, and abdomen examined separately using a stereo microscope with magnification of 200 \times .¹³ Infective third-stage larvae (L3) were identified according to the criteria of Nelson.¹⁴

Morphologic criteria alone were relied upon for identifying members of the *An. punctulatus* complex because of the rarity of the *An. farauti* morphotype in the study area. Allozyme analysis has revealed six species within the *An. punctulatus* group, but four of these belong to the *An. farauti* complex.¹⁵ According to Foley and others,¹⁵ specimens that

fit Belkin's description of *An. punctulatus*¹² were confined to this species and *An. farauti* No. 4. The presence of a sector spot, a condition never found in *An. koliensis*, was used to identify *An. punctulatus*. Fourteen specimens morphologically identified as *An. koliensis* from a nearby area were confirmed by allozyme analysis¹⁵ (performed by Jeffrey Hii, James Cook University, Townsville, Queensland, Australia).

Calculation of biting rates and transmission potentials.

The ABR, AIBR, and ATP were calculated based on the formula by Walsh and others.¹¹ These criteria, subsequently adopted by the World Health Organization,¹⁶ do not require determination of parity for calculations of ATP and AIBR. Accordingly, monthly collections of mosquitoes were dissected to determine infection status irrespective of their parity. This is in contrast to the means by which transmission potential of *O. volvulus* by *Simulium* is measured. In that situation, it is common practice to determine parity of fresh specimens as a means of selecting parous females for further dissection. Nulliparous female *Simulium* flies are considered negative.

Monthly transmission indices were first calculated. The monthly biting rate is the estimated number of female mosquitoes biting one person between 6:00 PM and 6:00 AM in one calendar month. The product of the percentage of mosquitoes in the monthly catch harboring L3 multiplied by the biting rate gives the infective biting rate, i.e., the entomologic inoculation rate. The monthly transmission potential is the estimated number of *W. bancrofti* L3 to which the same person would be exposed during one month. The ABR and ATP are the sums of each of the respective monthly values for 12 consecutive months. The AIBR and ATP calculations were based on L3 found anywhere in the body of the mosquito. To calculate annual values, we needed to estimate the monthly rates for October when no collections were performed. The best estimate was considered to be the mean of the September and November values.

Statistical methods. The significance of differences in mosquito infection rates was evaluated by the chi-square test.

RESULTS

Vectors of *W. bancrofti*. A total of 338 person-nights of landing collections were performed. This yielded 35,504 mosquitoes belonging to seven species (Table 1). *Culex annulirostris* was the predominant species, constituting 54.0% of all mosquitoes caught. Other culicine species were *Cx. quinquefasciatus* (0.5%) and *Mansonia uniformis* (0.8%). The numbers of *Cx. annulirostris*, *Cx. quinquefasciatus*, and *M. uniformis* dissected were 1,371, 127, and 255, respectively, but none was infected with filarial larvae. Therefore, *Culex* and *Mansonia* species were not considered to be involved in filariasis transmission in the villages surveyed.

Of a total of 15,873 *Anopheles* mosquitoes caught, 14,916 (94.0%) were *An. punctulatus* (Table 1). The other *Anopheles* species were *An. koliensis* (5.9%), *An. bancrofti* (0.06%), and *An. farauti* s.l. (0.09%). Seven *An. bancrofti* females were dissected, and one was infected with a single first-stage larvae (L1). *Anopheles punctulatus* and *An. koliensis* were the only species found to harbor L3 and were, therefore, the only species identified as vectors of *W. ban-*

TABLE 1
Wuchereria bancrofti infection rates of anthropophilic female mosquitoes caught in five villages in the East Sepik Province of Papua New Guinea using the all-night landing catch method*

| Species | No. caught | No. dissected | No. with L1, L2, or L3 larvae | No. with L3 larvae |
|-------------------------------|------------|---------------|-------------------------------|--------------------|
| <i>Anopheles bancrofti</i> | 10 | 7 | 1 (14.3) | 0 (0) |
| <i>Anopheles farauti</i> | 14 | 14 | 0 (0) | 0 (0) |
| <i>Anopheles koliensis</i> | 933 | 585 | 37 (6.3) | 9 (1.5) |
| <i>Anopheles punctulatus</i> | 14,916 | 9,551 | 699 (7.3) | 198 (2.1) |
| <i>Culex annulirostris</i> | 19,160 | 1,371 | 0 (0) | 0 (0) |
| <i>Culex quinquefasciatus</i> | 195 | 127 | 0 (0) | 0 (0) |
| <i>Mansonia uniformis</i> | 276 | 255 | 0 (0) | 0 (0) |
| Total | 35,504 | 11,910 | 737 (6.2) | 207 (1.7) |

* L1, L2, and L3 = first-, second-, and third-stage larvae, respectively. Values in parentheses are the % of the total caught.

crofti in the villages surveyed. *Anopheles punctulatus* was the only vector in four of the villages: Albulum, Ngahmbule, Peneng, and Yauatong.

Parallel indoor and outdoor landing catches performed in these villages between April and September 1993 showed that *An. punctulatus* was equally endophilic and exophilic. The total numbers of mosquitoes caught biting indoors and outdoors during this period were 3,201 and 3,174, respectively.

Anopheles koliensis was observed to be infected with *W. bancrofti* in Nanaha only. Similar numbers of *An. koliensis* were caught indoors (318) and outdoors (317).

Infection rates of *An. punctulatus* and *An. koliensis*. The monthly variation in the infection rates of *An. punctulatus* for the combined catches from all five villages is shown in Figure 1. Rainfall data is included for comparison. A total of 9,551 *An. punctulatus* were dissected and of these, 699 (7.3%) were infected (containing L1, L2, or L3) and 198 (2.1%) were infective (i.e., containing L3). The infection rate was lowest in November (3.2%) and highest in March (20.5%). Infective rates varied from 0.5% in November to 6.2% in June. The wet season (December to June) and dry season (July to November) infection rates were 15.3% and 3.8%, respectively, and the difference was statistically significant ($\chi^2 = 338.45$, degrees of freedom [df] = 1, $P < 10^{-8}$). The corresponding infective rates, 4.8% and 0.9%, were also significantly different ($\chi^2 = 151.48$, df = 1, $P < 10^{-8}$). The majority (72.9%) of *An. punctulatus* were caught during the dry season. Figure 1 mainly reflects observations in the villages of Albulum and Yauatong, which are located within 0.5 km of the Wara Krok Krok river, an important breeding site for *Anopheles*. The majority (85.5%) of mosquitoes dissected were caught in these two villages. The other three villages are located at a distance greater than 5 km from the Wara Krok Krok.

A detailed summary of the results of mosquito dissections according to village is presented in Table 2. The highest proportion of infected and infective mosquitoes were captured in Yauatong (11.7% and 3.5%, respectively), and the lowest in Ngahmbule (2.0% and 0.4%). Infected mosquitoes were caught in Ngahmbule only in August (7.7% infection rate), September (5.4%), and November (0.8%), and infective *An. punctulatus* exclusively in August (1.5%) and September (0.4%). At Peneng, infected mosquitoes were de-

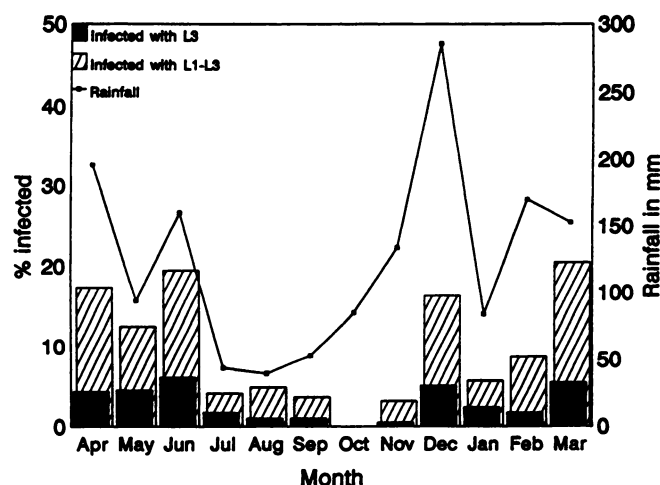


FIGURE 1. Monthly rainfall and infection (first-stage larvae–third-stage larvae [L1–L3]) and infectivity (L3 only) rates of female *Anopheles* mosquitoes in five East Sepik Province villages. Mosquitoes were collected between 6:00 PM and 6:00 AM using the landing catch method as described in the Materials and Methods.

tected in each monthly collection except for January. The highest infection and infective rates in this village were observed in February (21.2% and 3.9%, respectively). At Nanaha, the combined catches of *An. punctulatus* and *An. koliensis* were higher than the number of *An. punctulatus* caught in Ngahmbule or Peneng, but much lower than the number of this vector in Albulum or Yauatong. The small number of *An. koliensis* caught in villages other than Nanaha are not included in Table 2.

With respect to the level of infection and infectivity, the highest proportions of infected and infective *An. punctulatus* were captured in Yauatong (11.7% and 3.5% contained L1–L3 and L3, respectively). Maxima of 117 L1–L3 and 17 L3 were detected in a single mosquito captured in Albulum. One *An. punctulatus* caught resting inside a house in Yauatong contained 34 L3. For all villages combined, the mean larval density (L1–L3) per infected *An. punctulatus* was 6.2, and the mean number of L3 per infective *An. punctulatus* was 2.7.

With respect to *An. koliensis* captured in Nanaha, the maximum number of larvae (L1–L3) in a single infected *An. koliensis* was 15; the maximum number of L3 found in a

single female was six (Table 2). The mean larval densities (L1–L3) per infected *An. punctulatus* and *An. koliensis* were 4.8 and 3.8, respectively. The mean numbers of L3 per infective female (2.8) were the same for both mosquito species.

Statistical analyses of these data showed that differences in the infection rates of *An. punctulatus* among the five villages were highly significant. With respect to mosquitoes containing any larval stage, the *P* value was less than 10^{-8} ($\chi^2 = 145.47$; *df* = 4). A similar *P* value was calculated when mosquitoes containing L3 only were considered ($\chi^2 = 54.99$). At Nanaha, there was no significant difference between infection ($\chi^2 = 1.62$, *P* = 0.02; *df* = 1) and infective rates ($\chi^2 = 0.80$, *P* = 0.66) of *An. punctulatus* and *An. koliensis*.

Entomologic indices of transmission. Monthly entomologic indices of transmission according to village are presented in Table 3. At Albulum and Yauatong, people were exposed to L3 10 of the 11 months for which collections were performed. At Peneng and Nanaha, exposure occurred for eight months and at Ngahmbule for two months. The highest monthly transmission potential (MTP) was 683 L3/person/month, recorded for Yauatong in June in the wet season. At Albulum and Nanaha, the highest MTPs were 477 and 74 L3/person/month, both recorded in December (also in the wet season). At both Ngahmbule and Peneng, peak MTPs of 16 and 47 L3/person/month were recorded in September during the dry season.

The ABR for *An. punctulatus* ranged from 4,789 bites/person/year at Ngahmbule to 48,020 bites/person/year at Albulum (Table 4); AIBRs for the same vector ranged from 15 infective bites/person/year at Ngahmbule to 836 at Yauatong, and ATPs ranged from 31 L3/year at Ngahmbule to 2,340 in Yauatong. The values for Nanaha presented in Table 4 represent the total for the two species of mosquitoes. The ABRs for *An. punctulatus* and *An. koliensis* in this village were 5,909 and 4,421, respectively. The AIBRs and ATPs were 63 and 67, and 128 and 201, for *An. punctulatus* and *An. koliensis*, respectively.

DISCUSSION

Previous studies describing the entomologic aspects of *W. bancrofti* transmission in Papua New Guinea have shown that both anopheline and culicine mosquitoes are potential

TABLE 2
Summary of dissection results for *Anopheles punctulatus* and *An. koliensis**

| | Villages | | | | | | All villages |
|---|-----------|------------|----------|------------|----------|----------|--------------|
| | Albulum† | Ngahmbule† | Peneng† | Yauatong† | Nanaha† | Nanaha‡ | |
| No. of mosquitoes caught | 7,089 | 767 | 1,035 | 5,330 | 695 | 917 | 15,833 |
| No. dissected | 4,450 | 510 | 820 | 3,364 | 407 | 576 | 10,127 |
| No. (%) with L1, L2, or L3 larvae | 215 (4.8) | 10 (2.0) | 61 (7.4) | 395 (11.7) | 18 (4.4) | 37 (6.4) | 736 (7.3) |
| No. (%) with L3 larvae | 58 (1.3) | 2 (0.4) | 14 (1.7) | 119 (3.5) | 5 (1.2) | 9 (1.6) | 207 (2.0) |
| Mean no. of L1–L3 larvae per infected mosquito | 6.4 | 6.6 | 5.2 | 6.3 | 4.8 | 3.8 | 6.2† |
| Mean no. of L3 larvae per infective mosquito | 2.4 | 2.0 | 1.9 | 3.0 | 2.8 | 2.8 | 2.7† |
| Maximum no. of larvae per infected mosquito | 117 | 38 | 55 | 91 | 19 | 15 | 117† |
| Maximum no. of L3 larvae per infective mosquito | 11 | 3 | 4 | 17 | 8 | 6 | 17† |
| No. of person-nights | 68 | 68 | 66 | 68 | 68 | 68 | 406 |

* See Table 1 for definitions.

† *An. punctulatus* only.

‡ *An. koliensis* only.

TABLE 3

Monthly biting rates (MBR), monthly infective biting rates (MIBR), and monthly transmission potentials (MTP) in the villages of Albulum, Ngahmbule, Peneng, Yauatong, and Nanaha in the East Sepik Province of Papua New Guinea*

| Month | Albulum | | | Ngahmbule | | | Peneng | | | Yauatong | | | Nanaha | | |
|------------|---------|------|-------|-----------|------|-----|--------|------|-----|----------|------|-------|--------|------|-----|
| | MBR | MIBR | MTP | MBR | MIBR | MTP | MBR | MIBR | MTP | MBR | MIBR | MTP | MBR | MIBR | MTP |
| April 1993 | 120 | 11 | 11 | 68 | 0 | 0 | 236 | 4 | 4 | 979 | 53 | 93 | 848 | 24 | 42 |
| May | 1,511 | 40 | 76 | 54 | 0 | 0 | 558 | 9 | 21 | 1,868 | 149 | 440 | 62 | 0 | 0 |
| June | 173 | 15 | 19 | 68 | 0 | 0 | 365 | 4 | 12 | 2,595 | 180 | 683 | 484 | 7 | 41 |
| July | 1,407 | 20 | 20 | 248 | 0 | 0 | 108 | 0 | 0 | 856 | 20 | 30 | 600 | 7 | 7 |
| August | 5,549 | 41 | 77 | 446 | 7 | 7 | 527 | 7 | 20 | 2,825 | 34 | 75 | 225 | 7 | 57 |
| Sept | 8,325 | 74 | 135 | 1,388 | 5 | 16 | 1,178 | 30 | 47 | 5,171 | 64 | 153 | 694 | 0 | 0 |
| October | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Nov | 14,520 | 60 | 157 | 1,118 | 0 | 0 | 668 | 9 | 9 | 6,383 | 38 | 38 | 2,895 | 15 | 15 |
| Dec | 2,519 | 135 | 477 | 70 | 0 | 0 | 1,077 | 9 | 9 | 1,767 | 168 | 450 | 674 | 31 | 74 |
| Jan 1994 | 70 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 589 | 23 | 102 | 1,178 | 0 | 0 |
| Feb | 1,876 | 49 | 195 | 14 | 0 | 0 | 371 | 14 | 29 | 2,023 | 0 | 0 | 497 | 11 | 32 |
| March | 527 | 13 | 25 | 62 | 0 | 0 | 39 | 0 | 0 | 946 | 63 | 180 | 388 | 20 | 53 |
| Total | 36,597 | 458 | 1,192 | 3,536 | 12 | 23 | 5,150 | 86 | 151 | 26,002 | 792 | 2,244 | 8,545 | 122 | 321 |

* — = no data collections.

vectors.^{2,8} In the most recent published report from the Sepik area, Bryan observed that *An. punctulatus* in the village of Yauatong in East Sepik Province, a mosquito collection site in the current study, had an infection rate of 47.3% and infective rate of 3.4%.⁸ Because a major goal of the current research is to determine whether mass chemotherapy can sustainably diminish transmission of *W. bancrofti*, we performed detailed entomologic surveys in five East Sepik villages before drug distribution commenced.

Only *Anopheles* mosquitoes, *An. punctulatus* and *An. koliensis*, were observed to be infected with L1–L3, although culicine mosquitoes were abundant (Table 1). This is in contrast to the situation in West Sepik Province, located more than 50 km from the study villages, where *Cx. quinquefasciatus* was noted to be infected (no L3 were detected).⁸ In four of the five villages in the current study, *An. punctulatus* was the sole vector. This mosquito breeds along the edges of rivers formed during the wet season (November through June in the year of the current survey, see Figure 1). During the dry season, sections of the river dry up and form numerous sun-lit puddles of clear water which serve as additional breeding sites for *An. punctulatus*.¹⁷ Accordingly, human biting rates of *An. punctulatus* were generally higher during the dry than during the wet season. Albulum and Yauatong, which had higher mosquito densities than Peneng and Ngahmbule, are located within 1 km of the major river in this area. Peneng, Ngahmbule, and Nanaha are located more than 1.5 to approximately 5 km from this or other

rivers suitable for *Anopheles* breeding. Unlike Albulum and Yauatong, tributaries of rivers that flowed near Peneng and Ngahmbule were small and heavily shaded. At Nanaha, *An. koliensis* breed in streams at the margins of forest. In all five villages, people were equally exposed indoors and outdoors to bites of *An. punctulatus*.

Based on infective rates, *An. koliensis* and *An. punctulatus* appear to be equally capable of transmitting *W. bancrofti* (Tables 1 and 2). Little information on the natural infection rates of *An. koliensis* in this area is available. No infections were detected in 286 specimens collected in Maprik, East Sepik Province,¹⁸ but at Wansu, West Sepik Province, 14% of 300 specimens were infected and 0.67% were infective.⁸ The infection rates of landing catches of *An. punctulatus* observed in the current study are similar to those reported by others in Papua New Guinea but high compared with *Anopheles* mosquitoes in other areas of the world. For example, the highest rates were 6.8% infected and 1.6% infective for approximately 100 *An. gambiae* collected in Tanga, Tanzania.¹⁹ Less than 1.0% of *An. funestus* were infective in endemic areas of Tanzania^{20,21} and Kenya.²² High infection rates reported for *Anopheles* mosquitoes in the Pacific region were determined from resting catches, which tend to include a higher proportion of infected mosquitoes compared with landing collections.^{8,10}

Marked seasonal variation in mosquito infection rates was observed in the current study (Figure 1 and Table 3). The proportions of infected and infective mosquitoes were lowest in the dry season (approximately 50 mm rainfall in July through September), and highest at the end of the rainy season. The former is most likely due to the fact that there was an upsurge in the number of nulliparous mosquitoes during this time, when breeding sites near Albulum and Yauatong increased coincidental with formation of numerous puddles in the drying riverbed. Indeed, no significant seasonal differences in mosquito infection rates were noted in Peneng, which is not near a river.

Finally, the major entomologic indices of transmission, ABR, AIBR, and ATP, estimated for *An. punctulatus* are high (especially in Yauatong) compared with reports for *Anopheles* mosquitoes outside Papua New Guinea.²³ The

TABLE 4

Entomologic indices of *Wuchereria bancrofti* transmission in five villages in the East Sepik Province of Papua New Guinea

| Village | ABR* | AIBR† | ATP‡ |
|-----------|--------|-------|-------|
| Albulum | 48,020 | 525 | 1,338 |
| Ngahmbule | 4,789 | 15 | 31 |
| Peneng | 6,073 | 106 | 179 |
| Yauatong | 31,779 | 836 | 2,340 |
| Nanaha | 10,340 | 130 | 329 |

* ABR = annual biting rate, expressed as the number of bites/person/year.

† AIBR = annual infective biting rate, expressed as the number of bites by third-stage (L3)-containing mosquitoes/person/year.

‡ ATP = annual transmission potential, the number of L3 to which an individual is exposed per year.

highest published ABR was 25,439 bites/person/year for combined values of *An. gambiae* and *An. funestus* in a village near Tanga, Tanzania.¹⁹ The highest previous estimates of AIBR (350 infective bites/person/year) and ATP (1,000 L3/person/year) were reported by Brinkman²⁴ for mosquitoes in the Marshall territory of Liberia. In contrast, Burkot and others, working in Madang Province in Papua New Guinea, estimated an ABR of 90,000 bites/person/year and an AIBR of 1,443 infective bites/person/year for *An. punctulatus*.⁹ (Information describing the number of L3 found in infective mosquitoes was not provided in this report, thereby precluding an estimate of ATP.) We are currently examining how the exceedingly high entomologic indices of *W. bancrofti* transmission in East Sepik Province reported in the current study influence the microfilarial carrier rates and frequency of lymphatic pathology in the exposed human populations.

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