

LYMPHATIC FILARIASIS IN A HYPERENDEMIC REGION: A TEN-YEAR, FOLLOW-UP PANEL SURVEY

KAREN MYUNG, ACHILLE MASSOUGBODJI, SERGE EKOUE, PASCAL ATCHADE, VALENTINE KIKI-FAGLA, AND
AMY D. KLION

*Departments of Biochemistry and Internal Medicine, University of Iowa, Iowa City, Iowa;
Faculty of Health Sciences, University of Benin, Cotonou, Benin*

Abstract. The present study is a long-term panel survey of a population living in a previously identified *Wuchereria bancrofti*-endemic area of Benin. Unexpectedly, a marked decrease in the prevalence of microfilaremia (from 9.4% to 0.48%; $P < 0.001$) occurred over a 10-year period in the absence of chemotherapy or vector control measures. The percentage of patients with chronic pathology remained stable during the study period. The decrease in the prevalence of parasitemia could not be explained by environmental or sociologic changes in the region, or by differences between the two study populations. These data suggest that the epidemiology of lymphatic filariasis in an endemic region may change independently of recognized modulating factors.

The filarial parasite *Wuchereria bancrofti* is estimated to infect more than 100 million individuals worldwide.¹ It is the predominant etiologic agent of lymphatic filariasis, which is characterized by progressive debilitating swelling of the extremities, scrotum, or breast (elephantiasis) in a subset of infected individuals. Although aggressive vector control and mass chemotherapy programs have been successful in eliminating the parasite from some geographically isolated endemic foci, including Taiwan and South Korea,¹ lymphatic filariasis continues to cause significant morbidity in many developing countries of the tropics and subtropics. Furthermore, models of global climate change predict extension of the endemic areas of lymphatic filariasis during the next century.²

Lymphatic filariasis has been targeted by the International Task Force for Disease Eradication as one of six potentially eradicable infectious diseases.³ Assessment of the relative success of various control programs for lymphatic filariasis is dependent not only on sensitive and specific tools for surveillance of infection and transmission, but on an accurate understanding of the natural history of infection in endemic areas. However, since most studies of lymphatic filariasis have been cross-sectional or complicated by chemotherapeutic intervention,^{4,5} little is known about the stability of transmission in endemic areas over time.

The present study is a long-term follow-up survey of a population living in a previously identified *W. bancrofti*-endemic area of Benin in which neither chemotherapeutic nor vector control interventions have been attempted. The prevalence of filariasis, as determined by clinical and parasitologic criteria, was compared with the results of an epidemiologic study of this region performed 10 years earlier (Kiki M, University of Benin, unpublished data). In addition, environmental, behavioral, and sociologic factors that might influence disease prevalence were investigated.

MATERIALS AND METHODS

Study area. The study was conducted in the endemic region of the Plateau of Adja (total population: 116,468 in 1983) in southwest Benin. This region encompasses the communes of Bopa, Come, and Houeyogbe and is bordered on the east by Lake Aherne. The altitude of the plateau is approximately 90 meters above sea level. The climate is of

the subequatorial type with temperatures ranging between 27°C and 31°C and mean percentage relative humidity between 95% and 53% depending on the season. The mean annual rainfall is approximately 1,000 mm. There were no major aberrations in mean monthly temperature or rainfall in the region between 1974 and 1994, and the mean annual temperature and rainfall have not changed significantly during this time (Carmichael G, Center for Global and Regional Environmental Research, University of Iowa, unpublished data). In this area, *W. bancrofti* is a nocturnally periodic infection transmitted by *Anopheles funestus* and *An. gambiae*. Of note, no other human filarial pathogens have been identified in this region.

Subjects. The initial epidemiologic study of 1,129 inhabitants of the Plateau of Adja was conducted during June through August 1983 (Kiki M, University of Benin, unpublished data). Data from this study were reanalyzed using the individual patient records. Because microfilariae were not detected in the peripheral blood of any of the 564 individuals from the cities of Bopa, Come, or Houeyogbe or of any children less than 10 years of age, analysis of the 1983 study was restricted to data from the 455 participants more than 10 years of age from the rural villages of the communes of Akodeha, Ouedeme, Possotome, and Se. The distribution of study subjects by village in 1983 and 1994 is shown in Table 1.

Follow-up evaluation of 410 individuals from these same rural villages was performed between October and December 1994. In each village, all volunteers more than 10 years of age were recruited by local health care workers. Villagers live in adobe huts which have no mosquito screens, electricity, or waste disposal systems. Daily activities include maintenance of the home and fishing in the central lagoon. These conditions are essentially unchanged from 10 years ago. Individuals were assembled in the commune center at night and the study was explained in French with translation by local health care workers. Informed consent was obtained from all participants. The study was reviewed and approved by the Institutional Review Boards of the University Iowa and the Faculty of Health Sciences at the University of Benin.

Clinical and parasitologic evaluation. Clinical evaluations were conducted in the villages at the commune center, and included an extensive medical history, assessment of vital signs, auscultation of the heart and chest, and evalua-

TABLE 1
Characteristics of the study population

	1984	1993
Median age, years (range)	33 (10-76)	50 (12-100)
Median exposure, years (range)	31 (1-75)	45 (2-100)
Gender (M/F)	257/198	232/178
Village (no. of subjects)		
Akodéha	182	174
Ouédémé	134	56
Possotomé	90	72
Sè	49	108

tion of the skin, extremities, and lymph nodes. Male external genitalia were examined for abnormalities of the testes, epididymis, and spermatic cords. Social considerations precluded examination of the female genitalia in most cases. Participants answered questionnaires regarding age, length of residence in the endemic area, occupation, ethnic group, filarial status of family members, and previous treatment with antifilarial agents.

In the 1983 study, microfilarial densities were calculated using the saponin lysis method on 1 ml of blood drawn between 10:00 PM and 2:00 AM. Based on previous comparisons of the sensitivity of saponin lysis and the Nuclepore[™] filtration method (Nuclepore, Pleasantown, CA)⁶ for the detection of *Loa loa* microfilariae, we elected to use the latter method for the 1994 study. Briefly, 2 ml of anticoagulated venous blood drawn between 10:00 PM and 2:00 AM were filtered through a polycarbonate membrane (pore size 3 = μm). The membranes were then placed on a microscope slide, dried overnight, and stained with Diff-Quik stain (Baxter Scientific Products, Deerfield, IL). When no microfilariae were detected by filtration in the first group of 34 samples, both saponin lysis and Nuclepore[™] filtration were used to analyze the second group of 36 samples. One microfilaremic patient was identified by filtration and none by saponin lysis, consistent with our previous experience with *L. loa*. Subsequent samples were analyzed by Nuclepore[™] filtration alone. Leukocyte counts were performed with a manual differential

for calculation of eosinophil levels and serum was obtained for detection of circulating parasite antigen by capture ELISA based on monoclonal antibody AD12.1 (performed by Dr. Gary Weil, Washington University, St. Louis, MO).⁷

Statistical analysis. Because of differences in the prevalence of microfilaremia between villages in the 1983 study, Cochran-Mantel-Haentzel analysis with stratification by village was used to compare results from the 1983 and 1994 studies unless otherwise indicated. All computations were carried out using JMP Statistics Made Visual software (SAS Institute Inc., Cary, NC).

RESULTS

Although participants in both studies were recruited in a similar fashion, the 1983 study population was significantly younger ($P < 0.001$, by Mann-Whitney U test), with 30% of individuals in the 10-20-year-old age group (Table 1 and Figure 1). This age bias was also reflected in the duration of residence in the endemic area, which was longer in the 1994 study population (Table 1). There was no difference in gender distribution between the two studies (Table 1).

A total of 43 of 455 patients (9.4%) were found to have circulating microfilariae in 1983. Microfilarial densities ranged from < 50 to 1,050 microfilariae/ml blood (median = 100 microfilariae/ml) and were similar in all age groups. The prevalences of microfilaremia by village were 7% in Akodeha, 14% in Ouedeme, 10% in Possotome, and 4% in Se. Based on these estimates, we would have expected to find 31 microfilaria-positive individuals in the 1994 study. Surprisingly, only two participants (one each from Akodeha and Possotome) had detectable microfilariae (Table 2; $P < 0.001$, by Cochran-Mantel-Haentzel test). To confirm these results, circulating parasite antigen assays, a sensitive and specific marker of microfilaremia,⁷ were performed on serum samples from the 1994 study subjects. Sera from six individuals, including one of the microfilaria-positive patients, were positive in the circulating antigen assay, and four additional samples gave borderline positive results. Serum

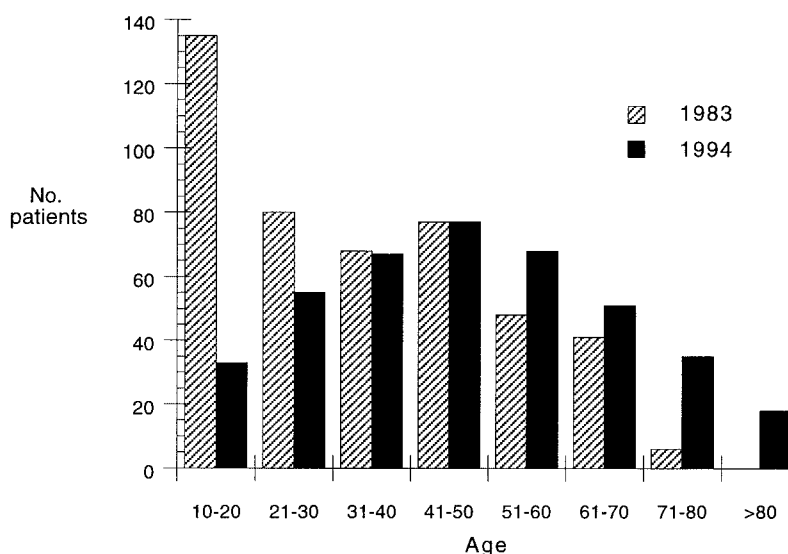


FIGURE 1. Age (years) distribution of the two study populations.

TABLE 2
Signs and symptoms of lymphatic filariasis

Symptom/sign	No. (%) of participants		<i>P</i> *
	1983	1994	
Microfilaremia	43 (9%)	2 (0.48%)	<0.001
Acute			
Lymphadenitis	117 (26%)	162 (40%)	<0.001
Orchitis	19 (4%)	23 (6%)	NS
Chronic			
Hydrocele	24 (5%)	24 (6%)	NS
Chyluria	35 (8%)	28 (7%)	NS
Elephantiasis	32 (7%)	43 (11%)	0.05
Any of the above	80 (18%)	78 (19%)	NS

* By Cochran-Mantel-Haenszel test. NS = not significant.

from the second microfilaria-positive patient was not available for confirmatory testing. Interestingly, all of the antigen-positive individuals had lived in the endemic area for > 35 years at the time of the study and three of the six had a history of chyluria.

Despite the observed decrease in the rate of microfilarial carriage over time, there was no parallel decrease in the prevalence of characteristic signs and symptoms of lymphatic filariasis in the study population (Table 2). In fact, the prevalence of both lymphadenitis and elephantiasis were higher in the 1994 study. The modest increase in elephantiasis is likely due to the differences in the age distribution of the two study populations, and fails to attain significance when the data is analyzed controlling for this variable. Lymphadenitis, on the other hand, is common in this population regardless of age due to cuts and abrasions of the lower extremities acquired while fishing barefoot in the lagoon. Although attempts were made in the 1994 study to distinguish individuals who had a history of lymphangitis extending distally (retrograde) from the lymph node, which is typical of lymphatic filariasis, from the more common proximal lymphangitis that may complicate bacterial or fungal cellulitis of other etiologies, this distinction was not generally appreciated by the study participants. No cases of retrograde lymphangitis and a single case of lymphadenitis with abscess were detected by physical examination in both the 1983 and 1994 studies.

By chance, seven of the 43 individuals who were microfilaremic in 1983 were included in the 1994 study. None of the seven had detectable microfilariae in 1994; however, progression of clinical disease was noted in two patients and one patient had detectable circulating parasite antigen.

To explore some of the factors that might explain the decrease in the prevalence of microfilaremia in this region, a detailed occupational and social history was obtained from all participants. There were no obvious differences in the use of personal protection measures, such as protective clothing, bed nets, screens, and insect repellents, between the two study populations, and no community-wide mosquito control programs were instituted during or for 10 years prior to the study period. The distribution of primary occupations was also similar between the two study populations, consisting primarily of fisherman, farmers, and homemakers. A moderate decrease in the number of students was observed (from 16% of the total in 1984 to 6% in 1994; $P < 0.001$,

by Fisher's exact test) and is most likely due to the higher percentage of school age children in the 1983 study, rather than a shift in the level of education in the community.

Six major ethnic groups exist in the plateau d'Adja: the Adja, the Ouatchi, the Pedah, the Mina, the Sahoue, and the Kotafon. With the exception of the Kotafon, who migrated into the region at the end of the 17th century, the ethnic groups in this region are descendent from a single lineage, and intermarriage between ethnic groups is fairly common. There was an excess of Kotafon in the 1994 study (22% versus 8% of the total study population; $P < 0.001$, by Fisher's exact test); however, microfilaremia was detected in members of all ethnic groups (including the Kotafon) in 1983. Although the numbers of microfilaremic individuals are too few to detect a statistical difference in prevalence of microfilaremia between the different ethnic groups, minor differences in genetic susceptibility are unlikely to account for the marked decrease in the prevalence of microfilaremia between the two studies.

All participants were questioned regarding the use of antifilarial chemotherapy (diethylcarbamazine [DEC], ivermectin, or albendazole) during the study period. Although mebendazole can be found in several of the local pharmacies and is used sporadically to treat symptomatic intestinal helminth infections, other anthelmintics are unavailable to the general population of the region. In fact, only one patient, who had a history of onchocerciasis acquired outside of the study region, had received prior antifilarial chemotherapy with DEC (administered at the University Hospital in Cotonou).

DISCUSSION

An understanding of the natural history of bancroftian filariasis in endemic regions is essential for the accurate interpretation of the efficacy of infection control measures. Because of the difficulties in obtaining long-term epidemiologic data, current control strategies have been based primarily on short-term (1–2 year) studies, which have suggested that the prevalence of microfilarial carriers remains remarkably stable over time in the absence of intervention.^{8,9} Consequently, reductions in the prevalence of infection have been attributed entirely to the institution of control measures, allowing comparisons to be made between interventions in different geographic areas.

Data from available long-term studies is less clearcut. In one study performed in the Cook Islands, there was a reduction in the prevalence of microfilaremia from 30% to 5% over a 17-year period.⁵ However, mass chemotherapy with DEC was administered between the initial evaluation and follow-up study and may have contributed to the observed decrease in parasitemia. In contrast, a community-wide followup survey in Tanzania showed no decrease in the prevalence of microfilaremia between 1975 and 1991, despite the intermittent use of control measures, including mass chemotherapy with DEC and vector control.⁴ However, the use of different methods of microfilarial detection in the initial and follow-up studies make the data somewhat difficult to interpret, since an overall decrease in the prevalence of microfilaremia in 1991 might be masked by an increase in the sensitivity of detection.

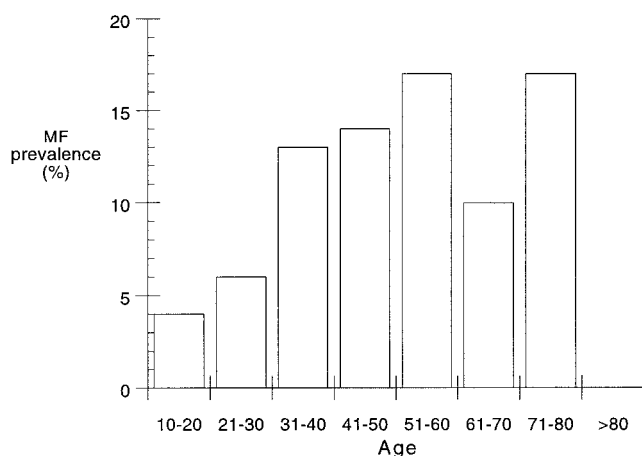


FIGURE 2. Age (years)-specific microfilarial (MF) prevalence in 1983.

In the present study, a marked decrease in the prevalence of microfilaremia (from 9.4% to 0.48%) occurred over a 10-year period in the absence of chemotherapy or vector control measures. This decrease was significant ($P < 0.0001$) despite the use of a more sensitive method of detection of microfilariae and confirmation by circulating parasite antigen assay (90% sensitive for the detection of active infection). It has been estimated from studies of transient residents of endemic areas that in the absence of reinfection, infected individuals lose their microfilaremia after 5–8 years.¹⁰ Interestingly, all seven of the microfilaremic individuals from the 1983 study who were included in the 1994 follow-up became amicrofilaremic.

Although the selection of participants was performed in an identical manner in 1983 and 1994, there were some differences in the distribution of age, village of residence, and ethnic background between the two study populations, which could have affected the observed prevalences of microfilaremia. Most epidemiologic studies have demonstrated an increase in the prevalence of microfilaremia with age that plateaus by young adulthood.^{4,10} A similar pattern was observed in the 1983 study in Bopa (Kiki M, University of Benin, unpublished data; Figure 2). Consequently, bias introduced by the shift in median age in the 1994 study would be expected to increase the prevalence of microfilaremia. Since the prevalence of microfilaremia in the four study villages varied considerably in 1983 and a greater proportion of the 1994 study population resided in villages of lower prevalence, all statistical comparisons (unless otherwise noted) were adjusted to account for the effect of village of residence. Whereas genetic differences have been postulated to affect the susceptibility to and clinical manifestations of lymphatic filariasis in some populations,^{11,12} the relationship between ethnic background and filariasis in this region is unknown. The fact that members of all ethnic groups were represented in the microfilaremic group in 1983, the degree of relatedness between the different ethnic groups, and the extent of intermarriage in the region all suggest that the observed differences in the proportion of Kotafon are unlikely to have biased the data significantly.

Despite the decrease in the prevalence of microfilaremia, there was no comparable decrease in the prevalence of

chronic pathology after adjustment for the difference in age between the two populations. This is not surprising in view of the length of time that it typically takes for chronic symptoms to develop in infected individuals. A positive correlation between the incidence of infection and the proportion of infected individuals that are microfilaremic has been proposed based on mathematical modeling and epidemiologic studies.¹³ This would imply a decrease in the incidence of infection in our study area. Although the apparent increase in the prevalence of acute manifestations of filariasis (i.e., lymphadenitis) would seem at first glance to be contradictory to this hypothesis, recent studies have implicated recurrent bacterial infections as an important factor in recurrent episodes of adenolymphangitis and progression towards chronic lymphedema and elephantiasis.^{1,14} Since such episodes are common in this population regardless of infection status due to minor trauma inflicted while fishing and farming barefoot, the increase in lymphadenitis may not reflect an actual increase in the incidence of infection. This is supported by the fact that none of the individuals with lymphangitis had detectable circulating parasite antigen.

The decrease in the prevalence (and potentially incidence) of lymphatic filariasis in the study region could not be explained by environmental or social change. Review of temperature and rainfall records showed no evidence of a major climatic change between 1980 and 1995, which could have led to a decrease in the vector population. Extensive questioning of study participants, health care workers, pharmacists, and market vendors of pharmaceuticals confirmed that antifilarial agents (including DEC, ivermectin, and albendazole) are not widely available in this region. Similarly, no obvious changes in socioeconomic status, level of education, or behavior of the population could be ascertained.

To our knowledge, this represents the first long-term epidemiologic survey of lymphatic filariasis in a hyperendemic region in the absence of chemotherapeutic intervention. Whether the observed decrease in prevalence will persist or whether lymphatic filariasis will recrudescence in this region remains to be seen. Nevertheless, the practical implication of the results is that the success or failure of control programs should be assessed cautiously, since the epidemiology in an endemic region may change independently of recognized modulating factors.

Acknowledgments: We thank the physicians and nurses of the plateau d'Adja for help with the field portion of the study, and Henri Essomé for assistance in the field and laboratory in Benin. We also acknowledge the laboratory of Dr. Gary Weil (Washington University, St. Louis, MO) for performing the circulating antigen assays.

Financial support. This work was supported by the Office of Research and Development (R & D), Department of Veteran's Affairs, and by the National Institutes of Health (grant AI-01170).

Authors' addresses: Karen Myung, Department of Biochemistry, University of Iowa, Iowa City, IA 52242. Achille Massougbodji, Serge Ekoue, and Pascal Atchade, Faculte des Sciences de la Sante, BP188, Cotonou, Benin. Valentine Kiki-Fagla, Cotonou, Benin. Amy D. Klion, Laboratory of Parasitic Diseases, National Institutes of Health, Building 4, Room 126, Bethesda, MD 20892.

REFERENCES

1994. *Lymphatic Filariasis Infection and Disease: Control Strategies*. Geneva: World Health Organization.

2. McMichael AJ, Haines A, Slooff R, Kovats S, 1996. *Climate Change and Human Health*. Geneva: World Health Organization.
3. CDC, 1993. Recommendations of the International Task Force for Disease Eradication. *MMWR Morb Mortal Wkly Rep* 42: 1–38.
4. Meyrowitsch DW, Simonsen PE, Makunde WH, 1995. A 16 year followup study on bancroftian filariasis in three communities of north-eastern Tanzania. *Ann Trop Med Parasitol* 89: 664–675.
5. Steel C, Guinea A, Ottesen EA, 1996. Evidence for protective immunity to bancroftian filariasis in the Cook Islands. *J Infect Dis* 174: 598–605.
6. Dennis DT, Kean BH, 1971. Isolation of microfilariae. Report of a new method. *J Parasitol* 57: 1146–1147.
7. Weil GL, Jain DC, Santhanam S, Malhotra A, Kumar H, Sethumadhavan KVP, Liftis F, Ghosh TK, 1987. A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. *J Infect Dis* 156: 350–355.
8. Jain DC, Menon PK, Sethumadhavan KV, Johny VM, Ghosh TK, 1989. Epidemiology of bancroftian filariasis in a semi-urban community of Kerala state. *J Commun Dis* 21: 265–271.
9. Day KP, Grenfell B, Spark R, Kazura JW, Alpers MP, 1991. Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *Am J Trop Med Hyg* 44: 518–527.
10. Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK, Bundy DAP, Grenfell BT, 1989. Estimation of age-specific rates of acquisition and loss of *Wuchereria bancrofti* infection. *Trans R Soc Trop Med Hyg* 83: 689–693.
11. Yazdanbakhsh M, Sartono E, Kruize YC, Kurniawan A, Partono F, Maizels RM, Schreuder GM, Schipper R, de Vries RR, 1995. HLA and elephantiasis in lymphatic filariasis. *Hum Immunol* 44: 58–61.
12. Chan SH, Dissanayake S, Mak JW, Ismail MM, Wee GB, Srinivasan N, Soo BH, Zaman V, 1984. HLA and filariasis in Sri Lankans and Indians. *Southeast Asian J Trop Med Public Health* 15: 281–286.
13. Bundy DAP, Grenfell BT, Rajagopalan PK, 1991. Immunoepidemiology of lymphatic filariasis: the relationship between infection and disease. *Immunol Today* 12: A71–A75.
14. Pani SP, Yuvaraj J, Vanamail P, Dhanda V, Michael E, Grenfell BT, Bundy DA, 1995. Episodic adenolymphangitis and lymphoedema in patients with bancroftian filariasis. *Trans R Soc Trop Med Hyg* 89: 72–74.