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LONGEVITY OF *WUCHERERIA BANCROFTI* VAR. *PACIFICA* AND MOSQUITO INFECTION ACQUIRED FROM A PATIENT WITH LOW LEVEL PARASITEMIA

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Abstract. Two cases are reported which illustrate important epidemiological aspects of lymphatic filariasis—prolonged longevity of the adult parasite and the possibility of transmission by individuals with ultra-low level microfilaremia. These cases demonstrate that people can remain carriers of microfilariae in the peripheral blood for many years without reinfection, and even those with a low level microfilaremia can constitute a significant reservoir of mosquito infection. Such cases represent one of the most serious obstacles to the eradication of lymphatic filariasis in regions where control is based on chemotherapy.

The duration of filarial fecundity and the minimum microfilarial density necessary for transmission of the disease are two epidemiological factors which are extremely important in the battle against lymphatic filariasis. In many areas the fight against this infection is based on mass chemotherapy.[1] In the absence of satisfactory drugs capable of destroying the adult worm without adverse effects to the patient it is necessary to reduce in a longlasting way the level of microfilaremia. However, microfilaricidal treatment has only a temporary action.

The maximum length of time classically given for the persistence of microfilariae in man is 10 years. Even when microfilarial densities are very low such persons still constitute a parasite reservoir which should not be underestimated. Indeed, such carriers of microfilariae seem to play a significant role in transmission,[2,3] and represent a serious obstacle to the eradication of filariasis.[4]

We report here two observations concerning *Wuchereria bancrofti* var. *pacifica* which illustrate this problem, the first showing greater parasite longevity than has been previously cited, the second demonstrating the possibility of disease transmission by subjects with very low level microfilaremia.

CASE REPORTS

Case 1

This patient, a female Tahitian of European origin born in 1900, presented with a first crisis of lymphangitis of the right leg at the age of 18 years. One year later involvement of the left lower limb occurred. Subsequently, these crises appeared approximately once every 2 years until, at the age of 35, the patient left Polynesia for France where they ceased.

From 1935–1975 the patient remained in France, her only trip abroad being an 8-month visit to Djibouti in 1937. On 5 July 1975 she returned to Tahiti and was seen in consultation on 24 July for regular follow-up of diabetes. Because of the past history of filariasis, six thick smears of 20 mm^3 of capillary blood were examined. They were negative, but concentration of 5 ml of venous blood according to the method of Ho Thi Sang and Petithory[5] showed two living *W. bancrofti* microfilariae.

Case 2

This patient, a 32-year-old Frenchman, arrived in Tahiti in 1972. This was his first trip abroad. At the beginning of January 1975 he presented with adenitis of the left axilla followed 3 days later by a lymphangitis of the left arm with the subsequent development of an abscess on the inner surface of the forearm which disappeared after incision. Repeated tests for microfilariae, consisting of five thick smears of 20 mm^3 of capillary blood, filtration of 1 ml of venous blood on millepore membranes, and concentration of 5 ml of venous blood, were all negative. Examination of pus collected from the abscess showed no filarial debris. Eosinophilia was 220/mm^3 (4% of 5,500 white blood cells). Passive hemagglutination, using a protein extract of *Dirofilaria*

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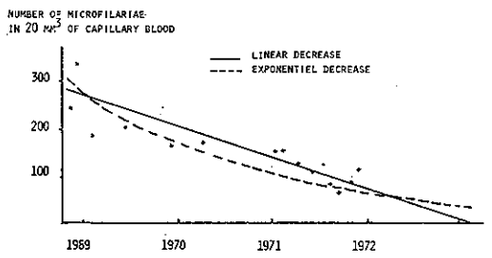

FIGURE 1. Decrease in microfilaremia in a subject living outside an endemic filarial zone. (After Brengues.[15])

immitis as antigen,[6] revealed a titer of 1:64. This was considered to be significant and led to the performance of "xenodiagnosis," using an original method which consisted of allowing non-parasitized mosquito vectors (100 laboratory-raised *Aedes polynesiensis*) to feed on the subject. Fifteen days later 75 mosquitoes were dissected and five infective larvae of *W. bancrofti* were found in one of them.

DISCUSSION

With respect to case 1, the possibility of passive transmission of microfilariae can be ruled out since she had not received any blood transfusions. The possibility of infection since her return to Tahiti is extremely unlikely since microfilariae were discovered 19 days after her arrival and this length of time is much too short for the development of microfilaremia.[7] Furthermore, the patient was staying in Papeete, an urban and administrative center of French Polynesia where filarial transmission is much reduced. The patient had visited Djibouti in 1937; however, this area has never been cited as an endemic zone of lymphatic filariasis.[8] Thus we can only conclude that the microfilaremia persisted for 40 years, a time greatly in excess of that which has been reported in the literature. Bancroft in 1879,[9] and Guptavanij and Harinasuta in 1971,[10] reported the period of longevity of *W. bancrofti* as 5 years. Slightly longer periods have been reported: 6 years by Jachowski et al.,[11] 7.5 years by Conn and Greenslit,[12] 8 years by Leeuwtin[13] and by Mahoney and Aiu.[14] In all these studies examination of the blood was carried out on one or two thick blood smears. It is possible that a longer period of fecundity would have been found if concentration methods had been used .

In order to illustrate this, we should like to cite the work of Brengues,[15] who observed the decrease of microfilaremia (*W. bancrofti*) in a carrier with a high level of microfilaremia who had left an endemic area. Estimation of the linear decrease showed negative microfilaremia 4.5 years after the beginning of the study. However, the observed evolution more closely followed an exponential curve (Fig. 1). Using this theoretical model, one can calculate that in Brengues' patient the time necessary to observe a density of two microfilariae, the number found in Case 1, in 5 ml of blood would be 32.3 years, a period of time similar to the 40 years observed in our patient.

The longevity of the adult filarial worm has been estimated by reference to epidemiological data or anatomical discovery of the adult worms, for example, during surgery. Figures given in the literature range from 10 years[16] to 17 years.[17]

In Case 2, although the most sensitive concentration methods were used microfilariae were not demonstrated; in spite of this the patient was potentially infective. We do not suggest that the method of xenodiagnosis described should be used routinely to demonstrate patients' infectivity. However, such tests, which cause no danger to the patient since the mosquitoes are uninfected, allow the study of the dynamics of mosquito infections with blood from humans with low level microfilaremia. Investigation of this aspect of filarial infection is important since in certain endemic regions of lymphatic filariasis where transmission is assured by *Aedes* there is an increase in parasite yield when the numbers of microfilariae present in man are low.[18] Furthermore, it seems that mosquitoes which feed on subjects with low level microfilaremia are capable of concentrating the microfilariae.[18,19] In our case, the volume of blood was about 225 mm^3 (each bite about 3 mm^3/75 mosquitoes dissected) for the mosquito feed which produced five microfilariae, and 1,000 or 5,000 mm^3 for concentration methods.

From the study of our two cases we can conclude that: 1) in the absence of reinfection a person can remain a carrier of microfilariae for 40 years; and 2) even with a very low level microfilaremia a person can be an efficient parasite

reservoir for the transmission of lymphatic filariasis. These two factors represent a notable obstacle in the eradication of this disease in French Polynesia, where eradication methods are based on mass chemotherapy with diethylcarbamazine which is not active against the adult worms at the doses utilized. The fact that the eradication of lymphatic filariasis in this region has not been successful even after 25 years of effort[20, 21] does not therefore necessarily imply that this is due to inefficiency in the application of treatment.

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EXPERIMENTAL TRANSMISSION OF *WUCHERERIA BANCROFTI* TO MONKEYS*

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Abstract. Infective larvae of *Wuchereria bancrofti* from laboratory-raised *Culex pipiens fatigans* and *Aedes togoi* mosquitoes fed on human volunteers in Jakarta, Indonesia (J strain) and Kinmen Island, China (K strain) were introduced into Taiwan monkeys (*Macaca cyclopis*) by subcutaneous inoculation, by foot puncture, or by permitting infected mosquitoes to feed weekly on the monkeys. Some animals were splenectomized and others were treated with varying regimens of immunosuppressants. Necropsy was done on monkeys that died or were killed and the entire bodies were examined for worms. A total of 78 monkeys (43 males and 35 females) were exposed to infection and parasites were found in 29% of the females and 63% of males. In infections of 38 days or less worms were recovered from the testes of males and the pelt, carcass and lymph nodes of both sexes, but after 42 days of infection most worms were in the testes of males, and a few were recovered from lymph nodes and carcasses of females. Worms recovered at 8–11 days were third-stage, those found between 14 and 38 days fourth-stage, and ones found between 42 and 103 days were young adults. After 148 days most were adults and microfilariae were seen in the uteri of female worms at 160 days and later. The parasites continued to grow in size with time. Microfilariae were detected in the blood of nine monkeys between 8 and 18 months and the patent period varied from 5–21 months. Microfilarial densities were low and erratic, and periodicity could not be determined. The effectiveness of methods of administering infections and the value of various treatment regimens seem uncertain; monkey antilymphocytic sera, however, appeared to have some influence. Parasites were found in 36% of the Taiwan monkeys given the J strain and 54% of those given the K strain. A limited number of *M. mulatta* (3), *M. irus* (*fascicularis*) (3) and *Aotus trivirgatus* (4) were also given infective larvae and adult *W. bancrofti* were recovered from the testes of one male *M. mulatta* and one male *M. irus*; uterine microfilariae were found in one female worm from the latter monkey. *A. trivirgatus* were negative. Low numbers of infective larvae recovered from mosquitoes fed on patent monkeys were introduced intermittently into seven clean monkeys and one became microfilaremic between 11 and 17 months postinoculation.

Although many attempts have been made to establish *Wuchereria bancrofti* in laboratory animals[1] it has only been in recent years that limited success has been obtained. In 1965 Dissanaike and Niles reported the recovery of a third-stage larva from a cat 20 days after infection with a periodic strain of *W. bancrofti* from Ceylon,[2] and in a series of studies from 1966–1970, Ramachandran reported the recovery of developing larvae from cats 7–84 days after infection with the Malayan rural strain of the parasite.[3–6] Using the Samoan subperiodic strain, Ash and Schacher demonstrated that *W. bancrofti* was able to com-

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