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REVIEW

Bancroftian filariasis: new understanding and strategies for control

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Lymphatic filariasis affects more than 120 million people in tropical countries and is considered the second most widespread cause of physical and social handicap. The development of both new, sensitive tools for monitoring filarial parasitism in communities, and of simple chemotherapy strategies raises the hopes of public health professionals for the elimination of this parasitic disease.

The parasitic disease lymphatic filariasis remains a major cause of social handicap and economic burden in 73 countries of the tropical and subtropical world, where at least 120 million people are parasitized [1]. Globally, more than 20% of the world population live in areas where they are at risk of infection. Lymphatic filariasis is primarily due to the filarial worm *Wuchereria bancrofti* (90% of infections, bancroftian filariasis), distributed throughout tropical areas, although one-third of the bancroftian filariasis cases occur in India and most of the remainder, in southeast Asia, Asia and the Pacific. *Brugia malayi* and *Brugia timori*, responsible for the remaining 10% of lymphatic filarial infections (brugian filariasis) are restricted to Asia (mainly China and India). Lymphatic filariasis affects people of both sexes and all ages, most of them living in poor conditions. Because *W. bancrofti* is transmitted in most endemic areas by the urban mosquito *Culex quinquefasciatus*, the prevalence of parasitism is increasing with the uncontrolled growth of tropical cities, creating numerous larval breeding sites for the vector.

The understanding and therefore the control of lymphatic filariasis have suffered for a long time from lack of research and funding. However, during the last decade, significant research advances have led to a better understanding of the severity and impact of the disease and to the development of efficient tools for diagnosis (in particular for bancroftian filariasis) and strategies of treatment. The development of feasible control measures, which can be applied on a community basis, the successes of recent programmes and increasing political commitment led the 50th World Health Assembly in May 1997 to pass a resolution identifying as a priority "the elimination of lymphatic filariasis as a public health problem" [2].

In this paper the most significant advances in research and control of bancroftian filariasis are reviewed, with particular reference to the contribution of the Institut Malardé, based in Tahiti, French Polynesia, where bancroftian filariasis is endemic.

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Aspects of the *Wuchereria bancrofti* life cycle

Cycle

Man is the only definitive natural host for *W. bancrofti*. Infection occurs during blood meal of a female mosquito when infective, L3 larvae, released from the mosquito proboscis, actively penetrate the mosquito-generated wound in the skin. L3 reach the subcutaneous tissues at the site of puncture and migrate towards the lymphatic channel by unknown mechanisms. Molting from L3 to L4 takes approximately 2 weeks. Differentiation to adults (males or females, 6-10 cm in length) takes several months, and the adult worms are estimated to persist for 4-6 years in the network of lymphatic nodes and vessels. The biology of *W. bancrofti* adult worms has been unknown for a long time due to their profound localization in the lymphatic system in humans and to the lack of a proper animal model for studying the different steps of the parasite/host interactions. Recent application of ultrasound technology to filarial patients in Brazil has enabled the visualization and localization of worms *in vivo* and the direct observation of the effect of drugs on their motility and/or survival [**3**].

After fecundation, adult females produce millions of immature microfilariae (Mf) (250-300 µm in length) that migrate to the blood, where they are picked up by mosquitoes. Microfilarial density in human blood may vary considerably between individuals, reaching more than 10,000 Mf/ml in some individuals.

The larval development in the mosquito lasts 10-14 days, according to temperature [**4**]. Only a proportion of ingested Mf cross the gut barrier, migrate to the thoracic muscles, where they molt twice until attaining the infective larva stage (L3 stage), and then reach the head, from where they are transmitted to the human during another blood meal.

Vectors

The urban mosquito *C. quinquefasciatus* is the vector of *W. bancrofti* in most endemic areas such as the Indian subcontinent, east Africa, Brazil and the Caribbean. However, locally, several other mosquito species can be vectors, e.g. *Anopheles punctulatus* and *A. koliensis* in Papua New Guinea [**5**], *A. gambiae* and *A. funestus* in most of the African continent or several *Aedes* species in the Pacific, leading to different epidemiological situations [**6**]. In French Polynesia, the main vector is *Aedes polynesiensis,* a rural mosquito [**7**]. *B. malayi* is mainly transmitted by *Mansonia* spp.

Periodicity

The number of Mf in the peripheral blood of an individual is a function of time of the day, and the most prevalent forms of *W. bancrofti* show a nocturnal periodicity, with significantly more Mf in peripheral blood during the night than during the day. There seems to be a correlation between the periodicity of the Mf and the feeding behaviour of the mosquito species, as most of these *W. bancrofti* forms are transmitted by the nocturnal mosquito *C. quinquefasciatus*. In contrast, in the Pacific (including Polynesia), where the vectors are daytime feeder *Aedes* mosquitoes, the Mf appears in the peripheral blood essentially during the day [**8**]. Despite the correlation between vector feeding behaviour and parasite periodicity, the reasons for that periodicity remain unclear.

CFA = circulating filarial antigen.
DEC = diethylcarbamazine.
ELISA = enzyme-linked immunosorbent assay.
L3 = third-stage larva.
mAb = monoclonal antibody.
Mf = microfilariae.
PCR = polymerase chain reaction.

Reassessing the clinical, social and economic incidences of parasitism

A considerable degree of concern has been raised about the social and economic impact of filariasis. In the 1995 World Health Report, lymphatic filariasis was identified as the second cause of permanent and long-term disability in the world [**9**]. About 44 million people suffer from visible manifestations of the disease. Adenolymphangitis is the common symptom of acute filariasis, with inflammation of lymph nodes and vessels, accompanied by fever episodes; the economic loss estimated for each patient is 30 days of work per year. About 16.2 million people suffer from lymphoedema and 15 million, elephantiasis of the limbs. Hydrocoele and scrotal pathology affect 27 million men. This profoundly disabling disease causes serious physical handicaps, inducing loss of work productivity and sometimes loss of employment. For example, weavers afflicted by filariasis had a loss of productivity of 27% compared with healthy individuals in southern India [**10**]. The physical handicaps, in particular hydrocoele or genital abnormalities in men, and lymphoedema of breasts or the genitals in women, in addition to the sexual dysfunction that they usually generate, induce serious psychological troubles and social consequences, including exclusion [**11**].

About 76 million people harbour circulating Mf without externally recognizable symptoms [**1**]. Until recently, these people were considered "asymptomatic". However, most of them suffer hidden damage to the lymphatics of the limbs, as observed by lymphoscintigraphy [**12**], or to the kidneys [**13**].

Moreover, the extent of endemicity is underestimated, in particular in Africa, where little data are available except from east Africa. Recent surveys in Ghana indicate that 41% of the population (>10 years old) harbour *W. bancrofti* Mf and that 3% suffer chronic filariasis [**14**]. It is likely that other foci exist on that continent. Furthermore, the estimation of filariasis endemicity is based on records concerning Mf carriers and diseased people. New diagnostic tools for detecting the presence of adult worms indicate that the records on parasitism are largely underestimated.

Tools for the diagnosis and monitoring of the epidemiological situation

Detecting the parasite in humans with community-based diagnostic tools

Microfilariae. Earlier, diagnosis of filarial parasites relied on sampling blood from humans and identifying the microfilariae under the microscope. This is a rather cumbersome technique, as in most areas, blood needs to be sampled at night (due to periodicity), a drawback which renders both patients and health workers very reluctant. In addition, the sensitivity of the diagnosis depends on the volume of blood sampled, and it was shown that analysing a drop of blood (20-60 µl) collected from the finger by finger-prick test underestimates the prevalence of microfilaraemia in comparison with the membrane filtration assay performed on 1 ml venous blood [**15, 16**]. There was therefore a need for more convenient and sensitive diagnostic tools for monitoring *W. bancrofti* parasitism.

Circulating filarial antigens as a marker of adult worm infection. A major breakthrough in the diagnosis of *W. bancrofti* was the development of two monoclonal antibodies (mAb) — AD12 and Og4C3 — which recognize circulating filarial antigen(s) (CFA) [**17, 18**]. They can be used in enzyme-linked immunosorbent assays (ELISAs) on blood from finger-prick blood samples (or venous blood samples) collected at any time of the day. Unfortunately, no similar test exists yet for *B. malayi.* It has been shown, by indirect evidence [**19**] or recently in laboratory infected monkeys [**20**], that the antigen(s) are of adult worm origin. An ELISA kit, using Og4C3 mAb, has been commercially available for a few years ("Trop-Ag *W. bancrofti* ELISA kit", JCU Tropical Biotechnology Pty. Ltd., Queensland, Australia); it is practical and can be used in most endemic countries, in a central laboratory. In addition, individual test cards using coated mAb have been developed for direct assessment of CFA in the field (ICT Diagnostics, Balgowlah, Australia), and are being evaluated. Both mAbs have been extensively studied, in ELISAs, in most endemic areas and present similar characteristics as diagnostic tools (reviewed in [**21**]). CFAs

can be detected in the blood at any time of the day, thus avoiding cumbersome night blood sampling. They are specific to *W. bancrofti* in humans. CFA assays detect most of the Mf carriers among those tested (because they harbour adult worms) and, in addition, a large proportion (18-40%) of individuals among those who are amicrofilaraemic, thus likely harbouring adult worms [**19, 22-25**]. Therefore, 3 groups of individuals can be distinguished in a *W. bancrofti* endemic population, regarding parasitism status (see fig. 1): i) Mf (and adult worm) carriers, contributing to the transmission of *W. bancrofti,* ii) Mf-negative but CFA-positive individuals, presumed to harbour adult worms only, and iii) individuals who are Mf- and CFA-negative, presumed to be free of parasite. Globally, the CFA prevalence in endemic populations is approximately twice the Mf prevalence. The reasons why individuals harbour only adult worms are unknown (Mf charge below the detection limit, lack of fecundity of the adult female, host immunity against Mf). Nevertheless, these individuals should be considered reservoirs and treated until worm clearance. In clinical filarial cases, CFA prevalence is also higher than Mf prevalence [**19, 21, 22**].

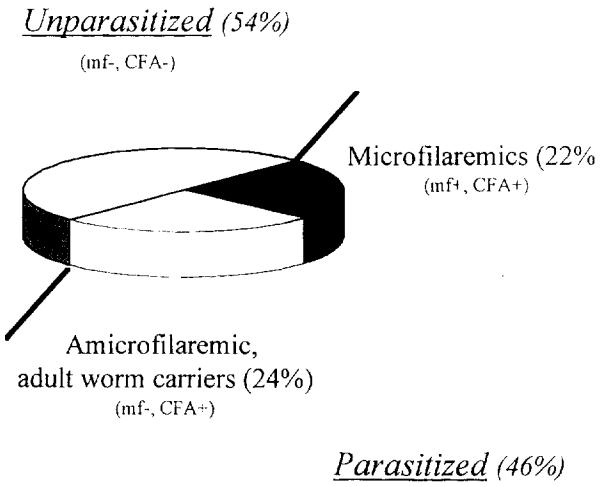

Fig. 1. Distribution of *W. bancrofti* parasitism in the population of Tahaa Island, French Polynesia.

Analysis was performed on 1,845 individuals (>20 years old) exposed to transmission (anti-filarial serology positive) and untreated for at least 10 years. Microfilaraemia was determined by membrane filtration assay on 1 ml venous blood collected during the day and CFA levels using the Og4C3-antigen ELISA.

Diagnosis by PCR. The second new diagnostic tool was the development of polymerase chain reaction (PCR) assays for the detection of *W. bancrofti* in human blood (and in mosquitoes; see later), with primers which amplifiy a DNA repeat sequence specific to *W. bancrofti* [**26**]. Several studies were done in different endemic areas for the detection of the parasite DNA in blood, but it was observed that PCR was correlated with the presence of Mf in the blood samples [**27**]. Therefore, there is still a controversy over its usefulness (regarding the cost and technicity of PCR) in areas where a single filarial species exists. However, in areas where multifilarial parasitism occurs, such as Central Africa or India, PCR on blood might be of help for identification of filarial agents because specific probes (and primers) now exist for *B. malayi* [**28**] and *Loa loa* [**29**] as well.

Monitoring *W. bancrofti* in mosquito vectors by PCR poolscreening

Monitoring the presence of *W. bancrofti* in mosquito populations is necessary for evaluating both the extent of transmission of the parasite and the efficacy of filariasis control programs. This is traditionally performed by dissection and individual microscopic observation of several hundreds of mosquitoes. Although this technique provides detailed information on the different stages of the larvae and their distribution within the mosquito body and allows the determination of long-term, established, entomological indices, it remains tedious work. Therefore, we have developed a strategy to evaluate the infection rate of a mosquito population by screening pools of vectors, instead of individuals, by PCR using *W. bancrofti*-specific primers [**26, 30**]. The PCR assay [**31**] is sensitive enough to detect the presence of as little as one parasite larva (even the smallest stage) in a pool of 50 mosquitoes. The percentage of positive individuals (infection rate) can be deduced from the percentage of pools which are

PCR-positive, using a computer program (Poolscreen Program™ [32]). An internal PCR standard has also been designed to avoid false-negative PCR results [33]. Although the *Ssp*I PCR assay is not stage-specific, it is very useful for monitoring infection rates, especially following chemotherapy campaigns, and is now currently used in chemotherapy trials in French Polynesia. In addition to the large numbers (several thousands) of mosquitoes which can be processed per day, the main advantages of the assay are that it can be used worldwide (and the standard as well) because the target DNA is ubiquitous to all *W. bancrofti* geographical isolates so far tested [26], and that it has been applied to the main vector species [31, 34, 35].

Control of filariasis

Drug treatments to kill the parasites

Control of *W. bancrofti* parasitism should rely on community-based distribution of filaricidal drugs, and whenever feasible, in vector control as a complementary tool. Three drugs have been shown to be safe and effective for large-scale application in filariasis control programs: diethylcarbamazine (DEC), used for 50 years [36], and recently ivermectin and albendazole. Fundamental shifts have occurred in chemotherapy strategies.

Decision-making in filariasis control programs (especially when to stop) has for a long time suffered from the lack of sensitive and convenient tools to monitor *W. bancrofti* parasitism in humans. In consequence, several programs have been stopped too early, leading to reemergence of endemicity a few years later. This happened in French Polynesia, where control programs with DEC, started in the 1950s, led to a sharp reduction of microfilarial prevalence [37]. However, ten years after interruption (in 1982), the incidence of the parasitism (microfilaraemia) reached precontrol levels (20-30% microfilaraemia in some islands), indicating that the adult worms had persisted [38].

Drug treatment should kill both microfilariae (for interrupting transmission) and adult worms to prevent reemergence of the parasitism. Treatment strategies have been simplified and now rely on ingestion of single doses of drugs once or twice a year instead of the formerly recommended 14-day course of DEC, which has no more effect than single-dose treatment (Cao *et al.*, in [39]). After its success in *Onchocerca volvulus* control in Africa, ivermectin was tried against *W. bancrofti* in French Polynesia [40] and shown to be microfilaricidal [41]. DEC and ivermectin, after ingestion, kill microfilariae within 1-4 h and keep the Mf level low until 6 months after treatment, but a major outcome was the discovery that treatment using 2 drugs is significantly more effective than single-drug treatments [42]. Indeed, chemotherapy assays carried out in French Polynesia showed that the best control is obtained by combining ivermectin and DEC in a single treatment, which maintains the Mf density <2% of the pretreatment value for at least 1 year (see fig. 2) [43]. Likewise, a study carried out in Sri Lanka showed that albendazole, a drug used against intestinal parasites, has a weak microfilaricidal effect when administrated alone but reduces the microfilaraemia by 99% for a year, when combined with ivermectin, as with ivermectin plus DEC (Ismail *et al.*, in [39]).

Field studies on the reduction (and clearance) of adult worm burden in patients are still scarce and remain an important area for future investigation. Annual treatments with DEC combined with ivermectin, or to a lesser extent DEC alone, reduce CFA levels significantly, while ivermectin alone has a poor effect (see fig. 2) [25, 43]. However, the number of treatments required to clear CFA depends on the initial CFA level [25, 44]. Community treatment of villagers with a combination of ivermectin plus DEC also has a better effect on reduction of the infectiosity of vectors, compared with results with DEC alone, as observed in Papua New Guinea (Bockarie *et al.*, in [39]). Albendazole has adulticidal effect against several nematode species, but not against *Onchocerca* [45]. Its effect against adult worms of *W. bancrofti* is under investigation.

Control programs should now be based on the treatment of the entire human population in a

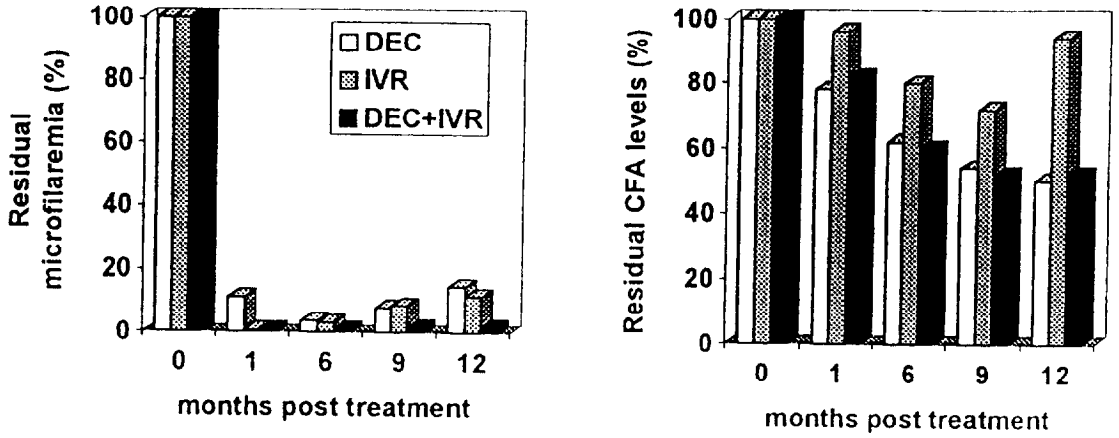

Fig. 2. Efficacy of 2-drug regimen versus 1-drug regimen annual treatment on reduction of microfilaraemia and CFA levels in microfilaraemic Polynesian individuals.

DEC=diethylcarbamazine at 6 mg/kg, IVR=ivermectin at 400 μg/kg. (Figure drawn from data of Moulia-Pelat *et al.*, 1995, ref. 43).

given endemic area (to avoid false-negative diagnosis). Mass community treatment should be applied yearly, for 4-6 years, with a combination of DEC 6 mg/kg and ivermectin 400 μg/kg, the latter also being active against other parasites such as intestinal worms, lice and scabies [**45**]. If DEC is used as the sole drug, treatment should preferentially be twice a year. Use of table/cooking salt fortified with DEC (0.2-0.4% w/w) has been tried successfully to interrupt transmission in several areas; however, this strategy implies the replacement of "normal" salt by DEC salt on the scale of the whole population [**46, 47**]). In areas where *W. bancrofti* coexists with *Onchocerca* and *Loa loa* (sub-Saharan Africa), DEC should be avoided, since it can induce severe adverse reactions, and therefore annual treatments of ivermectin (400 μg/kg) (or 200 μg/kg if associated with albendazole 400 mg) are recommended [**2**].

Reducing the disease

Severe clinical symptoms such as elephantiasis, lymphoedema and acute adenolymphangitis reflect in many situations the very poor hygiene conditions and the social isolation of patients. Drug treatments that kill the parasite have no direct effect on the clinical symptoms, but avoid further exacerbation by the worms. However, it was recently recognized that bacterial and fungal superinfections exacerbated the majority of adenolymphangitis episodes and that prevention of infection through simple local hygiene measures and antibiotherapy could decrease these damaging episodes.

Vector control

Parasitism and disease are related to intensity of transmission by the vectors [**48**]. Therefore, reduction of vector contact plays a significant role in interruption of transmission and should be implemented as a complementary tool to drug distribution. Practical approaches largely depend upon the vector biology. Populations of the main vector, *C. quinquefasciatus,* can be reduced by larvicides using the biological control agent *Bacillus sphaericus,* polystyrene beads or other chemicals, or by insecticide-impregnated mosquito bed-nets [**49, 50**]. In contrast, other vectors such as *A. polynesiensis* are

very difficult to control due to their widespread habitats.

Implementing control programs

The dramatic advances in chemotherapy and diagnostic tools (in particular CFA monitoring and analysis of vector infection by PCR) have considerably simplified the filariasis control programs and their chances of success (see fig. 3). Implementation of control programs does not require an elaborate management structure, particularly if they are integrated with other components of the health care system.

Epidemiological assessment to identify communities with filarial parasitism can be based on rapid diagnostic techniques such as review of existing health reports, clinical examination of adult males for hydrocoeles, examination of mosquito infections using traditional entomological techniques or more quickly, using PCR and on evaluation of CFA levels in daytime finger-pricks taken from sampled populations.

Drugs (1 or 2 regimen) must now be distributed once a year to the entire population of the endemic area for 4-6 years (which is the estimated life span of adult worms). Monitoring parasitism could be based primarily on the use of CFA detection in humans and also on evaluation of the rate of infection of vectors, by PCR poolscreening or more traditional entomological methods. Additionally, morbidity control methods, predictive mathematical models and vector control should be included when necessary.

The basic question of decision-making to interrupt treatment depends on the public health objectives. Basically, the program should be stopped when the endemic population has been cleared of circulating filarial antigen, and mosquitoes, of parasite larvae. Nevertheless, in view of past experience in filariasis control, surveillance programs should be established for monitoring the risks of reinfestation.

Key-words: Bancroftian filariasis, *Wuchereria bancrofti*; Chemotherapy, Public health, Diagnosis, Control; Review.

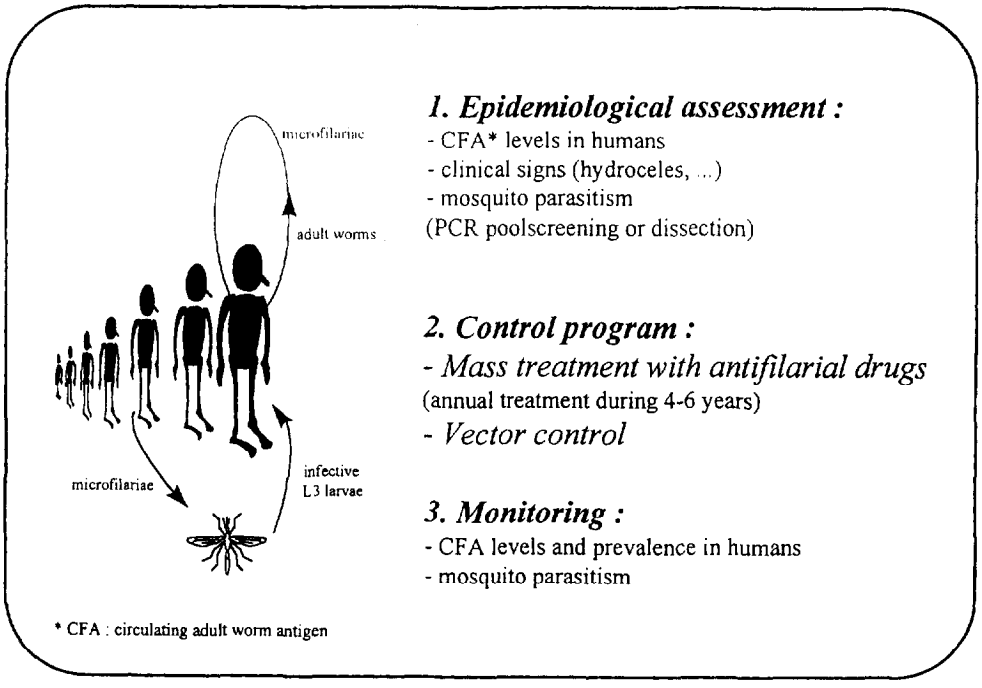

Fig. 3. Description of a bancroftian filariasis control program in an endemic area.

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Filariose de Bancroft: nouvelles stratégies de contrôle

Plus de 120 millions d'individus vivant en zone tropicale sont parasités par *Wuchereria bancrofti* et, dans une moindre mesure, par *Brugia malayi,* les deux agents de la filariose lymphatique. Alors que cette parasitose a été longtemps négligée, on reconnaît aujourd'hui qu'elle constitue la deuxième cause d'handicap physique et d'exclusion sociale dans le monde. Il y a quelques années, le diagnostic reposait sur l'observation des signes cliniques externes et sur la détection de microfilaires dans le sang, et le traitement, sur la prise répétée de diéthylcarbamazine (DEC), laquelle produisait des effets secondaires importants. Les porteurs de microfilaires, longtemps considérés «asymptomatiques», sont en fait atteints de troubles lymphatiques des membres et de dysfonctionnement rénal. Grâce à la mise au point d'anticorps monoclonaux permettant de détecter la présence d'antigènes circulants de vers adultes chez l'homme, on dispose maintenant de tests de diagnostic (ELISA ou cartes individuelles) beaucoup plus sensibles et simples d'utilisation à l'échelle de la communauté. Par ailleurs, pour évaluer la transmission, le suivi du taux de parasitisme dans les populations de moustiques vecteurs peut maintenant être réalisé par PCR (amplification enzymatique de gène) sur des lots de moustiques, grâce à des sondes d'ADN spécifiques. Bien que la DEC soit encore préconisée, le contrôle optimal repose sur la distribution, *à l'ensemble de la population,* d'une dose annuelle d'ivermectine associée à la DEC ou à l'albendazole, et cela pendant 4 à 6 ans, jusqu'à la clairance des antigènes de parasite dans la population. La lutte antivectorielle, bien qu'insuffisante à elle seule, est une approche complémentaire à la chimiothérapie.

Les progrès réalisés au cours des dix dernières années tant dans le domaine du diagnostic et de la chimiothérapie que dans la reconnaissance des graves conséquences socio-économiques de la filariose lymphatique, rendent maintenant possible l'élimination de cette parasitose, véritable problème de santé publique.

Mots-clés: Filariose de Bancroft, *Wuchereria bancrofti;* Chimiothérapie, Santé publique, Diagnostic, Contrôle; Revue.

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