Syarahan Inaugural

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Bertajuk

IMMUNODIAGNOSIS AND VACCINATION FOR BRUGIAN FILARIASIS:
Direct Rewards From Research Investments

▶ 6 June 2002

▶ Dewan Taklimat Tingkat Satu,
Bangunan Pentadbiran Universiti Putra Malaysia

PUTRA MALAYSIA

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ABSTRACT

Lymphatic filariasis is a field that has undergone a rapid expansion of knowledge, the direct reward of research investments made over the past 20 years. These advances have included the diagnosis of infection and monitoring of control efforts that have been made feasible by the development of tools based on antigen and DNA detection. The most important of the new tools for the control of lymphatic filariasis are simple, safe, inexpensive, conveniently delivered drugs that kill the microfilariae and adult worms. New diagnostic tools such as lymphoscintigraphy and ultrasonography for the examination and identification of hidden damage have also been developed that are far easier to use than anything previously. However, it is inopportune to conceive that problems of diagnosis of lymphatic filariasis as completely solved when there is still no antigen tests for the diagnosis of active or current infection with *Brugia malayi*. Brugian filariasis is zoonotic whereby man and animal reservoirs share the infection and is transmitted by the same species of mosquito vectors that are both zoophilic and anthropophylic. In endemic areas of subperiodic *B. malayi* where animal reservoir of the infection exists, filariasis control programme were adversely affected. Vaccination may form the basis of a possible future control method of public health importance in the elimination of microfilariae in animal reservoirs that would break the zoonotic transmission. Universiti Putra Malaysia in a deliberate attempt to join the international scientific community toward the control and possible eradication of lymphatic filariasis has embarked on research into the development of antifilarial nucleic acid vaccine i.e. to genetically engineered vaccines that can block zoonotic transmission of brugian filariasis. Filaricidal drugs unfortunately has no effect on the main pathological consequence of repeated infection, namely elephantiasis, for which in fact no drug treatment has any effect except possibly steroids in early cases. At Universiti Putra Malaysia, small scale preliminary studies into herbal remedy for chronic elephantiasis has been conducted and has shown encouraging results. Such herbal remedy undoubtedly will be of great service in restoring dignity, respect and health to the patients. Investment into this new research paradigm is therefore highly recommended. All these are exciting research findings but still more newer important scientific discoveries are still yet to come which will have far reaching implications for the future control of lymphatic filariasis and potentially its eradication.

INTRODUCTION

Lymphatic filariasis (elephantiasis) is one of the most debilitating and disfiguring of all diseases. One of the most prevalent of tropical diseases, it is also among the most neglected. It afflicts poor people in both urban and rural areas. Rarely fatal, it causes extensive disability, gross disfigurement and untold suffering for millions: young and old; man, women and children. In every community where it occurs, this disease remains a strong impediment to socioeconomic development.

Lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia malayi* remain a major public health problem in many tropical countries. More than 1.1 billion people i.e. 20 % of the world's population live in areas where they are at risk of infection, of which 90 % of the infections are with *W. bancrofti* and 10 % with *B. malayi*. A minimum of 120 million people in endemic countries worldwide is estimated to be infected (WHO, 1998). It is currently estimated that some 512 million people are at risk of infection in the sub-saharan Africa. The species *B. malayi* however is predominantly found in the Southeast Asian countries. Most control programme for lymphatic filariasis include mosquito control measures, diagnostic screening and drug therapy for suppression of microfilaremia.

The reference test, microscopy detection of circulating microfilariae, is insensitive and thus newer diagnostic methods have been developed, i.e., polymerase chain reaction (PCR) - based assays (Lizotte *et al.*, 1994; Wan Omar, A. *et al.* 1999) and detection of antifilarial antibodies and filarial antigenemia (Haarbrink *et. al.*, 1995, Wan Omar, A *et al.*, 1994; Wan Omar, A *et al.*, 2000; Wan Omar, A *et al.*, 2001). Immunodiagnostic assays are used to detect active infection, to replace the cumbersome night-blood examination, to distinguish filarial from non-filarial adenolymphangitis, to identify species of parasites in the mosquito vectors and to quantify worm burdens in infected persons. Serological tests that would permit the identification of microfilaremic individuals within populations in endemic areas, those tests that do not require night blood collection, tests that would detect all individuals with current active infections, or tests that could be used to quantify adult worm burdens following chemotheraphy would greatly facilitate filariasis surveys and constitute invaluable tools to monitor the impact of control programme. Some of the factors that have hampered the development of new serodiagnostic tests have now been partially overcome. The scarcity of parasite materials from species that infect humans has been alleviated somewhat by the ability to maintain complete life-cycles of several *Brugia* species in small rodents and, more recently, by the development of genomic and cDNA libraries from different stages of several filarial species that parasitize humans. A number of recombinant filarial antigens have now become available for testing. The specificity of newer immunodiagnostic assays for lymphatic filariasis has been substantially improved. However, it is inopportune to conceive of the problems of diagnosis of lymphatic filariasis as completely solved when there is still no equivalent antigen test for *B. malayi* active infection.

In lymphatic filariasis, it is generally hypothesized that in an endemic area the endemic normals, which are asymptomatic and amicrofilaremic, will include not only a proportion of individuals harbouring subclinical infections but also individuals with true protective immunity. It is perhaps less widely appreciated that possibly those with high microfilaria

loads may also possess effective immune response that protects them from superinfection in the face of continuing transmission of infective larvae. Protective immunity may be induced to antigens (on infective larval stages) that are not involved in the development of immunopathology, thus making the development of safe (i.e. non-pathogenic) vaccines a more feasible prospect. Attempts to confirm through newer powerful techniques of molecular immunology and molecular biology the protective potential of these antigens in animal models have started at Universiti Putra Malaysia. All these features have very significant implications for the potential for success of vaccine development efforts that may be subsequently of use in the control of the disease.

The critical components, from the perspective of the focus of this lecture are advances in the immunodiagnostics and the prospect of obliteration of zoonotic transmission of brugian filariasis through vaccination leading to better control and possibly elimination of lymphatic filariasis in Malaysia. All the advances and improvements made in the immunodiagnostics and vaccine development in lymphatic filariasis are truly direct rewards from research investments made in the past twenty years. Future research direction shall be highlighted focusing on the improvement of a rapid dipstick antigen test for brugian filariasis and the development of anti-*Brugia* genetic vaccine. Research into herbal remedy for the treatment of chronic elephantiasis by Universiti Putra Malaysia shall be addressed in brief.

LIFE CYCLE OF *W. BANCROFTI* AND *B. MALAYI*

The sheathed microfilariae circulate in the blood stream and in most parts of the world a marked nocturnal periodicity; they are found in the peripheral circulation from 22.00 to 02.00 hours. During the day these microfilariae hide in the lungs. Investigations on the mechanisms of microfilarial periodicity have shown that during the day, in the periodic form, the microfilariae are held-up in the small blood vessels of the lung, principly because of the difference in oxygen tension between the arterial and venous capillaries in the lungs. They can be stimulated to appear in the peripheral blood by giving a provocative dose of diethylcarbamazine citrate or by changing oxygen tensions in the lungs. Presumably the peripheral circulation represents an unfavourable environment and the microfilariae remain there for the minimum time necessary to maintain transmission.

When the microfilariae are ingested **(Fig. 1)** with a blood meal by a suitable species of mosquito they loose their sheath within 15 - 30 minutes in the stomach of the insect. A proportion manage to penetrate thestomach wall before the formation of a peritropic membrane and migrate to the thoracic muscles in 1 - 24 hours. Two days later they have metamorphosed into sausage shaped larvae, measuring 150 x 10 um. At the end of a week the alimentary canal is developed and the larvae then measure 250 x 25 um. During the second week the larvae grow much longer (1.2 - 1.6 mm) and finally migrate to the head where they enter tha labium and emerge through the tips of the labella while the mosquito is feeding. The infective third-stage larvae can be recognized, if the mosquito is dissected in a drop of saline, by the three subterminal papillae. The parasite cannot be transmitted from man to man or to animal and vice versa until the larvae have undergone this essential development to the infective stage in the mosquito.

Figure 1 :   Life cycle of *Wuchereria bancrofti* and *Brugia malayi*
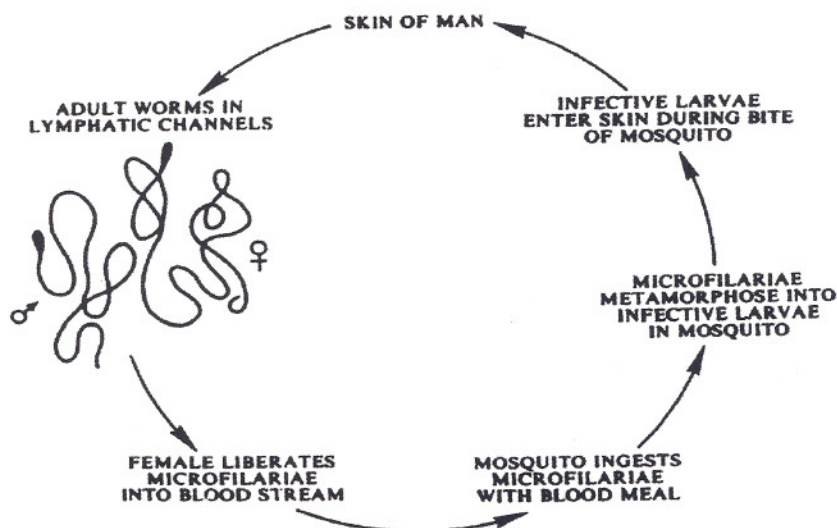
Optimal conditions for filarial development in mosquitoes are about 26 °C and over 90 % relative humidity. Many mosquitoes are killed by heavy infections and larvae sometimes fail to mature, so that the infection rate in the mosquitoes isusually low (below 0.5 %) though it can be high under some conditions.

Development in the mosquitoes takes at least 10 days; the infective larvae escape through the labella when the insect bites a new individual and enter the skin through the puncture wound. This is likely to be more successful in areas of high humidity where the skin will be moist, although the mosquito also secretes a drop of fluid on the skin before feeding. After entering the skin of man, the larvae migrate to the lymph glands where they moult twice, mature, and the female produce microfilariae in the blood in about a year or 82 days at the earliest. The adults may also be found in dilated lymphatic vessels, distal to lymphatic obstruction anywhere in the body. They can live for up to 17 years.

CLINICAL SPECTRUM OF BRUGIAN AND BANCROFTIAN FILARIASIS

The hallmark feature of lymphatic filariasis is the broad spectrum of disease manifestations occurring in individuals living together in an endemic area. Several reviews (Sasa, 1976;

Ottesen, 1980) have each divided this spectrum into five distinct syndromes: 1) asymptomatic with no detectable microfilaremia living in an endemic area and probably exposed; 2) asymptomatic and microfilaremic; 3) symptomatic, recurring short duration filarial fevers with lymphadenitis and / or lymphangitis, amicrofilaremic or microfilaremic; 4) symptomatic, lymphoedema (elephantiasis), usually amicrofilaremic and with previous episodes of filarial fevers; and 5) symptomatic, amicrofilaremic tropical pulmonary eosinophilia (TPE). In each of these syndromes the mechanisms of pathogenesis or resistance to lymphatic and pulmonary pathology remain unknown. It is obvious that these varieties of host responses offers the potential solutions to the problem of alleviating the suffering of individuals afflicted with the symptomatic infections.

Infection is transmitted during the bite of an infective mosquito. During the process of mosquito feeding, infective larvae (L3) are deposited on the skin surface near the site of the puncture wound. L3 actively migrate into the puncture wound and then into the lymphatic system. A successful uptake of this L3 will result in infection. The clinical manifestations of brugian filariasis are usually more distinct than those of bancroftian filariasis. Lymphadenitis occurs most frequently in the inguinal region, generally affecting one superficial node at a time. The attack seem to occur episodically, and is often said to be precipitated by hard labor in the field. The patient may be unable to work for several days but may remain ambulatory. Lymphadenitis usually resolves spontaneously without treatment. Sometimes, lymphadenitis is followed by a characteristic retrograde lymphangitis, although on rare occasions the infection has been observed to progress centripetally. The infected lymph vessel appears as a red streak, feel cord like and is often painful on palpation. The infection may spread to the surrounding tissues, producing cellulites, which may affect the whole thigh or even the entire limb. At this stage, the patient is usually bedridden with constitutional symptoms, and there is frequently slight lymphoedema of the foot and ankle. If the infected lymph node becomes an abscess, it may suppurate to form an ulcer. Constitutional symptoms usually resolve by rapid lysis or crisis once the abscess suppurates. Typically, the ulcer is relatively clean, in contrasts to those caused by bacterial infection, and heals spontaneously within a few days. The resulting scar tissues are closely related to the severity of infection of the affected node and the time between ulceration and examination.

The frequencies of episodic lymphadenitis vary from once to twice per year to several attacks per month. Occasionally, person spontaneously ceases to experience episodic lymphadenitis, in spite of residing in an endemic village. Lymphoedema is frequently observed during the course of adenolymphangitis, but it usually subsides completely after an acute attack.

With time the resolution of lymphoedema after each attack becomes less complete, and the chronic stage gradually develops. In brugian filariasis the leg below the knee is more frequently affected **(Fig. 2)**. Less frequently lymphoedema develops in the arm below the elbow. The skin of the affected extremity may vary from the normal texture with pitting oedema to a thickened state with little or no pitting oedema, or it may appear varicose. In most patients only the foot and the distal third of the leg are involved. The affected leg is usually less than twice its original size. Typically the swelling does not progress beyond the knee, and the normal contour of the affected knee is more or less preserved. Genital

involvement like hydrocoele and chyluria has not been reported except where brugian and bancroftian filariasis coexist.

Figure 2 :   In brugian filariasis the leg below the knee is more frequently affected.

In bancroftian filariasis, the male genitalia are most often affected during the acute stage, leading to funiculitis, epididymitis or orchitis. The cardinal features of infection are swelling, tenderness and pain, which is sometimes excruciating. After an acute episode of orchitis, the fluid in the tunica usually disappears completely, but after repeated attacks, resolution become less and less complete, and hydrocoele develops. It is the commonest sign of chronic bancroftian filariasis in most parts of the world. The hydrocoele fluid is usually clear yellow, with a faint reddish tint due to contaminated red blood cells. Microfilariae are sometimes found in hydrocoele fluid, even when they are absent in the blood. Sometimes the fluid appears turbulent and milky, and in some instances the patients may also complain of chyluria.

Lymphadenitis, lymphangitis, lymphoedema and elephantiasis of the extremities are less commonly observed in bancroftian than in brugian filariasis. They affect the leg, arm, scrotum, vulva and breast, in order of frequency. In contrast to brugian elephantiasis, bancroftian elephantiasis usually extends beyond the knee or elbow, affecting the whole leg or arm. The affected leg may enlarge to three times its original size.

PATHOLOGY

The concept that the clinical manifestations of filariasis result from immune responses of filarial antigens, and not from a direct toxic effect of the worms per se has emerged from studies on the pathology in humans and animals infected with lymphatic-dwelling filariae. Adult *Brugia* and *Wuchereria* filariae usually reside in the afferent lymphatics or in the corticle sinuses of lymph nodes. They first cause a dilatation of the lymphatic vessels, followed by the hypertrophy of the vessel wall. This is caused by the proliferation of endothelial and connective tissue and is associated with the formation of polyploid protrusions into the vessel lumen. The lymph vessels appear to remain patent as long as the worms they contain remain alive (Jamal, S, 1985; Tan, T.J., 1985). However as is the case with varicose veins, the valves of dilated, tortuous lymphatics become incompetent and allow back flow of the lymph that accumulates in distal portions of the affected limb. Clinically this result in pitting lymphoedema. If the infection is eliminated at this stage by drug treatment, or if the affected individual is removed from the endemic area, both the pathological and clinical manifestations are reversible.

The death of adult worms is associated with additional pathological events (O'Connor 1932; Rogers *et al.*, 1975). An area of necrosis develops around the dead parasite, resulting both from the dissolution of the worm and from degeneration of the host cells in the inflammatory exudates. This is followed by a granulamatous reaction containing foreign body-type giant cells as well as plasma cells and eosinophils, and the deposition of collagen around the degenerating parasite whose remains often become calcified. While the afflicted lymphatic becomes obstructed during this process, lymph flow is shunted via collateral lymph vessels. Recanalization of the obstructed lymphatic often takes place as the exudative and granulomatous reactions subside (K. Nawaz, *et al.*, 1985).

All manifestations of chronic lymphatic filariasis (elephantiasis of the limbs, genitalia or breast and chyluria) have a similar pathogenesis; it is the site of the pathological changes that determines what area of the body will be affected. Likewise orchitis, epididymitis and funiculitis, often associated with bancroftian filariasis, are the direct result of reactions induced by parasites lodged in the associated lymphatics. Phlebitis and lymph thrombi often accompany lymphatic inflammation in these regions. (F. Solti *et al*, 1985).

The lymphatic pathology in brugian and bancroftian filariasis is based mainly on human biopsy and autopsy material. Studies in experimentally infected animals have yielded additional information (S. A. Hines *et al.*, 1985). Pre-adults larval stages (L3 and L4) may be as effective as adult filariae in initiating inflammatory responses that result in lymphatic damage. Local lymphatic inflammation is correlated with the release of moulting fluids by juvenile larvae as well as with the deposition of unfertilized eggs and the secretion of other unidentified materials by adult worms (Wan Omar, A *et al.*, 1989: Wan Omar, A, 1991; Wan Omar, A *et al*, 1992). Lymphatic damage is not progressive after a certain peak of reaction is reached, except in repeatedly infected animals (Rogers and Denham, 1975). This explains the differences in the course and outcome of infections among local residents of endemic areas. However, what triggers the recurrent episodes of acute lymphatic inflammation in man remains unknown.

Finally, the most severe manifestations of filariasis such as elephantiasis and TPE often are accompanied by a certain degree of immunity, as is shown by the absence of detectable microfilaremia in these conditions (Wan Omar, A *et al*, 1994). This suggests that certain manifestations of filarial disease are unfortunate by products of immune responses by which the infected host presumably attempts to rid itself of the parasite. Whether the same antigens elicit "protective" and "pathogenic" immune reactions is yet undetermined.

ZOONOTIC FILARIASIS

It is not commonly realized that zoonotic filariasis does occur in Malaysia. Indeed, as early as 1939, Poynton and Hodgkin first described *Brugia malayi* - like microfilariae in a *Macaca fascicularis* from Perak. This finding stimulated further studies on filarial parasites in wild and domestic animals in Peninsular Malaysia and on the basis of finding of *B. malayi* like microfilariae in *M. fascicularis, Presbytis melalophos* and *Ncyticebus concang*, the domestic cat and dog, the possible importance of animal reservoirs of infection was first mooted (Edeson *et al*., 1955). Since then further epidemiological and experimental findings have confirmed these early observations.

Other than *B. malayi*, other filarial parasites of animals that live in close association with man are likely candidates for zoonotic transmission. Studies have shown that *B. malayi*, the most important human filarial parasite in Malaysia exist as strains, exhibiting biological and epidemiological differences. While the main vectors of the periodic strain are *Anopheles* spp., those of the subperiodic are *Mansonia* spp. mosquitoes. The intermediate strains are transmitted by both genera of mosquitoes (Mak, 1984). It is postulated that ecological changes through its effects on vector breeding and animal host, affect and induce changes in the parasite leading to the evolution of strains in various endemic localities. Experimental studies have shown that *B. malayi* can be transmitted between man and animals (Edeson and Wharton, 1957; Dondero *et al*., 1972). In addition, epidemiological studies have shown the impact of animal reservoirs on the transmission of the disease (Mak *et al*., 1980; Mak *et al*., 1982).

B. pahangi, a common filarial parasite of wild and domestic animals in South-East Asia, has been experimentally transmitted to humans and natural human infections have been reported in South Kalimantan, Indonesia (Palmeri *et al*., 1985). Although numerous searches have been made in Malaysia for human *B. pahangi* infection, they have not been successful. The eight infections reported to have *B. pahangi* microfilaremia were not passaged to animals, and that the parasite species were not confirmed by detailed analysis of adult morphological features but identified mainly by the staining characteristics of the acid phosphatase activity of the microfilariae (Mak J.W *et al*., 1987). Nevertheless, zoonotic *B. pahangi* infection should be considered a real possibility, especially in areas where animal reservoirs are present.

Non- human primates **(Fig. 3)** and domestic cats continue to be important reservoirs of subperiodic B. malayi infection. In subperiodic *B. malayi* endemic areas where such simian reservoirs of the infection exist, filariasis control programmes were adversely affected

(Mak *et al.*, 1982). Cats and dogs are infected by both *Brugia* spp. and *Dirofilaria* spp. Cats in Malaysia are probably infected with subperiodic *B. malayi* from man and that prevalence rates in these animals reflect the endemicity of a particular area. These domestic animals are also infected with other filarial parasites and thus pose a potential as reservoirs of zoonotic filariasis.

Figure 3 : In Malaysia non - human primates continue to be an important reservoir of subperiodic brugian filariasis.

IMMUNODIAGNOSIS

Diagnosis is still dependent on the detection of microfilariae or adult worms in specimens obtained from infected individuals. The most economical method for diagnosing lymphatic filariasis is detection of microfilariae in the peripheral blood. For diagnostic screening, 20 - 60 uL of capillary (finger-prick) blood can be dried on a slide, stained with Giemsa stain, and examined under microscope. However, the technique is insensitive, especially in regions where microfilariae density is low (Turner *et al.*, 1993). Furthermore, finger-

prick blood sampling can also grossly under-estimate the true prevalence of infection as the microfilariae that are present may be lost due to difficulty with fixation during staining procedure. Biopsies of other involved tissues, such as lymph nodes may demonstrate the occurrence of developing or mature adult worms, but this approach is invasive, impractical and therefore not acceptable. Ultrasound techniques have been successfully used to visualize rapidly moving ("dancing") adult worms of W. bancrofti in the dilated scrotal lymphatics of infected individuals (Amaral *et al.*, 1994). It is the only noninvasive method that directly monitors the macrofilaricidal efficacy of antifilarial drugs (Dreyer , 1998). This technique may be used to confirm infection in clinical cases but is limited in its application by the high cost and limited availability suitable ultrasound equipment Parasitological diagnosis is inadequate in cases of light infections, early prepatent infections, chronic obstructive disease and unisexual infection where there is no production of microfilariae. From this standpoint, immunological diagnosis is more practical since the clinical spectrum of the disease is so broad and often accompanied by allergic manifestations (Wan Omar, A 1987, PhD).

Serologic diagnosis was begun in 1916 when the first report of such was published by Rodhain *et al.* (1916). Since then numerous studies have been reported. However the use of immunodiagnostic techniques has fallen into disrepute. Guest et al (1967) concluded that such tests were not satisfactory because most published accounts of other aids to diagnosis, such as skin reactions and complement-fixation tests indicated that they were not sufficiently specific and that false-positives were common. Exhaustive reviews of immunologic methods for the diagnosis of human filariasis were made by Kagan (1963) and Wan Omar, A (PhD Thesis, 1988).

The problem of specificity may be considered from various aspects. By far, the most sustaining technical problem then and even now is the cross-reactivity between filarial infections with other helminthic infections which accounted for the high statistics of false-positive reaction in epidemiological surveys. Problem of cross-reaction in immunological diagnosis between diverse helminths is amplified in tropical countries where polyparasitism is rampant. In such countries therefore, more extensive control of the specificity of immunodiagnosis is of paramount importance. Common antigens shared by many different filariae of many different genera and species, are often present in other helminths (Wan Omar & Hammerberg, 1989) thus reducing the practical applications of these tests. However, shared filarial antigens can be an advantage, since cross-reactivity has permitted the use of animal parasites as a source of antigens for sero-diagnostic tests, for example the use of *D. immitis* antigens in the diagnosis of lymphatic filariaisis and the use of *Onchocerca gutterosa* from cattle for the diagnosis of human onchocerciasis (Kagan, 1963). A positive antibody test provides evidence of exposure to filarial infection while not indicating the species of filariid involved. This is an important consideration in areas where several human filariae are simultaneously present or where filariae that are specific to animals are a source of infective larvae to man. An example of these is the prevalence of titers to dirofilarial antigens among Australian aborigines and these correlate well with the prevalence of infections among dogs in the community (Welch and Dobson, 1974)

Specificity of any immunodiagnostic test is also important for the fact that filarial infections are of varying severity, and treatment with diethylcarbamazine causes side effects that are

not justified when the filariae present are non-pathogenic (*D. perstans*). Precise diagnosis can only be established by combining several methods, which seem to place the immunological tests as screening while parasitological demonstration of microfilariae still need to be conducted for confirmatory diagnosis. Several immunological tests were applied whose results complemented each other e.g intradermal tests, complement-fixation, passive haemagglutination, double gel diffusion or fluorescent antibody merely as screening tests followed by immunoelectrophoresis for specific precipitin arcs in order to establish species identification. With indirect fluorescent antibody tests, simultaneous use of specific antigens also leads to the same result. The results were still misleading with absence of specific arcs in immunoelectrophoresis and antibody titers were similar with all antigens in indirect fluorescent antibody tests.

For bancroftian filariasis, the immunochromatographic whole blood card test (ICT) has proved to be a rapid, highly sensitive and specific diagnostic test. Following results from a large-scale multi-country trial (**http://www.who.int/tdr/research/progress9900.htm**), where the test proved excellent in seven countries (Fiji, Ghana, Haiti, Kenya, Myanmar, Papua New Guinea, Tanzania) but not in India (where one-third of the global infected population lives), the test is undergoing re-assessment in India. For Brugian filariasis, two new diagnostic tests-deoxyribonucleic acid (DNA) diagnostics using polymerase chain reaction (PCR) enzyme-linked immunosorbent assay (ELISA) or DNA detection strips, and an antibody test-are being evaluated under field conditions.

The detection of soluble parasite materials circulating in host's blood was an approach introduced in the late seventies, which has many practical advantages; the main one was its ability in diagnosing active filarial infections. This approach stem from the pioneering work of Frank (1946) that demonstrated the presence of circulating parasite antigens in the blood of infected individuals by using whole blood as an antigen in the skin test. With a monoclonal antibody- based enzyme immunoassay technique, circulating parasite antigens have been detected in filarial and dirofilarial -infected animals (Wan Omar & Hammerberg, 1989) and humans with lymphatic filaraisis (Wan Omar, A *et al*, 1994; Wan Omar, A *et al*, 1994; Wan Omar, A *et al.*, 2000). Circulating antigen is present in blood both day and night and use of antigen assay would eliminate the need for nocturnal bleeding, which is usually required in parasitological diagnostic procedure.

Much effort has been devoted to the development of tests based on the detection of filarial worm antigens in biological specimens such as sera and urine. An ELISA method to detect filarial antigens in sera and urine has been described (Zheng, H *et al.*, 1987). Methods to detect filarial antigens in sera has been described in animals with prepatent or occult infections which are undetected by conventional parasitologic tests, give a more accurate indication of active infection than traditional sero-diagnostic tests described earlier (Wan Omar, A *et al.*,1992 ; Wan Omar, A *et al.*, 1993; Wan Omar, A *et al.*, 1993). Antigen test also has the ability of an indirect measurement of parasite burdens that can be used to monitor the efficacy of control programs or therapeutic interventions.

In a control study (Wan Omar & Hammerberg, 1989) on twelve dogs experimentally infected with *B. pahangi*, antigenemia was detected 30 days after initial infection with infective larvae **(Fig. 4)**. Antigen was not detected with pre-infection sera from these dogs. Serum

antigen content was significantly correlated (r = 0.99, p< 0.0001) with the number of *D. immitis* female worms recovered from the infected dogs at necropsy **(Fig. 5)**. Antigen was also detected in urine of these dogs. The correlation of parasite antigen levels with infection intensity is potentially important because it can establish the prognosis and outcome of clinical management of infected hosts (Wan Omar, A *et al.*, 1990; Wan Omar, A *et al.*, 1992). In canine dirofilariasis, only isolating the worms from the heart and directly counting them can establish worm burden. It is obvious that this is not practical for making prognosis in live infected animals. Cats and dogs infected with Brugia spp. are permissive for L3 maturation to fecund adult worms. In addition, these mammals develop immune response and lymphatic lesions reminiscent of those observed in human infection. Development of eosinophilic leukocytosis, antigenemia and parasite-specific antibody were studied in cats experimentally infected with *B. pahangi* (Wan Omar, A *et al.*, 1990; Wan Omar, A *et al.*, 1992).

Figure 4 : The detection of circulating antigens by MABXC3-ELISA. The dotted line represents the highest 414 nm absorbance reading detected with preinfection dog sera. The lower interrupted line represents the absorbance of the control dog sera with *Toxocara* and *Ancylostoma* infection and *Toxocara in vitro* products. All the sera were tested undiluted and the profile shown here are representative of three ELISA readings of the same frozen sera; a) A group of dogs which are offsprings of *B. pahangi* infected bitch and b) A group of dogs which are offsprings of a non-infected bitch.
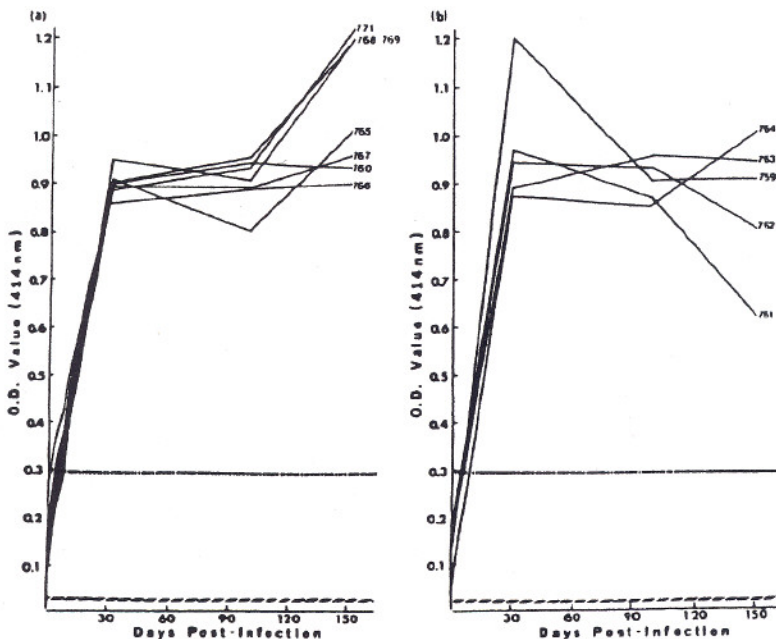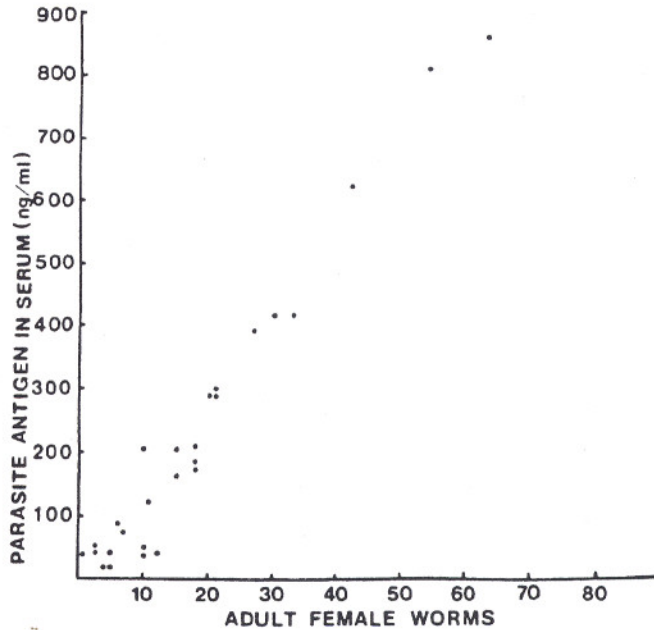
Figure 5 :   Serum parasite antigen content vs number of female *D. immitis* worms recovered at necropsy (r = 0.99, P ≤ 0. 0001).

A definite correlation between the onset of patency and increased eosinophilic leukocytosis (eosinophilia) was seen. Antigenemia was demonstrated as early as 30 days before patency. The cats demonstrating the highest anti-adult worm homogenate and anti-mf excretory-secretory titers were persistently amicrofilaraemic, and the most marked increase against all the three antigen sources upon reinfection occurred in low or amicrofilaraemic cats **(Table 1)**. These findings reconfirmed that the immunological changes demonstrated in these cats were in fact more or less similar seen in humans.

Table 1 : Parasite specific IgG titers of infected cats against three antigen preparations: *B. pahangi* adult worm homogenate (Ad. Hom), adult worm *in vitro* products (Ad. IVP)
And microfilaria *in vitro* products (Mf. IVP).

| CATS	Mf status		Ad. Hom	Ad. IV	PMf.lVP
CA	*Mf +	Pre	40,000	32,000	1,000
		Post	80,000	64,000	1,000
CB	Mf +	Pre	40,000	64,000	1,000
		Post	80,000	128,000	1,000
CC	Mf +	Pre	20,000	32,000	1,000
		Post	64,000	64,000	2,000
CD	Arnf	Pre	40,000	32,000	2,000
		Post	160,000	128,000	8,000
CE	Mf +	Pre	80,000	16,000	2,000
		Post	80,000	16,000	2,000
CF	**Amf	Pre	20,000	32,000	2,000
		Post	60,000	64,000	8,000
CG	Mf +	Pre	20,000	32,000	1,000
		Post	40,000	64,000	2,000
CH	Amf	Pre	40,000	32,000	1,000
		Post	160,000	32,000	8,000
CI	Mf +	Pre	80,000	16,000	2,000
		Post	80,000	16,000	2,000
CJ	Mf +	Pre	20,000	64,000	1,000
		Post	40,000	64,000	1,000
CK	Mf+	Pre	20,000	64,000	1,000
		Post	40,000	64,000	2,000
CF	Mf +	Pre	80,000	16,000	1,000
		Post	80,000	16,000	2,000
Controls			20,000	8.000	1.000

Mf denotes microfilaremic after post-initial infection.

** Amf denotes amicrofilaremic despite monthly repeated infection

Using a monoclonal antibody (MabXC3) based ELISA (MAbXC3-ELISA) filarial antigenemia was quantitated in various hosts including sera of Malaysian aborigines (Orang Asli) with acute lymphatic filariasis (Wan Omar, A *et al.*, 1993). In hosts infected with brugian filariasis and dirofilariasis, antigenemia quantitated range from 90 ng/ml to 960 ng/ml **(Fig. 6)**. None of the control animal and control human sera had antigenemia above the cut off value of 90 ng/ml. MAbXC3-ELISA and E-S antigens of *B. pahangi in vitro* products (B.pIVP) were also applied in several seroepidemiological surveys among household cats in Kuala Selangor (Wan Omar, A *et al*, 1992). Out of the 81 cats surveyed, 10 (12.35 %) and 5 (6.17%) were parasitologically positive for *B. pahangi* and *B. malayi* respectively. However, 21 (25.92%) were antigenemic, recording another six cats with active infections. Antifilarial

antibodies to B.p. IVP by direct ELISA showed very high cross reactivity with non-filarial gut worm infections, *Toxocara* spp. and *Ancylostoma* spp. Sixteen (19.75 %) cats had reciprocal titers ranging from 320 to 2,560. Only 1 (1.23 %) cat from this group was antigenemic.

Figure 6 :   Antigen levels in sera of animals infected with *Brugia, Dirofilaria* and non-filarial helminths (hookworm and ascarid) and sera of Orang Asli with acute lymphatic filariasis utilizing the MabXC3-based ELISA. C/B.P: cat experimentally infected with *B. pahangi*; NC/C: normal cat control; HUM/F: Orang Asli with acute lymphatic filariasis; NH/C: normal human control; G/B.P: gerbils infected with *B. pahangi*; NG/C: normal gerbil control; D/D.i: dog infected with *D. immitis*; ND/C:normal dog control; C/HEL: cats infected with hookworm and ascarid.
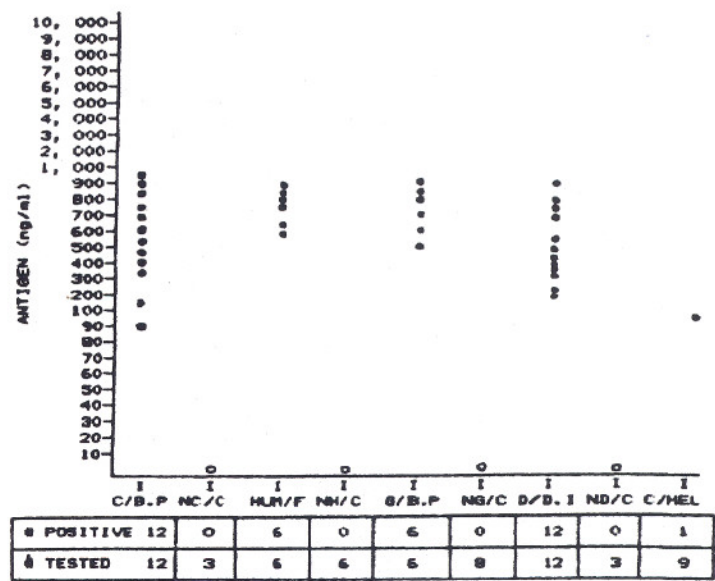
The prevalence of filarial antigenemia among local individuals (N= 990) was studied at Perak Tengah, an endemic area for brugian filariasis in Peninsular Malaysia (Wan Omar, A *et al.*, 1994). The MabXC3-based ELISA detected strongly positive antigenemia in six asymptomatic microcrofilaraemic individuals **(Fig. 7)**. Ten of the 12 sera from patients with clinical lymphangitis and lymphadenitis although amicrofilaremic were also positive. Antigen was detected in 5 of the 15 endemic normals that were asymptomatic and amicrofilaraemic; in 2 of the 6 patients with chronic elephantiasis and 3 of the 12 non-endemic normal individuals. This assay is specific since none of the patients with mix helminthic infections (ascariasis and trichurisis) was positive for filarial infection.

Figure 7 :   Scattergram of MAb.XC3 – based enzyme immunoassay of filarial antigen titers in serum samples of (A) asymptomatic microfilaremic patients; (b) amicrofilaremic patients with clinical lymphangitis and lymphoedema; (C) patients with chronic elephantiasis; (D) endemic normals; (E) non-endemic normals and (F) patients with mix helminthic infections.
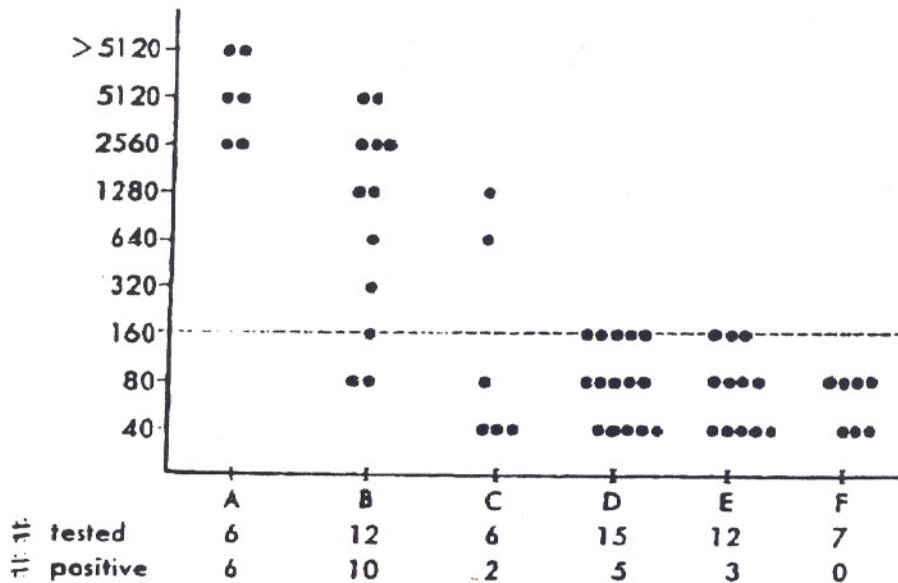
Parasitological and serological investigations for lymphatic filariasis were carried out on 450 immigrants detained at the immigration center at Semenyih, Selangor, Peninsular Malaysia (Wan Omar, A *et al.*, 2000). The countries of origin of these immigrants were Indonesia, the Philippines, Myanmar, Bangladesh, India and Pakistan. *B. malayi* adult worm homogenate (BmAH) antigen was used for the detection of antifilarial IgG and MabXC3-ELISA specific for filarial circulating antigens and non-phosphorylcholine reactive was used to detect antigenemia in these immigrants. Parasitologically 67 (14.89 %) were positive for *W. bancrofti* and 54 (12.0 %) for *B. malayi*. Serologically 63% had antifilarial IgG titre to BmAH antigen. One hundred and fifty five (34.44%) were found to be antigenemic **(Table 2)**. Antigenemic cases were high among the Indonesians and Bangladeshi. The highest titer (9600) was seen among 3 Indonesians and 2 Bangladeshi. All these immigrants originated from countries, which were endemic for lymphatic filariasis. While bancroftian filariasis is now unknown in Peninsular Malaysia, the potential of it to be reintroduced into Peninsular Malaysia by these immigrants should be of great concerned to the Malaysian government. Screening for lymphatic filariasis need to be conducted together with other communicable diseases on every immigrant entering Malaysia. If these are not quickly addressed Peninsular Malaysia will be at the risk of bancroftian filariasis transmission to the local population. In Malaysia *W. bancrofti* infection, especially in the cities has been eliminated. However, its vectors breed in abundance in the cities. With the influx of

immigrants and in relation to their occupational nature, the whole facet of bancroftian filariasis in Peninsular Malaysia may change.

Table 2 : Detection of antigenemic cases of lymphatic filariasis among illegal immigrants detained at Semenyih Immigration Detention Center.
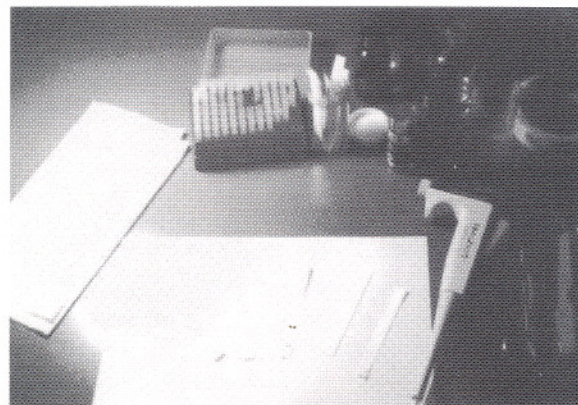
Country of Origin	No. tested	No. positive(%)	Reciprocal antigen titre					
			300	600	1200	2400	4800	9600
Indonesia	180	81 (113.0)	33	24	9	6	6	3
Phillipines	45	12 (2.67)	6	3	2	1	-	-
Myanmar	60	15 (3.33)	7	6	1	1	-	-
India	30	3 (0.66)	2	1	-	-	-	-
Bangladesh	120	42 (9.33)	15	9	7	6	3	2
Pakistan	15	2 (0.44)	1	1	-	-	-	-
Total	450	155 (34.44)	64	44	19	14	9	5

Sero-epidemiological and entomological studies into brugian filariasis were conducted at Kampung Toho, an interior aboriginal village in Negeri Sembilan (Wan Omar, A et al., 1994). A total of 150 aborigines (Orang Asli) were screened for filarial infection. Parasitologically, 2 (1.33%) were positive for *B. malayi*. Serologically 77 (51.33%) were positive for IgG antifilarial antibody against *B. malayi* adult worm homogenate. MAbXC3-ELISA detected filarial antigenemia in 25 (16.67%) individuals. It is not uncommon for antigen test to detect high positives because many would be false positives due to cross-reactivity of antibody responses to other non-filarial worm infections like ascariasis and trichuriasis. Results of antigen detection by MabXC3-ELISA are more specific because the problem of cross-reactivity has been circumvented. The monoclonal antibody (MabXC3) is non-reactive to phosporhlcholine. Nine mosquito species in five genera were caught. Anopheles maculates formed the largest number of mosquito species caught followed by *Mansonia* dives and *Aedes albopictus*. Although An. maculates was the dominant species caught and experimentally it can support the development of *W. bancrofti*, it is most unlikely to be of importance as a natural vector at this aboriginal settlement. *M. dives* was the commonest of the Mansonia mosquitoes caught. *M. uniformis* were caught on few occasion and it is the most probable vector for periodic *B. malayi* in the area although no filarial larvae were found in any of them.

The enzyme-linked immunosorbent assay (ELISA), which is widely in use, has limitations of its utility under field conditions. In such situations, a simple, inexpensive colorimetric dipstick assay using robust reagents and no instrumentation could have many diagnostic applications (Wan Omar, A et al., 1996; Wan Omar, A et al., 1996). We have recently reported that a **dipstick colloidal dye immunoassay (DIA)** that detects filarial antigens in human

serum is sensitive and specific for the diagnosis of active infecion of lymphatic filaraisis and dirofilariasis **(Fig. 8)**. This DIA was used in seroepidemilogical studies in various target populations in Peninsular Malaysia (Wan Omar, A *et al.*, 1997; Wan Omar, A *et al.*1999, Wan Omar, A, 1999; Wan Omar, A *et al.*, 2000). The target populations included in epidemiological screening using this DIA were the local population in endemic area, the Orang Asli population, immigrant population and animal reservoirs (Wan Omar, A *et al*, 1999).

Figure 8 : Materials used for dipstick colloidal dye immunological assay for the detection of antigenemic cases of lymphatic filariasis.

A total of 570 individuals from the state of Perak, Peninsular Malaysia were screened for **filarial antigenemia** (Wan Omar, A *et al.*1999). These individuals were divided into three groups viz, local residents of Perak Tengah district (n=240), foreign workers at oil palm estates (n=180) and **Orang Asli** (n=150) at Pos Piah Settlement. Antigenemia were detected in 36 (15 %) of **local population**; 45 (25.0 %) of the immigrant workers and 25 (16.66%) of the Orang Asli community. The prevalence of antigenemia was highest among the **immigrant workers**.

Field studies using this DIA were therefore extended into other estates, which employed Indonesian and Bangladeshi immigrant workers (N=630). At the oil palm estates at Hulu Trengganu District, Peninsular Malaysia (Wan Omar, A *et al.*, 2001), the DIA detected 96 (15.24 %) antigenemic cases, which comprised of all the microfilaremic cases and 15 (2.38 %) amicrofilaremic cases **(Table 3)**. The amicrofilaremic cases with filarial antigenemia consisted of 9 (1.43%) Indonesians and 6 (0.95 %) Bangladeshis.

Table 3 : Prevalence of filarial antigenemia among B. malayi (B.m MF), W. bancrofti (W.b MF) and amicrofilaremic (AMF) immigrants by dipstick colloidal dye immunoassay (DIA).

Nationality	No.tested	No.positive	B.m MF	Antigenemia W.b MF	AMF
Indonesian	390 (61.90)	66 (10.48)	42 (6.67)	15 (2.38)	9 (1.43)
Bangladeshi	240 (38.10)	33 (5.24)	9 (143)	18 (2.86)	6 (0.95)
TOTAL	630 (100.0)	96 (15.24)	51 (8.10)	33 (5.24)	15 (2.38)

* Percentages are in parantheses

In another sero-epidemiological studies (Wan Omar, A., 2001), filarial antigenemia were detected 16.0 % cats, 9.0 % dogs and 6.0 % long tailed macaque monkey, Macaca fascicularis. In addition, prevalence of dirofilarial antigenemia was 6.0% cats, 60.0 % dogs and 1.0 % monkeys.

We have used only 6 ul of the antisera, diluted (1:10) patients's sera per dipstick, which therefore conserve the diagnostic reagents a great deal. The DIA is rapid and can be read in approximately 2 hours. As the reaction developed into coloured dots, positivity can be deduced visually even by ordinary staff. Positive detection by the DIA denotes active infection. The DIA does not require sophisticated equipment or radioactivity and therefore suitable for field application (Wan Omar, A *et. al*, 2000).

RESISTENCE TO INFECTION AND ANTI- BRUGIA VACCINES

In the field of parasite vaccinology, protective immune response has been evoked using radiation attenuated helminth larvae. Such live vaccines have been successfully used against experimental helminthic infections with trichinella, ancylostoma, dictyocaulus, schistosomes and filariae. A commercial vaccine against Dictyocaulus viviparous has been available for over 20 years and the other vaccine against the dog hookworm has also reached the market (Cox, 1990). Studies on successful protection against filarial infection in cats, dogs and monkeys was reviewed by Wan Omar, A (1997).

In 1969, Fredericks and Ramachandran attempted to ascertain if protective immunity in filariasis could be achieved by vaccination with infective larval antigen. They rationalized that it would only be reasonable to assume that the vaccine most likely to be effective would be one derived from the infective stage of the parasite. The three types of antigens used were secretory and excretory infective larval antigen, infective larvae attenuated by incubation in immune serum obtained from a monkey which was proven refractory after several attempts at infection and x-irradiated infective larvae. They found that only x-irradiated infected larvae gave promising results for immunization trials, and irradiation *in the region of 20,000R appeared to be the best dose for maximum* attenuation of infective larvae. Wong et al. (1969) in a similar study found that persistent immunity to challenge infection was obtained in rhesus monkeys vaccinated with large numbers of infective larvae attenuated by x-irradiation at 20,000R. Persistent immunity was expressed as failture to cause microfilaremia and best dose was 200 irradiated larvae. Their studies suggested the effectiveness of a sufficient number of optimally attenuated infective larvae in eliciting functional antibody in a quantity sufficient to prevent patent infection. The exact nature of the so-called "functional-antigen" was still not clearly defined. As such, techniques, which were being developed at that time for harvesting, concentrating and characterizing antigens produced by different stages of the parasite in vitro, could undoubtedly be valuable in achieving this goal.

There are many shortcomings with radiation - attenuated vaccines. There are also several reasons why these irradiated vaccines have not come into greater use. The most important of these is the limited shelf life of most irradiated larval forms and the fact that live vaccine is likely to be applicable only in those diseases in which high level of immunity can be induced by natural infection.

Epidemiological observations indirectly suggest that exposure to parasite infections of animals may confer some degree of cross protection in man. The prevention and amelioration of a disease in man as a result of previous exposure to heterologous infections of animal origin has been termed zooprophylaxis (Nelson *et al.*, 1962). It is interesting to note that in studies on experimental immunization against *B. malayi* infection in rhesus monkeys found that vaccines infective larvae attenuated with 20,000R would produce immunity to challenge infections. It was noted however, that attenuated larval antigen load would not only have to be sufficiently large but need to survive long enough in the host to produce a substantial amount of functional antigens to induce immunity. Similarly, animal infective larvae would have to survive long enough in the human host to induce protective immune responses without causing disease.

There is little data on the successful use of dead worm material as vaccine (Wan Omar, A *et al.*, 1989; Wan Omar, A *et al.*, 1994). Direct proof of filarial infection in nature confers resistance to reinfection with the same parasite is almost non-existence. However, evidence for this phenomenon exists in several experimentally infected animal models. The Mongolian gerbil or jird (*Meriones unguiculatus*) has been shown to be a reliable host for *B. pahangi* and subperiodic *B. malayi*. The commercial availability of an inbred strain of jirds may further the use of this animal species in immunological studies on filariasis. We found that immunogen isolated from a monoclonal antibody (MAbXC3) affinity chromatography (MAP-AH) engendered significant protective immunity in jirds to challenge infections administered by either subcutaneous (s.c.) or intraperitoneal (i.p.) routes **(Table 4)**. Jirds vaccinated with MAP-AH intraperitoneally had 98.0 % reduction in mean worm burden (t-test, P<0.0001) compared to the control while 91.0 % reduction was observed in the group vaccinated with AH (Wan Omar, A *et al.*, 1993). Jirds vaccinated subcutaneously with MAP-AH had a mean worm burden of 2.25 ± 0.38, a 97.75 % reduction compared to the control mean of 27.75 ± 0.38, which is statistically significant (P<0.0001).

Table 4 :   Recovery of *B. pahangi* in normal control jirds and jirds vaccinated with either AH or MAP-AH intraperitoneally at 14 weeks interval between intraperitoneal challenge and autopsy.

| Treatment	No of jirds	Worms recovered / L3 injected	Range	Meani ± SEM	Percent reduction
Vaccinated with AH	12	108/1,200	9-12	9 ± 0.51*	91.0%
Vaccinated with MAP-AH	12	24/1,200	0-3	2 ± 0.53*	98.0%
Control	12	270/1,200	18-24	22.5 ± 0.53	-

Statistically significant difference from control (t-test, P<0.0001)

The i.p challenge provided numerous advantages over s.c challenge. It is well known that after a single i.p inoculation, *Brugia* infective larvae (L3) develop normally and rarely migrate out of the peritoneum of the jirds. On the other hand after s.c inoculation, the L3 reach the lymphatics and settle down at some other anatomical sites (e.g the hearts and the lungs). Since vaccination also works through subcutaneous route, the protection against challenge infection indicates that the resistance in this model was systemic rather than local in nature. This also suggests that other routes of vaccination might be feasible. It is possible that inoculation of vaccine inoculum into muscle would minimize any vaccine-induced lymphatic pathology.

The increase in anti- brugia antibody detected with ELISA in vaccinated jirds immediately after challenge was an important factor responsible in the vaccine induced killing of the challenged larvae mediated by the antibody. Interestingly, the jirds vaccinated with the MAP-AH registered the highest antibody build-up and the lowest percentage of worm recovery. Presumably the epitope recognized by MAb.XC3 was protective. The immunogen bearing this epitope can be concentrated many fold by affinity chromatography. This immunogen may be a suitable putative candidate for use as a non-pathogenic anti-brugia vaccine.

There was suppression of microfilaremia in *B. malayi* challenged cats after triple intramuscular vaccination with MAP-AH (Wan Omar, A *et al.*, 1994, Wan Omar, A *et al.*, 1996). Microfilaremia was detected at very low level at patency in all the vaccinated cats (6 mf/ml blood) and by the twentieth week post vaccination all the cats were free of microfilaremia **(Fig. 9)**. In contrast, increased microfilaraemia was observed in non-vaccinated cats (the average count at patency was 150 mf/ml blood).

Figure 9 :   Suppression of microfilaremia in cats vaccinated with MAP-AH (__) and non-vaccinated cats (📄). The arrow (↘) indicate the third intramuscular vaccination and the time of the challenge infection with *B. malayi* infective larvae.
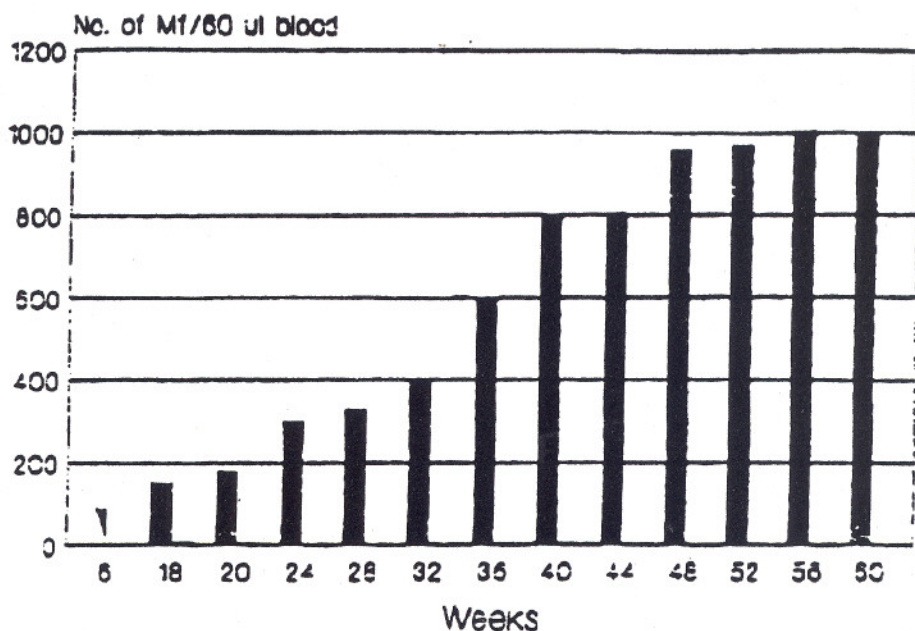
The antifilarial antibodies mounted against a penel of immunogens by cats vaccinated with MAP-AH was studied (Wan Omar, A *et al.*, 1994, Wan Omar, A *et. al.*, 2001). Four antigen preparations were used to provide the widest possible variety of antigens for the characterization of parasite specific antibody responses in cats vaccinated with the dead vaccine, MAP-AH **(Table 5)**. The four antigen preparations were *B. malayi* adult worm homogenate (AH), adult *in vitro* products (BmAdIVP), microfilariae in vitro products (MfIVP) and *B. malayi* L3 homogenate (L3Hom). Generally the vaccinated cats mounted significant antibody response to all these antigen preparations. Interestingly the MAP-AH vaccinated cats mounted relatively higher antibody titres to MfIVP. Similarly it has been observed that high anti-microfilariae antibodies in human patients strongly correlate with microfilaraemia suppression, which eventually lead to clearance. Silver staining of the electrophoresed MAP-AH revealed that the epitope of the immunogen were found on antigens of heterogeneous molecular weights.

Table 5 : IgG titers of vaccinated and non-vaccinated (with PBS) cats against four antigen preparations: Adult worm homogenate (AH), *in vitro* products of adult worms (Ad IVP), *in vitro* products of microfilariae (Mf IVP) and homogenate of infective larvae (L3Hom).

Cats		AH	AdIVP	MfIYP	L3Hom	Cats	AH	AdIVP	MfIVP	L3Hom
A	Pre	40	40	40	40	J	40	40	40	40
	Post	1280	320	640	640		1280	640	80	640
B	Pre	40	40	40	40	K	40	40	40	40
	Post	2560	640	1280	1200		1280	1280	80	1280
C	Pre	80	90	80	80	L	80	80	30	80
	Post	640	320	320	320		640	320	160	320
D	Pre	40	40	40	40	M	80	80	80	80
	Post	1280	1280	320	320		1280	640	160	160
E	Pre	40	40	40	40	N	40	40	40	40
	Post	640	640	1280	640		640	640	80	640
F	Pre	80	80	80	80	O	40	40	40	40
	Post	5120	1280	640	2560		2560	1280	80	1280
G	Pre	40	40	40	40		Control cats ***			
	Post	2560	640	640	1280	C1	40	40	40	40
H	Pre	40	40	40	40	C2	40	40	40	40
	Post	640	1280	1280	640	C3	40	40	40	40
I	Pre	80	90	80	80	C4	40	40	40	40
	Post	2560	1280	1280	2560	C5	40	40	40	40

Header spanning: Vaccinated* (AH, AdIVP, MfIYP, L3Hom) and Non-Vaccinated** (AH, AdIVP, MfIVP, L3Hom).

* The group of cats which were vaccinated with MAP-AH. Sera were taken pre- and one month after the third dose of vaccination.

** The group of cats which were not vaccinated with MAP-AH but receive PBS injection. Sera were taken pre- and 3 months after the challenge infection.

*** Control titers were obtained from 5 uninfected adult cats that reeeived no vaccination or PBS injection.

Field vaccination trials with MAP-AH were performed against the other panel vaccines derived from the above immunogens (Wan Omar, A *et al.*, 2000; Wan Omar, A *et al.*, 2001; Wan Omar, A et al., 2001). Six hundred cats at Perak Tengah, an endemic area for *B. malayi* were vaccinated and followed for a period of three years. Pre-vaccination microfilaremia occurred only in 198 cats (33.0 %). Out of these 600 cats, 594 (94 % mounted significant anti-MfIVP IgG titres. Relatively higher titers were observed in MAP-AH vaccinated cats. Microfilarial count ranged from 27 to 981 mf per 60ul blood. There were suppression and / or sharp drop of microfilaraemia in the vaccinated cats. By the twenty fourth weeks post-vaccination, all the vaccinated cats were cleared of microfilaremia. This amicrofilaremic state extended until the end of this three-year study suggesting a stable and lasting humoral protection conferred by the vaccine.

There has been a great deal of interest recently in the use of genetically engineered vaccines. Some researchers proved that genetically engineered vaccines are capable of eliciting both humoral and cellular immunity. Genetic engineering has made it possible the production of apathogenic organisms in a more predictable and stable manner. There are many advantages of genetically engineered vaccines such as the ability to insert multiple genes of protective antigens into a vector, safer because there is no clinical disease and the process does not involve the introduction or reliance upon the infectious organisms. Studies on the recombinant vaccines (Wan Omar, A *et al.*, 2001) capable of stimulating a protective immune response in *B. malayi* infected animals are in progress and some encouraging preliminary results have been obtained.

LYMPHATIC FILARIASIS RESEARCH AT UNIVERSITI PUTRA MALAYSIA

World Health Organization (WHO) in 1993 identified lymphatic filariasis as one of only six diseases that could be eradicated. The other five diseases identified as eradicable were: Guinea worm, polio, mumps, rubella, and pork tapeworm. There were three main biological and technical reasons: humans were only hosts, there was no multiplication of the parasite in the mosquito, the disease had a poor transmission system and good drugs and diagnostics were available. In May 1997, the World Health assembly passed a resolution making elimination of lymphatic filariasis a public health priority. A Lymphatic Filariasis Global Alliance was formed in 1997 (Malcolm Dean, 2001) comprising of three key international agencies (WHO, the World Bank, UNICEF), multiple numbers of non-governmental agencies, national aid agencies, leading academic institutions, plus the two drug giants (GSK and Merck & Co., Inc.) and all has come together as one to eliminate the disease. This Global Alliance has targeted elimination of lymphatic filariasis by 2020.

Universiti Putra Malaysia in a deliberate attempt toward the same goal of better control and possible elimination of lymphatic filariasis in Malaysia has embarked on three key areas of lymphatic filariasis research.

Our immunological and molecular biological research on lymphatic filariasis has contributed to a much -improved understanding of the make-up of the filarial worms,

and this is also having significant impact on immunodiagnostics. Those techniques, which employ a "labeled" reagent, dominate the field at present. ELISA use enzymes as labels are more suitable for large- scale screening and yields objective results. Although specific and sensitive, it requires the use of spectrophotometer, which is expensive and sophisticated that is not readily available in many underdeveloped areas of the world. Moreover ELISA is time consuming, requires technical skill and expertise to run. Other techniques that detect parasite antigens, such as radioimmunoprecipitation-PEG assays or immunoradiometric assays have added disadvantage of requiring radioactive reagents. The various dot-blot tests are simpler and less cumbersome use antigens or antibodies on paper or plastic supports to detect antibody or antigen which is then visualized using antiglobulins labeled with enzymes, colloidal dyes or gold. These dot-blot methods are more convenient, especially as dipstick techniques, which make them also suitable for large- scale epidemiological screening. Detecting circulating antigens of B. malayi is a new approach in the diagnosis of active infection. The DIA that was developed by our laboratory provides an inexpensive, simple, robust test for filarial antigen detection and can be performed without instrumentation that makes it suitable for a wide variety of field applications. Therefore future research is to subject this DIA to more rigorous field trials before it can be recommended as a diagnostic reagent for screening and as a tool for epidemiological studies.

Development of an effective, long-lasting vaccine that would prevent infection and block zoonotic transmission would clearly represent a major advancement in the control of brugian filariasis. This would be particularly useful in areas where it is not possible to treat animal reservoirs on a yearly basis, which unfortunately is the case for those countries where brugian filariasis is endemic.

Although, as noted above, there is no evidence of a protective immune response developing in nature, there is some optimism concerning the possible development of a vaccine based upon results in animal filarial infections and the power of the newer techniques of molecular immunology and molecular biology. Attempts at vaccine development by Universiti Putra Malaysia shall be focused upon two major approaches:

1. Development of an immune response directed against developing larvae, especially L3 and L4.

2. Development of an immune response against molecules that are critical for the survival of the parasite.

The main aim in the development of **antifilarial nucleic acid vaccines** is to genetically engineered vaccines for the control of brugian filariasis zoonotic transmission. These genetic vaccines shall be administered to common animal reservoir hosts like cats and dogs at the endemic areas. The administration of these vaccines is to suppress the development of microfilaremia in the reservoir animals that will in turn obliterate transmission to the mosquito vectors henceforth blocking zoonotic transmission to man (Wan Omar, A et. al., 2001; Wan Omar, A et al., 2001). Genetic vaccines offer several advantages compare to the conventional formulations. One such advantage is the stability and almost non-pathologic to the recipient host. Preliminary and indirect data concerning potential immunogens for

genetic vaccine formulations shall be collected in anticipation of trials in non- human primates and ultimately in humans.

A significant advancement in treating lymphatic filariasis has been achieved with introduction of single-dose treatment regimens with DEC alone or in combination with albendazole or ivermectin. Unfortunately these drugs has no effect on the main pathological consequence of repeated infection, namely elephantiasis, for which, in fact, no drug treatment has any effect except possibly steroids in early cases. Although not entirely satisfactory, there are measures for treatment of the oedema that precedes and accompanies elephantiasis of the limbs. Elevation of the affected limb and use of elastic stockings or pressure bandaging **(Fig. 10)** are obvious procedures in early stages of the disease. Surgical procedures for more advanced pathologic conditions to remove excess connective tissue may give short-term cosmetic benefits but long-term complications. Microvascular surgery in which small lymphatics are anastomosed to a lateral central vein, lymphaticovenous microsurgery, is physiologically more rational and can produce remarkable reduction in limb size. A further innovation of this surgery, nodoso-venous shunting, can yield even more impressive results. Unfortunately, such procedures require a very skilled plastic surgeon and are available to very few patients.

Figure 10 : Topical application of herbal preparation followed by pressure bandaging in an elephantiasis patient.

Small scale preliminary studies into **herbal remedy** for chronic elephantiasis has been conducted and encouraging results obtained (Wan Omar, A *et al*, 1995; Wan Omar, A *et al*, 1995). The logical extension from these studies is therefore to carry out the research on a bigger and wider scale. Investment into this new research paradigm on lymphatic filariasis by Universiti Putra Malaysia is not only prudent but also worthwhile because the potential for success of discovering herbal remedy for chronic elephantiasis is great. It will be very near in the future that herbal remedy for chronic elephantiasis shall be discovered.

CONCLUSIONS

The routine laboratory diagnosis for *B. malayi* infection still relies on the microscopy confirmation of microfilariae by examination of stained thick blood smears and blood concentration using Knott's method or membrane filtration. Although these methods are cheap and specific, they require night blood samples, which is very inconvenient. In addition, these parasitological techniques will not detect low microfilaremia levels, unisexual infections, and cases of tropical pulmonary eosinophilia, chronic obstructive lymphangiopathy and amicrofilaremic stages of infection. In the global programme for elimination of lymphatic filariasis, antigen detection assays for bancroftian filariasis are available as diagnostic and epidemiologic tools. Howeverever, no equivalent antigen test exists for brugian filariasis despite the many attempts aimed at its development. The diagnostic test successfully developed recently is an antibody test using recombinant proteins (Rahmah *et al.*, 2001) However, antibody tests can have some problems as discussed earlier. Although PCR-based assays can also be employed to detect *Brugia* infection effectively, they are probably not suitable for large-scale screening. The availability of a rapid dipstick colloidal dye test developed for antigen detection thus is an innovation toward the development of a practical diagnostic test for brugian filariasis.

There is an urgent need for better and sustainable methods to control lymphatic filariasis especially the zoonotic brugian filariasis. Drugs that are available may have limitations for wide scale use in some countries because of financial constraints. Measures aimed at interrupting transmission have rest heavily on vector control. Investigations into anti-brugia vaccines should therefore be made a high priority. Vaccination against brugian filariasis in reservoir animals can be integrated as one of the component control methods at the endemic areas and any progress, however small should be followed. A critical step has been made in the development of anti-brugia vaccine. The substantial task ahead include (1) improving the vaccines which has been described using powerful immunological and molecular biological techniques and (2) devising and testing these vaccines which may prove more efficacious first in laboratory animals before extended their use to reservoir animals. A monoclonal antibody-affinity purified immunogen has been used to formulate an anti-brugia vaccine designated as MAP-AH has been shown to induce protection against challenge infection with *Brugia* spp infective larvae. This vaccine evoked a strong antifilarial antibody response and what is most striking is suppression of microfilaraemia in cats infected with *B. malayi*. While its potentially useful for blocking transmission to or development of microfilariae within the intermediate host as well as for diminishing microfilarial-induced pathology, this vaccine would not be feasible or

acceptable for direct use in man. However, these animal models of protective immunity provide useful systems for identification of filarial-protective immunogens. No other vaccination protocol known to us can readily induce a protective response in a *Brugia*-susceptible host. Subsequent molecular cloning of genes that encode protective proteins may form the basis for the development of genetically-engineered antifilarial vaccine. These tasks also require a continuous interaction between laboratory and the field and interdisciplinary strategy that is linked to the health care delivery and promotion systems in the endemic areas. The pursuit of this approach and the achievements of the dipstick tests and anti-brugia vaccine to date allow us to foresee that widespread application of an efficacious diagnostic test and anti-brugia vaccine in areas where sizeable animal reservoirs exist to interrupt zoonotic transmission, eradication of lymphatic filariasis in Malaysia represents no longer an unrealistic target.

We are in a new millennium. The despair of elephantiasis can and must be turned to hope. What was taught to be untreatable is treatable. The basic nineteenth century rules of hygiene including washing, simple skin care, the elevation of affected limbs and research into herbal remedy in this new millennium all taken together not only may halt the progression of the disease, but may even reverse it, which ultimately contribute to the restoration of dignity, respect and health to patients. Hence, both the government, the private sector and the people must all work together to make certain that Malaysia can look forward to a brighter and healthier future, a future free of lymphatic filariasis.

ACKNOWLEDGEMENTS

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