IMMUNOLOGICAL REACTIVITY OF JIRD (*MERIONES UNGUICULATUS*) TO INFECTION OF *BRUGIA PAHANGI*

by

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ABSTRAK

Tindakbalas Imun Jird (Meriones unguiculatus) terhadap Jangkitan Brugia pahangi

Filariasis ialah jangkitan parasit kronik yang menjadi kepentingan kesihatan awam dan sosioekonomi di banyak negara tropika dan subtropika dan masih menjadi masalah kesihatan utama di banyak kawasan-kawasan lain di dunia. Kajian ini dilakukan untuk menerangkan berbagai mekanisme immunoregulator yang ditimbulkan dengan infeksi *B. pahangi* pada jerbil (*Meriones unguiculatus*).

Kami dapati jerbil yang terjangkit dengan *B. pahangi* menunjukkan mikrofilaremia lebih kurang 8-10 minggu selepas jangkitan yang bebas daripada bilangan larva infektif yang diinokulasikan. Ketumpatan mikrofilaria tergantung kepada bilangan larva infektif yang diinokulasikan pada masa-masa selepas jangkitan, tetapi ianya tidak tergantung kepada bilangan cacing dewasa yang depat dikutip daripada jerbil terjangkit. Purata cacing yang dikutip balik ialah 36, 28.4, 27.07 dan 22.53% dalam jerbil yang terjangkit dengan 5, 25, 75 dan 25 x 3 larva infektif masing-masing yang tidak menunjukkan perbezaan yang signifikan. Kebanyakkan daripada cacing dewasa dalam setiap kumpulan jerbil terjangkit terdapat pada limfatik korda sperma. Selainnya tertabur dalam jantung, paru-paru dan limfatik lain. Tiada cacing ditemui dalam limfatik servikal dan aksilari. Purata panjang cacing dewasa jantan dan betina yang dikutip daripada semua jerbil terjangkit juga tidak menunjukkan perbezaan signifikan. Ini bermakna inokulasi sebanyak 75 larva infektif tidak menyebabkan kesan kesesakan atau kerintangan kepada parasit untuk hidup dan menghasilkan mikrofilaria.

Eosinofilia darah periferi dapat dilihat dalam semua jerbil terjangkit. Terdapat dua kemuncak yang nyata semasa jangkitan, satu semasa 2-3 minggu selepas jangkitan dan satu lagi 7-10

minggu selepas jangkitan. Bilangan eosinofil berkadar terus kepada bilangan larva infektif yang diinokulasikan dan tidak pernah menurun bawah nilai jerbil kawalan sepanjang masa 16 minggu selepas jangkitan. Bilangan eosinofil dalam jerbil bermikrofilaria adalah lebih tinggi daripada jerbil yang tidak bermikrofilaria tetapi eosinofil tidak tergantung kepada jumlah mikrofilaria yang beredar dalam darah. Tidak ada perbezaan signifikan didapati dengan parameter hematologi lain.

Semua jerbil terjangkit dengan ***B. pahangi*** sedikit sebanyak menunjukkan imunosupresi humoral tak-spesifik sepanjang masa 16 minggu selepas jangkitan apabila dibandingkan dengan kumpulan kawalan tak terjangkit. Supresi ini bergantung kepada masa jangkitan dan menunjukkan supresi yang tinggi pada minggu-minggu awal jangkitan dan menurun perlahan-lahan apabila jangkitan berkembang. Hasil juga menunjukkan sel merekat plastik bertanggungjawab kepada bilangan tindak balas PFC dan pecahan sel tak merekat daripada jerbil terjangkit nampaknya boleh meransangkan sepresi dalam populasi sel kawalan.

Apabila cerakin blastogenik dilakukan, kami dapati imunosupresi sel tak-spesifix berlaku dalam sel nodus limfa dan limpa jerbil terjangkit tetapi ianya lebih jelas dalam sel limpa. Supresi ini tidak tergantung kepada kuantum jangkitan tetapi tergantung kepada masa jangkitan yang menunjukkan supresi maksimum pada masa paten jangkitan. Walau bagaimanapun, imunosupresi sel spesifix itu hanya berlaku dalam sel limpa jerbil yang dijangkiti dengan 25 dan 75 larva infektif manakala sel nodus limfa daripada semua jerbil terjangkit menunjukkan gerakbalas yang signifikan terhadap semua antigen. Keputusan menunjukkan kegiatan kawalatur terspesifikan enzim dan tak terspesifikan enzim dapat dikorilasikan dengan kehadiran mikrofilaria dalam darah dan supresi spesifik terhad secara anatomi kepada limpa. Apabila dilakukan pembiakan bersama dalam cerakin blastogenik, kami dapati sel limpa daripada jerbil terjangkit dapat mensupresikan gerakbalas PHA dan Con A sel limpa normal dan sel limpa terjangkit ini juga dapat mensupresikan semua gerakbalas antigen sel nodus limfa daripada jerbil terjankit. Sebaliknya, sel nodus limfa daripada jerbil terjangkit dapat mensupresikan gerakbalas PHA dan Con A sel nodus limfa normal. Untuk lebih menyatakan

lagi sel limpa yang bertanggungjawab untuk supresi. eksperimen peringkatan sel dilakukan. Kami dapati kehabisan sel limpa merekat plastik daripada jerbil terjangkit mengembalikan gerakbalas blastogenik terhadap PHA dan Con A. Sel limpa merekat plastik ini dapat mensupresikan gerakbalas PHA dan Con A sel limpatak merekat terjangkit dan sel nodus limfa normal. Walau bagaimanapun, kehabisan sel limpa merekat plastik daripada jerbil terjangkit tidak mengembalikan gerakbalas blastogenik terhadap semua antigen, tetapi sel limpa merekat plastik ini dapat mensupresikan semua gerakbalas antigen sel nodus limfa terjangkit.

Antibodi IgG dapat dikesan pertama kali pada sesetengah jerbil satu minggu selepas inokulasi dengan larva. Titer antibodi yang tinggi itu kekal sepanjang 16 minggu selepas jangkitan dalam semua kumpulan jerbil terjangkit. Tahab antibodi IgG dalam jerbil terjangkit dengan ***B. pahangi*** tergantung kepada masa selepas jangkitan dan kuantum larva yang diinokulasikan. Tidak terdapat perbezaan signifikan antara ketiga-tiga antigen itu, tetapi antigen dewasa disarankan untuk teknik ELISA kerana kespesifisitian dan kepekaannya yang tinggi memberikan hasil yang lebih tetap dalam mengesan antibodi IgG dalam jerbil yang terjangkit dengan ***B. pahangi***.

ABSTRACT

Filariasis is a chronic parasitic infection which is considerable public health and socioeconomic importance in many tropical and subtropical countries and remains a major health problem in many areas of the world. The present study was undertaken to elucidate the various immunoregulatory mechanisms elicited by different doses of subcutaneous infection with *B. pahangi* in jirds *(Meriones unguiculatus)*

We found that jirds infected with *B. pahangi* showed microfilaraemia at about 8-10 weeks post-infection. The microfilarial densities was dependent on the number of infective larvae inoculated and times post-infection, but it was not dependent on the number of adult worms recovered from the infected jirds. The average worm recovery were 36, 28.4, 27.07 and 22.53% in jirds infected with 5, 25, 75 and 25 x 3 infective larvae respectively and this was not significantly different. The majority of the adult worms in every group of the infected jirds were found in the lymphatics of spermatic cords. The rest of the worms were distributed in heart and lungs and other lymphatics. No worms were found in the lymphatics of cervical and axillary. The average length of both male and female adult worms recovered from all the infected jirds also did not show any significant difference. This means that the inoculation of up to 75 infective larvae did not cause any crowding effect or resistance to the pârasite growth and microfilariae production.

Peripheral blood eosinophilia was observed in all infected jirds. There were two distinct peaks during the course of infection, one occurred at 2-3 weeks post-infection and the other occurred at 7-10 weeks post-infection. The number of eosinophils appeared to be directly proportional to the number of infective larvae inoculated and were never below the values of control jirds throughout the period of 16 weeks post-infection. The number of eosinophils in microfilaraemic jirds were higher than in amicrofilaraemic jirds but they were not dependent on the total number

of circulating microfilariae. Analysis of the other haematological parameters showed no significant changes.

All the jirds infected with *B. pahangi* expressed some degree of non-specific humoral immunosuppression throughout the period of 16 weeks post-infection when compared to the uninfected control group.The suppression was time dependent, it is higher in the early weeks of infection and dropped gradually with the evolution of the infection. It was also found that plastic adherent cells were responsible for the number of PFC reaction and non-adherent cell fraction from infected jirds seemed to be able to induce suppression in control cell population.

When *in vitro* blastogenic assays were performed, we found that non-specific cellular immunosuppression occurred in both spleen and lymph node cells of infected jirds but it was more prominent in the spleen cells. The suppression was not dependent on the quantum of the infections but dependent on the times' post-infection; the maximum suppression occurred at about the time of patency. However, the specific cellular immunosuppression occurred only in the spleen cells of jirds infected with 25 and 75 infective larvae while lymph node cells from all the infected jirds exhibited significant responses to all the antigens. There was no significant difference in the responses among the three antigens. The results showed that both antigen specific and non-specific regulatory activities were correlated with the presence of circulating microfilariae and that specific suppression was only anatomically restricted to the spleen. When co-cultivation were performed in blastogenic assay, we found that spleen cells from the infected jirds were able to suppress the PHA and Con A responsiveness of normal spleen cells and that these infected spleen cells were also able to suppress all the antigens responsiveness of lymph node cells from the infected jirds. In contrast, lymph node cells from the infected jirds were unable to suppress both PHA and Con A responsiveness of normal lymph node cells. Further characterize the spleen cells responsible for the suppression, showed that depletion of plastic adherent spleen cells from the infected jirds restored the blastogenic response to PHA and Con A. These plastic adherent spleen cells were able to suppress PHA and Con A

responses of the infected non-adherent spleen cells and normal lymph node cells. However, depletion of plastic adherent spleen cells from the infected jirds did not restore the blastogenic responses to all the antigens, but these plastic adherent spleen cells were able to suppress all the three antigen responses of the infected lymph node cells.

The IgG antibody could be first detected in some infected jirds at one week post infection, with the highest titer at about the time of patency. The high titers of the antibody persisted throughout the period of 16 weeks post-infection in all groups of the infected jirds. The levels of IgG antibody in jirds infected with *B. pahangi* were dependent on times of post-infection and the quantum of the larvae inoculation. There were no signfiicant difference among the three antigens, but adult worm antigen was recommended for the ELISA technique due to its high specificity, sensitivity and consistency in the detection of specific antifilarial IgG antibody.

CHAPTER 1

GENERAL INTRODUCTION

Filariasis was probably known as early as 600 to 250 B.C. as people with elephantiasis were excluded from the Buddhist priesthood (Laurençe, 1967). It is a disease caused by long, thin, thread-like nematodes belonging to the Superfamily Filarioidea, Family Onchocercidae (Chabaud and Anderson, 1959). The parasites known to cause human infections belong mainly to the genera ***Wuchereria, Brugia, Onchocerca, Loa, Dipetalonema***, and ***Mansonella.*** They can be classified as lymphatic dwelling, ***Wuchereria bancrofti***, ***Brugia malayi*** and ***Brugia timori***; tissue dwelling, ***Onchocerca volvulus***, ***Loa loa*** and ***Dipetalonema streptocerca;*** and body cavity filariids, ***Dipetalonema perstans*** and ***Mansonella ozzardi.***

The biology of filarial parasites within the infected human host differs markedly from that of insect-borne protozoan parasites, such as those causing malaria. Whereas protozoan parasites can multiply directly within the host, adult filariae can not. Moreover, the maturation of infective larvae into adult worms, which occurs over a period of several months, is inefficient. Consequently, even a brief exposure to biting malarious mosquitoes may initiate a rapidly progressive, potentially fatal malarial infection, but the development of a filarial infection with detectable microfilaraemia may require thousands of bites from insects bearing infective filarial larvae (Weller and Liu, 1990). Finally, the manifestations of filariasis develop slowly, usually over years or even decades, especially in lifelong residents of areas of endemic infection. Thus, filarial infections are gradually evolving, chronic diseases and prevalent in locales in which the population is continually exposed to infected vectors. Filariae are ovoviviparous nematodes producing microfilariae (the advanced embryonic or prelarval stage) into blood circulation or tissue of the vertebrate host depending on the filarial species.

Variable periodicity may be found among microfilariae that circulate in the blood. Microfilariae are incapable of further development unless taken up by specific blood-sucking insects in which they migrate to certain sites or organs to complete two moultings and become infective third stage larvae. The diseases are transmitted by biting of the infective blood-sucking insects and infective larvae (L_3) actively migrate into the puncture wound.The intermediate host of all human filariae are haematophagous insects of the order Diptera which belong to the following families:

Culicidae (mosquitoes) :***W. bancrofti, B. malayi*** and ***B. timori***

Simuliidae (black flies) : ***O. volvulus***

Tabanidae (horse flies) :***L. loa***

Ceratopogonidae (biting midges) :***D. streptocerca, D. perstans*** and ***M. ozzardi***

The infective larvae escape from the mouth parts of the insect while it is feeding. They are deposited on the skin surface near the site of the puncture wound made by the insect and enter the definitive host by penetrating through the wound which remains after the infected intermediate host has completed its blood meal. In the definitive host, the larvae moult twice during the migration to favoured site to become adults and produce microfilariae (Fig. 1.1).

The most important filarial worms of man are ***O. volvulus*** which causes severe skin disease (subcutaneous nodules) and river-blindness and ***W. bancrofti*** which causes elephantiasis, hydrocoele and chyluria. Onchocerciasis is limited to tropical Africa, Central and South America and foci in the Eastern Mediterranean Region while bancroftian filariasis is found throughout the wet tropical world such as Africa, Asia, Oceania, Latin America and Caribbean . The other serious pathogens of elephantiasis are ***B. malayi*** and ***B. timori. B. malayi*** is confined to regions of Asia, whereas ***B. timori*** is restricted to parts of Indonesia. Other human filarial parasites ***L. loa, D. streptocerca, D. perstans*** and ***M. ozzardi*** are present in countries in Africa, Central and South America and have not been reported in Asian Region. ***Dirofilaria immitis*** is

a filarial parasite of dogs (common heart worm of dogs). The parasite has been found in human on several occasions (Yoshimura and Yokogawa, 1970; Takeuchi *et al.*, 1981; Ciferri, 1982). The worldwide distribution of lymphatic filariasis is shown in Figure 1.2.

Lymphatic filariasis presents a spectrum of clinical features. Many infected persons are asymptomatic, even when large numbers of microfilariae are circulating in their blood. The inflammatory manifestations include "filarial fevers"-recurrent debilitating episodes marked by a triad of fever, lymphadenitis and retrograde lymphangitis. Chronic lymphatic obstruction, resulting in elephantiasis of the extremities, hydrocoele, chyluria or chylous effusion, develops in some patients after years of infection. Finally, a minority of infected persons have tropical pulmonary eosinophilia (TPE), a distinctive syndrome marked by nocturnal asthma, pulmonary and peripheral eosinophilia, and obstructive, followed by restrictive, pulmonary disease (Weller and Liu, 1990). TPE is believed to be due to a hyperimmune response to filarial parasites, mainly that against microfilariae. The disease may cause infection of eyes and so far four cases of adult *Brugia* in the conjunctival cysts of man have been reported (Dissanaike *et al.*, 1974; Mak *et al.*, 1974 and Mak *et al.*,1983) and may also cause pulmonary infarction (Beaver *et al.*, 1971; Beaver and Cran, 1974). Lymphatic filariasis is responsible for considerable disability and disfigurement, due to acute adenolymphangitis and chronic lesions like elephantiasis and hydrocoele (Fig. 1.3).

The other major human filarial parasites are localized in different anatomical sites in the host and elicit distinct clinical manifestations. Loiasis is due to infection with ***L. loa***. The adult worms reside in subcutaneous tissues, and the microfilariae circulate in the blood. Although most infected persons are asymptomatic, migrating adult ***L. loa*** can cause episodic, edematous subcutaneous lesions (Calabar swellings) and can occasionally present in dramatic fashion by subconjunctival passage across the eye. Onchocerciasis is caused by ***O. volvulus*** and adult parasites are localized in subcutaneous tissues where they may cause subcutaneous nodules, whereas the

microfilariae are found in the skin, subcutaneous tissues, and eyes. In onchocerciasis, unlike lymphatic filariasis and loiasis, the microfilariae rather than the adult worms are primarily responsible for serious and disabling manifestations, which include pruritic dermatitis and progressive ocular disease (river blindness). Although fatality is rare, filarial infections are major causes of morbidity in the affected populations.

Classical obstructive filariasis is easy to recognize but many infections are inapparent. Laboratory confirmation of the infection will therefore be necessary. Definitive diagnosis of filarial infection has always required the direct demonstration of the parasite in the human tissues or fluids. This is not easy in view of the fact that filariae can be present in small numbers or sequestered in inaccessible sites. Furthermore the means to detect them parasitologically are relatively insensitive and inconvenient such as the need to sample night blood for lymphatic filariasis and to take several skin snips for onchocerciasis. Therefore development of new immunodiagnostic techniques are needed to facilitate routine diagnosis. The advantage of serodiagnosis is the ability to detect prepatent and postpatent infections as well as patent infection. The ideal serologic test should also provide information on the immune status of the patient in terms of reinfection and the development of disease. The improvements in immunoassays now promise better approaches to diagnosis of filarial infection and more precise definition of risk factors for the disease. In some instances, histopathological technique can be used for the diagnosis as the various stages of filarial parasites may be seen in tissue sections. Therefore, there are three ways available for the diagnosis of filariasis even in infected cases who do not show any sign or symptom.

Control measures in filariasis can be directed towards three main targets. They are the reduction of the reservoir of infection, the control of vectors and finally the reduction of human-vector contact by promoting personal protection from the bites of insect vectors. The last strategy is effective alone but the three measures are best applied together.

Prevention of lymphatic filariasis needs the avoidance of being bitten by infective mosquitoes. In many endemic areas this would not be possible due to the numerous chances of vector-host contact. Anti-mosquito repellants, the use of mosquito nets and wearing of protective clothings will decrease the chances of mosquito bites. Vector control may be feasible. For populations at high risk to infection as in those living in areas of intense zoonotic transmission, a prophylaxis will be extremely useful. Over the last 40 years diethylcarbamazine (DEC) has been the drug of choice for treating lymphatic filariasis but its main disadvantages are that multiple doses over time are required and that death of parasites during treatment frequently causes unpleasant side-effects. Recently, Ottesen *et al.* (1990) reported that ivermectin should become a useful medication for the control of bancroftian filariasis by its practical advantages of single-dose administrations which caused rapid clearance of microfilariae from the blood and producing a prolonged suppression of microfilaraemia. Elephantiasis, chyluria and hydrocoele associated with filariasis also require surgical treatment, although the results are not always satisfactory in patients with elephantiasis.

Although there have been several successful programmes to control lymphatic filariasis, the disease continues to be one of considerable public health and socioeconomic importance in many tropical countries and remain a major health problem in many areas of the world. It is a disease affecting people in rural areas as well as an increasing number of those living in urban areas with poor sanitation. The disease is endemic in many tropical and subtropical countries of Africa, Central and South America, Asia and Oceania, covering worldwide a surface area inhabited by an estimated 2700 million people (56 % of the world's population). Nine hundred and five million people live in endemic areas are at direct risk of infection and about 90 million people are actually infected with ***W. bancrofti*** (81.6 million), ***B. malayi*** and ***B. timori*** (8.6 million)(WHO, 1984). Onchocerciasis is endemic in 34 countries, 26 in Africa, 6 in Latin America and 2 in the Eastern Mediterranean, with a total population of 585 million; and 78 million, 5 million and 2 million of the populations, respectively, are at direct risk of infection.

Presently, 17.6 million people are actually infected and just under 336,000 are blind as a result of the disease (WHO, 1986).

Brugian filariasis was first recognized as clinically distinct from bancroftian filariasis by Lichtenstein (1927). Although elephantiasis of the leg is one of the sequelae common to both of them, the brugian filariasis is normally confined to those parts of the legs below the knee while bancroftian filariasis involves the entire leg (Plate 1.1, 1.2). It is noticeable that the elephantiasis of the scrotum, breast, hydrocoele and chyluria caused by bancroftian filariasis, are very rare or absent in Brugian filariasis (Plate 1.3). Brug (1927, 1929) confirmed that these microfilariae were morphologically different from those of ***W. bancrofti*** and called them ***Filaria malayi.*** The adult worms were described by Rao and Maplestone (1940) from materials collected in India and the species of ***Wuchereria malayi*** was established. Buckley (1958) compared the morphology of ***W. bancrofti*** with ***W. malayi*** and two other animal filarial parasites, ***W. pahangi*** and ***W. patei*** and concluded that ***malayi, pahangi*** and ***patei*** were closely related but distinct from ***bancrofti*** and he placed them into a new genus called ***Brugia*** after Brug.

There are two strains of ***B. malayi*** differing in the periodicity of microfilariae found in peripheral blood. These strains are the nocturnally periodic and the nocturnally subperiodic strains. The highest concentration of microfilariae in the peripheral blood occurs during the period that the local mosquito vectors are most actively feeding (Fig. 1.4). Wharton (1963) suggested that filarial nematodes adapted their periodicity patterns to the mosquito vector circadial biting patterns to facilitate their transmission. The two strains of ***B. malayi*** in man were first described by Turner and Edeson (1957). The periodic strain is endemic in open rice fields or swamp areas and is transmitted mainly by the genus ***Anopheles*** while the subperiodic strain is endemic in forest areas and is transmitted by certain ***Mansonia*** mosquitoes. Laing *et al.* (1960) showed that the subperiodic form is a zoonosis which also occurs in wild and domestic animals. It

can be transmitted experimentally from man to cats and *vice versa*. The periodicity patterns of each form are variable in different host species.

There is another species of ***Brugia*** infecting man on the islands of Timor, Flores, Alor and Roti in Indonesia (Oemijati and Tjoen, 1966; Kurihara and Oemijati, 1975; Wheeling *et al.*, 1975; Lim, 1986). It was first reported by David and Edeson (1965) from Portuguese Timor that the nocturnally periodic microfilariae found in human blood were different from ***B. malayi*** microfilaria in morphological characteristics and their staining reactions and it is known as "Timor" microfilaria. Partono *et al.* (1977) described the morphology of the adult worms which was obtained from experimentally infected Mongolian jirds and established a new species ***B. timori.*** It causes elephantiasis of the lower limbs below the knee and only parasitic to man (Dennis *et al.*, 1976; Partono *et al.*, 1978)

Other species of ***Brugia*** are parasites of domestic and wild animals: ***B. pahangi*** (Buckley and Edeson, 1956), ***B. patei*** (Buckley *et al.*, 1958), ***B. buckleyi*** (Dissanaike and Paramananthan, 1961), ***B. ceylonensis*** (Jayewardene, 1962), ***B. beaveri*** (Ash and Little, 1964), ***B. guyanensis*** (Orihel, 1964) and ***B. tupaiae*** (Orihel, 1966) (Table 1.1). All of these species have similar life cycles in mosquitoes and vertebrates and are parasites of the lymphatic system except ***B. buckleyi,*** which lives in the heart and blood vessels of the Sri Lankan hare.

B. pahangi was found in cats and dogs in the same endemic area as subperiodic ***B. malayi*** (Buckley and Edeson, 1956). This species is capable of developing into mature adults when inoculated into human volunteers (Buckley, 1958; Edeson *et al.*, 1960) and may cause human infections in nature (Palmieri *et al.*, 1985). Spencer *et al.* (1981) found cross-reactions between ***B. pahangi*** and ***B. malayi*** by using the ELISA test. Both species have shown closely homologous surface antigens in all stages, adult worms, infective larvae and also microfilariae (Maizels *et al.*, 1983). Jirds *(Meriones*

unguiculatus) infected with ***B. pahangi*** develop lymphatic lesions that histologically resemble the human filariasis lesions in which dilation of the lymphatic vessels, fibrosis and granulomatous inflammation occur (Ah and Thompson, 1973; Vincent *et al.*, 1980). These findings indicate its close relationship between ***B. pahangi*** and the human species ***B. 'malayi*** and because ***B. pahangi*** is successfully transmitted to many laboratory animals especially infection in the jirds, it is therefore most widely used as a model in a variety of studies in chemotherapy, immunology and host-parasite relationship, especially to elucidate the immunoregulatory mechanisms in lymphatic filarial infection which is difficult to achieve in human. Although laboratory animals like cats and jirds can harbour ***B. malayi,*** they are not as easily infected as with ***B. pahangi*** (Laing *et al.*, 1961; Wilson and Ramachandran, 1971; Ash, 1973 a; Denham, 1974). Other human filariids are not easily transmitted to laboratory animals. A small laboratory animal model needs to be established in order to have a practical, inexpensive system in which to carry out a variety of immunological and chemotherapeutic studies. ***L. loa*** can be transmitted to some African and South American monkeys, some strains of ***O. volvulus*** to chimpanzees (Dissanaike, 1979) and ***W. bancrofti*** to monkeys (Laing *et al.*, 1961; Cross *et al.*, 1979; Dissanaike and Mak, 1980; Harinasuta *et al.*, 1981; Palmieri *et al.*, 1982, 1983). Partial development of ***W. bancrofti*** is reported in Mongolian jirds (Ash and Schacher, 1971; Suswillo *et al.*, 1977; Cross *et al.*, 1981) hamsters (Dissanaike, 1974; Cross *et al.*, 1981) and cats (Laing *et al.*, 1961; Ramachandran *et al.*, 1966; Ramachandran and Sivanandam, 1970), where an adult male worm was recovered from a jird after 92 days; and advanced fourth-stage larvae or young adults were recovered from hamsters after 60 days. This failure to provide a suitable laboratory model for the parasite, may be due either to an inhibitory factor from the host which is actively suppressing further development of the worm, or it lacks of an essential nutrient required by the parasite for continued maturation.

There have been many attempts to introduce ***Brugia*** into common laboratory rodents because experimental animals like cats, dogs and monkeys are very expensive to use and also difficult to handle. Infections of the mouse for which inbred strains as well as a variety of biochemical and immunological reagents are available, are not very successful (Laing *et al.*, 1961; Chong and Wong, 1967; Furman and Ash, 1983; Howells *et al.*, 1983). Similarly difficulty is observed in another species of jird ***(M. libycus)***, wood rat ***(Neotoma lepida)*** and kangaroo rat ***(Dipodomys merriami)*** (Ash and Riley, 1970 a, b). Successful results were obtained from infections of ***B. pahangi*** in T-cell deprived mice and nude (athymic) mice (Suswillo *et al.*, 1980, 1981; Vincent *et al.*, 1982); white rat (Sucharit and Macdonald, 1972, 1973; Harbut, 1973); Lewis rat (Vincent and Vickery, 1983); multimammate rat, ***Mastomys natalensis*** (Petranyi and Mieth, 1974; Sanger *et al.,* 1981; Reddy *et al.*, 1984); golden hamster, ***Mesocricetus auratus*** (Laing *et al.*, 1961; Edeson *et al.*, 1962; Sucharit and Macdonald, 1972; Harbut, 1973; Malone *et al.*, 1974; Carraway and Malone, 1985), in which microfilariae and adult worms were found. The most successful attempts have been made with infections of ***B. pahangi, B. malayi*** and ***B. patei*** in Mongolian jirds, ***Meriones unguiculatus*** (Ash and Riley, 1970 a, b; Ash, 1973 b). Adult worms are found in the heart, pulmonary arteries, lymphatics, testes and occasionally in the peritoneal cavity when infective larvae are inoculated subcutaneously (Ash and Riley, 1970 a, b; Ash, 1971, 1973 a; El Bihari and Ewert, 1971; Ah and Thompson, 1973). The parasite develop a chronic systemic lymphatic infection that resembles human filariasis (Ah and Thompson, 1973; Vincent *et al.*, 1980). If the larvae are given intraperitoneally the majority of adult worms are found in the peritoneal cavity with the exception of a few which penetrate the lymphatics, this route of infection is a rich source of all stages of the worm found in the definitive host (Ah and Thompson, 1973; Mc Call *et al.*, 1973). Ash (1971) also described the preferential susceptibility of male compared with female jirds to infection of ***B. pahangi.*** Eighty two percent of males and 24% of females become patent after subcutaneous inoculation of ***B. pahangi*** larvae. El Bihari and Ewert (1973) found a similar difference between the two sexes when they were infected with ***B. malayi,*** 96% of males and 43%

of females become patent after the inoculation of *B. malayi* larvae. Because of the reliability of ***Brugia*** infections in the jirds ***(Meriones unguiculatus)***, this system is now being used in a variety of studies, especially to elucidate the immunoregulatory mechanisms in lymphatic filarial infection. Ottesen (1980) also suggested that in humans infected with lymphatic dwelling filariae, ***W. bancrofti*** and ***B. malayi***, immunoregulatory mechanisms play an important role in detrmining both the parasitological and clinical outcome of infection. However, studies to elucidate the immunoregulatory mechanisms that operate in filarial infections are difficult to achieve in humans. More information about the parasite development and the immunological reactivity of the infected host are still needed to better understand the infection.

Therefore, the following objectives have been drawn up for the present study. 1. To investigate parasitologically the infection of ***B. pahangi*** in jirds inoculated subcutaneously with different numbers of the infective larvae. 2. To determine the levels, durations and pattern of eosinophilia and some other haematological parameters in jirds infected with the various infective dose of ***B. pahangi*** by using the quantitation eosinophil count, total WBC count and differntial WBC count. The blood picture, haematocrit and microfilarial densities were also examined. 3. To detect the number of antibody producing cells and the immune regulatory mechanisms in the spleen of jirds infected with different quantum of ***B. pahangi*** at different times of post-infection by using Plaque Forming Cell Assay (PFCA) to SRBC. 4. To further investigate and analyse the immunoregulatory events responsible for producing the alterations in mitogens and parasite specific antigens reactivity of lymph node and spleen cells from jirds infected with different quantum of ***B. pahangi*** at different times of post-infection by using ***in vitro*** lymphocyte blastogenesis assay. 5. To determine the IgG antibody levels in sera of all the infected jirds by using ELISA technique with different sources of homologous antigens.

Table 1.1 The species, natural mammalian hosts, sites of infection and geographic distribution of the genus *Brugia* (After Denham and McGreevy, 1977).

Species	Natural Mammalian Hosts	Site of Infection	Distribution
Brugia malayi	Man *(Homo sapiens)*	Lymphatics	Asia
(Brug, 1927)	Long-tailed macaque	and	
Buckley, 1958	*(Macaca irus)*	Lymph nodes	
	Dusky leaf-monkey		
	(Presbytis obscurus)		
	Banded leaf-monkey		
	(Presbytis melalophos)		
	Silvered leaf-monkey		
	(Presbytis cristatus)		
	Cat *(Felis catus)*		
	Wild cat *(Felis bengalensis)*		
	Wild cat *(Felis planiceps)*		
	Civet cat *(Paradoxurus*		
	hermaphroditus)		
	Civet cat *(Arctogalidia*		
	trivirgata)		
	Pangolin *(Manis javanica)*		

Table 1.1 continued.

Species	Natural Mammalian Hosts	Site of Infection	Distribution
Brugia pahangi (Buckly and Edeson, 1956)	Dusky leaf-monkey *(P. obscurus)* Slow loris *(Nycticebus coucang)*	Lymphatics and Lymph nodes	West Malaysia
Buckley, 1958	Cat *(F. catus)* Dog *(Canis familiaris)* Tiger *(Panthera tigris)* Wild cat *(F. bengalensis)* Wild cat *(F. planiceps)* Civet cat *(P. hermaphroditus)* Civet cat *(A. trivirgata)* Civet cat *(Viverra zibetha)* Civetcat *(Arctictus binturong)* Otter *(Lutra sumatrana)* Pangolin *(M. javanica)* Moom-rat *(Echinosorex gymnurus)* Giant-squirrel *(Ratufa bicolor)*		

Table 1.1 continued.

Species	Natural Mammalian Hosts	Site of Infection	Distribution
Brugia patei (Buckley, Nelson and Heisch, 1958) Buckley, 1958	Dog *(C. familiaris)* Cat *(F. catus)* Genet cat *(Genetta tigrina)* Bush baby *(Galago crassicaudata)*	Lymphatics and Lymph nodes	Kenya
Brugia buckleyi Dissanaike and Paramananthan, 1961	Ceylon hare *(Lepus nigricollis singhala)*	Heart, Pulmonary arteries, hepatic veins, vena cava	Sri Lanka
Brugia ceylonensis Jayewardene, 1962	Dog *(C. familiaris)*	Lymph nodes and associated tissues	Sri Lanka
Brugia beaveri Ash and Little, 1964	Raccoon *(Procyon lotor)*	Lymph nodes "skin and body soakings"	U.S.A (Louisiana)
Brugia guyanensis Orihel, 1964	Coatimundi *(Nasua nasua vittata)*	Lymphatics	Guyana
Brugia tupaiae Orihel, 1966	Tree shrew *(Tupaia glis)* Tree shrew *(Tupaia tana)*	Lymphatics	Malaysia, Thailand, North Borneo

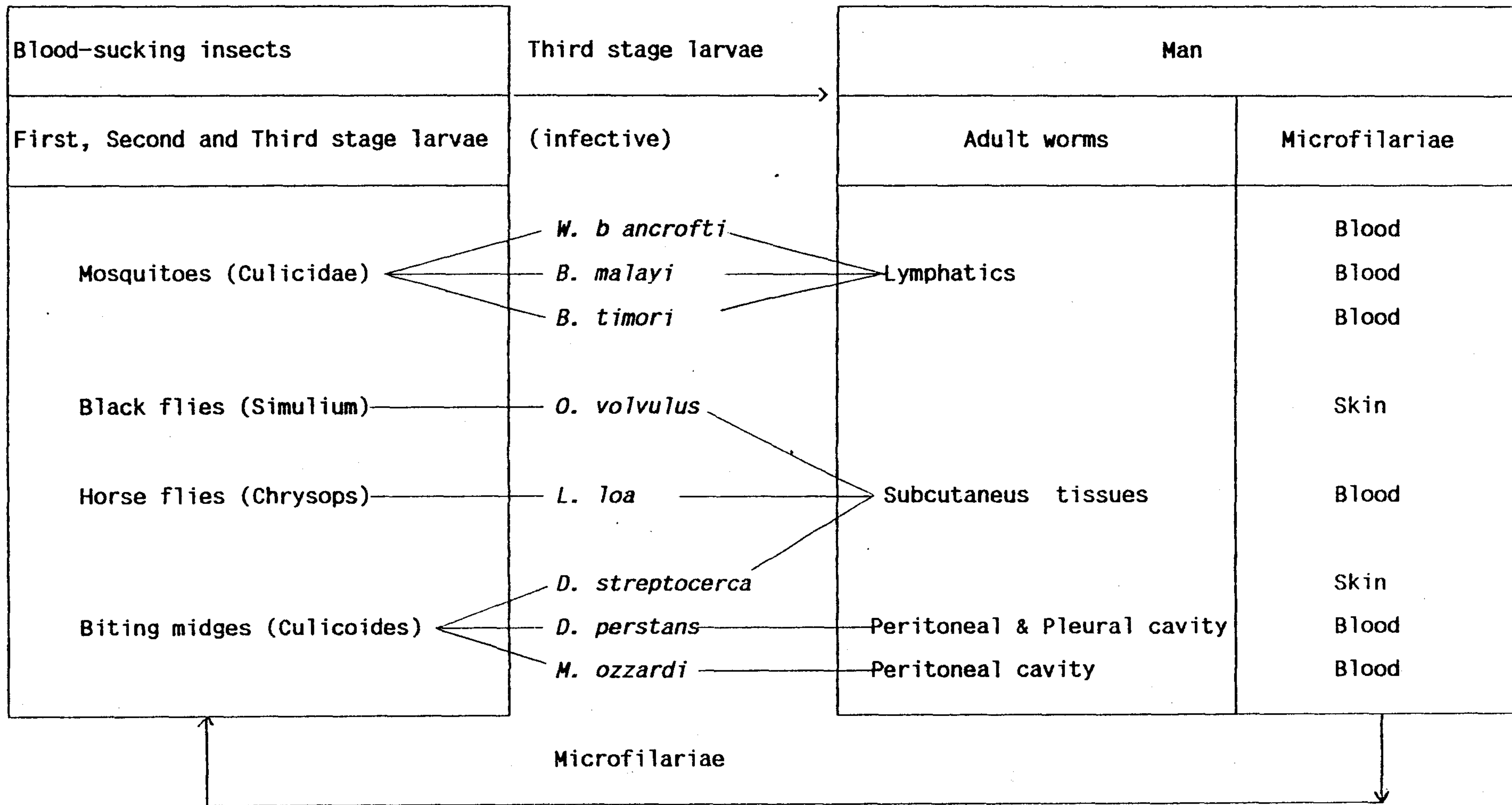

Figure 1.1 Life cycle diagram of human filariae

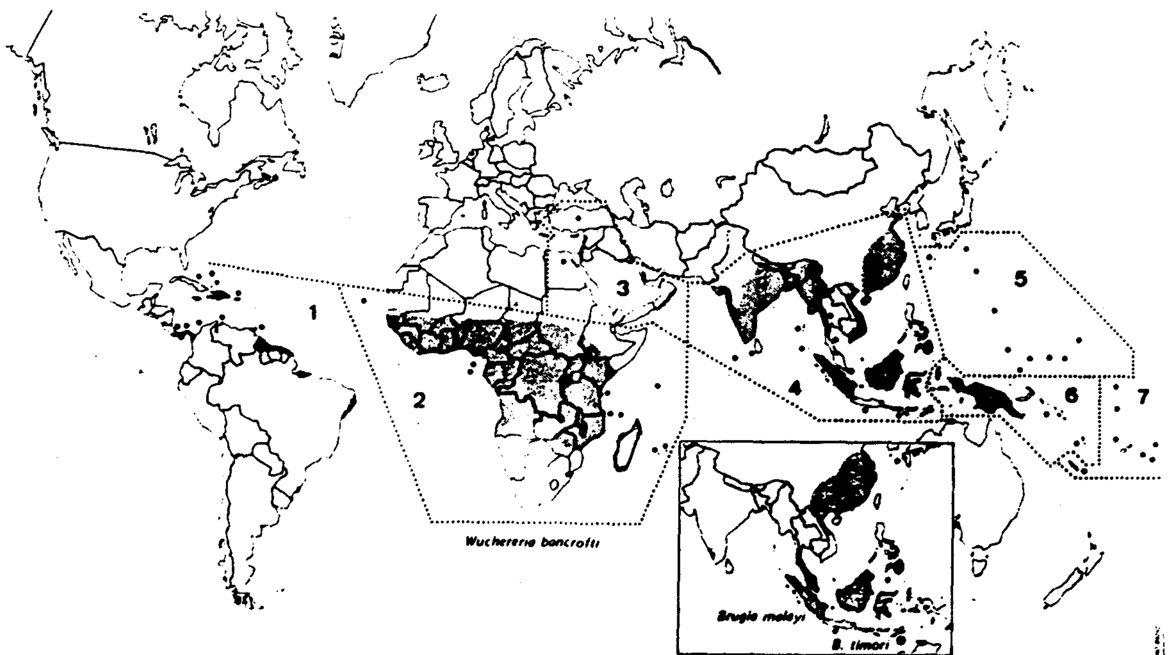

Figure 1.2 Distribution of the lymphatic filariasis.

(From Lymphatic Filariasis, Fourth report of the WHO Expert Committee on Filariasis, WHO Technical Report Series 702, 1984).

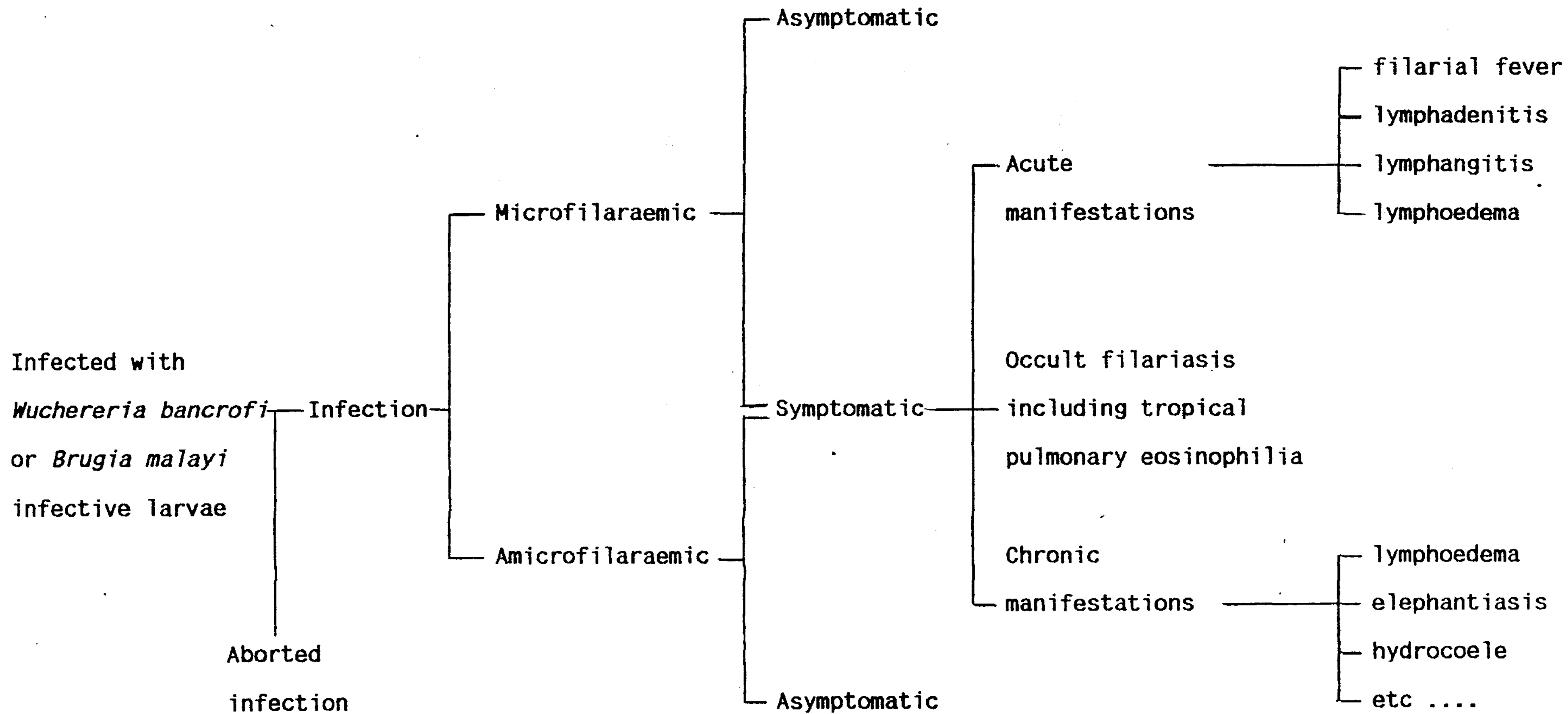

Figure 1.3 Spectrum of clinical outcomes due to infection with *Wuchereria bancrofti* or *Brugia malayi*

Figure 1.4 Circadian periodicity of the microfilariae of B. malayi in different hosts expressed as a proportion of the maximum parasitaemia. (a) Nocturnally periodic strain in man (Turner and Edeson, 1957). (b) Nocturnally subperiodic strain in man (Turner and Edeson, 1957). (c) Subperiodic strain in cats ("A" from Edeson, 1959; "B" from Burren, 1972). (d) Subperiodic strain in two species of monkeys (Edeson, 1959).
(After Denham and McGreevy, 1977).

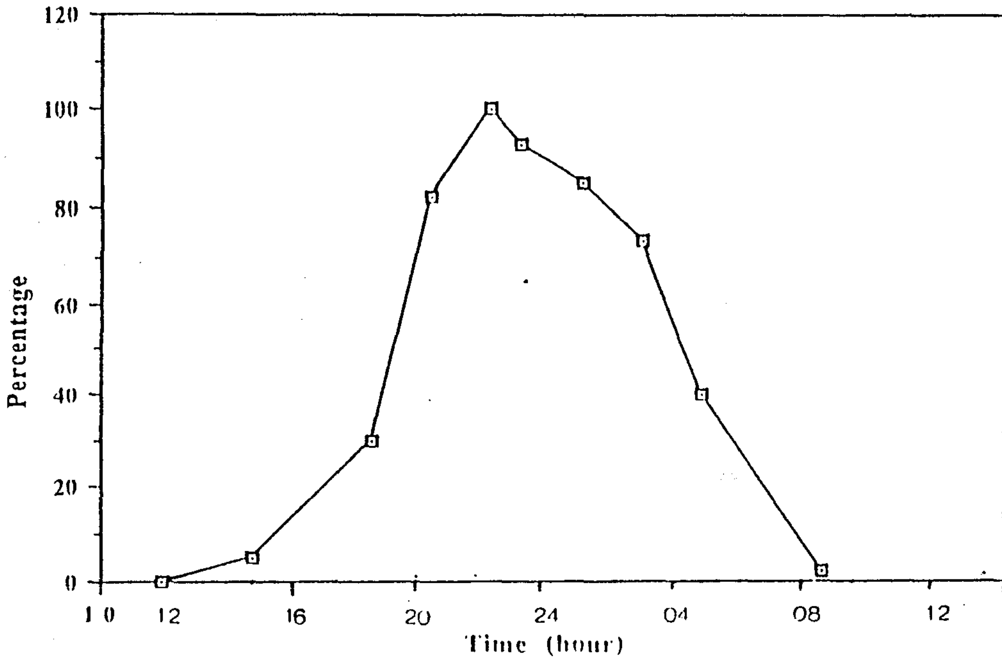

Fig. 1.4 a

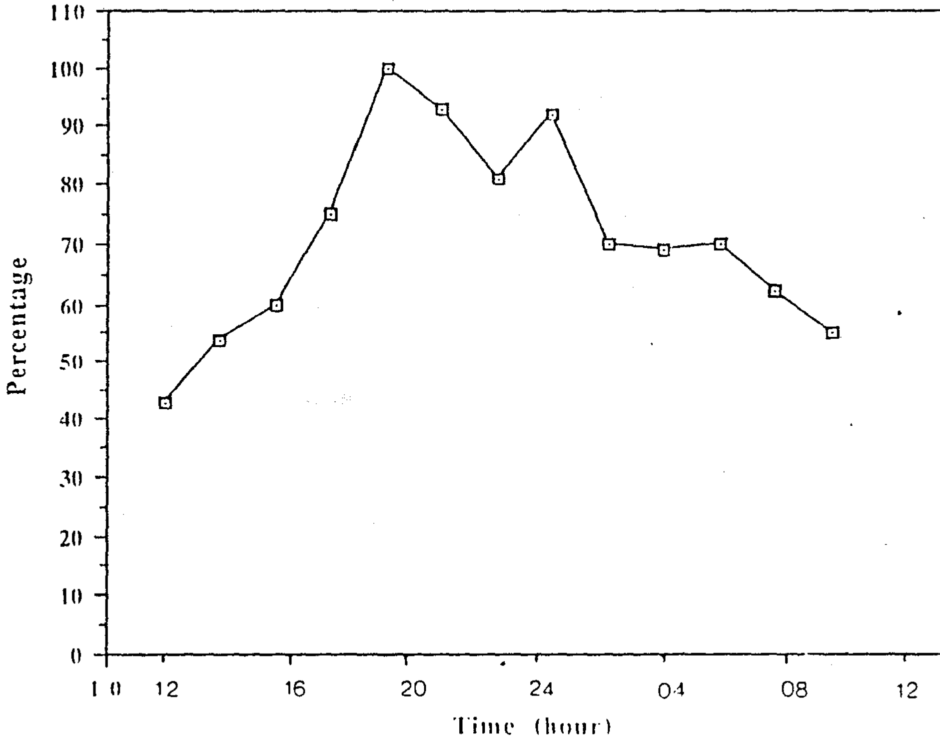

Fig. 1.4 b

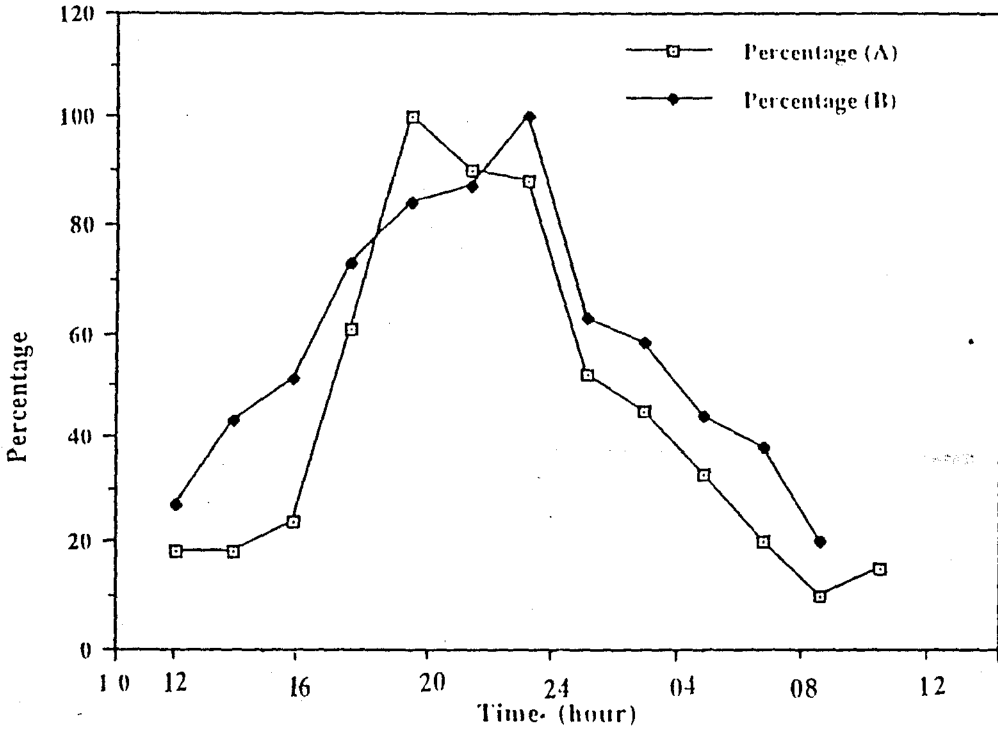

Fig. 1.4 c

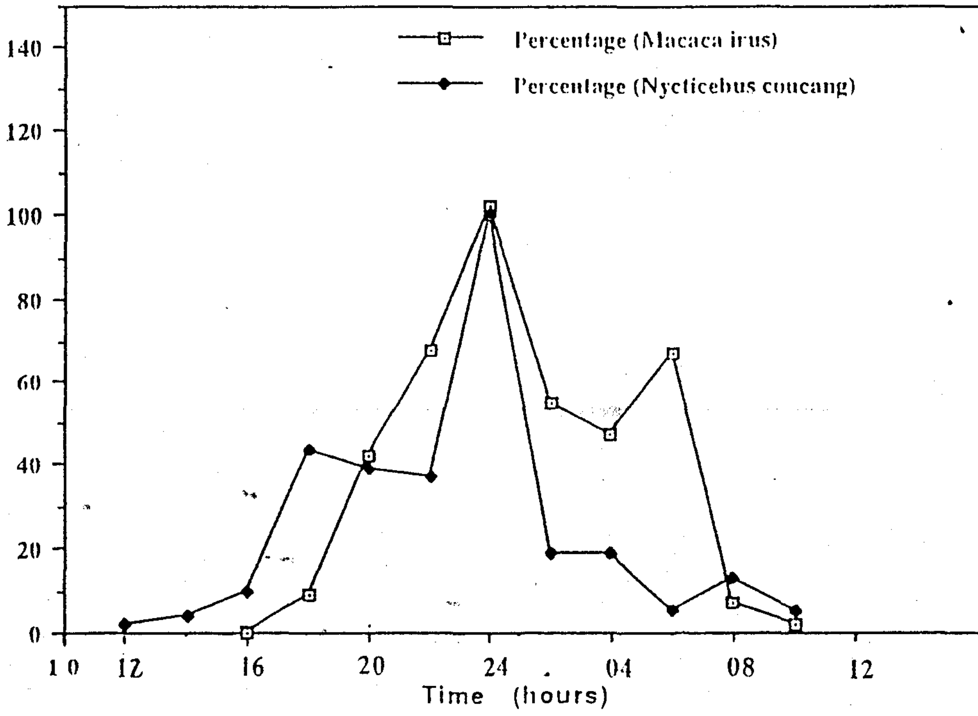

Fig. 1.4 d

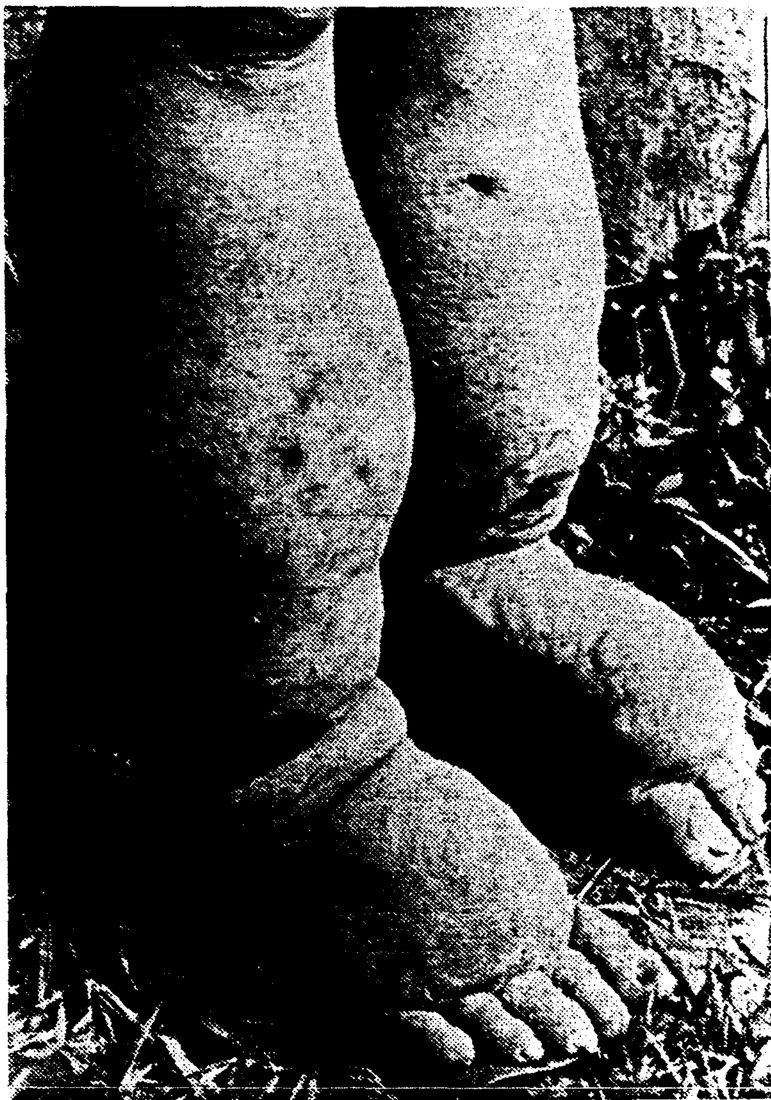

Plate 1.1 Elephantiasis caused by *Brugia malayi.*

Photo courtesy of Dr. Mak Joon Wah, Institute for Medical Research, Kuala Lumpur (From Parasitologi Perubatan by Vijayamma Thomas, 1983).

Plate 1.2 Elephantiasis caused by *Wuchereria bancrofti.*

Photo courtesy of the Wellcome Trust (From TDR news No. 32, June 1990).

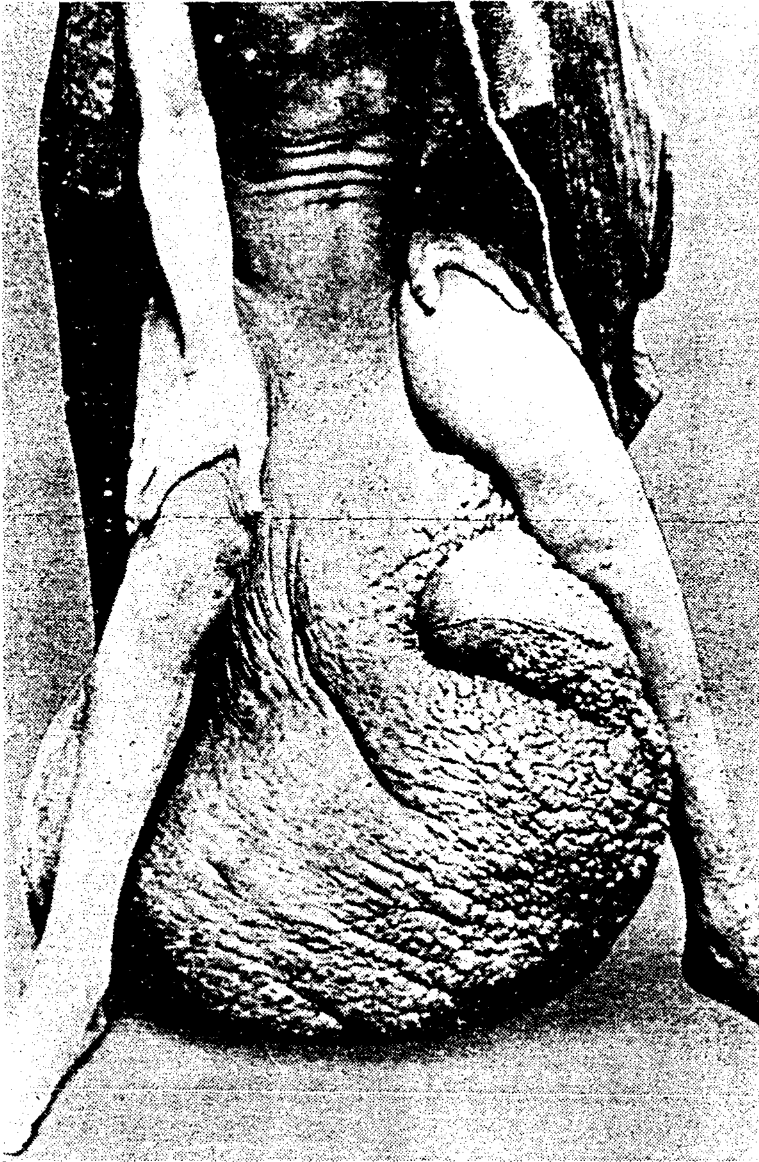

Plate 1.3 Elephantiasis of the scrotum due to *Wuchereria bancrofti.*

(From Craig and Faust's Clinical Parasitology, Eight Edition, 1977).

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 PARASITE

The strain of ***Brugia pahangi*** was originally obtained from the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia and maintained in local domestic cats in our animal unit at School of Medical Sciences, University Sciences Malaysia, Kelantan, Malaysia for use throughout this study.

2.2 EXPERIMENTAL ANIMALS

2.2.1 Cat

Local domestic cats were caught from the areas around the campus of University Sciences Malaysia, Penang, Malaysia and screened for any possible infection by blood examination for microfilariae and stool examination for intestinal parasites. The worm-free cats were used as reservoir hosts to maintain the infection of ***Brugia pahangi*** and served as a source of microfilariae to mosquito vectors. Cats were fed twice a day with rice and fish at morning time and cat's food in package at evening time, the water was available ***ad libitum.***

2.2.2 Jird

Mongolian jirds ***(Meriones unguiculatus)***, (Plate 2.1), male 6 - 8 weeks - old were used throughout the study. The single strain of jirds were originally obtained from Institute for Medical Researeh (IMR), Kuala Lumpur, Malaysia and were bred in our animal unit. They were inbred and golden brown in colour. The animals were maintained at room