GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) OF CTLA-4 AND FOXP3 GENES IN LYMPHATIC FILARIASIS AND SCHISTOSOMIASIS

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GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) OF *CTLA-4* AND *FOXP3* GENES IN LYMPHATIC FILARIASIS AND SCHISTOSOMIASIS

by

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LIST OF ABBREVIATIONS

	Description	Abbreviations
1	Asymptomatic amicrofilareamia	AsAm
2	Asymptomatic microfilaraemia	AM
3	Base pair(s)	bp
4	Celcius	^{o}C
5	Chi-square test	χ^2
6	Confident interval	CI
7	Conventional polymerase chain reaction	cPCR
8	Cytotoxic T-lymphocyte antigen 4	CTLA-4
9	Cluster of differentiation	CD
10	Deoxyribonucleotide triphosphate	dNTP
11	Deoxyribonucleic acid	DNA
12	Double strand deoxyribonucleic acid	dsDNA
13	Diethylcarbamazine	DEC
14	Ethylenediaminetetraacetic acid	EDTA
15	Forkhead box P3	FoxP3
16	Gram(s)	*g*
17	Hardy-Weinberg equilibrium	HWE
18	Heterozygous forward primer	HF
19	Heterozygous reverse primer	HR
20	Immunoglobulin G4	IgG4
21	Interleukin	IL
22	Liter(s)	L
23	Luria Bertani	LB
24	Linkage disequilibrium	LD
25	Lymphatic filariasis	LF
26	Microgram(s)	µg
27	Microliter(s)	µl
28	Micrometre(s)	µm
29	Microfilaria	mf
30	Milligram(s)	mg
31	Milliliter(s)	ml
32	Millimetre(s)	mm
33	Millimolar(s)	mM
34	Minor groove binder	MGB
35	Molar	M
36	Mutant forward primer	MF
37	Mutant reverse primer	MR
38	National Centre for Biotechnology Information	NCBI
39	Nanometer	nm
40	Non-template control	NTC
41	Odd ratio	OR
42	Picomole	pmol
43	Polymerase chain reaction	PCR
44	Probability	*P*
45	Real-time polymerase chain reaction	qPCR

46	Registered	®
47	Single nucleotide polymorphism	SNP
48	The Single Nucleotide Polymorphism Database	dbSNP
49	Trademark	™
50	Unit	U
51	Wild type forward primer	WF
52	Wild type reverse primer	WR
53	World Health Organization	WHO

PENGGENOTIPAN POLIMORFISME NUKLEOTIDA TUNGGAL (SNP) PADA GEN *CTLA-4* DAN GEN *FOXP3* DALAM LIMFATIK FILARIASIS DAN SCHISTOSOMIASIS

ABSTRAK

Filariasis limfatik (LF) merupakan sejenis penyakit jangkitan parasit yang disebabkan oleh cacing halus daripada spesis *Brugia* dan *Wuchereria* yang hidup di dalam sistem limfatik manusia. Penyakit ini adalah penyumbang terbesar kepada ketidakmampuan dan perubahan kekal kepada pesakit yang tinggal di kawasan endemik. Manakala penyakit schistosomiasis pula yang disebabkan oleh cacing dari spesis *Schistosoma*, merupakan salah satu daripada penyakit jangkitan parasit yang paling kerap berlaku di dunia; yang menyebabkan kerosakan pada organ dalaman dan menyekat kadar pembesaran kepada kanak-kanak. Kecenderungan terhadap infeksi dan keterukkan penyakit berhubung kait dengan kepelbagaian variasi genetik manusia. Walau bagaimanapun, perkaitan ini masih belum dikaji terutamanya pada gen *CTLA-4* dan gen *FoxP3* yang diekspreskan oleh Tregs, untuk fungsi dan sumbangan gen-gen ini terhadap risiko infeksi helminth yang kronik. Pendekatan penggenotipan SNP telah digunakan dalam kajian ini bagi mengenalpasti SNP dalam LF yang disebabkan oleh *Brugia malayi* dan schistosomiasis yang disebabkan oleh *Schistosoma haematobium*. Secara ringkasnya, kaedah kajian ini melibatkan pengestrakkan dan pengkuantitian sampel-sampel DNA daripada kumpulan terinfeksi parasit dan kumpulan kawalan sihat daripada kawasan endemik yang terlibat. Kemudian, dengan menggunakan lima SNP daripada gen *CTLA-4* dan satu SNP daripada gen *FoxP3*, sampel-sampel bagi kawalan positif telah dipilih dengan menjalankan penggenotipan berskala kecil dengan real-time PCR terhadap sepuluh sampel dan seterusnya diikuti dengan analisa pengesahan jujukan dan penjajaran DNA. Selepas itu, penggenotipan

berskala besar telah dilakukan ke atas semua sampel. Akhirnya, analisis terhadap genotip telah dijalankan menggunakan beberapa kaedah statistik. Pengesahan genotip melalui penjujukan ke atas tiga kawalan positif iaitu 6944W, 6725H dan 20M didapati mempunyai keputusan yang konsisten dengan keputusan yang diperolehi daripada real-time PCR. Empat daripada enam sampel kawalan positif (6725H, 6944W, 8209H dan 20M) yang tidak mencukupi sumber DNA telah berjaya diklonkan. Analisis penjujukan dan kromatogram plasmid telah mengesahkan genotip yang dijangka. Penggenotipan berskala besar telah berjaya dijalankan ke atas 571 sampel pesakit dan kawalan. Dengan menggunakan ujian chi-square bagi menganalisa frekuensi alel, signifikasi yang rendah di lihat ke atas pesakit LF yang membawa rs733618G dibandingkan dengan kawalan sihat (*P*= 0.032). Didapati hanya rs16840252 yang tidak signifikan daripada lima SNPs dalam kumpulan schistosomiasis apabila dibanding dengan kumpulan kawalan sihat. Analisa taburan genotip menunjukkan perbezaan yang signifikan (*P*= 0.013) di antara pesakit LF dengan kawalan sihat bagi rs733618. Sementara itu bagi sampel daripada kawasan endemik schistosoma, julat 'odd ratio' (OR) bagi taburan genotip untuk semua SNP kecuali bagi rs16840252 dan rs231775 adalah menaik dari 0.61 ke 5.65 bagi pesakit apabila dibandingkan dengan kawalan sihat (*P*<0.05). Kesemua SNP di dalam empat set data yang berbeza adalah di dalam keseimbangan Hardy-Weinberg (HWE) (*P*>0.05), kecuali bagi rs11091253 dalam set data pesakit schistosomiasis. Kajian hubung kait pesakit LF yang membawa genotip rs733618 AG (*P*= 0.02) dan pembawa alel minor (AG + GG) (*P*= 0.01) menunjukkan penurunan perbezaan yang sedikit terhadap risiko LF, setelah dibandingkan dengan genotip AA. Dikalangan kes asimptomatik amikrofilaraemik (AsAm), kaitan positif yang rendah diperolehi untuk setiap genotip dan varian bagi rs733618 dengan julat OR iaitu 0.27 – 0.45. Di dalam kes asimptomatik mikrofilaraemik (AM) pula, rs231775 telah menunjukkan penurunan risiko yang sedikit signifikan dengan julat OR iaitu 0.50 – 0.57. Dalam kumpulan schistosomiasis pula, rs733618 telah menunjukkan peningkatan yang

signifikan terhadap risiko infeksi (*P*= 0.012). Kesemua kohort telah memberikan empat haplotip yang serupa (>5%) apabila dilakukan pengiraan dengan Expectation-Maximization (EM)-Algorithm. Walau bagaimanapun, tiada hubung kait yang signifikan diperolehi antara haplotip dengan LF mahupun schistosomiasis. Kajian ini telah mengenalpasti SNP di dalam promoter gen *CTLA-4* yang mungkin secara fungsinya mempunyai hubungan dengan kecenderungan kepada LF dan schistosomiasis.

GENOTYPING OF SINGLE NUCELOTIDE POLYMORPHISMS (SNPs) OF *CTLA-4* AND *FOXP3* GENES IN LYMPHATIC FILARIASIS AND SCHISTOSOMIASIS

ABSTRACT

Lymphatic filariasis (LF) is a chronic parasitic disease caused by thread-like worms of *Brugia* and *Wuchereria* species that live in the human lymphatic system. It is a major cause of permanent disability and disfigurement in the endemic areas. Schistosomiasis is another chronic condition caused by schistosoma species which remains as a prevalent parasitic disease worldwide; it causes damage to internal organs and impair growth in children. Susceptibility to infections and disease severities are associated with a wide range of human genetic variations. However such association has not been well investigated especially with regard to *CTLA-4* and *FoxP3* genes expressed by Tregs, for their functional roles and contributions to the risk of chronic helminth infections. SNP genotyping approach was used in this study to identify potential SNPs in LF caused by *Brugia malayi* and schistosomiasis caused by *Schistosoma haematobium*. Briefly the methodology involved extraction and quantification of DNA samples from both parasitic diseases and healthy controls from the endemic areas. Subsequently, using five SNPs in *CTLA-4* gene and one SNP in *FoxP3* gene, samples for positive controls were selected by performing small scale real-time PCR genotyping on ten samples followed by confirmation by DNA sequencing. This was followed by large scale genotyping of all samples. Finally, analyses of the genotypes were performed using several statistical methods. Genotype confirmation by direct sequencing of three positive controls namely 6944W, 6725H and 20M was found to be identical with real-time PCR. Out of six positive control samples, four samples (6725H, 6944W, 8209H and 20M) with insufficient DNA material were successfully cloned. Plasmid sequencing and chromatogram analysis confirmed the expected genotypes. Large scale genotyping

was successfully performed on 571 patient and healthy control samples. Using chi-square test for allele frequency analysis, significant increase of rs733618A carriers was found in patients with LF compared with healthy control individuals (*P*=0.032). Out of five SNPs from schistosomiasis group, rs5742909 was found not be statistically significant when compared with healthy controls. Genotype distributions showed significant difference (*P*= 0.013) between LF patients and healthy controls for the rs733618. Meanwhile for samples from the schistosomiasis endemic area, the odd ratios (ORs) for the genotype distributions of all SNPs except rs16840252 and rs231775 increased from 0.61 to 5.65 in patients when compared with healthy controls (*P*<0.05). All SNPs in four different datasets were in Hardy-Weinberg Equilibrium (HWE) (*P*>0.05) except for the rs11091253 in schistosomiasis patients. Association studies revealed that LF carriers of the rs733618 AG genotypes (*P*= 0.02) and minor allele carriers (AG + GG) (*P*= 0.01) showed significant decrease risk for LF. Among the asymptomatic amicrofilaraemic (AsAm) cases, positive associations were found for all genotypes and variants of rs733618 with ORs ranging from 0.27 to 0.45. In the asymptomatic microfilaraemic (AM) cases, rs231775 showed significant decrease in risk with ORs ranging from 0.50 to 0.57. In schistosomiasis population, rs733618 showed significant increase in the risk for infection (*P*= 0.012). All cohorts harbored the same four common haplotypes (>5%) when computed with Expectation Maximization (EM) Algorithm. Nevertheless, there was no statistical significant association between haplotypes and LF or schistosomiasis. The study has identified SNPs in *CTLA-4* promoter gene that may be functionally linked with susceptibility to LF and schistosomiasis.

CHAPTER ONE

INTRODUCTION

1.1 Introduction to lymphatic filariasis

1.1.1 Lymphatic filariasis

Commonly known as elephantiasis, lymphatic filariasis (LF), is a devastating parasitic infection spread by mosquitoes. LF is caused by thread-like parasitic worms that can live for years inside human body and thrive in human lymphatic systems. Although it does not kill, the 1998 World Health Report ranked LF as the fourth leading cause of permanent disability (WHO, 1998). This disease is widespread and occurs throughout the tropical and sub-tropical regions of the world, such as Asia, the Pacific, Africa and the Americas (Figure 1.1). It is endemic in over 80 countries and 1.1 billion people are at risk of infection, 120 million people worldwide are already affected by the disease and about 40 million of those affected suffer debilitating disabilities (WHO, 1997). Statistics have shown that over 25 million men suffered from the genital form of the disease, while more than 15 million of people suffered from lymphoedema or elephantiasis of the leg (WHO, 2009). Out of this, 90% of the cases are caused by *Wuchereria bancrofti* whereas *Brugia malayi* accounts for about 10% (or 13 million) of the infected people especially in South and Southeast Asia, South Korea, and parts of China. However, *Brugia timori* is only restricted to Timor Leste and a few islands in Indonesia (Michael, 2000). The World Health Organization

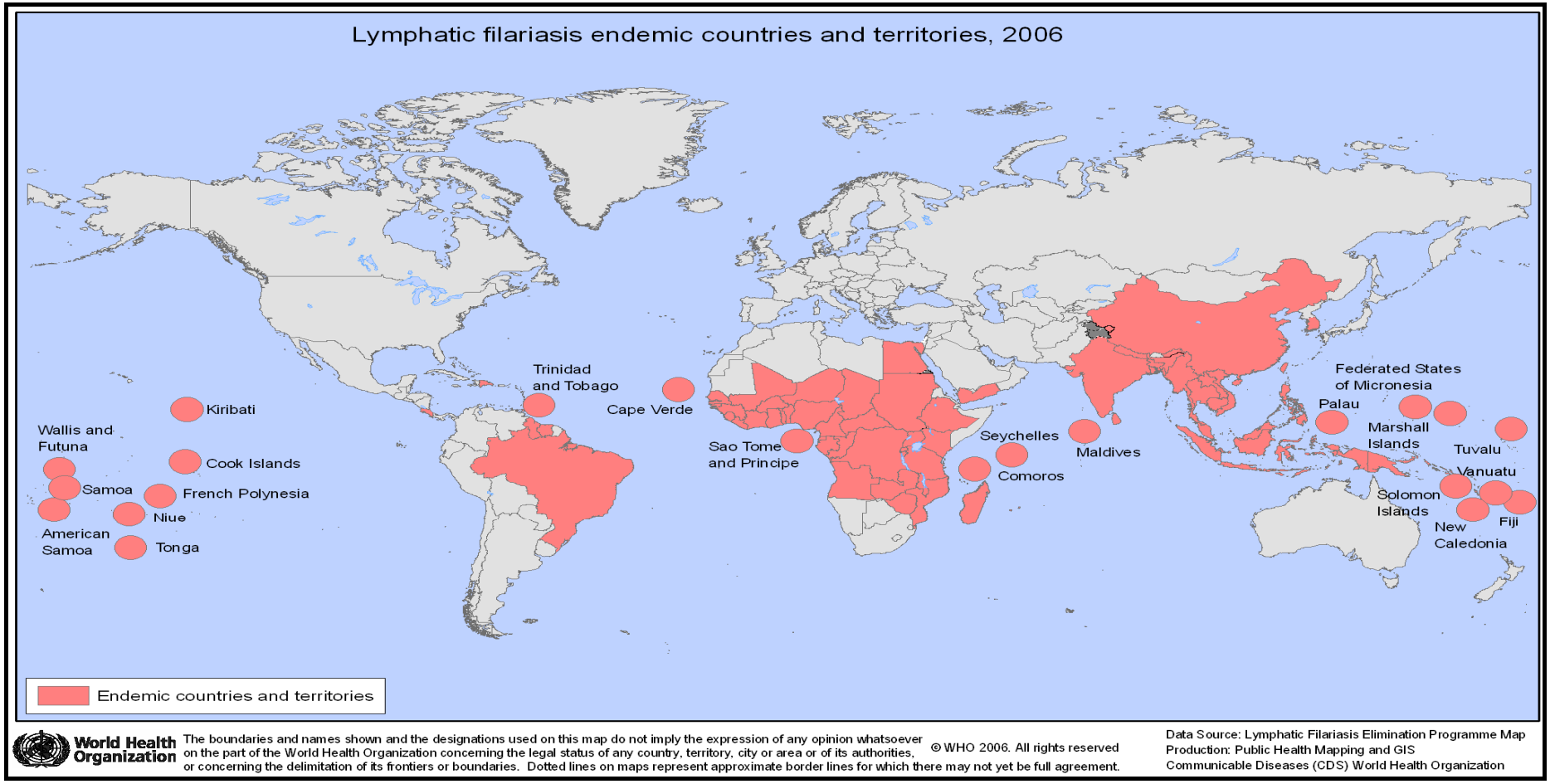

Figure 1.1 World endemic countries for lymphatic filariasis in 2006

Source:http://gamapserver.who.int/mapLibrary/app/searchResults.aspx

(WHO) in 1998 announced that LF is one of only six eradicable or potentially eradicable diseases and this was the stimulus that led to the current global campaign against LF (WHO, 1998). Consequently, a global programme for elimination of the disease known as the 'Global Programme to Eliminate Lymphatic Filariasis' (GPELF) was initiated in year 2000.

Patients with chronic manifestation are prevented from experiencing normal working and social life because of physiological consequences and obvious physical handicap. It is primarily a disease of the poor because of its prevalence in remote rural areas and the underprivileged periurban and urban areas and consequently reduces ability people to work, resulting in loss of family income. Thus, LF imposes an economic burden on infected individuals, their household, and the communities in which they live. Ultimately, LF is a disease that perpetuates the poverty cycle.

1.1.2 Transmission and life cycle

There are three lymphatic filarial that infect human namely *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori.* The disease is being transmitted to human by the bite of more than 70 species and subspecies of mosquitoes mainly *Anopheles, Aedes*, *Culex* and *Mansonia*; hence it is referred to as a vector-borne disease (Stone *et al*., 1959; Nanduri & Kazura, 1989). The three lymphatic filariae have similar biphasic life cycle where larval development takes place in mosquito (intermediate host) and adult development takes place in the human (definitive host). Basically, there are four different stages of the life cycle of lymphatic filaria as depicted in Figure 1.2.

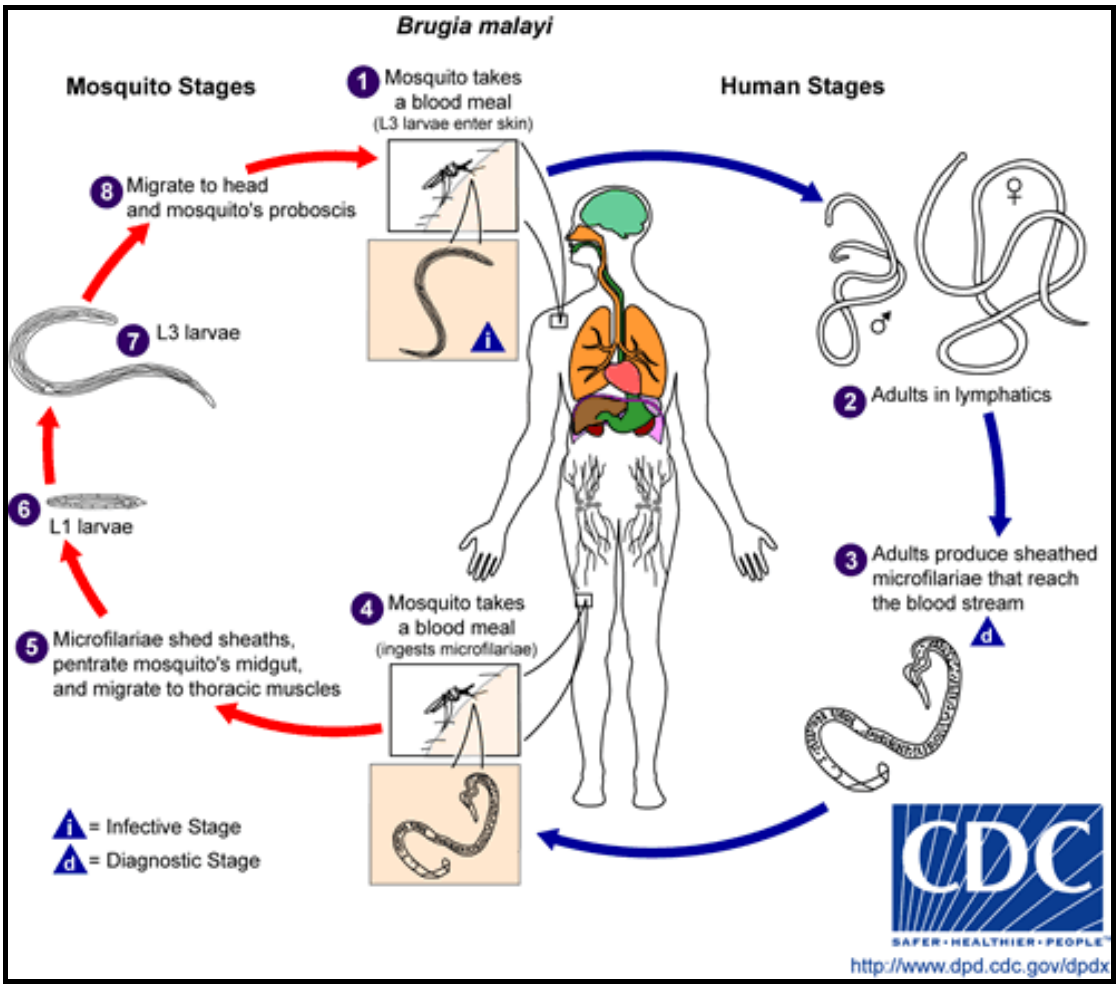

Figure 1.2 The life cycle of *Brugia malayi*

Source: www.dpd.cdc.gov/dpdx

Mosquito ingests microfilariae (mf) as part of its blood meal. Within a few hours, the mf penetrates the mosquito gut wall, migrates to the flight muscles and molts to the second larval stage (L_2). The L_1 and L_2 molt takes place between days 6 to 10 depending on the environmental conditions. After several days, the parasite undergoes an additional molt to the L_3 parasite. The L_3 then migrates from the flight muscles to the mouth parts of the vector, where they are positioned to be passed on to the vertebrate host during a subsequent blood meal (Scott, 2000).

Infection is initiated by the deposition of the third stage larvae (L_3) on the skin of human host following a bite by an infective mosquito. In the definitive host, the larval undergo an additional molt to the fourth larval stage (L_4) between days 9 and 14 post-infection as they mature into the lymphatic-dwelling adult male and female worms to complete the life cycle. Adult female parasites can remain reproductively active for more than 5 years. The lymphatic-dwelling filariae are diecious and undergo ovoviviparous reproduction resulting in the release of fully formed, sheathed first stage larvae (L_1 or mf) from the female. Then, the mf enter the peripheral circulation of the human host where they are available to be ingested by the vector during a blood meal (Scott, 2000).

The unique characteristic of the mf stage of the parasites is that they have “periodicity” that restricts their appearance in the blood to only certain periods of the day. In general, the mf can be classified into two main groups based on their circadian rhythms; i) the noctural periodic strain, ii) the nocturnaly

subperiodic strain. In the nocturnally periodic strain, they virtually disappear from the peripheral circulation and are found predominantly in the blood vessels of lungs and deep tissues during the daytime. Conversely, during the night time (especially between 10 pm until 2 am), the mf can be found in the peripheral blood. Meanwhile, for the nocturnally subperiodic strain, the mf is present in the blood both day and night but the density is much more higher during the night and lose their sheath in the process of drying on microscope slides (Bowman *et al.,* 2002). In addition to this basic strain, there is also nonperiodic or diurnally subperiodic strain of mf found in the South Pacific where it is transmitted mainly by day-biting mosquitoes of genus *Aedes* (Nanduri & Kazura, 1989).

In Malaysia, the predominant strain is the nocturnal subperiodic *B. malayi*. Due to its predominant appearance in blood at night, conventional diagnostic method is by microscopic examination of night blood. Studies of *Wuchereria* and *Brugia* species suggested that their periodicity is due to the difference in the oxygen tension between the arterial and venous blood in the lungs (Hawking & Gammage, 1968; Burren, 1972; Nanduri & Kazura, 1989). They suggested that if the difference in the arterial-venous oxygen tension is <50 millimeters of mercury (mm Hg), mf were accumulated in lungs and when oxygen tension exceeds 50 mm Hg during sleep, mf migrated from the pulmonary vasculature and appeared in the peripheral circulation. However, Wang and Saz (1974) suggested that these parasites have adapted their periodicity to the vector feeding behavior, possibly to facilitate their transmission (Nanduri & Kazura, 1989).

1.1.3 *Brugia malayi*

Brugian filariasis is caused by *Brugia malayi* and *Brugia timori* infection and it is endemic in parts of India, China, Indonesia, Philippines, Thailand, Vietnam, South Korea and Malaysia (WHO, 1998). However, China and South Korea have been recently certified as having eliminated the disease. This species was first identified by Lichtenstein and named by Brug in 1927. The *B. malayi* species is classified under the family of Filariidae and the genus of Brugia. The distribution of *B. malayi* is very similar to that of *W. bancrofti.* However, the differences between *W. bancrofti, B. malayi* and *B. timori* reside in their epidemiology, vectors, reservoirs, symptoms, but mainly the species morphology.

The brugia female worm measures 43 to 55 mm in length by 130 to 170 μm in width, and male worm measures 13 to 23 mm in length by 70 to 80 μm in width. Adult worms produce microfilariae, measuring 177 to 230 μm in length and 5 to 7 μm in width (Figure 1.3), which are sheathed (*http://www.dpd.cdc.gov*). The adult male and female worms of *B. malayi* inhabit primarily the lumen of lymphatics whereby the microfilariae usually migrate from the lymphatics into the blood stream (Nanduri & Kazura, 1989).

In Malaysia, repetition subperiodic strains of *B.malayi* is principally transmitted by mosquitoes such as *Mansonia annulata*, *Mansonia annulifera*, Mansonia *longiplapis* and *Mansonia annulifera* acting as secondary vectors (Lim, 2005). Generally, brugian filariasis leads to symptoms and manifestations that may include recurrent fever, lymphatic damage, renal

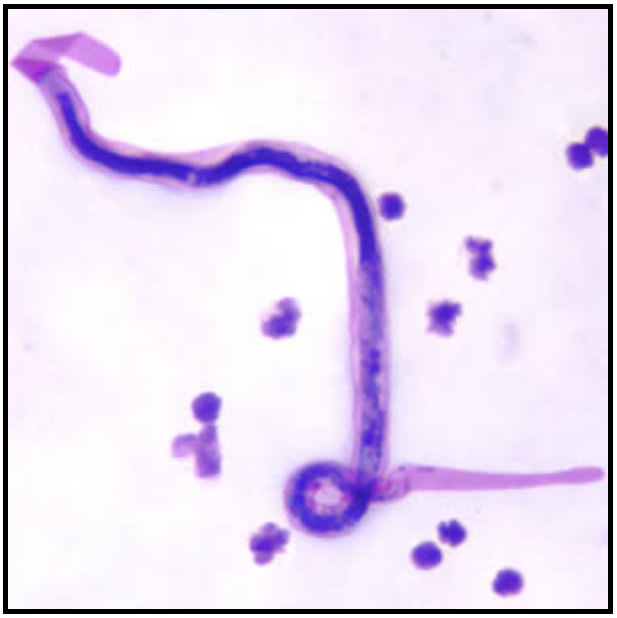

Figure 1.3 Microfilaria of *Brugia malayi*

Source : http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Filariasis_il.htm

damage, adenolymphangitis, lymphoedema, elephantiasis and pulmonary disease. However, the infection is usually limited to legs below the knee and upper limbs distal to elbow.

1.1.4 Clinical manifestation

Lymphatic filariasis is characterized by a wide range of clinical presentation. Clinical features and pathology of lymphatic filariasis depend on the sites occupied by developing and mature worms, the number of worms present, length of infection and the immune responses of the host especially to damaged and dead worms. In areas where LF is endemic, infections can be asymptomatic, acute and chronic. The clinical manifestations of LF vary from one endemic area to another and also differ to some extent on the species of the parasite.

Asymptomatic amicrofilaraemia individuals in the endemic community show no clinical manifestations or circulating microfilariae. This includes individuals with prepatent infection or adult worm infection without microfilaraemia. Microfilaraemic individuals are those with microfilariae circulating in their blood, and they may develop clinical manifestations (symptomatic microfilaraemia) or have no obvious clinical manifestations (asymptomatic microfilaraemia). Some of asymptomatic microfilaraemia individuals may remain microfilaraemic and asymptomatic for years or even for the rest of their lives. In contrast, obvious symptomatic filarial disease manifests itself in both acute and chronic forms, and maybe with or without infection.

In the acute form of lymphatic filariasis, there are recurrent attacks of fever (filarial fever) often described as adenolymphangitis (ADL) with painful inflammation of the lymph nodes (lymphadenitis) and lymph ducts (lymphangitis). The lymphatics involved are those of the limbs, genital organ (spermatic cord) and breasts. In bancroftian filariasis, the lymph glands in the groin and lymphatics of the male genitalia are frequently affected. Inflammation of the spermatic cord and repeated attacks can lead to blockage of the spermatic lymph vessels, leading to accumulation of fluid in the scrotal sac which becomes distended (hydrocele). In brugian filariasis, the affected lymph nodes are mostly situated in the inguinal and axillary regions with inflammation of distal lymphatics (Cheesebrough, 2000).

Chronic lymphatic filariasis is characterized by hydrocele, lymphoedema and elephantiasis. Hydrocele is the most common chronic manifestation in bancroftian filariasis. Microfilariae are rarely found in the blood of patients with hyrdocele or elephantiasis but can be found occasionally in hydrocele fluid. On the other hand, chronic lympoedema progressing to elephantiasis is a complication of advanced lymphatic filariasis and most commonly affect the legs. The arms, scrotum, penis, vulva and breasts are more rarely affected. Following recurrent episode of acute attacks, first pitting oedema and then chronic non-pitting oedema with loss of skin elasticity and fibrosis develops. In the leg, loss of contour is first observed around the ankle. Over several years the oedema becomes non-pitting with thickening and loss of skin elasticity. Further progression leads to evident elephantiasis with skin folds, dermatosclerosis and papillomatus lesions. Secondary bacterial and fungal

infections are common in the lymphoedematous skin, and probably exarcerbate the progression of elephantiasis. In severe case pus may ooze from chronis ulceration in the effected part, which may also emanate a foul smell (Simonsen, 2008).

1.1.5 Diagnosis of lymphatic filariasis

The diagnosis of brugian LF caused by *B. malayi* and *B. timori* can be performed using conventional laboratory methods such as thick blood smear technique, DNA-based laboratory assay using the PCR technique, ultrasonography and immunoassays. For thick blood smear technique, 60 µl of blood is spread onto the slide, dried, stained and examined under the microscope for the presence of microfilariae. This method is insensitive for active infections; it misses people with low mf counts and those with amicrofilaremic infections; these individuals have the potential to contribute to future transmission. In addition, night blood collection is troublesome to both the staff and villagers and impractical in some endemic areas (Weil & Ramzy, 2006). The sensitivity of this technique can be improved by membrane filtration of 1-5 ml of blood prior to microscopy.

Molecular diagnostics by PCR is available for LF diagnosis. The main obstacle with the PCR method is that it requires sophisticated laboratory equipment and trained personnel to perform the analysis; and requires several hours to obtain the results. Thus, it is not practical to be used with large number of samples and for field screening. In addition, PCR generally

do not detect people with amicrofilaraemic infection and still requires night blood smear for maximum sensitivity.

Ultrasonography, the detection is based on 'filarial dance sign' which refers to live adult worm inside the lymphatic vessels. Other than not suitable for large scale studies, it is not very useful for brugian filariasis in which the adult worms are not found in the peripheral lymphatics.

Immunoassays detect the presence of specific antigens or antibodies in the blood of individuals. Antigen detection assay is not available for brugian filariasis. With regard to detection of specific antibodies, it was recognized that the elevated levels of IgG4 is a marker of active infection rather than past exposure and it does not cross-react with antigens from non-filarial helminthes (Lal & Ottesen, 1988; Kwan-Lim *et al.*, 1990; Turner *et al.*, 1993; Haarbrink *et al.*, 1995, Chanteau *et al*., 1995; Terhell *et al.*, 1996; Rahmah *et al.*, 1998). To date, there are two commercialized rapid tests for diagnosis of brugian lymphatic filariasis which is based on detection of anti-filarial IgG4 antibody test namely Brugia Rapid^{TM} and PanLF Rapid^{TM}. The latter can be used for detection of both brugian and bancroftian filariasis.

Brugia Rapid^{TM} is a novel test for rapid detection of brugian filariasis. The test is based on the use of the *Bm*R1 recombinant antigen derived from the specific *Bm*17DIII gene of *B. malayi*. In laboratory evaluations and field studies, Brugia Rapid^{TM} has been shown to be highly sensitive (>95%) and specific (≥99%) for detection of *B. malayi* and *B. timori* infections, although it

also cross-reacts with some *W. bancrofti* positive sera (Rahmah *et al*., 2001, Lim *et al.*, 2001, Rahmah *et al*., 2003, Lammie *et al*., 2004, Supali *et al.*, 2004, Jamail *et al*., 2005, Fischer *et al.,* 2005, Melrose *et al*., 2006, Shenoy *et al*., 2007). In a field trial in Malaysia, which is a low endemic area, Brugia Rapid™ detects about 9-10 times more positive cases than parasitological diagnosis through use of night blood smear (Jamail *et al*., 2005), while in the high endemic area of Indonesia, Brugia Rapid™ shows an increase in detection of about three times (Supali *et al*., 2004).

Similar to the Brugia Rapid™ for IgG4 antibodies detection, the PanLF Rapid™ test combined two recombinant antigens namely *Bm*R1 and *Bm*SXP; the latter is derived from *SXP1* gene isolated from *B. malayi* adult worm cDNA library for detection of bancroftian filarial infections. Therefore, it can be used to detect all three species of filariasis i.e *B. malayi*, *B. timori* and *W. bancrofti*. A multicenter international evaluation of PanLF Rapid™ conducted at five institutions showed an overall high sensitivity (96.5%) and overall specificity of 99.6% (Rahmah *et al*., 2007).

1.1.6 Treatment of lymphatic filariasis

The chemical 1-diethylcarbamyl-4-methylpiperazine hydrocholide was first synthesized in 1947 and first used as a treatment for lymphatic filariasis (Santiago-stevenson *et al*., 1947). Now known as diethylcarbamazine (DEC), it was quickly accepted as the standard treatment for lymphatic filariasis and until today, DEC still remains the best drug that is generally available for the treatment and prevention with filariasis (WHO, 1995). It has both

microfilaricidal and macrofilaricidal activities, but some adult worms may survive even after repeated courses of therapy.

The drug is taken orally (tablet 50 mg, 100 mg) in the form of dihydrogen citrate under the brand names Hetrazan, Banocide and Notezine, and is reported to be stable even under extreme heat such as autoclaving or cooking (Gelband, 1994). It is almost completely absorbed after oral administration and is widely distributed in non-fatty tissue. It is rapidly and extensively metabolized and the residuum is recovered unchanged in the urine within 48 hours. The plasma half-life is usually within 6-12 hours (WHO, 1995). DEC's mode of action is still poorly understood. Somehow, it alters the surface membranes of supposedly unrecognizable microfilariae in the blood into antigenic foreign bodies easily detectable by the host immune system (Lim, 2005).

Addiss and Dreyer (2000) recommended that DEC should be part of the treatment regiment given to all patients who exhibit clinical manifestation(s) of lymphatic filariasis. It is widely accepted that total cumulative dosage of 72 mg/kg are necessary to eliminate *W. bancrofti* infections. Lower dosages are recommended by some authorities for treating *B. malayi* and *B. timori* infections. Annual mass treatment with single-dose DEC and albendazole is the central strategy for the Global Programme to Eliminate Filariasis (GPELF). Full course DEC should be reserved for patients who have evidence of active infection with lymphatic filariasis, including those who are asymptomatic. For both the acute and chronic manifestations of lymphatic

filariasis, supportive or specific clinical care is important (Addiss & Dreyer, 2000).

1.2 Introduction to schistosomiasis

1.2.1 Schistosomiasis

Schistosomiasis also known as bilharzias, bilharziosis or snail fever, remains one of the most prevalent parasitic diseases in the world. It is caused by helminth parasites of the genus *Schistosoma*. More than 200 million individuals infected, of whom over half suffer from related morbidity (Steimann *et al*., 2006). Whilst the global burden of schistosomiasis has been estimated at 1.7 to 4.5 disability-adjusted life years (DALYs) (WHO 2002, WHO 2004), new research suggests this is a considerable underestimation of the 'true' burden of schistosmoniasis (King *et al*., 2005; Jia *et al*., 2007). This disease is commonly found in Africa, the Caribbean, eastern South America, East Asia and in the Middle East, especially in areas where the water contains numerous freshwater snails, which may carry the parasite (Figure 1.4). *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma intercalatum* are distributed throughout Africa, while *Schistosoma japonicum* is found in Southeast Asia and parts of China. Another species namely *Schistosoma mekongi* is mainly found in Cambodia and Laos. An estimated 85% of the world's cases of schistosomiasis are in Africa, where prevalence rates can exceed 50% of local populations (Chitsulu *et al*., 2000). Statistics have shown that 70 million may have heamaturia associated with *S. haematobium* infection, 18 million major bladder wall pathology and 10 million hydronephrosis. It is estimated that kidney failure

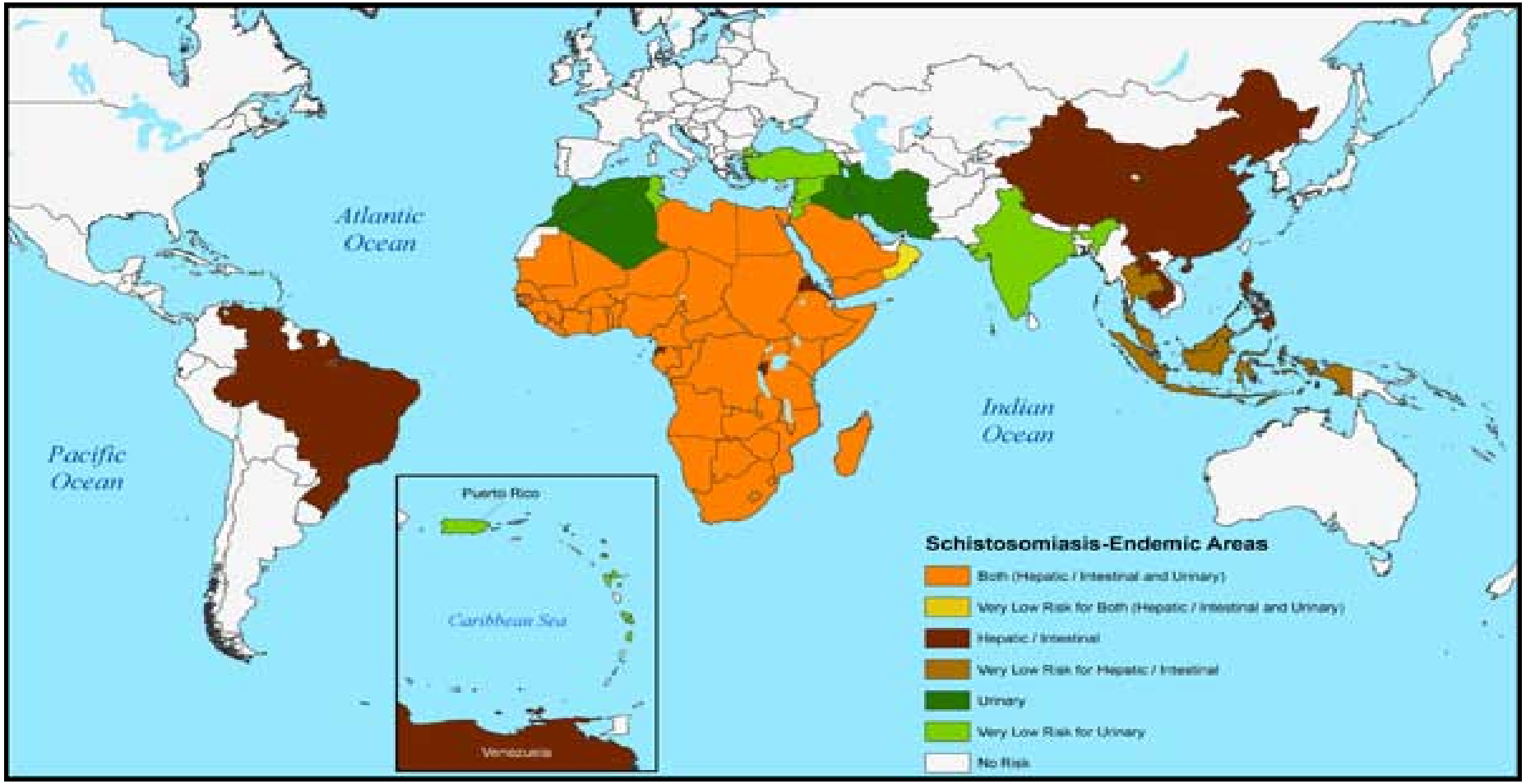

Figure 1.4 World endemic countries for schistosomiasis

Source: http://wwwnc.cdc.gov/travel/yellowbook/2010/chapter-5/schistosomiasis.aspx

due to *S. haematobium* causes 150 000 deaths each year; and portal hypertension due to *S. mansoni* produces 130 000 fatalities annually (WHO, 2006).

School-age children are most likely to become infected with this silent, destructive disease because it is easily contracted while playing, bathing and swimming in water contaminated with the *Schistosoma* parasite, which is shed from snails and infects by penetrating human skins. However, schistosomiasis also can be transmitted simply through contact with contaminated water while performing daily chores such as washing laundry and herding animals. The 400 million school-age children who are infected (WHO, 2001) are often physically and intellectually compromised by anemia, leading to attention deficits, learning disabilities, school absenteeism and higher drop out rates. The failure to treat school-age children therefore hampers child development, yields a generation of adults disadvantaged by irreversible sequelae of infection, and compromises the economic development of communities and nations. For communities already burdened by poverty and ravaged by scourges such as malaria and HIV/AIDS, schistosomiasis is especially devastating.

1.2.2 Transmission and life cycle

There are three main species of *Schistosoma* namely *S. haematobium*, *S. mansoni* and *S. japonicum* which are responsible for the majority of schistosomiasis infection in human while the other two species, *S. mekongi* and *S. intercalatum* parasitize humans to a much lesser extent and more

localized geographically. This disease is being transmitted through contact with fresh water that contains infective cercariae released from an intermediate host snail. The snail intermediate hosts are species-specific; *Bulinus* spp. in *S. haematobium*, *Biomphalaria* spp. in *S. mansoni* and *Oncomelania* spp. in *S. japonicum* (Webber, 2009). The life cycle of all five human schistosomes, as shown in Figure 1.5, are broadly similar where larvae development takes place in freshwater snails (intermediate host) and adult development takes place in the human (definitive host).

Parasite eggs are released into the environment from infected individuals, rupturing on contact with fresh water to release the free-swimming miracidium. Miracidia infect fresh-water snails by penetrating the snail's foot. After infection, close to the site of penetration, the miracidium transforms into a primary (mother) sporocyst. Germ cells within the primary sporocyst will then begin dividing to produce secondary (daughter) sporocysts, which migrate to the snail's hepatopancreas. Once at the hepatopancreas, germ cells within the secondary sporocyst begin to divide again, this time producing thousands of new parasites, known as cercariae, which are the larvae capable of infecting mammals.

Cercariae emerge daily from the snail host in a circadian rhythm, dependent on ambient temperature and light. Young cercariae are highly motile, alternating between vigorous upward movement and sinking to maintain their position in the water. Cercarial activity is particularly stimulated by water turbulence, shadows and human skin chemicals. Penetration of the human

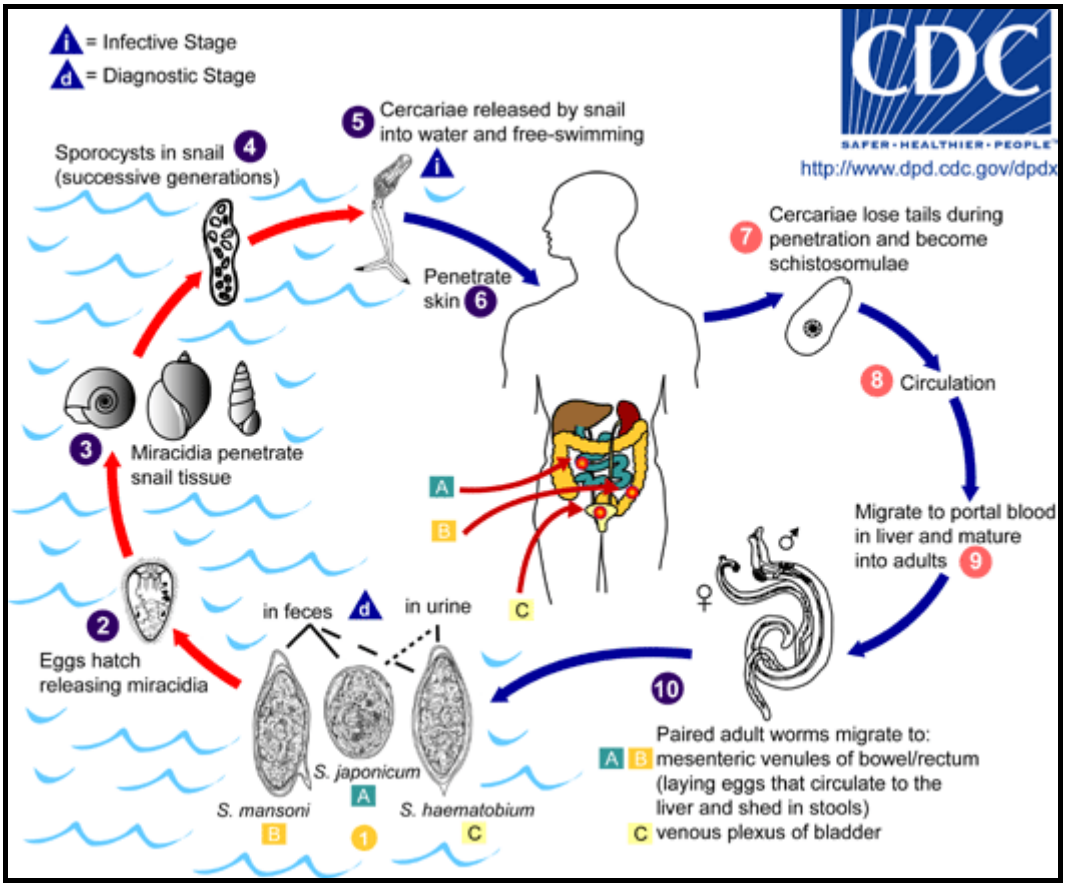

Figure 1.5 Life cycle of *Schistosoma* spp.

Source:http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm