

# Molecular Diagnosis and Monitoring of Benzimidazole Susceptibility of Human Filariids

Adisak Bhumiratana<sup>1,2,3</sup>, Apiradee Intarapuk<sup>3</sup>, Danai Sangthong<sup>3</sup>,  
Surachart Koyadun<sup>4</sup>, Prapassorn Pechgit<sup>1</sup> and Jinrapa Pothikasikorn<sup>5</sup>

<sup>1</sup>Department of Parasitology and Entomology, Faculty of Public Health,  
Mahidol University, Bangkok

<sup>2</sup>Center for EcoHealth Disease Modeling and Intervention Development Research,  
Faculty of Public Health, Mahidol University, Bangkok

<sup>3</sup>Environmental Pathogen Molecular Biology and Epidemiology Research Unit,  
Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok

<sup>4</sup>Ministry of Public Health, Department of Disease Control, Office of  
Disease Prevention and Control 11 Nakhon Si Thammarat

<sup>5</sup>Department of Microbiology, Faculty of Science, Mahidol University, Bangkok  
Thailand

## 1. Introduction

Lymphatic filarial nematode parasites, mainly *Wuchereria bancrofti* and *Brugia malayi*, are causing agents of lymphatic filariasis in humans, which can be effectively treated with antifilarial drugs including diethylcarbamazine (DEC) and ivermectin. Albendazole, an effective benzimidazole compound, acts as a broad-spectrum anthelmintic drug, and when combined with either one of antifilarial drugs, it exerts synergistic effects on reduction of peripheral microfilaremia in lymphatic filariasis cases. However, the varying parasite infection levels in those treated with DEC or ivermectin alone or in combination with albendazole are due to differences in drug responses. The additional clearance of infection with albendazole relative to what is observed with DEC or ivermectin alone suggests that albendazole has different parasite target(s). The homologous  $\beta$ -tubulin gene of human and veterinary filariids that  $\beta$ -tubulin homologs have conserved domains structurally related to other orthologs among the nematodes, cestodes, trematodes and vertebrate hosts, is responsible for benzimidazole susceptibility. The genetic inheritance of resistance in nematode parasites can undergo under selection of benzimidazole compounds in a way that albendazole resistance mechanism involves one of two single amino acid substitutions from phenylalanine to tyrosine in parasite  $\beta$ -tubulin at position 167 or 200. This genetically-stable marker has shown promise for molecular diagnosis and monitoring of *W. bancrofti* infections that carry responsible genotypes associated with benzimidazole susceptibility or resistance. In particular, this approach can augment the surveillance and monitoring of mass treatment impacts on the parasite populations in target areas where long-running elimination programs for lymphatic filariasis are implemented at a large-scale by using a regionally-adopted combination therapy with antifilarial drugs, recommended by the World Health Organization.

## 2. Lymphatic filariasis towards elimination

### 2.1 Factors that favor elimination

Lymphatic filariasis (LF) is a mosquito-borne parasitic disease caused by three main species of thread-like filarial nematode parasites belonging to the superfamily Filarioidea, which are *Wuchereria bancrofti* and *Brugia malayi*, and lesser extent by *Brugia timori*. These endoparasitic nematode lifestyles have a highly conservative life-cycle development sequence in which they develop their metamorphosis in both human and mosquito (Fig. 1). The adult worms that cause clinical manifestations of the disease are dioecious. Male and female worms have separate reproductive system in their pseudocoelomatic body cavity and they live in the

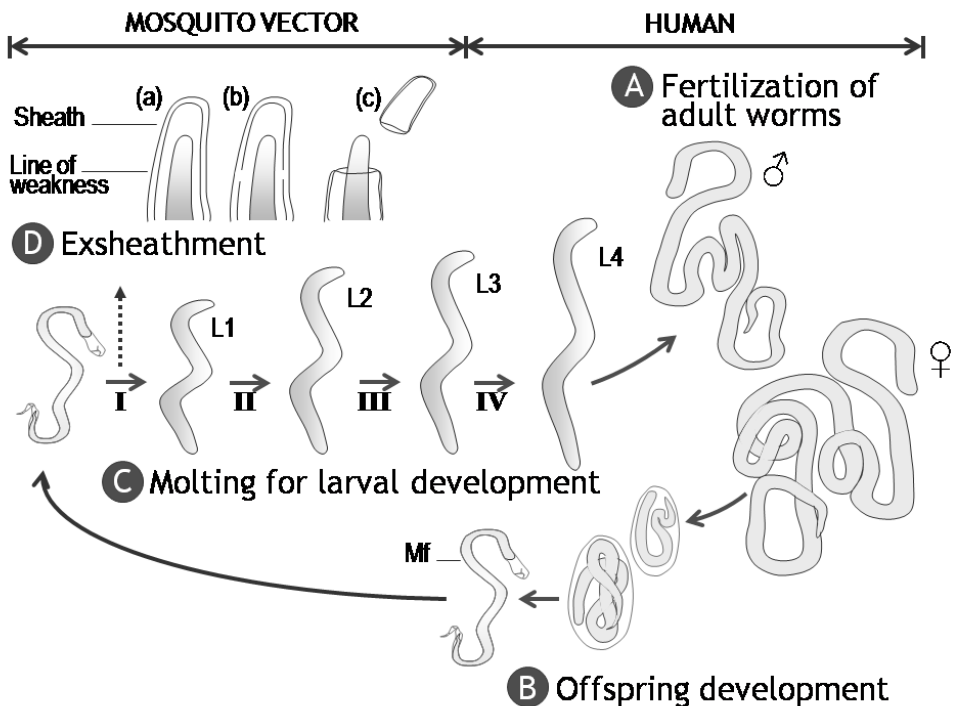


Fig. 1. A development sequence of filarial nematode parasite in human and mosquito (A-D). After the induction of L3 infection in susceptible human host, the mature filarial female worm (A) that possesses fecundicity and fertilization can produce microfilariae (Mf). These offsprings develop from fertilized eggs (B) in the uterus of female worm. They are ingested during the bite of mosquito vector, and consequently, a 5-stage molting progression (B) initially starts after exsheathment of the Mf in mosquito's midgut (C). In this regard, the probable mechanisms involve: (a) proposed anterior of line of weakness, (b) internal digestion of the sheath, and (c) exsheathment of anterior end. Third stage larvae (L3) possess post-infective stage development in mosquito thoracic muscle and then migrate to the proboscis. They are transmitted by infective mosquito during a blood meal, and that they become the L4 (or L5) and mature adult worms in the lymphatic system in human.

human lymphatic system, i.e., lymphatic vessels, with 5-15 years of life expectancy (Table 1). Only when its fecundic lifespan is capable of mating does the lymphatic-dwelling female worm produce advanced stage of sheathed larvae called "microfilariae". These short-living offsprings then penetrate the blood circulation. For a complete life cycle, the microfilariae are ingested by susceptible female mosquito during a blood meal in which they can develop further into larval stages L1-L2 to infective L3 stage. Transmission occurs when the infected mosquito transmits this L3 stage to susceptible persons during other blood meals. The naturally acquired transmission is associated with both intrinsic and extrinsic factors that can regulate the parasitic worm burdens in an endemic population (Fig. 2).

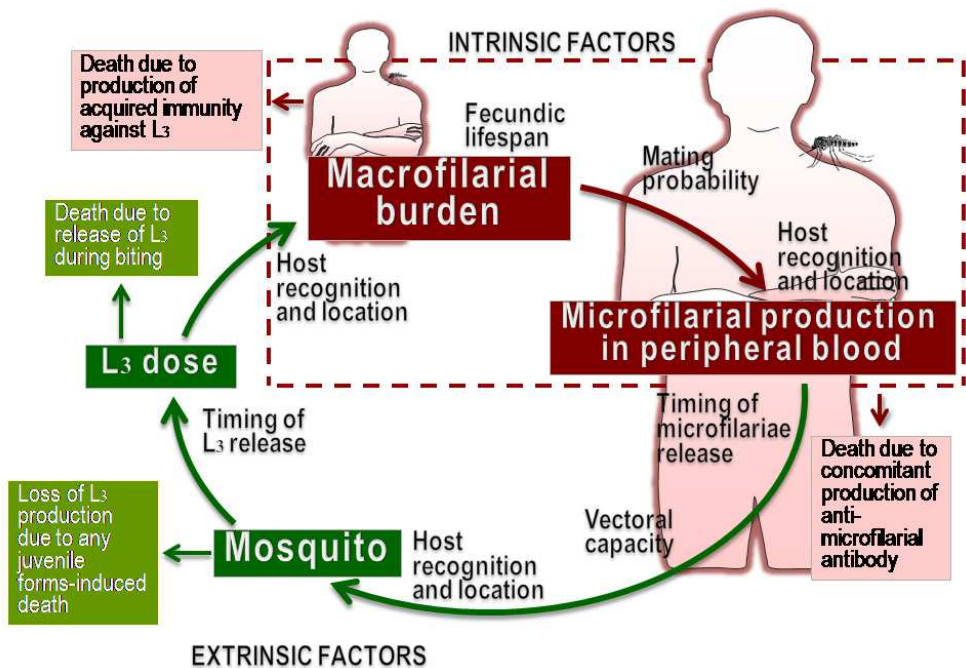


Fig. 2. An explanatory model of human-parasite-vector interactions and favorable factors that influence its adaptation in hosts. The lymphatic filarial infections in humans depend on extrinsic factors of incoming L3 inocula and host recognition and location, and a proportion of adult worms with fecundity and mating probability. Transmission is influenced by host-vector combination (host location and recognition of mosquito feeding habits), microfilarial loads in blood with timing of microfilariae release, and vectorial capacity (longevity and low refractoriness). Long lifespan of the mosquito and timing of L3 release are favorable factors of transmission.

As such, understanding of how these filarial nematode parasites can be removed from the human hemisphere is to understand basically the biology of their life cycle. How do the parasite taxa succeed their complete life cycle in the certain conditions under which they evoke host exploitation strategies? The biology of the parasitic symbionts can disclose the parasite diversity and fitness shaped by hosts and environmental constraints that the

parasites can evolve the adaptation, i.e., the ability to control the physiology and behavior of their host for their own benefit. It has been known so far their distribution among hosts and strategies of host exploitation are restricted in number of host species. The *W. bancrofti*, which is highly host-specific parasite, is sessile to human while other zoonotic *Brugia* taxa exploit other non-human reservoir hosts. This host-specificity variation does not account for the origin of the parasitism as they succeed their parasitism and share common transmission pattern and complex life cycle in vertebrate hosts and arthropods. The geographical variation, on the other hand, can be restrained by selective pressures from the hosts, or the physio-chemical environments such as therapeutic agents and insecticides, or due to phylogenetic constraints. Such this selection can be explained by the experimental infections, mainly using *Brugia* species in rodents (e.g., jirds, hamsters, rats and mice), dogs, ferrets, cats and monkeys. All of which were proposed not just for understanding vertebrate immunology, pathology and chemotherapy, but also for exploring the interactions of the parasite and host. Among these host-parasite systems, *B. pahangi* that infects naturally cats in the Southeast Asia is experimentally appropriate for studies of infection and disease dynamics (Table 1).

Parameter	Cat	Human
Parasite	<i>Brugia pahangi</i>	<i>Wuchereria bancrofti</i> <i>B. malayi</i> , <i>B. timori</i>
Longevity (years)	7-8	5-15
Mature female worm length (cm)	3-7	5-10
Location of :		
Adult	Lymphatics	Lymphatics
Microfilariae	Blood	Blood
Vector	Mosquito	Mosquito
Typical infection/disease sequelae <sup>a</sup>	Infection - Loss of infection - Pathology	Infection - Loss of infection - Pathology

<sup>a</sup>For both host-parasite systems, infection patterns encapsulate a spectrum ranging from asymptomatic persistent microfilaremia to symptomatic amicrofilaremia or complete refractoriness to infection. Adapted from Grenfell et al (1991).

Table 1. Correlation between feline and human lymphatic filariasis

Because of their conservation of complex life-cycle development between feline and human lymphatic filarial parasites, it induces the infection and clinical sequelae in susceptible cats, and hence, resembles that of *Brugia* and *Wuchereria* in humans. However, the parasite does not always succeed their population diversity by increasing its fitness in the hosts (Fig. 2). Its population dynamic is primarily influenced by naturally acquired immunity (i.e., a type of concomitant immunity), which plays a significant role in host selection pressure to restrict a parasitic worm burden (Bundy et al, 1991; Grenfell et al, 1991; Mitchell, 1991; Grenfell and Micheal, 1992). This immunity against incoming L3 cannot remove them from the infection in humans but restrict a number of L3 by a production of concomitant immunity. Variability of adult worm burden results in a proportion of microfilaremic and amicrofilaremic persons in the population. The circulating microfilariae have short life cycle in human blood circulation; a proportion of the microfilariae can be removed by anti-microfilarial antibody

(Simonsen et al, 1996; 2008; Ravindran et al, 2003). They develop two molts (L1 and L2) and induce the infection in susceptible mosquito vectors while the infected mosquitoes can regulate melanization involved in innate immune defense and wound healing to the penetration of filarial nematodes (Zou et al, 2010; Castillo et al, 2011). In blood-engorged mosquitoes that harbor high microfilarial density, they can induce death due to the vector intolerance against development of juvenile forms, and consequently, this leads to loss of L3. Furthermore, release of the L3 does not always permit a passage at an equal number during other blood meal taken by the infective mosquito due to the vector tolerance. Thus, they are naturally killed by host immune, vector barrier, and physical environment; these contributing factors can favor the LF elimination in humans.

As was, LF becomes one of six potentially eradicable diseases of which the criteria for assessing their eradicability (CDC, 1993) are based not only on the scientific feasibility of understanding the biological information mentioned earlier and practical use of the public health interventions and other methods to be applied or used in existing national control program, but also on the political will or popular support of executing the implementation of LF control strategies. First, the scientific feasibility depends basically on the disease vulnerability. Unlike the *W. bancrofti* that exists only in humans, the *B. malayi* spends an enzoonotic life-cycle in which the parasite is transmitted by the potent mosquito and thrive in domestic cats as non-human reservoir, and a *per se* epizoonotic cycle in which the parasite is transmitted and thrive through which the mosquito vector takes blood meal from infected cats, and subsequently, transmit the infective stage to humans during other blood meal. Controversially, the humans carrying *B. malayi* infection can serve as a source for the infection to permit a passage to the animal reservoir through bites of the mosquito vector. Nonetheless, both diseases do not always ease their spread into the population as a result of the naturally-induced immunity and duration of microfilarial production in susceptible individuals or communities (Maizels and Lawrence, 1991; Ottesen, 1992) (Fig. 2). More important, the infections can be both easily diagnosed using advanced tools and effectively treated with the antifilarial drugs that are safe, inexpensive and easily deployed. The feasibility of elimination has been shown that the operation of the pragmatic diagnostic methods or interventions is demonstrable at a large scale in the target populations. Last, the political will/popular support is pivotal for the program manager to capture information required to analyze situations of the perceived burden of LF that is figured out by a large number of suffering and disabled persons worldwide (WHO, 2002; 2008; 2010): the details are available at the websites of the Global Program to Eliminate Lymphatic Filariasis (GPELF) ([http://www.who.int/lymphatic\\_filariasis/disease/en/](http://www.who.int/lymphatic_filariasis/disease/en/)) and Global Alliance to Eliminate Lymphatic Filariasis (<http://www.filariasis.org>). Also, the National Program to Eliminate Lymphatic Filariasis (PELF) by which the resource mobilization and funding structure are administered must be allied, by adopting the GPELF's strategies, to practical considerations of interventions, methods, logistic supplies (medical and field-work), delivery processes, expenditures (unit costs and cost-effectiveness), integration of control activities into existing health systems services (or other health development programs), socio-economic impacts on gaining health benefits, community awareness and acceptance, and ecological disturbances to target disease, non-target disease and the environment. For the resource-limited countries as allied nations, a national budget plan that notifies outsources and fund raising needs the subsidy of the internationally collaborative program.

## 2.2 Mass chemotherapy as elimination strategy

Ideally, the success of controlling the disease depends definitely on the objectives and ultimate goals of disease control spectrum (CDC, 1993); with this regard, the rational management of LF elimination differs with control (Table 2). As recommended by the World Health Organization (WHO), two pillars of global elimination strategies emphasize interruption of transmission and elimination of the infection in humans, and the other large-scale morbidity control to prevent disease and disability (WHO, 1999a; 2002; 2008; 2010). Principle outcomes of the GPELF reduce numbers of microfilaremic persons and disease cases as preventing new infection introduced among the population at risk of, or affected with, the infection. To meet this criteria, the GPELF proposes a mainstay of elimination strategy effectively available for mass drug administration (MDA) in target population; a combined treatment with diethylcarbamazine citrate (DEC) 6 mg/kg plus 400 mg albendazole (in *W. bancrofti* transmission areas where *Onchocerca volvulus* is not coendemic), or with 200 µg ivermectin plus 400 mg albendazole (in *W. bancrofti* transmission areas where *O. volvulus* is coendemic) (Ottesen et al, 1997; 1999; Ottesen, 2000; WHO, 1999a; 2000; 2001; 2002). An annual MDA with coverage of 60-80% for 4-5 years is considered to be effective enough to interrupt transmission in control areas in the absence of vector control (Gyapong et al, 2005). Also, new options for mass treatment of at risk population are effectively available: DEC-fortified salt for 1 year; and a combination of single annual dose of albendazole plus DEC, followed by DEC-fortified salt (Weaver et al, 2011). The ample supply and distribution of DEC-fortified salt can be administered in some countries.

## 2.3 Surveillance and monitoring systems

As for presenting dynamics of the infection and disease in nature (Grenfell et al, 1991; Sridivya et al, 1991; Ottesen, 1992; Meyrowitsch et al, 1995), the parasites cause a wide spectrum of clinical manifestations in the affected population that are characterized by asymptomatic microfilaremia, acute lymphatic inflammation and chronic lymphatic pathology (WHO, 1992a; 1992b; 1994). Susceptible persons develop clinically LF as a result of prolonged exposure to multiple infective bites of potent mosquitoes from several months to years. On the other hand, the people living for at least 6 months are at the greatest risk for the infection. The chronic filariasis cases represent a tip of the iceberg, as microfilaremic carriers are the reservoir of the infection to others. The prevalence and intensity of the infection in humans depends mainly upon a number of microfilaremic persons and a geometric mean of microfilarial loads in the affected population. These proximate measures are indicative of the degrees of endemicity. The prevalence and geographical distribution of the disease are important not just for determination of its potential transmission in mosquito, but also for diagnosis and surveillance in different endemic settings. Therefore, the recognition of what is the filarial origin of the disease and where the affected individuals or communities are is fundamental for public health importance to identify the solution and control rationale. Diagnostic approaches that emphasize the detection and specific identification of microfilaremic infection in the individual by using most standard microscopic methods are important component of LF control program, which is aimed to reduce number of microfilaremic persons. The suitable blood collection and microscopic diagnosis in health settings can be effectively available to identify anyone infected, and subsequently, treated with antifilarial drug regimens. However, this specific objective of control is less important as the elimination is desired not just to interrupt transmission and clear human infection on a large-scale, but also to reduce the morbidity attributed to the

disease and hence improve both personal-focused hygiene and health care to all beneficiaries in target population at risk (Table 2).

	<b>Control</b>	<b>Elimination</b>
<b>1. Definition</b>	- Reduction of microfilaremia prevalence	- Reduction of microfilaremia prevalence as arbitrarily qualitative or quantitative level of control as no longer a public health problem
<b>2. Outcome indicators as options for monitoring tools</b>	- Annual infection prevalence; microfilaremia rate (a microfilaremic number surveyed in endemic population) - Disease rate (a number of disease cases surveyed in endemic population) - Annual transmission potential (ATP) (i.e., a number of infective bite per annum which acquire the infection) as mosquito infection rate is not useful indicator	- Degrees to which transmission is interrupted; microfilaremia rate mosquito infection rate and antigenemia rate - Disease rate is not useful indicator
<b>2.1 Objective</b>	- <b>To assess whether the control program achieves its goals or desired outcomes</b> (e.g., reduction in microfilaremia and/or disease prevalence)	- <b>To assess whether the elimination program achieves its goals or desired outcomes†</b> (e.g., reduction in microfilaremia prevalence, antigenemia prevalence)
<b>3. Process indicators as options for monitoring and evaluation tools</b>	- Coverage of selective treatment and follow-up, as mosquito net utilization, insecticide spraying and reservoir control can be useful indicators	- Mass treatment coverage, drug compliance coverage (e.g., adverse drug reactions prevalence and severe adverse event reports), KAP survey
<b>3.1 Objective</b>	- <b>To assess how well the various components of the control program are functioning</b> (e.g., the number of newly and follow-up microfilaremic persons treated with antifilarial drugs)	- <b>To assess how well the various components of the elimination program are functioning†</b> (e.g., the number of new infections, the number of drug tablets distributed)

Adapted from the GPELF ([http://www.who.int/lymphatic\\_filariasis/disease/en/](http://www.who.int/lymphatic_filariasis/disease/en/)).

†Outcome indicators can be optionally used for monitoring systems; longitudinal surveillance of populations in sentinel sites, cross-sectional “spot checks” in other sites, and auxiliary “background” surveillance.

KAP - Knowledge, attitudes and practices.

Table 2. Rational approaches to lymphatic filariasis control and elimination

Tools	Advantages for the applications	Chief obstacles when applied or used in demonstrated areas	References
ICT Filariasis	<ul style="list-style-type: none"> <li>• Simple-to-use rapid diagnostic test kit commercially available for use in qualitative detection of <i>W. bancrofti</i> adult worm circulating antigens present in whole blood/serum/plasma samples; stronger positive test line equivalent to higher antigen levels;</li> <li>• Sensitive and specific for anytime-of-day determination of active infection (antigenemic) whether microfilaremia is present, or the treatment is given;</li> <li>• Highly reproducible and practical when lots of large-scale blood samples (100 µl each) are analyzed either under the field conditions by not-well-trained field workers or in laboratory settings (with 100 µl each of serum/plasma samples freshly prepared or frozen) by laboratory personnel in order to assess the human infection rates in areas known as endemic for <i>W. bancrofti</i> or respective areas of emergence/reemergence;</li> <li>• Suitable for rapid assessment survey to detect the early infection in endemic carriers including migrants, refugees, any persons who work in endemic areas (mine in rainforest and border) for years, or visitors to the areas;</li> <li>• Suitable to monitor and evaluate the infection in humans inhabiting in risk areas in initial surveillance before MDA; drug responses during MDA; and the new infection in post-MDA areas whether they are certified as eliminated areas</li> </ul>	<ul style="list-style-type: none"> <li>• Costly;</li> <li>• Indicate, but not quantify, the level of the circulating antigens; somehow, provides false-negative identifications with the infections harboring very low antigen titers;</li> <li>• Cannot differentiate the status between infection and disease, occurrence and recurrence, or sensitivity and resistance</li> <li>• Primarily requires standardization and quality control of lots of samples (finger-prick blood) that are collected and analyzed</li> </ul>	<p>Weil &amp; Liffis, 1987 Weil et al, 1987; 1996; 1997 Freedman et al, 1997 Ramzy et al, 1999 Bhumiratana et al, 1999; 2002; 2004; 2005 Nguyen et al, 1999 Phantana et al, 1999 Simonsen et al, 1999 Omar et al, 2000 Pani et al, 2000; 2004 Sunish et al, 2002; 2003 Braga et al, 2003 Engelbrecht et al, 2003 Koyadun et al, 2003 Nuchprayoon et al, 2003 Siriaut et al, 2005 Ruberanziza et al, 2009 Foo et al, 2011</p>



Tools	Advantages for the applications	Chief obstacles when applied or used in demonstrated areas	References
<b>Og4C3 ELISA</b>	<ul style="list-style-type: none"> <li>• Commercially available diagnostic test kit, and principally the same when use in qualitative and quantitative detection of active <i>W. bancrofti</i> infection, but more sensitive and specific than the ICT Filariasis;</li> <li>• Highly reproducible when lots of large-scale serum/plasma samples (100 µl each of freshly prepared or frozen samples) are analyzed in the public health reference laboratory or research institutes in order to assess the human infection rates, and to monitor and evaluate the infection or drug responses in individuals or in the target population under the circumstances described above;</li> </ul>	<ul style="list-style-type: none"> <li>• Costly, labor-intensive and intrusive</li> <li>• Cannot differentiate the status between infection and disease, occurrence and recurrence, or sensitivity and resistance</li> <li>• Primarily requires standardization and quality control of lots of venous blood samples (subsequently prepared for sera or plasma) that are collected, transported, stored and analyzed;</li> <li>• Also requires well-trained field workers and laboratory personnel</li> </ul>	<p>More &amp; Copeman, 1990            Turner et al, 1993            Chanteau et al, 1994a; 1994b            Lammie et al, 1994            McCarthy et al, 1995            Rocha et al, 1996            Nicolas, 1997            Ismail et al, 1998            Eberhard, 1997            Simonsen and Dunyo, 1999            Nuchprayoon et al, 2003            Bhumiratana et al, 2004; 2005</p>
<b>Polymerase chain reaction (PCR)</b>	<ul style="list-style-type: none"> <li>• Very highly sensitive and specific for the microfilaremic infections with <i>W. bancrofti</i> and <i>B. malayi</i> in humans and mosquitoes distinguishable from other filarial nematode parasites such as <i>O. volvulus</i>, <i>D. immitis</i>, <i>D. repens</i> and <i>B. pahangi</i>;</li> <li>• Highly reproducible and practical when lots of samples as low as 20 µl human blood, individual mosquito (dissected or whole body), or mosquito pool, are analyzed in the public health reference laboratory or research institutes in order to assess the human and</li> </ul>	<ul style="list-style-type: none"> <li>• Too costly, labor-intensive, intrusive, in-house developed tool</li> <li>• Primarily requires standardization and quality control of lots of samples (finger-prick or venous blood, or pooled wild-caught mosquitoes) that are collected, transported, stored and analyzed;</li> <li>• Also requires well-trained field workers or</li> </ul>	<p>Chanteau et al, 1994c            Lizotte et al, 1994            McCarthy et al, 1996            Siridewa et al, 1996            Williams et al, 1996            Zhong et al, 1996            Ramzy et al, 1997            Nicolas et al, 1999            Cox-singh et al, 2000            Thanomsub et al, 2000            Pradeep Kumar et al, 2002</p>

Tools	Advantages for the applications	Chief obstacles when applied or used in demonstrated areas	References
	<p>mosquito infection rates, and to monitor and evaluate the infections or drug responses in individuals or in the target population under the circumstances described above;</p> <ul style="list-style-type: none"> <li>• Can differentiate the status between infection (microfilaremia) and disease, occurrence (new infection) and recurrence, or sensitivity and resistance;</li> <li>• Detection limit of as low as one Mf per blood volume tested (up to 1 ml), or one juvenile larva (L1, L2 or L3) per pooled mosquitoes (up to 100)</li> </ul>	<p>laboratory personnel and accessory equipments for DNA preparation and analysis of PCR products;</p> <ul style="list-style-type: none"> <li>• Cannot differentiate larval stages (L1, L2 or L3) in infected mosquitoes</li> </ul>	<p>Fischer et al, 2003            Kanjanavas et al, 2005            Nuchprayoon et al, 2005; 2007            Rao et al, 2006            Mishra et al, 2007            WHO, 2009            Bhumiratana et al, 2010            Pechgit et al, 2011            Takagi et al, 2011</p>

Table 3. Direct determination tools for use as part of the Global Program to Eliminate Lymphatic Filariasis

To meet this objective, a large-scale transmission control requires a current magnitude and geographical distribution of the disease in the at-risk population. To understand the extent to which the target population needs to be designed for MDA and monitored whether the MDA implementation is effective, such surveillance and monitoring systems are required. To identify the communities with, or at risk of, the infection, for instance, the direct assessment techniques (Table 3) are required for practical use both in initial surveillance for filarial infection and in monitoring and evaluating the effectiveness of mass treatment, as part of the GPELF (WHO, 1999a; 1999b; Ottesen, 2000). In this regard, the mass treatment with more effective antifilarial drug regimens as well as the availability of other existing and alleviating control measures has been deliberately implemented to meet such these highly achievable objectives of the elimination. Nonetheless, in addition to what is recommended by WHO, the GPELF requires for ground-breaking development of systems, protocols and tools that will be able to be convincingly applied to or routinely used in the PELF to fix undesirable events of mass treatment impacts in different complex epidemiological settings (Kyelem et al, 2008; Ottesen et al, 2008; WHO, 2008; 2010).

### 3. Parasite infection and drug responsiveness

#### 3.1 Microfilaremia and drug-responsive microfilaremia

In an endemic population that represents the infection and disease dynamics in nature, the asymptomatic microfilaremia is a stage of the active infection with filarial adult worms (Srividya et al, 1991; Meyrowitsch et al, 1995), which regulate host immune responses (hypoimmune responsiveness) in infected individuals (Bundy et al, 1991; Grenfell et al, 1991; Maizels and Lawrence, 1991; Mitchell, 1991; Grenfell and Micheal, 1992; Ottesen, 1992;

Simonsen et al, 1996; 2008; Ravindran et al, 2003). This phenomenon results in immunotolerance, i.e., a prolonged induction of the balance of immune defense to the parasites stimulated by the adult worms, in most asymptomatic microfilaremic persons. The female adult worm involves in regulation of the host microfilaremia. The fecundicity (a period of pregnancy) allows its fertilization to produce a diverse number of the offspring microfilariae. Although the proportion of microfilariae can be removed from the blood circulation in patients, there are the plenty of microfilariae, which circulate in the peripheral blood and show the appearance both nocturnally and diurnally. This microfilarial periodicity or circadian cycle of the parasite in humans is clinically unimportant for treatment but very important for its epidemiologic implication, which plays a significant role in diagnosis, surveillance and epidemiology. The parasite infection is the foundation for the processes that not only determine the infection prevalence but also monitor and evaluate the effectiveness of the treatment with the antifilarial drugs in infected individuals. In this regard, the amount of microfilaremia seems to be a function of naturally-acquired infection loads, which refers to as the most viable microfilariae, and drug-responsive microfilaremia refers to as the affected parasite population that harbors a diverse range of viable and non-viable microfilariae (Pechgit et al, 2011). These outcome indicators are useful for monitoring and evaluating the benzimidazole susceptibility of the filarial nematode parasites in the population in areas of the PELF implementing the MDA 2-drug regimen either albendazole plus DEC or albendazole plus ivermectin.

However, the MDA 2-drug regimen is not the only factor that shapes the parasite population under complex epidemiological settings. Of note, the *W. bancrofti* populations have ability to provoke the genetic variability that shows the important implications in the endemic populations targeted by the MDA (Pradeep Kumar, 2000). The existence of genetic diversity of *W. bancrofti* populations that has greater heterogeneity under DEC therapy and vector control gives rise to questioning about the development of drug resistance in LF, which possibly occurs in the target populations. The selection pressure is an intensity of selection affecting the frequency of genes in a parasite population. The selection that increases or decreases the susceptibility of the parasite population depends on the frequency of the alleles involved. The genetic polymorphism occurring in the *W. bancrofti* population under the selection pressure(s) may evoke gradually under specified conditions to yield the fitness, which can be determined by a genotype in the parasite population. The increase in the parasite fitness can be estimated by the equilibrium frequencies of the alleles (genotypes) at heterozygote advantage in a hypothetical population. That is, rapid establishment of advantageous alleles in the *W. bancrofti* population, called "selection sweep", may evoke with advantageous drug-resistant genotypes epidemiologically linked to other factors shaped by the host and environment (Schwab et al, 2006; 2007; Churcher et al, 2008). Eventually, it may reduce the genetic variation in the population.

### 3.2 Benzimidazole-susceptibility of the parasite

This chapter emphasizes microfilaremia responsiveness in the population under the suppression of the PELF implementing MDA 2-drug regimen, 6 mg/kg DEC plus 400 mg albendazole. The microfilaremia responses against the DEC are the foundations of understanding how the albendazole exerts the effects on the parasite population in addition to what is observed by DEC alone. The DEC is known as the oldest of the antifilarial drugs used in the LF control. The single-dose drug acts as microfilaricide as does the effective ivermectin (de Silva et al, 1997; Ottesen, 2000; Molyneux et al, 2003; Ottesen et al, 2008)

while its macrofilaricidal activity is not definitely effective against adult worms (Eberhard et al, 1991; Norões et al, 1997; Dreyer et al, 1998; Rajendran et al, 2002; 2004; Oliveira-Menezes et al, 2007). The adult worm loads that are age-dependent (Lammie et al, 1994; Rajendran et al, 2002) are susceptible to treatment with the DEC alone or even combination with albendazole (Norões et al, 1997; Rajendran et al, 2002). It was seen that DEC alone disturbs the microfilarial sheath of some filarial species while it has effects on the oogenesis and fertilization of female adult worms. Nonetheless, little is known about the filarial nematode parasites whether they evoke resistance mechanism against DEC due to the lack of deepening its mechanism of action, particularly the availability in parasite tissues and the selectivity on parasite targets. Oliveira-Menezes et al (2007) demonstrated that DEC has minor effects on alterations of the cuticle or surface of both male and female adult worms; these responsible parasites were collected from the *W. bancrofti*-infected patients treated with DEC, as compared to those isolated from the untreated patients. Additionally, such alterations are seen in adult worms recovered from the patients treated with DEC plus albendazole. The possible explanation is that the potential adulticidal effect of albendazole relative to what is observed by the DEC alone. The subtle alterations imply the distinct morphologic characteristics of the parasite itself or the complex host-parasite interactions, and if implemented and continuously prolonged, the annual mass treatment with DEC plus albendazole has not yet become an apparent issue, particularly the impacts on the parasite population adaptation. Of note, the *O. volvulus* parasite develops the mechanism involved in resistance to ivermectin (Awadzi et al, 2004). Do the filarial nematode parasites have mimicry in the resistance mechanisms to ivermectin and albendazole? However, detailed study of the parasite resistance to albendazole has not been established.

Focus is on the MDA 2-drug that acts as effective microfilaricide while its aberrant activity that influences microfilaremia response to its efficacy in microfilaricidal activity. Provided this phenomenon occurs, the expected outcomes of such drug failure will impact on solving the solutions and paving the implications of how they will adapt under the certain circumstances and how we will also mitigate their adaptation. Most studies enlightened the understanding of this effective deworming MDA 2-drug, which plays the significance of reduction of the infection prevalence. A single-dose combined treatment with DEC plus albendazole has short- and long-term effects on *W. bancrofti* microfilaremics in the endemic populations (Ismail et al, 1998; Ottesen et al, 1999; El Setouhy et al, 2004; Rajendran et al, 2004). Compared to those receiving DEC alone, an additional benefit of the combined drugs results in decline in annual cyclic infection prevalence due to progressive reduction in density of *W. bancrofti* microfilaremia. Although its macrofilaricidal effect on clearance of *W. bancrofti* antigenemia has been reported (McCarthy et al, 1995; Eberhard, 1997; Rajendran et al, 2002; 2004; Koyadun et al, 2003; Bhumiratana et al, 2004; Siriaut et al, 2005; Bhumiratana et al, 2005; Yongyuth et al, 2006), the DEC alone or co-administered with the albendazole does not clear rapidly the antigenemia. The MDA with the DEC alone will recover an increase in the antigenemia prevalence of *W. bancrofti* unless there is yearly-round MDA in the population (Rajendran et al, 2002). A 400 mg single oral-dose albendazole regimen is broad-spectrum effective against helminthiases (Albonico, 1994; de Silva et al, 1997; Beach et al, 1999; Ottesen et al, 1999; Horton, 2000) and, as co-administered orally with DEC, a synergistic and long-term effect on geohelminths has been proven useful for 'beyond-lymphatic filariasis' elimination program (Ottesen et al, 1997; 1999; Ismail et al, 1998; Horton et al, 2000; Ottesen, 2000; Mani et al, 2002; 2004; Molyneux et al, 2003; Yongyuth et al, 2006).

Few reports established the evidence that the human filarial nematode parasites provoke molecular mechanism involved in benzimidazole sensitivity/resistance until recently findings of the genetically-induced resistance against benzimidazole compounds have been well documented in veterinary nematode parasites (Beech et al, 1994; Kwa et al, 1995; Humbert et al, 2001; Bennett et al, 2002; Drogemuller et al, 2004; Robinson et al, 2004; Cole et al, 2006; Ghisi et al, 2007). Resistance to albendazole in veterinary nematodes is known to be caused by either one of two single amino acid substitutions from phenylalanine to tyrosine in parasite  $\beta$ -tubulin at position 167 or 200. The genetically stable *W. bancrofti*  $\beta$ -tubulin gene responsible for a molecular mechanism of drug resistance has been proposed as that of the veterinary helminth parasites is performed under selection of albendazole and ivermectin. The *W. bancrofti* population isolated from the patients treated with a combination of albendazole and ivermectin had significantly higher genotypic frequencies associated with resistance at position 200 (Schwab et al, 2005). A resistance mutation was not detected at position 167. Hoti et al (2003, 2009) reported that the polymorphism in the codon of this residue in *W. bancrofti* populations representing geographically distant areas of India, through sequencing exon 5 region of  $\beta$ -tubulin isotype 1 gene. The nucleotide sequence data showed that *W. bancrofti* isolates from wide geographic areas of India had codon for Phe (TTC) at position 200, suggesting that the parasite might be genetically sensitive to benzimidazole. Similarly, Bhumiratana et al (2010) and Petchgit et al (2011) demonstrated that the *W. bancrofti* population recovered from the dynamic cross-border migrant population from areas that have been targeted by the MDA 2-drug regimen (300 mg DEC plus 400 mg albendazole) elicits the genetic background of benzimidazole susceptibility; a resistance mutation has not been observed at position 167 or 200. However, the albendazole, anthelmintic benzimidazole, is being co-administered with an antifilarial drug such as DEC, part of the PELF implementing in many endemic countries. But this drug is known to result in the faster development of drug resistance in the veterinary nematode parasites and hence it is necessary to monitor drug sensitivity among the responsible *W. bancrofti* populations.

#### 4. Molecular diagnosis and monitoring of benzimidazole susceptibility

##### 4.1 Parasite beta-tubulin encoding gene as molecular marker

Molecular mechanisms of benzimidazole resistance in nematode parasites are hypothesized. However, detailed study of benzimidazole resistance in trichostrongylids found that the  $\beta$ -tubulin encoding gene involved in benzimidazole susceptibility is responsible for the genetic inheritance of resistance in the veterinary nematode parasites under selection with benzimidazole that involves one of two single amino acid substitutions from phenylalanine (Phe) to tyrosine (Tyr) in parasite  $\beta$ -tubulin at position 167 or 200 (Beech et al, 1994; Kwa et al, 1993; 1994; 1995; Roos et al, 1990; Elard et al, 1996; Elard and Humbert, 1999; Humbert et al, 2001; von Samson-Himmelstjerna et al, 2002; Winterrowd et al, 2003; Drogemuller et al, 2004; Cole et al, 2006; Ghisi et al, 2007). The potential point mutation occurs at the DNA level by nucleotide substitution for the codon for amino acid position 200 of the  $\beta$ -tubulin gene, a substitution of TTC (Phe) with TAC (Tyr). This irreversible change brings about distinguishment of the responsible parasite population between benzimidazole-sensitive and -resistant nematodes. This principal mechanism for benzimidazole resistance is postulate to involve changes in the selectivity of the benzimidazoles on the primary structure of  $\beta$ -tubulin molecules, a building block of the microtubule in the parasites (Lacey, 1988; Lacey and Gill, 1994; Robinson et al, 2004).



Intriguingly, such mimicry in molecular mechanism for benzimidazole resistance in the filarial nematode parasites has been increasingly investigated, based basically on the molecular characterization of the homologous  $\beta$ -tubulin gene retained in their genome and the advantageous fitness of benzimidazole-resistant genotypes in the population (Roos et al, 1995; Elard et al, 1998; Elard et al, 1999; Silvestre et al, 2001; Silvestre and Humbert, 2002).

The nematode parasites possess the single-copy homologous  $\beta$ -tubulin (*tubb*) gene that encodes a  $\beta$ -tubulin polypeptide, 448 amino acids (Met1 to Glu448). Hypothetically similar to that of trichostrongylids, the binding of benzimidazoles to conserved domains (of the exons 4 to 6) leads to blocking an assembly of tubulin (TUBB), and thus disrupting structural formation of microtubule (cytoskeleton protein) in the nematode parasites. The nucleotide sequences of the homologous  $\beta$ -tubulin gene as molecular marker and other related TUBB gene family of the nematode parasites can be retrieved from the genome databases: the GenBank at the National Center for Biotechnology Information (NCBI), <http://www.ncbi.nlm.nih.gov/genbank/>, the European Molecular Biology Laboratory (EMBL) <http://www.ebi.ac.uk/>, and the DNA DataBank of Japan (DDBJ) <http://www.ddbj.nig.ac.jp/>. The website of nematode and neglected genomics (<http://www.nematodes.org/fgn/index.html>) establishes genome database, especially for the filarial genome project (FGP), which includes published complete *B. malayi* genome. Meanwhile, the homologous sequences of *B. malayi*  $\beta$ -tubulin gene can be obtained from the TIGR genome database (<http://www.tigr.org/tdb/e2k1/bma1>).

The structural organization of homologous *tubb* genes of two filarial nematode parasites, *B. pahangi* (Guenette et al, 1991) and *D. immitis* (Bourguinat et al, 2011), has been shown for the establishment of complete coding sequences that span 9 discrete exons: exon 1 (Met1 to Lys19), exon 2 (Phe20 to Asp55), exon 3 (Gly56 to Gln131), exon 4 (Gly132 to Lys174), exon 5 (Val175 to Leu228), exon 6 (Val229 to Gln292), exon 7 (Met293 to Arg324), exon 8 (Glu325 to Thr386) and exon 9 (Ala387 to Glu448). The homology is 78% at DNA level due to bias of codon usage and insertion/deletion of intron sequences (Fig. 3). Among these, the exons 4 and 5 confer hypothetical point mutation at amino acid positions Phe167Tyr (or TTT/TAT) and Phe200Tyr (or TTC/TAC), based only on the second nucleotide base changed in the codons. In the homologous segment of its closely related taxa, *W. bancrofti*  $\beta$ -tubulin (*Wbtubb*) gene that possesses two distinct exons, 4 (Gly132 to Lys174) and 5 (Val175 to Leu228), with flanking intron sequences (Fig. 3) shares the homology at DNA level with *B. malayi* and *B. pahangi* (93% similarity), compared to *O. volvulus* and *D. immitis* (76% similarity) (Bhumiratana et al, 2010; Pechgit et al, 2011). This target DNA has been proved useful for designing *Wbtubb* locus-specific primers to discriminate between *Wbtubb* and other homologs of human and animal filariids. Based on its usefulness in molecular diagnosis and monitoring of the infection carrying the benzimidazole-sensitive or resistant phenotypes, the PCR applications of this molecular marker for *W. bancrofti* have been well documented (Hoti et al, 2003; 2009; Schwab et al, 2005; Bhumiratana et al, 2010; Pechgit et al, 2011).

#### 4.2 Polymerase chain reaction-based approaches

In contrary to the antigen detection methods such as ICT Filariasis and Og4C3 ELISA that provide the proof of *W. bancrofti* antigenemic infection in human blood, the microfilarial DNA detection by PCR provides the evidence of *W. bancrofti* microfilaremic infection in human blood and mosquito (Table 3). As a result of the existence of genetically stable

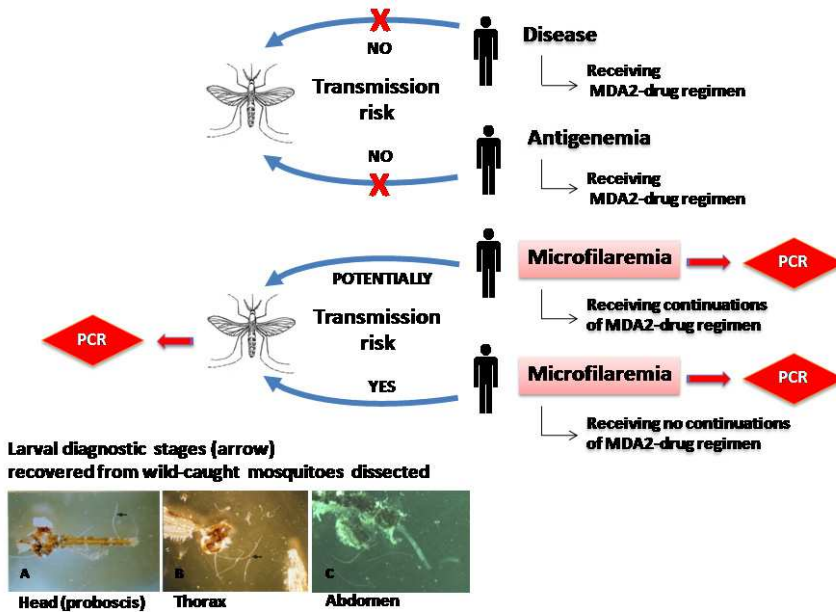


Fig. 4. A proposed scheme for PCR detection of *W. bancrofti* benzimidazole-susceptible isolates in human blood and mosquito.

Primer Name	Sequence (5' to 3')	Direction	Length (bases)	Expected amplicon size (bp)	Hypothetical nucleotide positions	Susceptible(S)/resistant(R) genotypes investigated	Reference
BT9	CAGGTACAGATT GCTACAGT	Forward	20	607 <sup>c</sup>	(TTT)167(TAT)	S/Phe167	Bhumiratana
BT12	GCGATTTAAACC CGACAGC	Reverse	19		(TTC)200(TAC)	S/Phe200	et al (2010)
BT121 <sup>a</sup>	GGATCCGTATCA GATGTTGTG	Forward	21	174 <sup>d</sup>	(TTC)200(TAC)	S/Phe200	Bhumiratana
BT122 <sup>b</sup>	GAATCCAAGTG GTTGAGGTCG	Reverse	22				et al (2010)
Wt2F	GTATCAGATGTT GTGTTG	Forward	18	475 <sup>e</sup>	(TTC)200(TAC)	S/Phe200	Hoti et al
Wt2R	ACGACTTGAATG AGTTGTC	Reverse	19				(2003)
Wbbt2 F	TATCAGATGTTG TGTTGG	Forward	18	475 <sup>f</sup>	(TTC)200(TAC)	S/Phe200	Hoti et al
Wbbt2 R	CTGTTGAG AAGTTCAGCA	Reverse	19				(2009)

5' modifications with additional recognition sequences: <sup>a</sup>*Bam*HI (GGATCC) and <sup>b</sup>*Eco*R I (GAATTC).

<sup>c</sup>Retrieved *Wuchereria bancrofti* genome accession nos.: AY705383 and GU190718–24.

<sup>e</sup>Retrieved *Brugia pahangi* genome accession nos.: M36380.

<sup>f</sup>Retrieved *Wuchereria bancrofti* genome accession nos.: EF190199-190209, EF492870-492878.

Table 4. The  $\beta$ -tubulin isotype 1 gene-specific primers used in the PCR amplification of *W. bancrofti* benzimidazole-susceptible isolates



*W. bancrofti*  $\beta$ -*tubulin* gene, the nested PCR amplification can work well with the microfilaremic infection that responds to treatment with MDA 2-drug regimen (DEC plus albendazole) (Pechgit et al, 2011). This newly developed PCR assay in addition to promising advanced tool (Hoti et al, 2003; 2009; Bhumiratana et al, 2010) has the potential benefits in the molecular diagnosis and monitoring of the infection, as compared to the other PCR amplification methods previously described elsewhere (Table 3). The concepts for PCR assays based on the *Wbtubb* locus-specific primers (Table 4) have been proposed in two applicable formats: the locus-specific nested PCR and allele-specific nested PCR. These applications have established the advantages on how to circumvent some common counterintuitive problems of conventional PCR with regards to both parasite genome analysis and low-copy gene detection; such detailed study has been well established by Pechgit et al (2011). The *W. bancrofti* microfilarial DNA detection methods depends much on the purity and quantity of the microfilariae recovered from different blood sample preparations. The purified aggregate parasite number in the absence of human host white blood cells, for example, are ideal for the quality of DNA extract, which serves as target sequences in the PCR reactions. In general, most PCR methods for the detection of *W. bancrofti* distinguishable from other filarial nematode parasites in human and mosquito is based on the repetitive *Ssp I* sequences, which are highly copy number per haploid genome. However, PCR amplification based on this *Ssp I* locus provides the positive identifications of the parasite infection existed in specimens of choice. The assay does not determine the infection that responds to benzimidazole sensitivity/resistance; such responsible *W. bancrofti* parasite population is amplified based on the  $\beta$ -*tubulin* gene which is single copy in haploid genome. Therefore, the amplification is performed using the *Wbtubb* locus-specific nested PCR and allele-specific nested PCR that provides the proof of the *W. bancrofti* infection carrying benzimidazole-sensitive/resistant phenotypes; methodologically, the technical requirements for their applications have been described by Pechgit et al (2011) and Hoti et al (2009). More specific, based on our experience, the *Wbtubb* locus-specific nested PCR with thermocycling modifications using touchdown and touchup cycles has been applied or used in detection and characterization of *W. bancrofti* infection both in human blood from patients untreated or treated with DEC plus albendazole and in wild-caught mosquito, provided such infections carrying benzimidazole-sensitive/resistant strains are the same source of the parasite population (Fig. 4). Hypothesis is that whether the parasite infection is genetically predisposed to the MDA 2-drug regimen (DEC plus albendazole) in areas under suppression of PELF, it will have frequencies of benzimidazole-susceptible homozygous allele (*SS*) greater than benzimidazole-susceptible heterozygous allele (*Sr*) and homozygous resistant allele (*rr*), which are associated with albendazole resistance, unless the parasite fitness is increased. This also permit the monitoring and evaluation of the parasite fitness to better understand theoretically and hypothetically evolutionary biology and ecology of the parasite, by which the human hosts play a key as a major source of selective pressures on the adaptation of parasite population constrained by environmental conditions.

## 5. Future perspectives

The GPELF has been deployed into the endemic countries implementing MDA 2-drug regimes (i.e., single annual doses of albendazole in combination with DEC or ivermectin) to reduce microfilaremia prevalence to levels low enough (principally lower than transmission threshold) to interrupt transmission of the disease in the absence of vector control. Based on scientific information on drug resistance to anthelmintics, the issue of albendazole resistance

in the *W. bancrofti* parasite has assumed increasing importance since the GPELF is implemented on a large-scale in at-risk populations in different complex epidemiological settings, and predictably, the implementation phase of the program will increase. Many studies have shown some vulnerability in how the parasite has the ability to evoke molecular mechanisms for resistance to anthelmintics as the nematodes of veterinary importance have developed resistance against both albendazole and ivermectin. Furthermore, there have been lines of evidence that the vulnerability of helminthiasis control programs that employ the MDA with these drugs is associated with several factors that facilitate the promotion of drug-resistant strains. At the same time, the factors are considered concerning the new options of drug combinations with different parasite targets or modes of actions to keep active shelf-life of DEC because the filarial nematode parasites have long life-span and complex life-cycle development. Likewise, to better understand what is relative to achieve MDA's goal to confront growing trend of drug resistance, it is essential for the development of molecularly diagnosing and monitoring the benzimidazole sensitivity/resistance in areas of long-running PELF program implementation using albendazole plus DEC or albendazole plus ivermectin. More applicable tools which will be validated in effective manner are also required to explore the genetic basis for resistance to anthelmintics.

## 6. References

- Albonico, M. (1994). A randomized controlled trial comparing mebendazole and albendazole against *Ascaris*, *Trichuris* and hookworm infections. *Trans R Soc Trop Med Hyg* 88, 585–589.
- Awadzi, K., Boakye, D.A., Edwards, G., Opoku, N.O., Attah, S.K., Osei-Atweneboana, M.Y. et al. (2004). An investigation of persistent microfilaridermias despite multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. *Ann Trop Med Parasitol* 98, 231–249.
- Beach, M.J., Streit, T.G., Addiss, D.G., Prospere, R., Roberts, J.M., Lammie, P.J. (1999). Assessment of combined ivermectin and albendazole for treatment of intestinal helminth and *Wuchereria bancrofti* infections in Haitian schoolchildren. *Am J Trop Med Hyg* 60, 479–486.
- Beech, R.N., Prichard, R.K., Scott, M.E. (1994). Genetic variability of the beta-tubulin genes in benzimidazole-susceptible and resistant strains of *Haemonchus contortus*. *Genetics* 138, 103–110.
- Bennett, A.B., Anderson, T.J., Barker, G.C., Michael, E., Bundy, D.A. (2002). Sequence variation in the *Trichuris trichiura* beta-tubulin locus: implications for the development of benzimidazole resistance. *Int J Parasitol* 32, 1519–1528.
- Bhumiratana, A., Koyadun, S., Suvannadabba, S., Karnjanopas, K., Rojanapremsuk, J., Buddhirakkul, P., et al. (1999). Field trial of the ICT Filariasis for diagnosis of *Wuchereria bancrofti* infections in an endemic population of Thailand. *Southeast Asian J Trop Med Public Health* 30, 562–568.
- Bhumiratana, A., Wattanakull, B., Koyadun, S., Suvannadabba, S., Rojanapremsuk, S., Tantiwattanasup, W. (2002). Relationship between male hydrocele and infection prevalences in clustered communities with uncertain transmission of *Wuchereria*

- bancrofti* on the Thailand-Myanmar border. *Southeast Asian J Trop Med Public Health* 33, 7–17.
- Bhumiratana, A., Siriaut, C., Koyadun, S., Satitvipawee, P. (2004). Evaluation of a single oral dose of diethylcarbamazine 300 mg as provocative test and simultaneous treatment in Myanmar migrant workers with *Wuchereria bancrofti* infection in Thailand. *Southeast Asian J Trop Med Public Health* 35, 591–598.
- Bhumiratana, A., Koyadun, S., Srisuphanunt, M., Satitvipawee, P., Limpairojn, N., Gaewchaiyo, G. (2005). Border and imported bancroftian filariases: baseline seroprevalence in sentinel populations exposed to infections with *Wuchereria bancrofti* and concomitant HIV at the start of diethylcarbamazine mass treatment in Thailand. *Southeast Asian J Trop Med Public Health* 36, 390–407.
- Bhumiratana, A., Pechgit, P., Koyadun, S., Siriaut, C., Yongyuth, P. (2010). Imported bancroftian filariasis: Diethylcarbamazine response and benzimidazole susceptibility of *Wuchereria bancrofti* in dynamic cross-border migrant population targeted by the National Program to Eliminate Lymphatic Filariasis in South Thailand. *Acta Trop* 113, 121–128.
- Bourguinat, C., Keller, K., Prichard, R.K., Geary, T.G. (2011). Genetic polymorphism in *Dirofilaria immitis*. *Vet Parasitol* 176, 368–373.
- Braga, C., Dourado, M. I., Ximenes, R. A. D. A., Alves, L., Brayner, F., Rocha, A., et al. (2003). Field evaluation of the whole blood immunochromatographic test for rapid bancroftian filariasis diagnosis in the northeast of Brazil. *Rev Inst Med Trop Sao Paulo*, 45, 125–129.
- Bundy, D.A.P., Grenfell, B.T., Rajagopwlan, P.K. (1991). Immunoepidemiology of Lymphatic filariasis: the relationship between infection and disease. *Immunol Today* 12, A71–75.
- Castillo, J.C., Reynolds, S.E., Eleftherianos, I. (2011). Insect immune responses to nematode parasites. *Trends Parasitol* (in press), doi:10.1016/j.pt.2011.09.001.
- CDC. (1993). Recommendations of the International Task Force for Disease Eradication. *Morb Mortal Wkly Rep* 42(RR16), 1–38.  
[<http://www.cdc.gov/mmwr/preview/mmwrhtml/00025967.htm>]
- Chanteau, S., Moulia-Pelat, J.P., Glaziou, P., Nguyen, N.L., Luquiaud, P., Plichart, C., et al. (1994a). Og4C3 circulating antigen: a marker of infection and adult worm burden in *Wuchereria bancrofti* filariasis. *J Infect Dis* 170, 247–250.
- Chanteau, S., Glaziou, P., Luquiaud, P., Plichart, C., Moulia-Pelat, J.P., Cartel, J.L. (1994b). Og4C3 circulating antigen, anti-*Brugia malayi* IgG and IgG4 titers in *Wuchereria bancrofti* infected patients, according to their parasitological status. *Trop Med Parasitol* 45, 255–257.
- Chanteau, S., Luquiaud, P., Failloux, A.B., Williams, S.A. (1994c). Detection of *Wuchereria bancrofti* larvae in pools of mosquitoes by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* 88, 665–666.
- Churcher, T.S., Schwab, A.E., Prichard, R.K., Basáñez, M.G. (2008). An analysis of genetic diversity and inbreeding in *Wuchereria bancrofti*: implications for the spread and detection of drug resistance. *PLoS Negl Trop Dis* 2, e211.

- Cole, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmeltjerna, G., Silvestre, A., et al. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 136, 167–185.
- Cox-Singh, J., Pomrehnb, A.S., Wolfec, N.D., Rahmand, H.A., Lua, H., Singha, B. (2000). Sensitivity of the nested-polymerase chain reaction (PCR) assay or *Brugia malayi* and significance of 'free' DNA in PCR-based assays. *Int J Parasitol* 30, 1177–1179.
- de Silva, N., Cuyatt, H., Bundy, D.A. (1997). Anthelmintics: a comprehensive review of their clinical pharmacology. *Drugs* 53, 769–788.
- Dreyer, G., Addiss, D., Santos, A., Figueredo-Silva, J., Norões, J. (1998). Direct assessment in vivo of the efficacy of combined single-dose ivermectin and diethylcarbamazine against adult *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg* 92, 219–222.
- Drogemuller, M., Schieder, T., von Samson-Himmeltjerna, G. (2004). Beta-tubulin complementary DNA sequence variations observed between cyathostomins from benzimidazole-susceptible and -resistant populations. *J Parasitol* 90, 868–870.
- Eberhard, M.L. (1997). Clearance of *Wuchereria bancrofti* antigen after treatment with diethylcarbamazine or ivermectin. *Am J Trop Med Hyg* 57, 483–486.
- Eberhard, M.L., Lammie, P.J., Dickinson, C.M., Roberts, J.M. (1991). Evidence of nonsusceptibility to diethylcarbamazine in *Wuchereria bancrofti*. *J Infect Dis* 163, 1157–1160.
- Elard, L., Cabaret, J., Humbert, J.F. (1999). PCR diagnosis of benzimidazole-susceptibility or -resistance in natural populations of the small ruminant parasite, *Teladorsagia circumcincta*. *Vet Parasitol* 80, 231–237.
- Elard, L., Comes, A.M., Humbert, J.F. (1996). Sequences of beta-tubulin cDNA from benzimidazole-susceptible and -resistant strains of *Teladorsagia circumcincta*, a nematode parasite of small ruminants. *Mol Biochem Parasitol* 79, 249–253.
- Elard, L., Humbert, J.F. (1999). Importance of the mutation of amino acid 200 of the isotype 1  $\beta$ -tubulin gene in the benzimidazole resistance of the small-ruminant parasite *Teladorsagia circumcincta*. *Parasitol Res* 85, 452–456.
- Elard, L., Sauve, C., Humbert, J.F. (1998). Fitness of benzimidazole-resistant and -susceptible worms of *Teladorsagia circumcincta*, a nematode parasite of small ruminants. *Parasitology* 117, 571–578.
- El Setouhy, M., Ramzy, R.M.R., Ahmed, E.S., Kandil, A.M., Hussain, O., Farid, H.A., et al. (2004). A randomized clinical trial comparing single- and multi-dose combination therapy with diethylcarbamazine and albendazole for treatment of bancroftian filariasis. *Am J Trop Med Hyg* 70, 191–196.
- Engelbrecht, F., Oettl, T., Herter, U., Link, C., Philipp, D., Edeghere, H., et al. (2003). Analysis of *Wuchereria bancrofti* infections in a village community in northern Nigeria: Increased prevalence in individuals infected with *Onchocerca volvulus*. *Parasitol Int* 52, 13–20.
- Fischer, P., Boakye, D., Hamburger, J. (2003). Polymerase chain reaction-based detection of lymphatic filariasis. *Med Microbiol Immunol* 192, 3–7.
- Foo, P.K., Tarozzic, A., Mahajan, A., Yoong, J., Krishnan, L., Kopf, D., et al. (2011). High prevalence of *Wuchereria bancrofti* infection as detected by immunochromatographic

- card testing in five districts of Orissa, India, previously considered to be non-endemic. *Trans R Soc Trop Med Hyg* 105, 109–114.
- Freedman, D.O., de Almeida, A., Miranda, J., Plier, D.A., Braga, C. (1997). Field trial of a rapid card test for *Wuchereria bancrofti*. *Lancet* 350, 1681.
- Ghisi, M., Kaminsky, R., Maser, P. (2007). Phenotyping and genotyping of *Haemonchus contortus* isolates reveals a new putative candidate mutation for benzimidazole resistance in nematodes. *Vet Parasitol* 144, 313–320.
- Grenfell, B.T., Micheal, E., Denham, D.A. (1991). A model for the dynamics of human lymphatic filariasis. *Parasitol Today* 7, 318–323.
- Grenfell, B.T., Micheal, E. (1992). Infection and disease in lymphatic filariasis: an epidemiological approach. *Parasitology* 104(suppl), S81–90.
- Guenette, S., Prichard, R.K., Klein, R.D., Matlashewski, G. (1991). Characterization of a beta-tubulin gene and a beta-tubulin gene products of *Brugia pahangi*. *Mol Biochem Parasitol* 44, 153–164.
- Gyapong, J.O., Kumaraswami, V., Biswas, G., Ottesen, E.A. (2005). Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert Opin Pharmacother* 6, 179–200.
- Horton, J. (2000). Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology* 121(suppl), S113–132.
- Horton, J., Witt, C., Ottesen, E.A., Lazdins, J.K., Addiss, D.G., Awadzi, K., et al. (2000). An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. *Parasitology* 121, S147–160.
- Hoti, S.L., Subramanian, K., Das, P.K. (2003). Detection of codon of amino acid 200 in isotype 1 beta-tubulin gene of *Wuchereria bancrofti* isolates, implicated in resistance to benzimidazole in other nematodes. *Acta Trop* 88, 77–81.
- Hoti, S.L., Dhamodharan, R., Subramanian, K., Das, P.K. (2009). An allele specific PCR assay for screening for drug resistance among *Wuchereria bancrofti* populations in India. *Indian J Med Res* 130, 193–199.
- Humbert, J.F., Cabaret, J., Elard, L., Leignel, V., Silvestre, A. (2001). Molecular approaches to studying benzimidazole resistance in trichostrongylid nematode parasites of small ruminants. *Vet Parasitol* 101, 405–414.
- Ismail, M.M., Jayakody, R.L., Weil, G.J., Nirmalan, N., Jayasinghe, K.S.A., Abeyewickrema, W., et al. (1998). Efficacy of single dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* 92, 94–97.
- Kanjanavas, P., Tan-ariya, P., Khawsak, P., Pakpitcharoena, A., Phantana, S., Chansiri, K. (2005). Detection of lymphatic *Wuchereria bancrofti* in carriers and long-term storage blood samples using semi-nested PCR. *Mol Cell Probes* 19, 169–172.
- Koyadun, S., Bhumiratana, A., Prikchu, P. (2003). *Wuchereria bancrofti* antigenemia clearance among Myanmar migrants after biannual mass treatments with diethylcarbamazine, 300 mg oral-dose FILADEC tablet, in Southern Thailand. *Southeast Asian J Trop Med Public Health* 34, 758–767.

- Kwa, M.S.G., Veenstra, J.G., Van Dijk, M., Roos, M.H. (1995).  $\beta$ -Tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. *J Mol Biol* 246, 500–510.
- Kwa, M.S.G., Veenstra, J.G., Roos, M.H. (1993). Molecular characterisation of beta-tubulin genes present in benzimidazole-resistant populations of *Haemonchus contortus*. *Mol Biochem Parasitol* 60, 133–143.
- Kwa, M.S.G., Veenstra, J.G., Roos, M.H. (1994). Benzimidazole resistance in *Haemonchus contortus* is correlated with a conserved mutation at amino acid 200 in beta-tubulin isotype 1. *Mol Biochem Parasitol* 63, 299–303.
- Kwa, M.S.G., Veenstra, J.G., Van Dijk, M., Roos, M.H. (1995).  $\beta$ -Tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. *J Mol Biol* 246, 500–510.
- Kyelem, D., Biswas, G., Bockarie, M.J., Bradley, M.H., El-Setouhy, M., Fischer, P.U., et al. (2008). Determinants of success in national programs to eliminate lymphatic filariasis: a perspective identifying essential elements and research needs. *Am J Trop Med Hyg* 79, 480–484.
- Lacey, E. (1988). The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. *Int J Parasitol* 18, 885–936.
- Lacey, E., Gill, J.H. (1994). Biochemistry of benzimidazole resistance. *Acta Trop* 56, 245–262.
- Lammie, P.J., Hightower, A.W., Eberhard, M.L. (1994). Age specific prevalence of antigenemia in a *Wuchereria bancrofti*-exposed population. *Am J Trop Med Hyg* 51, 348–355.
- Lizotte, M., Supali, T., Partono, F., Williams, S.A. (1994). A polymerase chain reaction assay for the detection of *Brugia malayi* in blood. *Am J Trop Med Hyg* 51, 314–321.
- Maizels, R.M., Lawrence, R.A. (1991). Immunological tolerance: the key feature in human filariasis? *Parasitol Today* 7, 271–276.
- Mani, T.R., Rajendran, R., Munirathinam, A., Sunish, I.P., Abdullah, S.M., Augustin, D.J. et al. (2002). Efficacy of co-administration of albendazole and diethylcarbamazine against geohelminthiasis: a study from South India. *Trop Med Int Health* 7, 541–548.
- Mani, T.R., Rajendran, R., Sunish, I.P., Munirathinam, A., Arunachalam, N., Satyanarayana, K. et al. (2004). Effectiveness of two annual, single-dose mass drug administrations of diethylcarbamazine alone or in combination with albendazole on soil-transmitted helminthiasis in filariasis elimination programme. *Trop Med Int Health* 9, 1030–1035.
- McCarthy, J.S., Guinea, A., Weil, G.J., Ottesen, E.A. (1995). Clearance of circulating filarial antigen as a measure of the macrofilaricidal activity of diethylcarbamazine in *Wuchereria bancrofti* infection. *J Infect Dis* 172, 521–526.
- McCarthy, J.S., Zhong, M., Gopinath, R., Ottesen, E.A., Williams, S.A., Nutman, T.B. (1996). Evaluation of a polymerase chain reaction-based assay for diagnosis of *Wuchereria bancrofti* infection. *J Infect Dis* 173, 1510–1504.
- Meyrowitsch, D.W., Simonsen, P.E., Makunde, W.H. (1995). Bancroftian filariasis: analysis of infection and disease in five communities of north-eastern Tanzania. *Ann Trop Med Parasitol* 89, 653–663.

- Mishra, K., Raj, D.K., Hazra, R.K., Dash, A.P., Supakar, P.C. (2007). The development and evaluation of a single step multiplex PCR method for simultaneous detection of *Brugia malayi* and *Wuchereria bancrofti*. *Mol Cell Probes* 21, 355–362.
- Mitchell, G.F. (1991). Co-evolution of parasites and adaptive immune responses. *Immunol Today* 12, A2–5.
- Molyneux, D.H., Bradley, M., Hoeraul, A., Kyelem, D., Taylor, M.J. (2003). Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends Parasitol* 19, 516–522.
- More, S.J., Copeman, D.B. (1990). A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Trop Med Parasitol* 41, 403–406.
- Nguyen, N.L., Plichart, C., Esterre, P. (1999). Assessment of immunochromatographic test for rapid lymphatic filariasis diagnosis. *Parasite* 6, 355–358.
- Nicolas, L. (1997). New tools for diagnosis and monitoring of bancroftian filariasis parasitism: the Polynesian experience. *Parasitol Today* 13, 370–375.
- Nicolas, L., Luquiaud, P., Lardeux, F., Mercer, D.R. (1999). A polymerase chain reaction assay to determine infection of *Aedes polynesiensis* by *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg* 90, 136–139.
- Norões, J., Dreyer, G., Santos, A., Mendes, V.G., Mendeiros, Z., Addiss, D. (1997). Assessment of the efficacy diethylcarbamazine on adult *Wuchereria bancrofti* in vivo. *Trans R Soc Trop Med Hyg* 91, 78–81.
- Nuchprayoon, S., Porksakorn, C., Junpee, A., Sanprasert, V., Poovorawan, Y. (2003). Comparative assessment of an Og4C3 ELISA and an ICT filariasis test: A study of Myanmar migrants in Thailand. *Asian Pac J Allerg Immunol* 21, 253–257.
- Nuchprayoon, S., Junpee, A., Poovorawan, Y., Scott, A.L. (2005). Detection and differentiation of filarial parasites by universal primers and polymerase chain reaction-restriction fragment length polymorphism analysis. *Am J Trop Med Hyg* 73, 895–900.
- Nuchprayoon, S., Junpee, A., Poovorawan, Y. (2007). Random amplified polymorphic DNA (RAPD) for differentiation between Thai and Myanmar strains of *Wuchereria bancrofti*. *Filaria J* 6:6, doi:10.1186/1475-2883-6-6.
- Oliveira-Menezes, A., Lins, R., Norões, J., Dreyer, G. (2007). Comparative analysis of a chemotherapy effect on the cuticular surface of *Wuchereria bancrofti* adult worms in vivo. *Parasitol Res* 101, 1311–1317.
- Omar, M.S., Sheikha, A.K., Al-Amari, O.M., Abdalla, S.E., Musa, R.A. (2000). Field evaluation of two diagnostic antigen tests for *Wuchereria bancrofti* infection among Indian expatriates in Saudi Arabia. *Southeast Asian J Trop Med Public Health* 31, 415–418.
- Ottesen, E.A. (1992). Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* 104(suppl), S71–79.
- Ottesen, E.A. (2000). The global programme to eliminate lymphatic filariasis. *Trop Med Int Health* 5, 591–594.
- Ottesen, E.A., Duke, B.O.L., Karam, M., Behbehani, K. (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Org* 75, 491–503.

- Ottesen, E.A., Ismail, M.M., Horton, J. (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* 15, 382–386.
- Ottesen, E.A., Hooper, P.J., Bradley, M., Biswas, G. (2008). The global programme to eliminate lymphatic filariasis: health impact after 8 years. *PLoS Negl Trop Dis* 2, e317. doi:10.1371/journal.pntd.0000317.
- Pani, S.P., Hoti, S.L., Elango, A., Yuvaraj, J., Lall, R., Ramaiah, K.D. (2000). Evaluation of the ICT whole blood antigen card test to detect infection due to nocturnally periodic *Wuchereria bancrofti* in South India. *Trop Med Int Health* 5, 359–363.
- Pani, S.P., Hoti, S.L., Vanamail, P., Das, L.K. (2004). Comparison of an immunochromatographic card test with night blood smear examination for detection of *Wuchereria bancrofti* microfilaria carriers. *Natl Med J India*, 17, 304–306.
- Pradeep Kumar, N., Patra, K.P., Hoti, S.L., Das, P.K. (2002). Genetic variability of the human filarial parasite, *Wuchereria bancrofti* in South India. *Acta Trop* 82, 67–76.
- Pechgit, P., Intarapuk, A., Pinyoowong, D., Bhumiratana, A. (2011). Touchdown-touchup nested PCR for low-copy gene detection of benzimidazole-susceptible *Wuchereria bancrofti* with a *Wolbachia* endosymbiont imported by migrant carriers. *Exp Parasitol* 127, 559–568.
- Phantana, S., Sensathein, S., Songtrus, J., Klagrathoke, S., Phongnin, K. (1999). ICT filariasis test: a new screening test for Bancroftian filariasis. *Southeast Asian J Trop Med Public Health* 30, 47–51.
- Rajendran, R., Sunish, I.P., Mani, T.R., Munirathinam, A., Abdullah, S.M., Arunachalarm, N. et al. (2002). The influence of the mass administration of diethylcarbamazine, alone or with albendazole, on the prevalence of filarial antigenaemia. *Ann Trop Med Parasitol* 96, 595–602.
- Rajendran, R., Sunish, I.P., Mani, T.R., Munirathinam, A., Abdullah, S.M., Arunachalarm, N. et al. (2004). Impact of two annual single-dose mass drug administrations with diethylcarbamazine alone or in combination with albendazole on *Wuchereria bancrofti* microfilaraemia and antigenaemia in South India. *Trans R Soc Trop Med Hyg* 98, 174–181.
- Ramzy, R.M.R., Farid, H.A., Kamal, I.H., Ibrahim, G.H., Morsy, Z.S., Faris, R., et al. (1997). A polymerase chain reaction-based assay for detection of *Wuchereria bancrofti* in human blood and *Culex pipiens*. *Trans R Soc Trop Med Hyg* 91, 156–160.
- Ramzy, R.M.R., Helmy, H., El-Lethy, A.S.T., Kandil, A.M., Ahmed, E.S., Weil, G.J., et al. (1999). Field evaluation of a rapid format kit for the diagnosis of bancroftian filariasis in Egypt. *East Mediterr Health J* 5, 880–887.
- Rao, R.U., Atkinson, L.J., Ramzy, R.M.R., Helmy, H., Farid, H.A., Bockarie, M.J., et al. (2006). A real-time PCR-based assay for detection of *Wuchereria bancrofti* DNA in blood and mosquitoes. *Am J Trop Med Hyg* 74, 826–832.
- Ravindran, B., Satapathy, A.K., Sahoo, P.K., Mohanty, M.C. (2003). Protective immunity in human lymphatic filariasis: problems and prospects. *Med Microbiol Immunol* 192, 41–46.
- Robinson, M.W., McFerran, N., Trudgett, A., Hoey, L., Fairweather, I. (2004). A possible model of benzimidazole binding to beta-tubulin disclosed by invoking an inter-domain movement. *J Mol Graph Model* 23, 275–284.



- Rocha, A., Addiss, D., Ribeiro, M.E., Norões, J., Baliza, M., Medeiros, Z., et al. (1996). Evaluation of the Og4C3 ELISA in *Wuchereria bancrofti* infection: infected persons with undetectable or ultra-low microfilarial densities. *Trop Med Int Health* 1, 859–864.
- Roos, M.H., Boersema, J.H., Borgsteede, F.H.M., Cornelissen, J., Taylor, M., Ruitenbergh, E.J. (1990). Molecular analysis of selection for benzimidazole resistance in the sheep parasite *Hemonchus contortus*. *Mol Biochem Parasitol* 43, 77–88.
- Roos, M.H., Kwa, M.S.G., Grant, W.N. (1995). New genetic and practical implications of selection for anthelmintic resistance in parasitic nematodes. *Parasitol Today* 11, 148–150.
- Ruberanziza, E., Mupfasoni, D., Karibushi, B., Rujeni, N., Kabanda, G., Kabera, M., et al. (2009). Mapping of lymphatic filariasis in Rwanda. *J Lymphoedema* 4, 20–23.
- Schwab, A.E., Boakye, D.A., Kyelem, D., Prichard, R.K. (2005). Detection of benzimidazole resistance-associated mutations in the filarial nematode *Wuchereria bancrofti* and evidence for selection by albendazole and ivermectin combination treatment. *Am J Trop Med Hyg* 73, 234–238.
- Schwab, A.E., Churcher, T.S., Schwab, A.J., Basáñez, M.G., Prichard, R.K. (2007). An analysis of the population genetics of potential multi-drug resistance in *Wuchereria bancrofti* due to combination chemotherapy. *Parasitology* 134(Pt 7), 1025–1040.
- Schwab, A.E., Churcher, T.S., Schwab, A.J., Basáñez, M.G., Prichard, R.K. (2006). Population genetics of concurrent selection with albendazole and ivermectin or diethylcarbamazine on the possible spread of albendazole resistance in *Wuchereria bancrofti*. *Parasitology* 133(Pt 5), 589–601.
- Silvestre, A., Cabaret, J., Humbert, J.F. (2001). Effect of benzimidazole under-dosing on the resistant allele frequency in *Teladorsagia circumcincta* (Nematoda). *Parasitology* 123, 103–111.
- Silvestre, A., Humbert, J.F. (2002). Diversity of benzimidazole-resistance alleles in populations of small ruminant parasites. *Int J Parasitol* 32, 921–928.
- Simonsen, P.E., Dunyo, S.K. (1999). Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Trans R Soc Trop Med Hyg* 93, 278–282.
- Simonsen, P.E., Lemnge, M.M., Msangeni, H.A., Jakobsen, P.H., Bygbjerg, I.C. (1996). Bancroftian filariasis: The patterns of filarial-specific immunoglobulin G1 (IgG1), IgG4, and circulating antigens in an endemic community of northeastern Tanzania. *Am J Trop Med Hyg* 55, 69–75.
- Simonsen, P.E., Meyrowitsch, D.W., Jaoko, W.G., Malecela, M.N., Michael, E. (2008). Immunoepidemiology of *Wuchereria bancrofti* infection in two East African communities: Antibodies to the microfilarial sheath and their role in regulating host microfilaremia. *Acta Trop* 106, 200–206.
- Siriaut, C., Bhumiratana, A., Koyadun, S., Anurat, K., Satitvipawee, P. (2005). Short-term effects of a treatment with 300 mg oral-dose diethylcarbamazine on nocturnally periodic *Wuchereria bancrofti* microfilaremia and antigenemia. *Southeast Asian J Trop Med Public Health* 36, 832–840.

- Siridewa, K., Karunanayake, E.H., Chandrasekharan, N.V. (1996). Polymerase chain reaction based technique for the detection of *Wuchereria bancrofti* in human blood samples, hydrocele fluid, and mosquito vectors. *Am J Trop Med Hyg* 54, 72–76.
- Srividya, A., Pani, S.P., Rajagopalan, P.K., Bundy, D.A.P., Grenfell, B.T. (1991). The dynamics of infection and disease in Bancroftian filariasis. *Trans R Soc Trop Med Hyg* 85, 255–259.
- Sunish, I.P., Rajendran, R., Mani, T.R., Gajanana, A., Reuben, R., Satyanarayana, K. (2003). Long-term population migration: an important aspect to be considered during mass drug administration for elimination of lymphatic filariasis. *Trop Med Int Health* 8, 316–321.
- Sunish, I.P., Rajendran, R., Mani, T.R., Munirathinam, A., Tewari, S.C., Hiriyana, J., et al. (2002). Resurgence in filarial transmission after withdrawal of mass drug administration and the relationship between antigenaemia and microfilaraemia - A longitudinal study. *Trop Med Int Health* 7, 59–69.
- Takagi, H., Itoh, M., Kasai, S., Yahathugoda, T.C., Weerasooriya, M.V., Kimura, E. (2011). Development of loop-mediated isothermal amplification method for detecting *Wuchereria bancrofti* DNA in human blood and vector mosquitoes. *Parasitol Int* (in press), doi:10.1016/j.parint.2011.08.018.
- Thanomsub, B.W., Chansiri, K., Sarataphan, N., Phantana, S. (2000). Differential diagnosis of human lymphatic filariasis using PCR-RFLP. *Mol Cell Probes* 14, 41–46.
- Turner, P., Copeman, D.B., Gerisi, D., Speare, R. (1993). A comparison of the Og4C3 antigen capture ELISA, the knott test, an IgG4 assay and clinical sign, in the diagnosis of bancroftian filariasis. *Trop Med Parasitol* 44, 45–48.
- von Samson-Himmelstjerna, G., Pape, M., von Witzendorff, C., Schnieder, T. (2002). Allele-specific PCR for the beta-tubulin codon 200 TTC/TAC polymorphism using single adult and larval small strongyle (Cyathostominae) stages. *J Parasitol* 88, 254–257.
- Weaver, A., Brown, P., Huey, S., Magallon, M., Bollman, E. B., Mares, D., et al. (2011). A low-tech analytical method for diethylcarbamazine citrate in medicated salt. *PLoS Negl Trop Dis* 5, e1005, doi: 10.1371/journal.pntd.0001005.
- Weil, G.J., Liffis, F. (1987). Identification and partial characterization of a parasite antigen in sera from humans infected with *Wuchereria bancrofti*. *J Immunol* 138, 3035–3041.
- Weil, G.J., Jain, D.C., Santhanam, S., Malhotra, A., Kumar, H., Sethumadhavan, K.V.P. et al. (1987). A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis. *J Infect Dis* 156, 350–355.
- Weil, G.J., Ramzy, R.M.R., Chandrashekar, R., Gad, A.M., Lowrie Jr., R.C., Faris, R. (1996). Parasite antigenemia without microfilaremia in bancroftian filariasis. *Am J Trop Med Hyg* 55, 333–337.
- Weil, G.J., Lammie, P.J., Weiss, N. (1997). The ICT Filariasis Test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitol Today* 13, 401–404.
- Who. (1992a). Lymphatic filariasis: disease and its control. Fifth Report of the WHO Expert Committee on Filariasis. Geneva: World Health Organization. *WHO Tech Rep Ser* 821, p. 1–71.

- Who. (1992b). Informal consultation on evaluation of morbidity in lymphatic filariasis, Tuberculosis Research Centre, Madras 10-11 February 1992. Geneva: World Health Organization. Mimeographed document WHO/TDR/FIL/92.3.
- Who. (1994). Lymphatic filariasis infection and disease: control strategies. Report of a consultative meeting held at the University Sains Malaysia, Penang, Malaysia, August 1994. Geneva: World Health Organization. Mimeographed document WHO/TDR/CTD/FIL/PENANG/94.1.
- Who. (1999a). Informal consultation on epidemiologic approaches to lymphatic filariasis elimination: initial assessment, monitoring, and certification, Atlanta, Georgia, USA 2-4 September 1998. Geneva: World Health Organization. Mimeographed documented WHO/FIL/99.195.
- Who. (1999b). Guidelines for certifying lymphatic filariasis elimination (including discussion of critical issues and rationale), following from Informal consultation on epidemiologic approaches to lymphatic filariasis elimination: initial assessment, monitoring, and certification, Atlanta, Georgia, USA 2-4 September 1998. Geneva: World Health Organization. Mimeographed documented WHO/FIL/99/197.
- Who. (2000). Elimination of lymphatic filariasis. Report of informal consultative meeting on lymphatic filariasis in SEA region, Bhubaneswar, Orissa, India, 23-25 February 2000.
- Who. (2001). Regional strategic plan for elimination of lymphatic filariasis (2000-2004). South-East Asia Regional Office, New Delhi: World Health Organization. Mimeographed documented SEA/FIL/28.corr.1.
- Who. (2002). Global programme to eliminate lymphatic filariasis: Annual report on lymphatic filariasis 2001. Geneva: World Health Organization. Mimeographed document WHO/CDS/CPE/CEE/2002.28.
- Who. (2008). Global programme to eliminate lymphatic filariasis. *Wkly Epidemiol Rec* 83, 333–341.
- Who. (2009). Report: The role of polymerase chain reaction technique for assessing lymphatic filariasis transmission. Geneva: World Health Organization, p.1–55. Mimeographed document WHO/HTM/NTD/PCT/2009.1.
- Who. (2010). Progress report 2000–2009 and strategic plan 2010–2020 of the global programme to eliminate lymphatic filariasis: halfway towards eliminating lymphatic filariasis. Geneva: World Health Organization, p.1–93. Mimeographed document WHO/HTM/NTD/PCT/2010.6
- Williams, S.A., Nicolas, L., Lizotte-Waniewski, M., Plichart, C., Luquiaud, P., Nguyen, L.N., et al. (1996). A polymerase chain reaction assay for the detection of *Wuchereria bancrofti* in blood samples from French Polynesia. *Trans R Soc Trop Med Hyg* 90, 384–387.
- Winterrowd, C.A., Pomroy, W.E., Sangster, N.C., Johnson, S.S., Geary, T.G. (2003). Benzimidazole-resistant  $\beta$ -tubulin alleles in a population of parasitic nematodes (*Cooperia oncophora*) of cattle. *Vet Parasitol* 117, 161–172.
- Yongyuth, P., Koyadun, S., Jaturabundit, N., Sampuch, A., Bhumiratana, A. (2006). Efficacy of a single-dose treatment with 300 mg diethylcarbamazine and a combination of 400 mg albendazole in reduction of *Wuchereria bancrofti* antigenemia and

concomitant geohelminths in Myanmar migrants in Southern Thailand. *J Med Assoc Thai* 89, 1237–1248.

Zhong, M., McCarthy, J.S., Bierwert, L., Lizotte-Waniewski, M., Chanteau, S., Nutman, T.B., et al. (1996). A polymerase chain reaction assay for detection of the parasite *Wuchereria bancrofti* in human blood samples. *Am J Trop Med Hyg* 54, 357–363.

Zou, Z., Shin, S.W., Alvarez, K.S., Kokoza, V., Raikhel, A.S. (2010). Distinct melanization pathways in the mosquito *Aedes aegypti*. *Immunity* 32, 41–53.



## **Current Topics in Tropical Medicine**

Edited by Dr. Alfonso Rodriguez-Morales

ISBN 978-953-51-0274-8

Hard cover, 564 pages

**Publisher** InTech

**Published online** 16, March, 2012

**Published in print edition** March, 2012

Tropical Medicine has emerged and remained as an important discipline for the study of diseases endemic in the tropic, particularly those of infectious etiology. Emergence and reemergence of many tropical pathologies have recently aroused the interest of many fields of the study of tropical medicine, even including new infectious agents. Then evidence-based information in the field and regular updates are necessary. Current Topics in Tropical Medicine presents an updated information on multiple diseases and conditions of interest in the field. It includes pathologies caused by bacteria, viruses and parasites, protozoans and helminths, as well as tropical non-infectious conditions. Many of them are considering not only epidemiological aspects, but also diagnostic, therapeutical, preventive, social, genetic, bioinformatic and molecular ones. With participation of authors from various countries, many from proper endemic areas, this book has a wide geographical perspective. Finally, all of these characteristics, make an excellent update on many aspects of tropical medicine in the world.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Adisak Bhumiratana, Apiradee Intarapuk, Danai Sangthong, Surachart Koyadun, Prapassorn Pechgit and Jinrapa Pothikasikorn (2012). Molecular Diagnosis and Monitoring of Benzimidazole Susceptibility of Human Filariids, Current Topics in Tropical Medicine, Dr. Alfonso Rodriguez-Morales (Ed.), ISBN: 978-953-51-0274-8, InTech, Available from: <http://www.intechopen.com/books/current-topics-in-tropical-medicine/molecular-diagnosis-and-monitoring-of-benzimidazole-susceptibility-of-human-filariids>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.