FACTORS ASSOCIATED WITH BRUGIAN LYMPHATIC FILARIASIS INFECTION IN TERENGGANU, 2010-2019

by

DR AHMAD SYAIFY BIN BERAHIM

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LIST OF SYMBOLS

%	Percent
°C	Celsius
=	Equal to
≥	More than and equal to
≤	Less than and equal to
>	More than
<	Less than

LIST OF ABBREVIATION

AOR	Adjusted Odds Ratio
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
MDA	Mass Drug Administration
MOH	Ministry of Health
OR	Odds Ratio
SPSS	Statistical Package for the Social Sciences
TAS	Transmission Assessment Survey
WHO	World Health Organisation

ABSTRAK

FAKTOR-FAKTOR BERKAITAN DENGAN KEJADIAN KES LIMFATIK FILARIASIS BRUGIA DI NEGERI TERENGGANU, 2010-2019

Latar belakang: Filariasis limfatik adalah sejenis penyakit tropika bawaan nyamuk yang yang berpunca daripada cacing nematoda *Wuchereria bancrofti, Brugia malayi* dan *Brugia timori* dengan *Brugia malayi* selaku spesies utama di Terengganu. Filariasis limfatik menyebabkan morbiditi teruk dalam jangka panjang yang boleh mempengaruhi faktor penentu sosial kesihatan yang lain. Penduduk Terengganu di lokaliti endemik telah menjalani pemberian ubat beramai-ramai (MDA) bermula dari tahun 2004 sehingga tahun 2008 dan telah lulus ketiga-tiga tinjauan penilaian jangkitan (TAS). Walau bagaimanapun, Terengganu masih melaporkan kes sporadik melalui survelan aktif pengambilan darah filaria pada waktu malam.

Objektif: Tujuan utama kajian ini adalah untuk mengenalpasti faktor-faktor yang berkaitan dengan kejadian jangkitan filariasis dari tahun 2010 hingga 2019 di negeri Terengganu di samping menentukan nisbah dan taburan kes *Brugia malayi* yang telah didaftarkan.

Metodologi: Kajian ini adalah kajian kawalan-kes (case-control). Kajian ini menggunakan data sekunder dari eVekpro atas talian, pengkalan data Jabatan Kesihatan Negeri Terengganu, Jabatan Meteorologi Malaysia dan Unit Entomologi, Jabatan Kesihatan Negeri Terengganu. Persampelan rawak mudah digunakan bagi pengambilan sampel yang telah dikenalpasti menepati kriteria kajian yang telah ditetapkan. Data kategori diterangkan menggunakan frekuensi dan peratusan sementara min dan sisihan piawai untuk data berangka. Data-data dianalisa dengan menggunakan *Multiple Logistic Regression* untuk menilai perkaitan antara faktor-faktor dengan kejadian jangkitan filariasis.

Keputusan: Prevalen jangkitan filariasis yang diperoleh daripada kajian ini adalah 33.7 per 1,000,000 populasi pada tahun 2010 dan menurun menjadi 5.6 per 1,000,000 populasi pada tahun 2019. Data yang diperoleh dianalisa menggunakan Multiple Logistic Regression. Terdapat kaitan antara faktor umur ≥ 40 tahun (AOR:0.395, 95%CI: 0.209, 0.747), min bulanan taburan hujan (mm) (AOR: 1.003, 95%CI: 1.000, 1.005), min bulanan kelembapan (%) (AOR: 0.846, 95%CI: 0.745, 0.961), kepadatan vektor (%) (AOR: 1.030, 95%CI: 1.012, 1.048) dan kehadiran vektor *Mansonia* (AOR: 0.083, 95%CI: 0.034, 0.205) dengan berlakunya kejadian jangkitan filariasis.

Kesimpulan: Kajian ini mendapati faktor-faktor yang boleh dikaitkan dengan kejadian jangkitan filariasis di Terengganu dari tahun 2010 sehingga tahun 2019 (selepas pemberian ubat beramai-ramai) adalah umur, min bulanan taburan hujan (mm), min bulanan kelembapan (%), kepadatan vektor (%), dan kehadiran vektor *Mansonia*. Nisbah jangkitan mikrofilariasis di Terengganu sebelas tahun selepas MDA adalah kurang daripada satu peratus. Kajian setempat berkaitan vektor filariasis perlu dipertingkatkan untuk memastikan vektor di lokaliti endemik dan juga lokaliti-lokaliti yang dikenalpasti sebagai berisiko tidak mengandungi cacing filariasis. Penduduk yang berusia muda harus diberi tumpuan semasa program saringan dan program promosi kesihatan kerana mereka lebih rentan terhadap jangkitan filariasis disebabkan oleh aktiviti yang dijalankan oleh mereka semasa waktu aktif vektor menggigit. Perubahan cuaca harus dijadikan petunjuk oleh pegawai kesihatan daerah akan kemungkinan peningkatan jangkitan filariasis semasa program saringan di samping input daripada kajian entomologi yang harus dianalisis dengan sewajarnya. Walaupun kajian ini mendapati tiada kaitan yang signifikan antara lokaliti tidak endemik dengan kejadian kes sporadik, lokaliti bersebelahan dengan lokaliti endemik dicadangkan agar tetap diberi perhatian khusus kerana lokaliti ini tidak terlibat dalam program MDA tetapi mempunyai faktor cuaca dan persekitaran vektor yang hampir sama dengan lokaliti endemik yang mempunyai kaitan signifikan dengan kejadian kes filariasis.

Kata kunci: filariasis limfatik, tinjauan penilaian penularan, pemberian ubat beramai-ramai, *Brugia malayi*

ABSTRACT

FACTORS ASSOCIATED WITH BRUGIAN LYMPHATIC FILARIASIS CASES IN TERENGGANU, 2010-2019

Background: Lymphatic filariasis is a neglected mosquito-borne tropical disease which is caused by nematodes *Wuchereria bancrofti, Brugia malayi* or *Brugia timori* with *Brugia malayi* as the main species in Terengganu. Lymphatic filariasis cause severe morbidity rather than mortality which can influence other social determinants of health. Terengganu had undergone mass drug administration (MDA) from year 2004 until year 2008 and has passed all three transmission assessment surveys (TAS). Nevertheless, sporadic cases were still reported through active surveillance of night blood survey.

Objectives: The main objectives of this study were to determine the associated factors of registered *Brugia malayi* infection from year 2010 until 2019 in Terengganu state and to describe its proportion and distribution.

Methodology: This study was a case-control study and it utilized secondary data from eVekpro online, Terengganu State Health Department, Malaysia Meteorology Department and Entomology Unit, Terengganu State Health Department databases. Simple random sampling applied to the samples which fit to the assigned criteria. The categorical data were described using frequency and proportion while mean and standard deviation for numerical data. Data were analysed using Multiple Logistic Regression to look for associations.

Result: The prevalence of filariasis infection from this study was 33.7 per 1,000,000 populations in year 2010 and decrease to 5.6 per 1,000,000 population in year 2019. The data which were analysed using Multiple Logistic Regression found associations of age ≥ 40 years old (AOR:0.395, 95%CI: 0.209, 0.747), mean monthly rainfall (mm) (AOR: 1.003, 95%CI: 1.000, 1.005), mean monthly humidity (%) (AOR: 0.846, 95%CI: 0.745, 0.961), vector density (%) (AOR: 1.030, 95%CI: 1.012, 1.048) and presence of *Mansonia* vector (AOR: 0.083, 95%CI: 0.034, 0.205) with the occurrence of filariasis infection.

Conclusion: The associated factors of filariasis infection in Terengganu state from year 2010 until year 2019 (after Mass Drug Administration) were age, mean monthly rainfall (mm), mean humidity (%), vector density (%) and *Mansonia* species. The proportion of filariasis infection in Terengganu eleven years post MDA was still lower than one percent as being targeted for filariasis elimination. More local studies on filariasis vectors are needed to ensure that vectors in endemic localities as well as localities identified as at risk do not contain filariasis worms. Young age group should be given special attention during screening and health promotion program as they were more prone to infection due to their activities during active biting time of the vectors. Change of weather should alert the district health officer of possibilities of increase in filariasis infection detection during screening program whereas the data input from entomology study should be analysed accordingly. Although this study found no significant association between non-endemic localities with sporadic case occurrence, localities adjacent to endemic localities are recommended to remain special attention because these localities were not involved in the MDA program but their weather and environmental vector factors are similar to endemic localities which have significant association with the occurrence of filariasis cases.

Keywords: lymphatic filariasis, transmission assessment survey, mass drug administration, *Brugia malayi*

CHAPTER 1

INTRODUCTION

1.1 Background

According to the World Health Organization [WHO] (2020a), lymphatic filariasis is a neglected mosquito-borne tropical disease which caused by nematodes *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori*. Centers for Disease Control and Prevention [CDC] (2019b) states that an adult worm which lives in the human lymph vessels, has a life expectancy of about five to seven years and will mate and release millions of microfilariae in the blood stream throughout its life. The vector for this disease includes mosquitoes from genera *Anopheles*, *Mansonia*, *Culex* and *Aedes* in which the distribution, ecology, biology and transmission potential for each genera vary greatly (WHO, 2013). Environmental factors influence disease occurrence indirectly by influencing the vector's preference for survival. The main host or reservoir for this disease is human while animals' reservoir is of less epidemiologic importance (Centres for Disease Control and Prevention, 2019b). Nevertheless, in certain areas, zoonotic transmission had been reported and its potential as a true reservoir in the future still controversial.

Chronic infection leads to severe morbidity and disability from progressive, irreversible swelling of the limbs and genitals due to lymphatic dysfunction (Dickson *et al.*, 2017; Misra *et al.*, 2016). The disease seldom cause mortality but cause major debilitating effects to the patients and result in 3.3 million DALYs (disability-adjusted life years) (Kwarteng and Ahuno, 2017). Initial goal of eliminating this disease globally by year 2020 has been rescheduled to year 2030 because it was no longer possible to be achieved (World Health Organisation, 2020b).

Eliminating this disease does not only reduce suffering but also contribute to the reduction of poverty (World Health Organisation, 2020a). Poverty is one of the major social determinants of health globally and improving this factor will improve the overall public health. However, majority of individuals who were infected with lymphatic filariasis parasites were asymptomatic for years, with relatively few progressing to acute and chronic stages (Nutman, 2003; CDC, 2018). This makes it difficult to detect this disease in time to avoid chronic infection and complications in the future. Therefore, a good surveillance should be made available to ensure asymptomatic persons to be diagnosed and treated early to halt the transmission. Mass drug administration is still the best strategy for endemic area due the natural history of the disease.

In year 2000, over 120 million people were infected by filariasis parasites, with about 40 million become deformed and in year 2018, 893 million people in 49 countries were living in areas that require preventive chemotherapy to stop the spread of infection while 25 million men already affected with hydrocele and over 15 million people with lymphoedema (WHO, 2020a). Severe lymphoedema is under-reported in Africa, while reporting from South East Asia has increased over the past few years (CDC, 2019b). Increase awareness and improve surveillance provide better view of the burden of the disease as well as providing better knowledge for control program.

Around the globe, 90% of lymphatic filariasis cases were caused by *Wuchereria bancrofti* and the remainder were caused by *Brugia malayi* and to the lesser extent *Brugia Timori* (World Health Organisation, 2020a). *Brugia malayi* mainly distributed in India, Indonesia and South East Asia (WHO, 2010). Other countries with *Wuchereria bancrofti* lymphatic filariasis have been successful in eliminating the disease. However, countries with *Brugia malayi* infection such as Malaysia still striving to stop the transmission, thus, more study should be conducted to search for any possibility of different approach to achieve the elimination target.

Locally, WHO reports indicate that 116 of the 994 implementation units (sub-districts and districts) are endemic for filariasis across Peninsular Malaysia, Sabah and Sarawak with 1.12 million people at risk to get the infection. Sub-periodic *Brugia malayi* accounts for the majority of cases, with 2% caused by *Wuchereria bancrofti*. Government data suggest microfilaria prevalence had decreased from 1.0% to 0.2% in Peninsular Malaysia and 5.5% to 1.5% in Sabah and Sarawak from the 1980s until the commencement of the National Mass Drug Administration (MDA) program in 2004. However, other pre-MDA studies of the local population in Peninsular Malaysia, Sabah and Sarawak found a prevalence of *Brugia malayi* microfilaria of 0.26–23.85%, 20.69% and 0.90%, respectively (Dickson *et al.*, 2017). Although the prevalence has been decreasing over the years, improved surveillance, prevention and control should be strengthened due to the chronicity nature of the disease. There will be chances those asymptomatic carriers have not been diagnosed and keep transmitting the disease to other people and manifest as lymphoedema or elephantiasis later in their life.

Further studies are required to access current infection prevalence and the need for extra cycles of MDA especially around the border of endemic area. Limited data on the prevalence of lymphatic filariasis associated morbidity in the local Malaysian population were found in studies. Several South East Asian countries, including Cambodia, Thailand, and Vietnam, have already eliminated lymphatic filariasis, albeit with a different species distribution of *Wuchereria bancrofti* (Dickson *et al.*, 2017). The uniqueness of *Brugia malayi* infection provide opportunity for Malaysia to lead on the research for this disease in this region. In contrary, Malaysia is still working toward the elimination and more data is needed to support the evidence of transmission of lymphatic filariasis has been stopped in this country.

Terengganu state is one of the endemic states for lymphatic filariasis in Peninsular Malaysia with *Brugia malayi* as the main agent (Al-Abd *et al.*, 2014; WHO, 2017b). Terengganu had conducted mapping process to determine the endemicity of its sub-district in the year 2002 and subsequently 19 sub-districts had been recognized as red implementation unit (IU) (endemic area). The population within the localities had undergone MDA from year 2004 to 2008 (WHO, 2017b). Certain areas in Malaysia still did not reach the target of one percent prevalence such as in Tangkarason, Sabah whereby in year 2014 their microfilaria rate was 2.4%, but compared to Indonesia, this rate still lower as their national disease prevalence was 4.7% in year 2016 (WHO, 2017b).

Nevertheless, even countries which had achieved elimination should continue their surveillance. WHO reported that out of 17 countries which had reached the target, only six countries reported surveillance activities and it proposed that without extensive post validation activities, transmission can remain undetected and subsequently the number of infection can increase to previous level (World Health Organisation, 2020b). Therefore, a country like Malaysia which is on the verge of eliminating the disease should determine the factors associated with *Brugian* infection in order to strengthen the surveillance system post elimination as the study before MDA program regarding associated factors of this disease in Malaysia was also limited.

1.2 Problem Statements

Terengganu is one the endemic filarial state in Malaysia and the main lymphatic filariasis species in Terengganu are *Brugia malayi*. In South East Asia countries where the lymphatic filariasis had been eliminated, the species were primarily *Wuchereria bancrofti* with *Brugia malayi* as the secondary species. Therefore, it will be useful to look deeper onto *Brugia malayi* infection as many studies were focusing on *Wuchereria bancrofti* species as they were the major species globally while in fact the main species in Malaysia and Terengganu state specifically were from *Brugia malayi* species.

Although Mass Drug Administration (MDA) was implemented in 19 red implementation units in Terengganu started from year 2004 to year 2008 until completed 5 cycles, Terengganu still reported sporadic cases of lymphatic filariasis infection to date (JKNT, 2014). Nevertheless, there are limited study to date concerning associated factors of *Brugia malayi* filariasis infection in Malaysia as well as its current prevalence.

The presence of filariasis infection in spite of less than one percent microfilaria rate had been achieved is not an absolute indicator to reduce surveillance activities as the areas which were adjacent to endemic areas were not included in the transmission assessment survey and may harbour infection due to the nature of vector borne diseases and increase the number of sporadic cases later on.

There were many associated factors related to lymphatic filariasis infection which may be altered compared to before MDA if the environment, dynamicity of vector, agent or human host in term of their daily activities has evolved over time due development in the area. However, there were limited studies after the Mass Drug

Administration Program in year 2008 in Peninsular Malaysia while in fact, it is important to re-evaluate the current situation after completion of the intervention in addition to transmission assessment survey as Malaysia yet to achieve the aim of lymphatic filariasis elimination by year 2020.

1.3 Rationale of the study

By studying the associated factors of *Brugia malayi* among all registered filaria cases in Terengganu from year 2010 to 2019 (post MDA), it will help to re-evaluate the situation of filariasis infection for the past ten years and prepare necessary resources for better transmission control. This is important to ensure that even though Terengganu state had passed all the three Transmission Assessment Survey (TAS) in year 2011, 2015 and 2017, the survey was only included primary school children aged seven years old as the subjects and did not involve any subject from the adjacent area to the endemic area which can be risky as a source of transmission as well. Due to the nature of vector borne diseases, it is important to identify whether the associated factors are significant factors and also present in the areas adjacent to the endemic areas and warrant further survey at those areas. The knowledge will help to determine whether an infection will likely to occur in those previously classified as non-endemic areas.

It will also expand the knowledge about *Brugia malayi* species which mainly infest population from South Asia and South East Asia. There were limited data or publications regarding *Brugia malayi* infection in this region epidemiologically. *Brugia malayi* is not the dominant species for lymphatic filariasis globally but it is an important species in this region particularly Malaysia as most of the filariasis infection were from this species. Therefore, by conducting this study, it can contribute to knowledge sharing particularly in Peninsular Malaysia by focusing on Terengganu state. The findings at other South East Asea country such as Indonesia in which there were possibilities of zoonotic transmission of *Brugian* filariasis increased the need for further evaluation post MDA to identify possibilities of other reservoirs.

This study is also believed to benefit stakeholders in decision making and policy development towards elimination of lymphatic filariasis. In any elimination program, it is important to involves multiple agencies. By conducting this study, the significant associated factors can help to convince the stakeholders to decide on the issue. For example, if the cases were significantly occurred in the non-endemic areas, additional measures need to be taken to stop the transmission as these areas were excluded from MDA previously.

1.4 Research questions

i. What are the proportion and distribution of registered *Brugia malayi* lymphatic filariasis cases in Terengganu, 2010-2019?
ii. What are the associated factors of registered *Brugia malayi* lymphatic filariasis cases in Terengganu, 2010-2019?

1.5 Objectives

1.5.1 General objective

To study associated factors of *Brugia malayi* among all registered lymphatic filariasis cases in Terengganu from 2010-2019.

1.5.2 Specific objectives

i. To describe the proportion and distribution of registered *Brugia malayi* cases in Terengganu, 2010-2019.
ii. To determine associated factors of registered *Brugia malayi* in Terengganu, 2010-2019.

1.6 Research hypotheses

There are associations between sociodemographic (age, sex, ethnicity), environmental factors (humidity, rainfall, temperature) and vector density with registered *Brugia malayi* cases in Terengganu.

CHAPTER 2

LITERATURE REVIEW

The literature search was conducted via multiple databases including Scopus, PubMed, Wiley Online Library, Directory of Open Access Journal (DOAJ) and Google scholar. Keywords used in the search include filariasis, *Brugia malayi,* factors, filariasis vectors, elimination, mass drug administration, pathophysiology and transmission.

2.1 Pathophysiology and transmissibility of lymphatic filariasis infection

Lymphatic filariasis is a vector-borne disease. The commonest vector for filariasis in Southeast Asia is mosquito species in the genera *Mansonia* and *Aedes* (Centres for Disease Control and Prevention, 2019a).

The third-stage filarial larvae are introduced into the susceptible host during the vector blood meal. The larvae on the skin of the human host will penetrate into the bite wound into the body and develop into adults that usually live in the lymphatic system (Kwarteng and Ahuno, 2017). Female worms measure 43mm to 55mm in length by 130µm to 170µm in width, and males measure 13mm to 23mm in length by 70µm to 80µm in width. Sheathed microfilariae will be produced by female adult worms and have nocturnal periodicity or sub-periodicity in some places. Their size ranges from 177µm to 230µm in length and 5µm to 7µm in width. They will migrate in the lymph to enter the blood stream where another vector consumes them and transmits to another susceptible host after undergone transformation stage within the mosquitoes (Centres for Disease Control and Prevention, 2019a).

The accumulation of adult worm and microfilariae and repetitive inflammation process within the lymphatic system led to the development of severe lymphedema of the limbs (elephantiasis) and occasionally genitalia (hydrocele) due to dysfunction of lymphatic vessels. This will manifest as grossly swollen limbs, thick and pitted skin and may present with secondary infection. They may also present with tropical pulmonary eosinophilia, a chronic syndrome whereby it involves peripheral hypereosinophilia, wheezing, chest pain, splenomegaly, and bloody sputum and more frequently found in South and Southeast Asia (Centres for Disease Control and Prevention, 2019a).

2.2 Towards eliminating filariasis

In year 2010, WHO published the strategic plan of the Global Programme to Eliminate Lymphatic Filariasis 2010–2020 but targeting filariasis elimination in 2020 has been hardly achieved and new target has been set to year 2030. By the end of year 2011, 53 out of 73 endemic countries were implementing mass drug administration, of which 12 had moved to the surveillance phase (WHO, 2013). Under this program, until 2019, over 8.2 billion cumulative treatments has been delivered to more than 923 million people since 2000 with the objective of eliminating the parasites in the blood of infected people and prevent the risk of transmission in the community and as a result, infection level has been decreasing to the level no further MDA is needed (WHO, 2020).

Even though Malaysia has come close to filariasis elimination, surveillance still should be continued to avoid re-emergence of the disease post elimination. Sri Lanka which achieved elimination in year 2016, has reported increase of filariasis cases which being identified due to the migrant microfilaria carriers from neighbouring endemic regions (South India) or indigenous transmission of the zoonotic strain of *Brugia malayi* that is prevalent among the canine population of the country (Chandrasena *et al.*, 2016). A study in Belitung, Indonesia also found that, even though it had completed its MDA cycle in year 2010 and had passed three transmission assessment survey (TAS), the microfilaria rate had increased to more than one percent which was above the threshold indicated by WHO to prove transmission of the disease had been stopped in a locality (Santoso *et al.*, 2020).

2.3 Risk Factors for Lymphatic Filariasis Infection

According to Dickson et al., (2017), in spite of significant burden of lymphatic filariasis in South East Asia, there are limited reliable data on the current prevalence of infection and morbidity. In many studies, there are several factors which are associated with lymphatic filariasis infection and it can be divided into three main domains which are sociodemographic factors, environmental factors and the factor of the vectors for disease transmission.

2.3.1 Sociodemographic Factors

In sociodemographic factors, male gender and adult age has a strong association with lymphatic filariasis infection. A case control study in Andra Pradesh, India found that higher numbers of microfilaria cases were found in males than in females particularly in the age groups of 26 to 40 followed by 41 to 60 years old (Mutheneni *et al.*, 2016). This is also similar to a study in American Samoan whereby the risk of lymphatic filariasis was strongly associated with male gender and older age

(Graves *et al.*, 2020). This shows that these groups were more exposed to the filariasis vector possibility due to their activities during active biting period.

Another factor which has strong association with this infection is agricultural related job, lower socioeconomic status and middle income. In India, higher number of filarial cases were reported among persons who engaged in agriculture than in other occupations and who were in middle income group (Mutheneni *et al.*, 2016). This was supported by another study which stated risk of lymphatic filariasis infection was strongly associated with lower socioeconomic status (Graves *et al.*, 2020). This was probably because lower socioeconomic group were mainly among who involved in agricultural sectors in developing countries.

Between year 2004 to 2008, countries which were endemic for lymphatic filariasis underwent Mass Drug Administration (MDA) Program with the aim to stop the transmission. However, within the country itself the areas were further divided into endemicity according to Implementation Unit and not all the populations in the specific countries were involved in the program. A study found that a higher number of filaria infections was observed among those who did not participated in MDA programs and lack of knowledge regarding mosquito transmission (Ikhwan *et al.*, 2016; Mutheneni *et al.*, 2016; Graves *et al.*, 2020). Therefore, this study is important to suggest whether previous non-endemic areas also need the MDA in view of dynamicity of a vector borne disease.

2.3.2 Environmental Factors

In environmental factors, higher rainfall, high humidity and high temperature are strongly associated with lymphatic filariasis infection. This can be related to the abundance of vector within optimum condition and increase probability of transmission. Higher rainfall (>700 mm per year) contributed to expansion of vector habitats and population (Burgert-Brucker *et al.*, 2020). Possibility for filariasis disease occurrence increase with mosquito density which can be found more in places that has suitable temperature and humidity. The development of lymphatic filariasis parasites in the mosquito takes two weeks at 27°C and 90% humidity (Edirisinghe, 2017; Ikhwan *et al.*, 2016; Pratiwi *et al.*, 2019).

Type of house, proximity to swamp area and use of mosquito nets also associated with lymphatic filariasis infection. Higher number of microfilaremia cases were recorded from tiled house than the hut and reinforced cement concrete (RCC) houses. The breeding habitats like cesspit, cesspool and open drainage contributed to high vector density which increase likelihood of transmission of filariasis (Mutheneni *et al.*, 2016). Among variables most associated to filariasis incidence in Bintan District, Indonesia were mosquito-net use and distance to swamp (Ikhwan *et al.*, 2016).

2.3.3 Vector factors

In filariasis vector domain, the main vectors for *Brugia malayi* with nocturnal periodicity are mosquitoes from genera *Anopheles* and *Mansonia* which available in India and South-East Asia; *Brugia malayi* with nocturnal sub-periodicity, mosquitoes from genera *Mansonia* in South-East Asia; and *Brugia malayi* with diurnal subperiodicity also from genera *Mansonia* in Thailand (WHO, 2013). Other study found that periodic *Brugia malayi* is principally transmitted by *Anopheles campestris* mosquitoes, with *Mansonia uniformis* and *Mansonia annulifera* acting as secondary vectors (Kwa, 2008). The main vector for transmission will depends on the areas which are suitable for the species survival. Thus, as there were few species that can transmit the disease, entomology studies may yield different result when the areas being developed for other purposes. The transmission intensity is influenced by differing vector transmission dynamics, including vector biting rates and competence, and the number of individuals with microfilaria (Burgert-Brucker *et al.*, 2020). Other study found that transmission of lymphatic filariasis is significantly influenced by vector density (Pi-Bansa *et al.*, 2019).

2.4 Factors associated with failures of Mass Drug Administration

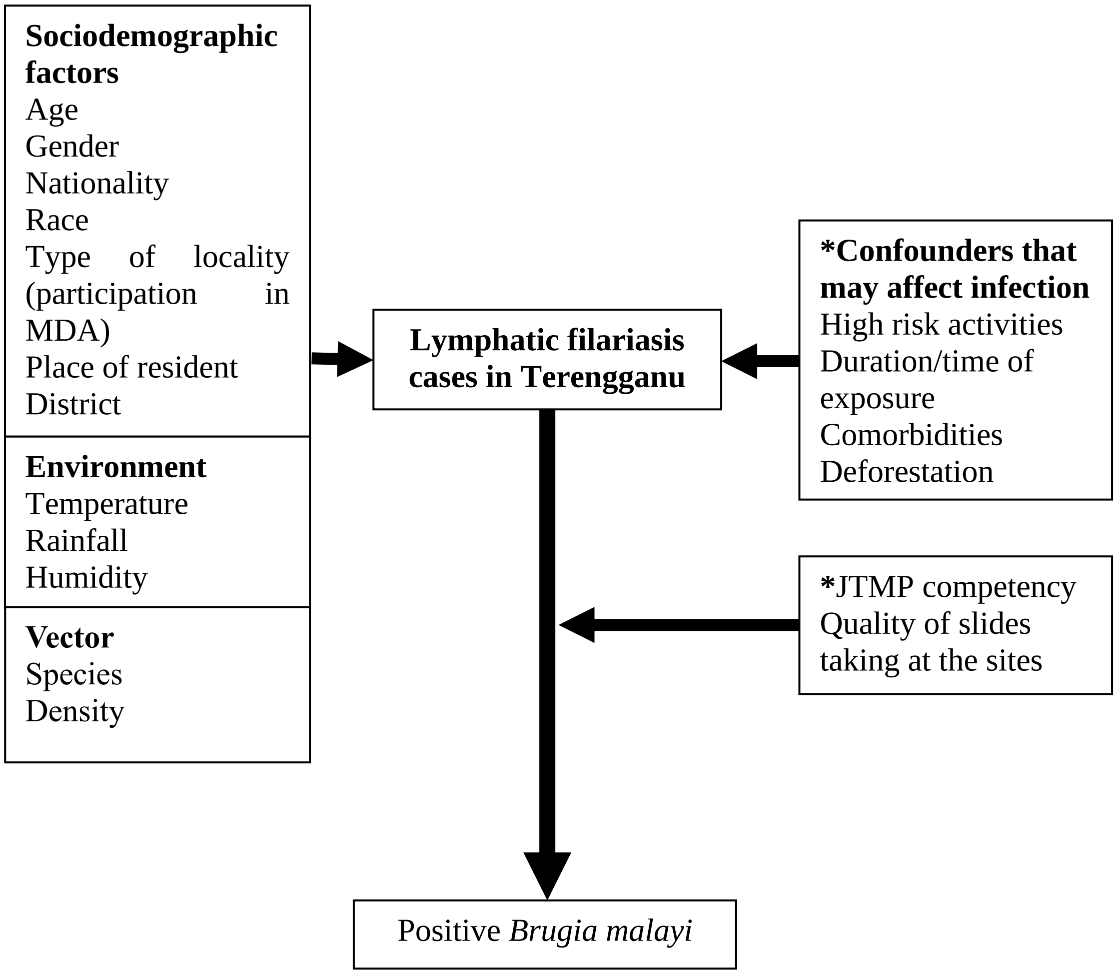
There were few countries which reported failure of mass drug administration even though this is the best approach proven by countries which had eliminated this disease. In some countries, the failure to stop transmission was not directly due to MDA itself but rather the main failure was due to inability to reach coverage of at least 80% of population in the implementation unit. In spite of six to seven rounds of MDA in few endemic areas, microfilaria prevalence was still above the one percent threshold and thus it was suggested that additional efforts by programs authorities were important to improve the coverage and better monitoring as well as evaluation procedures (Srividya *et al.*, 2019). A weak chain of medication supply during MDA also contribute to maldistribution and wastage (Gyapong *et al.*, 2018). As WHO recommended of at least 80% coverage of MDA in order to reach very minimal to zero transmission of filariasis infection, strategies on medication distribution in order to stop the transmission should be strengthened.

Other than that, environmental factor also played roles by providing suitable habitat for vector breeding. Therefore, in a very high endemic of filariasis infection, it was still recommended for vector control to reduce its density (Aye *et al.*, 2018). Swampy area may not be associated with infection in certain places but it is worth to look at in areas where *Mansonia* species were identified as the main vector. A study in Banyuasin, Indonesia where microfilaria rate was 2.02% found that the area were surrounded by swampy and rubber or palm plantations which were suitable for *Mansonia* larvae development (Pratiwi *et al.*, 2019). This type of areas needs a good management approach according to environmental guidelines available as swampy areas are also important ecologies for other animal species as well. Ecological balance should not be disrupted to avoid other unintended consequences. Clearance of water plants shall be a good solution for this kind of areas.

Possibilities of zoonotic transmission of *Brugia malayi* has been proposed in some researches. This was based on the findings of filariasis infection occurred in other animal species whereby the area had undergone MDA and already passed the transmission assessment survey but few years later, there was resurgence of infection with microfilaria rate more than one percent being reported (Santoso *et al.*, 2020). This finding was very important as zoonotic transmission will need further measures in controlling activities as well as strengthening the collaboration with other agencies such as veterinary department. As the microfilaria can be carried by few vector species, entomology studies are very important to ensure that there will be no cross transmission between urban and rural areas. Urban filariasis had been reported in certain areas and certainly it will change the management of disease control in the future (Simonsen and Mwakitalu, 2013).

2.5 Conceptual framework

Figure 2.1 is a conceptual framework which shows the association between possible sociodemographic factors, environmental factors and vector domains with the occurrence of filariasis infection by using night blood slides to detect for presence of microfilariae. Confounders cannot be analysed due to restricted availability of secondary data.



*Factors not included in study

Figure 2.1: Conceptual Framework Factors Associated with *Brugia Malayi* Infection

CHAPTER 3

METHODOLOGY

3.1 Study design

This study was a case-control study. This design was chosen in view of this study was intended to identify the multiple factors that led to one outcome which was positive filariasis infection. The low prevalence of the disease complements the appropriateness of choosing this study design.

3.2 Study area

The study area was in Terengganu which is one of the states in Malaysia. It is located at the East Coast of Peninsular Malaysia. It consists of eight districts namely Kuala Terengganu, Dungun, Marang, Kemaman, Hulu Terengganu, Besut, Setiu and Kuala Nerus. According to *Unit Pembangunan Ekonomi Negeri Terengganu* [UPENT] (2017), there were 99 sub-districts and 1,017 villages with total area of 1,295,757.6 Ha and number of populations of 1,221,600 in year 2017. All collected data were covering all the districts.

Figure 3.1 shows the map of Terengganu state with Hulu Terengganu as the biggest district and Kuala Terengganu as the smallest district (adapted from (UPENT, 2017)). This study utilised databases of lymphatic filariasis cases from Terengganu Health State Department, Entomology Unit Terengganu Health State Department and Malaysia Meteorology Department.

(Source: *Data Asas Unit Perancang Ekonomi Negeri Terengganu 2017* (UPENT, 2017, p. ix)

Figure 3.1: Map of Terengganu State

3.3 Study period

The study was conducted between January 2021 to May 2021.

3.4 Reference population

The reference population was all registered *Brugia malayi* filariasis cases in Terengganu.

3.5 Source population

The source population was all registered *Brugia malayi* filariasis cases in Terengganu from 2010-2019 in eVekpro online

3.6 Criteria for case selection

3.6.1 Inclusion Criteria

All lymphatic filariasis cases in eVekpro online with positive thick blood smear for *Brugia malayi*, 2010-2019.

3.6.2 Exclusion Criteria

i. Duplicated registered lymphatic filariasis cases.
ii. Imported/foreign cases (other species than *Brugia malayi* infection)
iii. Dataset which was not complete

3.7 Criteria for control selection

3.7.1 Inclusion criteria

All first close contact of cases in eVekpro online with negative thick blood smear for lymphatic filariasis, 2010-2019.

3.7.2 Exclusion criteria

i. Duplicated lymphatic filariasis registries.

ii. Dataset which was not complete.

3.8 Sample size calculation

Sample size was calculated based on specific second objective which was to determine associated factors of registered *Brugia malayi* in Terengganu, 2010-2019 by using two proportions formula by PS Software. Conventionally, the power of the study was set at 80% with α=0.05. Table 3.1 illustrated the sample size calculation for each variable including an additional 20% drop out rate. Sample size for objective 1 was not calculated as it only involved descriptive study. The largest total sample size calculated for objective 2 was 208 samples. Therefore, the final sample size taken for the study was 104 for each case group and control group with the ratio of case to control one to one basis.

Table 3.1 : Sample Size Calculations

No	Factors/ Variables	Po	P1	m	n (n X 2) + 20%	Literature Review
1.	Age (Adult)	0.330	0.15	1	208	(Mutheneni *et al.*, 2016)
2.	Sex (Male)	0.610	0.30	1	94	(Ikhwan *et al.*, 2016)
3.	Type of locality (Participate in MDA program)	0.860	0.25	1	22	(Graves *et al.*, 2020)
4.	Vector density (high)	0.154	0.70	1	29	(Pi-Bansa *et al.*, 2019)
5.	Rainfall (>700mm)	0.065	0.15	1	207	(Burgert-Brucker *et al.*, 2020)
6.	Temperature (high)	0.641	0.40	1	159	(Pratiwi *et al.*, 2019)

Po, the proportion of factors among controls (from literatures); **P1**, the estimated proportion among cases; **m,** ratio between the cases to controls; **n**, number of patient's data require, determine by PS software; +20%, considering estimated 20% drop out.

3.9 Sampling method and subject recruitment

Evekpro online system was developed by Ministry of Health as a database for vector borne diseases. It was started to be used effectively in year 2010. All data were entered by health inspectors and being monitored by epidemiology officers at district, state and ministry level.

The required data were downloaded and kept in a set of proforma in Microsoft Excel. Simple random sampling was applied to both case and control groups from eVekpro online database after data cleaning. Initial raw data revealed 179 filariasis cases occurred from year 2010 until year 2019. After data cleaning, 142 cases were eligible for the study. A total of 104 samples were selected randomly by using excel formula from 142 cases and 108 controls respectively. All cases and controls were transferred into SPSS 26 for data analysis.

3.10 Research tools and Variables

A proforma using Microsoft Excel was created and all required data from eVekpro online database will be keyed-in before being transferred to SPSS version 26 for data analysis. The variables gathered from eVekpro online includes:

i. Year and month for cases and controls
ii. Place of stay for cases and controls
iii. Age for cases and controls
iv. Sex for cases and controls
v. Nationality for cases and controls
vi. Ethnicity for cases and controls
vii. Type of locality (endemic or non-endemic locality) for cases and controls

The data collected from secondary weather excel database:

i. Year and month of data collection
ii. Name of weather sentinel site
iii. Mean monthly temperature
iv. Mean monthly rainfall
v. Mean monthly humidity

The data collected from secondary entomological excel database:

i. Year and month of entomological study
ii. Locality of entomological study
iii. Total number of vectors catch
iv. Total number of all mosquitoes catch

3.11 Operational definition

i. Lymphatic filariasis is diagnosed by the night blood survey (NBS) result either positive or negative.
ii. Night blood survey (NBS) is the collection of thick blood smear from the suspected population at night by health personals and will be examined under microscope by lab personal to diagnose the presence of microfilariae.
iii. *Brugia Rapid Test* is a rapid test used to detect antibodies against *Brugian* microfilariae.
iv. Mass drug administration (MDA) is one method of controlling lymphatic filariasis whereby annual treatments are given to the endemic area population for at least five cycles either using ivermectin (150–200 mg/kg) or diethylcarbamazine (6 mg/kg) and albendazole (400mg) depending on the endemic region (Kwarteng and Ahuno, 2017). In Terengganu, albendazole and diethylcarbamazine were used. All population were given the