IMPORTED HUMAN MALARIA CASES IN PENINSULAR MALAYSIA AND ITS ASSOCIATED FACTORS, 2015-2019

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by

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LIST OF ABBREVIATIONS

AdjOR	Adjusted Odds Ratio
CI	Confidence Interval
BFMP	Blood Film for Malaria Parasite
MOH	Ministry of Health Malaysia
NMRR	National Medical Research Registry
OR	Odds Ratio
PCR	Polymerase Chain Reaction
P. falciparum	*Plasmodium falciparum*
P. knowlesi	*Plasmodium knowlesi*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
WHO	World Health Organization

LIST OF SYMBOLS

=	Equal to
<	Less than
α	Alpha
β	Beta
%	Percentage
n	Number of samples
m	Ratio between two groups

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ABSTRAK

Sebanyak 3061 kes malaria manusia telah dilaporkan di Malaysia pada tahun 2015 sehingga 2019, yang mana 77% daripadanya adalah kes import. Walaupun terdapat penurunan trend jangkitan tempatan malaria manusia, terdapat peningkatan kes-kes jangkitan import malaria manusia. Kajian kami dijalankan bertujuan untuk mengkaji taburan dan faktor-faktor yang mempunyai kaitan dengan jangkitan import malaria manusia di Semenanjung Malaysia dari tahun 2015 sehingga 2019. Kajian ini dilaksanakan menggunakan data survelan Kementerian Kesihatan Malaysia. Kes-kes malaria manusia di Semenanjung Malaysia dari tahun 2015 sehingga 2019 yang dilaporkan di dalam Vekpro Online yang memenuhi kriteria kajian telah dipilih. Kaedah persampelan secara rawak telah diguanakan dalam kajian ini. Kesemua data yang telah diambil dari Vekpro Online direkodkan ke dalam proforma. Kesemua kes diklasifikasikan kepada jangkitan tempatan malaria manusia atau jangkitan import malaria manusia. Analisa regresi logistik digunakan untuk menentukan faktor yang berkaitan dengan jangkitan import malaria manusia. Sebanyak 716 kes telah dimasukkan ke dalam kajian, yang mana 89.4% adalah lelaki. Kebanyakan kes (85.3%) adalah dari kumpulan umur 18-59 tahun, diikuti dengan kumpulan umur bawah 18 tahun (11.2%). Majoriti kes adalah warganegara Malaysia (68.4%), bekerja di sektor perladangan, pembalakan dan perikanan (55%), menghidapi jangkitan *P. vivax* (56%), mempunyai gametosit (62.7%), mempunyai jangkitan yang tidak teruk (91.1%), dan tinggal di kawasan luar bandar (59.4%). Hubungkait yang signifikan dengan jangkitan import malaria manusia adalah kumpulan umur 18-59 tahun (AdjOR 8.687; 95% CI: 3.631, 20.782, *p*-value <0.001), 60 tahun dan ke atas (AdjOR 7.844; 95% CI: 1.308, 47.024, *p*-value=0.024), lelaki (AdjOR 8.029; 95% CI: 3.497, 18.434,

p-value<0.001), warganegara asing (AdjOR 7.412; 95% CI: 3.009, 18.260, *p*-value<0.001), bekerja dalam sektor perladangan (AdjOR 2.680; 95% CI: 1.297, 5.536, *p*-value=0.008), menghidapi jangkitan *P. vivax* (AdjOR 0.300; 95% CI: 0.125, 0.595, *p*-value=0.001), mempunyai gametosit (AdjOR 0.300; 95% CI: 0.158, 0.569, *p*-value<0.001), dan tinggal di kawasan luar bandar (AdjOR 0.055; 95% CI: 0.016, 0.190, *p*-value<0.001). Kesemua penemuan ini akan membantu Kementerian Kesihatan Malaysia untuk merumuskan strategi baru dan memperkukuhkan strategi yang sedia ada dalam menangani malaria manusia import dan seterusnya mencegah pengenalan semula malaria ke dalam negara.

Kata kunci: malaria, import, jangkitan tempatan, faktor berkaitan

ABSTRACT

A total of 3061 human malaria cases have been reported in Malaysia between 2015 and 2019, and 77% of cases were imported. Despite the downward trend of locally acquired human malaria cases, there is an increasing trend of imported human malaria cases. Our study aimed to study the distribution and associated factors for imported human malaria cases in Peninsular Malaysia from 2015 until 2019. The study was conducted using surveillance data at the Ministry of Health Malaysia. Human malaria cases in Peninsular Malaysia from 2015 until 2019 reported in Vekpro Online that fulfil subject criteria were included. A simple random sampling technique was used for sampling. All data extracted from Vekpro Online were recorded in proforma. All cases were classified into locally acquired and imported human malaria. Multiple logistic regression was used to determine the factors associated with imported human malaria cases. A total of 716 cases were included, of which 89.4% were males. Most of the cases (85.3%) were in the age group 18-59 years, followed by less than 18 years (11.2%). The majority of the cases were Malaysian (68.4%), working in the agriculture, forestry, and fishing sectors (55%), having *P. vivax* infection (56%), have the presence of gametocyte (62.7%), having an uncomplicated infection (91.1%) and living in the rural area (59.4%). Significant association with imported human malaria were found in the age group 18-59 years (AdjOR 8.687; 95% CI: 3.631, 20.782, *p*-value <0.001), 60 years and above (AdjOR 7.844; 95% CI: 1.308, 47.024, *p*-value=0.024), male (AdjOR 8.029; 95% CI: 3.497, 18.434, *p*-value<0.001), foreigner (AdjOR 7.412; 95% CI: 3.009, 18.260, *p*-value<0.001), working in agricultural sector (AdjOR 2.680; 95% CI: 1.297, 5.536, *p*-value=0.008), infected with *P. vivax* (AdjOR 0.300; 95% CI: 0.125, 0.595, *p*-value=0.001), presence of gametocytes (AdjOR 0.300; 95% CI: 0.158, 0.569, *p*-value<0.001), and living in the rural area (AdjOR 0.055; 95%

CI: 0.016, 0.190, *p*-value<0.001). These findings will aid the Ministry of Health Malaysia in formulating new strategies and strengthen the existing one in managing imported malaria and preventing the reintroduction of malaria into the country.

Keywords: malaria, import, locally acquired, associated factor

CHAPTER 1 : INTRODUCTION

1.1 Background

Malaria is a vector-borne disease caused by *Plasmodium* species. Five Plasmodium species can infect human; namely, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, which are transmitted by female *Anopheles* mosquito. The first four species are classified as human malaria, while *P. knowlesi* is classified as zoonotic malaria because there is no evidence of human-to-human transmission (WHO, 2019a). *P. knowlesi* has the morphological features of the early trophozoites that resemble those of *P. falciparum*. The growing trophozoites of *P. knowlesi* are similar to the band-form trophozoites of *P. malariae*, rendering it inaccurate in diagnosing it based on microscopy alone (Singh & Daneshvar, 2013).

It is estimated that there are 228 million cases of malaria worldwide in 2018, down from 251 million in 2010 and 321 million in 2017. Almost 85% of them are located in India and sub-Saharan Africa, and *P. falciparum* and *P. vivax* remain the most common species in the world. Approximately 405 000 deaths worldwide are due to malaria, of which 67% are children under five years of age (WHO, 2020). World Health Organization (WHO) identifies 11 countries as high burden countries, namely Burkina Faso, Cameroon, the Democratic Republic of the Congo, Ghana, Mali, Mozambique, Niger, Nigeria, Uganda, United Republic of Tanzania and India. The mortality and morbidity caused by malarial infection considerably affect the economies of developing countries. Most underdeveloped nations are still with a greater prevalence

of malaria, and the infection sustains a vicious cycle of disease, poverty and poor hygiene (Amegah *et al*., 2013).

Malarial infection in Malaysia was documented late in 1960, where approximately 0.24 million infected individuals were reported throughout the country. The prevalence was enormously declined up to 40,000 individuals late in 1980 with subsequent employment and implementation of effective strategies to combat the infection caused by malaria (Rahman, 1982). Late in 2008, the incidence was further condensed to 7000 individuals annually. Along with the declined prevalence, the mortality rate was also reduced in Malaysia, with around 50 deaths annually (0.09%). These figures indicate that Malaysia has produced effective strategies and public health programs in order to control and eliminate malarial infection across the country. The etiological agents involved in the malarial disease in Malaysia in 2011 were reported, *P. vivax* with 45.6%, *P. falciparum* with 18.3% and *P. malariae* with 17% (Ministry of Health Malaysia *et al*., 2015).

Following the adoption of the Global Technical Strategy for Malaria 2016-2030, with the vision of "A World Free of Malaria" and A Framework for Malaria Elimination, an increasing number of countries are progressing towards the elimination of malaria, including Malaysia (WHO, 2015, 2019c; WHO and Global Malaria Programme, 2017). Malaysia is one of the countries identified by the WHO that has the potential to achieve elimination status by 2020, which is characterised by zero indigenous human malaria cases for three consecutive years (WHO, 2016, 2019b).

Between 2015 and 2019, a total of 3061 human malaria cases have been reported in Malaysia, with imported cases accounting for 77% of all cases reported. Despite a downward trend in the number of cases of locally acquired human malaria, which is shown in Figure 1.1, there is an upward trend in the number of imported human malaria cases (Ministry of Health Malaysia, 2020). This issue was addressed by the WHO (2019b) in its report, mentioning that Malaysia is currently facing the threat of imported human malaria.

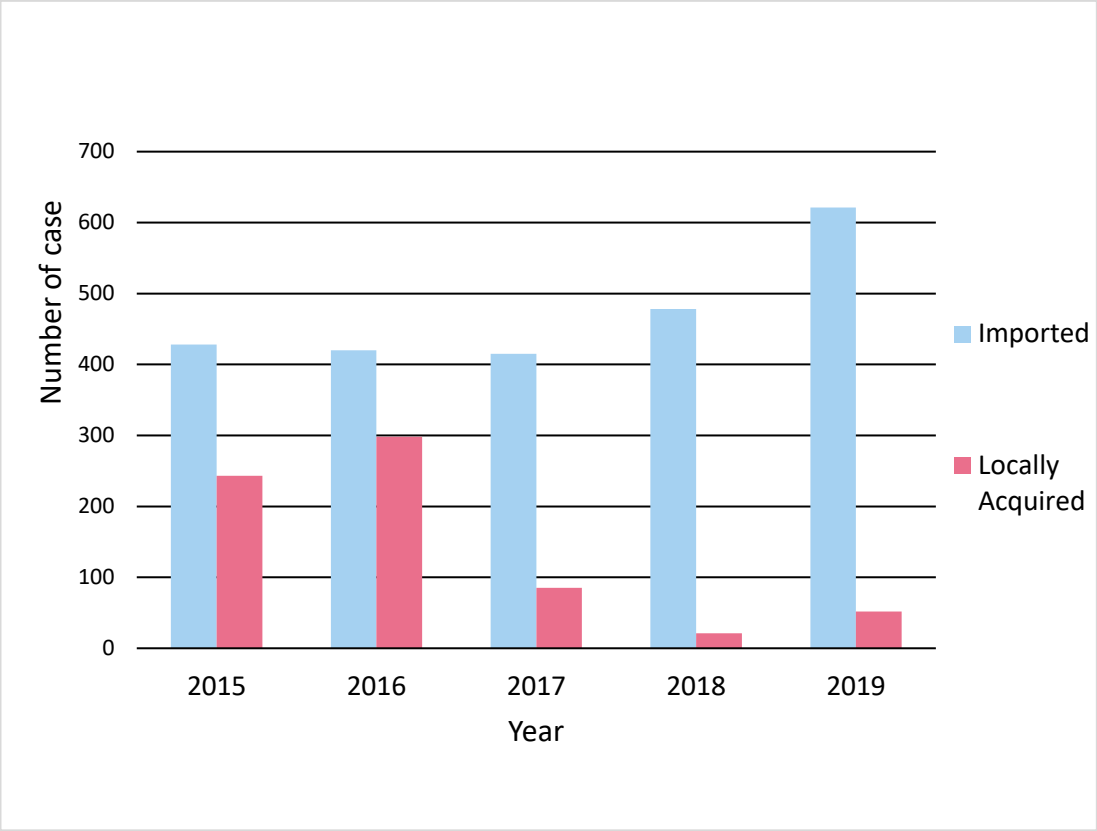


Figure 1.1: Classification of human malaria cases in Malaysia, 2015-2019

Malaria importation can be classified based on human movements, while eliminating region, during transit, in the endemic area and after returning to eliminating country (Sturrock *et al*., 2015). Non-immune migrants, mobile populations and travellers are among the groups with a considerably higher risk of contracting malaria, and having severe disease, than others (Agomo & Oyibo, 2013; WHO, 2021).

Despite the fact that only 22.3% of cases of malaria occur in Peninsular Malaysia, and the majority of them are concentrated in Selangor, Kelantan, Perak, Pahang and Johor, as mentioned in the study by Hussin *et al*. (2020), there is a noticeable difference in patterns and characteristics of malaria cases between Peninsular Malaysia, and Sabah and Sarawak. The most common age group in Peninsular Malaysia was 20 to 29 years compared to 30 to 39 years in Sabah and Sarawak. *P. vivax* is the most typical species found in Peninsular Malaysia, while in Sabah & Sarawak, *P. knowlesi*. The same study also shows that 31% of the total malaria cases recorded in Malaysia are due to human malaria.

1.2 Problem statements

There are still limited studies on imported human malaria in Peninsular Malaysia and how it varies from locally acquired human malaria. The knowledge gap is still present, as most recent studies focus more on zoonosis malaria, especially in Sabah and Sarawak. Although imported human malaria cases do not directly affect the elimination status in Malaysia, there is a potential risk of subsequent local transmission if our current preventive and control measures have not addressed this issue. Furthermore, despite the increasing trend of imported human malaria infection in Malaysia, there is still no study done on the factors associated with it.

1.3 Rationale of the study

This knowledge gap warrants further study in the understanding of imported human malaria in Peninsular Malaysia, including its proportion, trends and distribution, and attributed factors because they play a vital role in the reintroduction of malaria in the

country. In addition, it will also provide an opportunity to strengthen interagency and international cooperation in the management of imported human malaria. This knowledge will allow policymakers to prioritise resource allocation and reinforce existing preventive and control measures.

1.4 Research question

1. What is the distribution of imported cases of human malaria in Peninsular Malaysia in 2015-2019 as compared to locally transmitted human malaria?

2. What are the factors associated with imported human malaria infection in Peninsular Malaysia in 2016-2020?

1.5 Objectives

1.5.1 General objective

To study the distribution and associated factors for imported human malaria cases in Peninsular Malaysia in 2015-2019.

1.5.2 Specific objectives

1. To describe the sociodemographic, clinical, parasite and environmental factors for imported and locally transmitted human malaria cases in Peninsular Malaysia in 2015- 2019.
2. To determine the associated factors for imported human malaria infection in Peninsular Malaysia in 2015-2019.

1.6 Research hypothesis

There are significant sociodemographic, parasite and environmental factors associated with imported human malaria cases in Peninsular Malaysia in 2015-2019.

CHAPTER 2 : LITERATURE REVIEW

The search of papers in this study was done using online search engines and databases, including PubMed, Science Direct, Scopus, Springer link and Google Scholar. Several search strategies were applied, including the use of Boolean operators, “AND”, “OR” and “NOT”. The keywords used were malaria, import, indigenous, locally acquired, associated factor and epidemiology.

2.1 Transmission of human malaria

Malaria transmission can occur when a host, vector, and agent interact in a suitable environment. Malaria parasites have two hosts, the *Anopheles* mosquito and the human. There are approximately 515 *Anopheles* mosquito species worldwide, but only 70 are capable of transmitting malaria. Only 40 of these are considered to be vectors of major importance. In Peninsular Malaysia, the primary vector is *An. maculatus*, whereas, in East Malaysia, the primary vector is *An. balabacencis* (Vythilingam *et al*., 2018). Human is the intermediate host for human malaria, where the Plasmodium asexual life cycle occurs. The life cycle of *Plasmodium* species is illustrated in Figure 2.1 below.

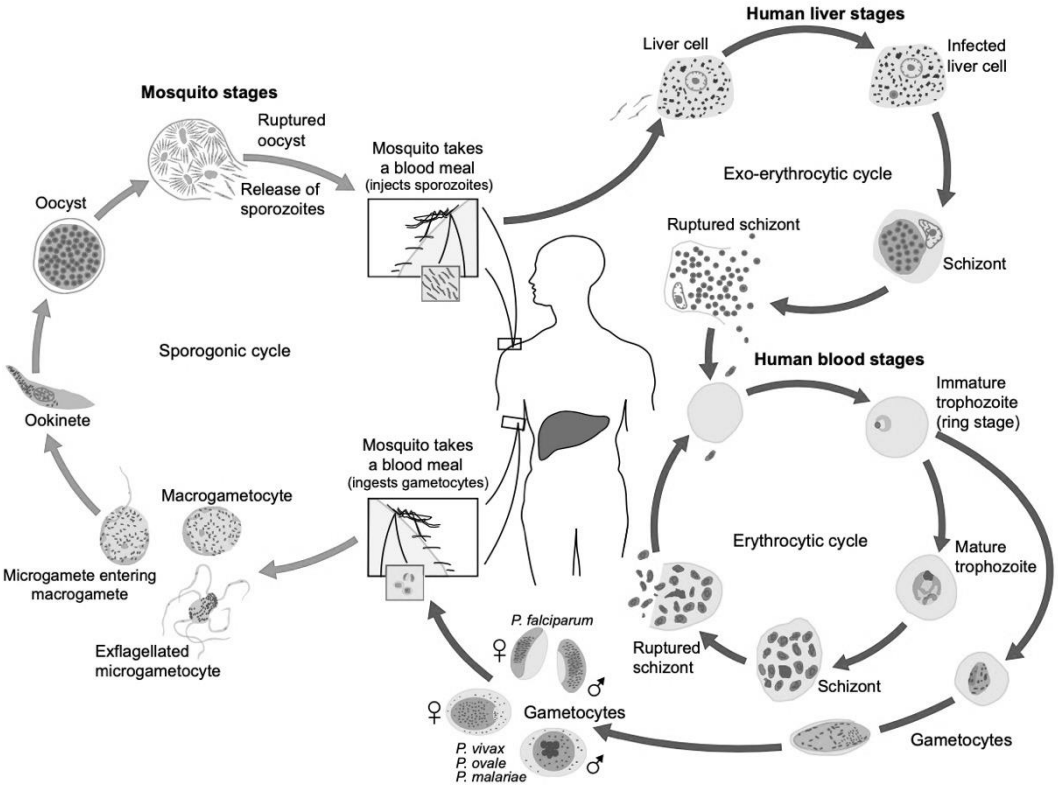
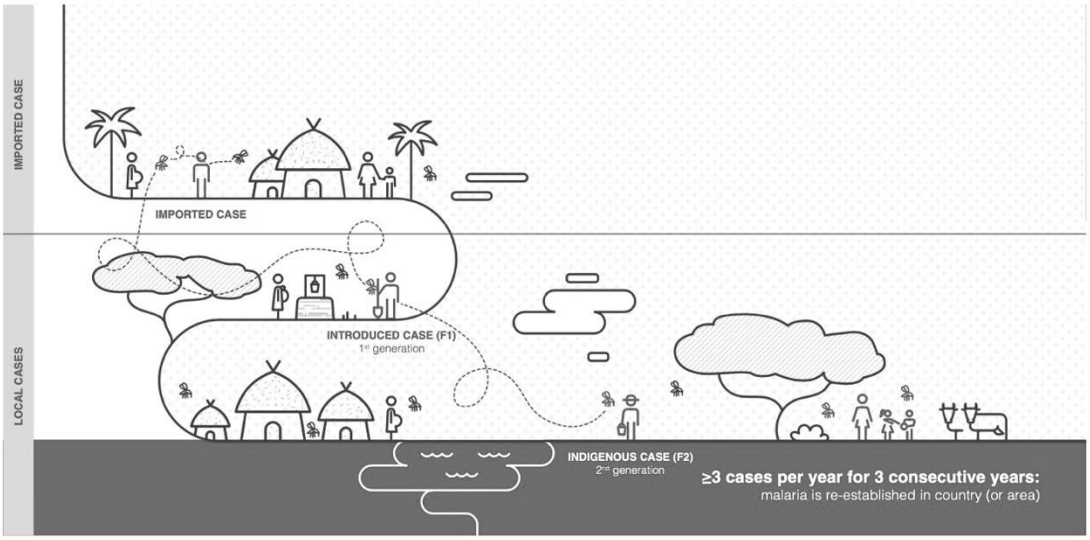


Figure 2.1: Malaria transmission cycle. Adapted from WHO (2015)

The term sporogony refers to the development of malaria parasites in the Anopheles mosquito, which involves a variety of stages in various insect organs. Male and female gametocytes mate after being ingested by a blood-feeding anopheline mosquito. The zygotes develop into ookinetes, which migrate across the stomach of the mosquito to form oocysts. The rupture of oocysts results in the release of hundreds of sporozoites. The sporozoites migrate and accumulate in the salivary glands, from which they are injected into the human body when an infective mosquito bites a human for a blood meal. The time between gametocyte ingestion and sporozoite release is referred to as the extrinsic incubation period, which varies between 8 and 10 days depending on the species.

Once inside the host's bloodstream, the sporozoites invade hepatocytes. The sporozoites then undergo exo-erythrocytic schizogony, a process in which the parasite nucleus repeatedly divides over several days; at the end, the schizont bursts, releasing thousands of merozoites into the bloodstream. Certain sporozoites in *P. vivax* malaria become dormant as hypnozoites for periods ranging from 3 to 18 months and rarely up to 5 years. Merozoites then invade erythrocytes, where they undergo repeated growth cycles, rupture, release, and reinvasion of new red cells. Malaria's entire clinical spectrum is attributed to this erythrocytic schizogony. Certain merozoites mature and develop into male or female gametocytes, which the mosquito consumes during the blood meal.

Mosquito-borne malaria transmission can be divided into imported and locally acquired (WHO & Global Malaria Programme, 2017). Imported malaria is a case in which the infection is contracted outside the country. Locally acquired malaria is further divided into introduced, indigenous, relapse and recrudescence. Introduced malaria is the first generation of locally transmitted malaria, with a solid epidemiological link with the imported case whereas, indigenous malaria is a case with no importation evidence. It also has no direct epidemiological link to the imported case. Relapse is the activation of *P. vivax* or *P. ovale* hypnozoite in a previously cured infection. Recrudescent is the recurrence of the illness with the same genotype due to incomplete parasite clearance after antimalarial treatment. The difference between imported, indigenous and introduced is illustrated in Figure 2.2.



(source: WHO & Global Malaria Programme, 2017)

Figure 2.2: The differences between imported, introduced and indigenous case

2.2 Clinical presentation of human malaria

Malaria has a variable incubation period, but on average, it lasts about 10–14 days (Ministry of Health Malaysia, 2014). Malaria's early symptoms are non-specific and resemble those of a mild systemic viral infection, including headache, lassitude, fatigue, abdominal discomfort, and muscle and joint aches. Then, fever, chills, perspiration, anorexia, vomiting, and worsening malaise typically follow. A high fever most frequently manifests malaria. The classical paroxysm begins with a 'cold stage,' characterised by dramatic rigors during which the patient visibly shakes. This progresses through a 'hot stage,' during which the patient's temperature exceeds 40°C, he or she may become restless and excitable, and may vomit or convulse, to a 'sweating stage,' during which the fever subsides, and the patient may fall asleep. This paroxysm may last 6–10 hours in untreated patients and is followed by a prolonged asymptomatic period followed by additional rigors. If prompt and effective treatment is administered during the early stages of malaria infection, and there is no evidence of vital organ

dysfunction, the patient can experience a complete and rapid recovery. If ineffective medications are administered, or treatment is delayed, the parasite burden continues to grow every 24 or 48 hours, resulting in severe malaria and possibly death.

Severe malaria is typically characterised by coma (cerebral malaria), metabolic acidosis, severe anaemia, hypoglycemia, acute renal failure, or acute pulmonary oedema. At this stage of the illness, the case fatality rate for patients receiving treatment ranges between 10% and 20%. However, severe malaria is almost always fatal if left untreated. Long-term cognitive impairment is also a common complication of cerebral malaria in children (Ministry of Health Malaysia, 2014).

2.3 Diagnosis and notification of malaria

Malaria is diagnosed by blood film for malaria parasite (BFMP) testing. Each positive slide will be sent to state-level laboratory for rechecking and PCR testing to minimise the error in diagnosis and parasite identification (Ministry of Health Malaysia, 2014). It is mandatory to notify confirmed case of malaria to the nearest District Health Office without any delay (Prevention and Control of Infectious Diseases Act 1988, 1988).

2.4 Factors associated with imported human malaria

In Malaysia, approximately 17% of the total malaria cases occur among foreigners (Hussin *et al*., 2020). The proportion of foreigners with imported human malaria is much lower in China, accounting for just six percent (Lei *et al*., 2019). It is due to most of the imported cases are local people who work in malaria-endemic countries,

especially in Africa and Southeast Asia, and have been infected there. This finding is in contrast with the study from Spain. The majority of the imported human malaria cases in Spain are among immigrants and those who are visiting friends and relatives in malaria-endemic countries (Herrador *et al*., 2019).

Numerous studies have reported that sex and age are ominously related to infection from malaria. It was investigated by William and Menon (2014) involving all malarial cases in Malaysia between 2007 and 2011. The data shows that middle-aged people are mainly affected by malaria infection, with a median of 31 years old. The frequency of malaria infection also was higher in this age group who travelled to malaria-endemic countries. In a study conducted in China by Lai *et al*. (2019), it has been shown that the majority of the imported cases of human malaria are young adult, as compared to locally acquired malaria. The same study also indicates that there is strong male predominance found in cases of imported human malaria compared to locally acquired malaria, and this finding is supported by Yu *et al*. (2020).

Moreover, in another study, a significant association was demonstrated between malaria and the lower-income group. It was found that people with less income and wooden houses are at greater risk of developing the infection when compared to those with higher income and living in concrete houses (Yadav *et al*., 2014). The vector for malaria infection, *Anopheles* species, could be attracted towards the assembly of wooden houses as it looks like their innate habitat. Spatial analysis by Bi and Tong (2014) shows that poverty may be one of the drivers for malaria transmission in China. A study in India by Kumar *et al*. (2014) have shown that lower-income groups are more vulnerable to malaria infection compared to higher income groups. This result is similar to Sabah, where most malaria cases are in agricultural sectors such as farming, plantation workers, and forestry activities (Barber *et al*., 2013). Poverty is also one

factor for the migration of international foreign workers to Malaysia, as described by Hamzah *et al*. (2020).

Malaria infection has been reported to occur in both rural and urban regions, with a greater incidence reported in rural areas (Ramdzan *et al*., 2020). Recently, semi-urban people have been exposed to malaria infection due to active deforestation (Nath *et al*., 2012). Besides, rural societies are at a high risk of being infected by malaria, mostly those living in the surrounding jungle areas. Lack of proper education at these vicinities has contributed to poor environmental preventive practices, and this lead to a growing number of malaria cases in these areas (Deressa *et al*., 2008). Braima and his colleagues reported that 35% of malaria cases occur in people who lived in the vicinity of the jungle and declared these people at higher risk of developing malaria infection (Braima *et al*., 2013).

Occupation in agricultural sectors such as farmers, loggers and forestry workforces involves in deforestation and land exploration are associated with malarial infection in Peninsular Malaysia because of exposure of human with the mosquitos that carry the infection over a period of time, which leads to the elevated risk of the malarial infection (Fornace *et al*., 2019; Muehlenbein *et al*., 2015; William & Menon, 2014). Overnight travelling and outdoor sleeping are also factors associated with malarial infection. Generally, all these with outdoor agriculture activities pave the way for the increased risk of malarial infection. Together with these, changes in the biological habitats may influence the mosquito vector that favours elevated level of blood-feeding on humans which facilitate the increased risk of the infection across Peninsular Malaysia (Barber *et al*., 2017). In Malaysia, around 25 percent of workers in the agricultural sector and one-third of workers in the service sector are migrants, and they can bring the malarial parasite into the country (World Bank, 2020).

Other important factors contributing to the re-emergence of locally acquired malaria from imported human malaria cases are the presence of Anopheles species vectors and favourable climate conditions (Hertig, 2019). *Anopheles Cracens* and *Anopheles maculatus* have been reported as important vectors responsible for the transmission of malaria in Peninsular Malaysia. *Anopheles maculatus* is the vector for transmission in human, while *Anopheles cracens* is a reported vector for simian malaria, including *Plasmodium knowlesi* (Vythilingam *et al*., 2014). Suitable climate and the presence of *An. maculatus* as the primary malaria vector for human malaria in Peninsular Malaysia indicates that Peninsular Malaysia is at risk of local human malaria transmission from imported malaria (Alias *et al*., 2014; Khormi & Kumar, 2016).

The majority of imported cases in China have been infected with *P. falciparum*, while *P. vivax* infection is mainly found in locally acquired malaria infection (Lai *et al*., 2019). A study by Rainova *et al*. (2018) in Bulgaria found that most of the cases originating from Africa were caused by *P. falciparum*, while the majority of cases originating from Asia were caused by *P. vivax*. In the United Kingdom, from 1996 to 2003, there is a decrease in imported malaria cases annually by >30%. There was no decline in imported malaria cases from 2004 to 2016. For most imported malaria infections, *P. falciparum* is responsible for 66.8% of the cases, followed by *P. vivax*, 16.1%, *P. ovale* and *P. malariae,* 7.6% and 4%, respectively. Most of the imported malaria infections were documented in people arriving from West Africa (Sakwe *et al*., 2019).

In a study by Zhang *et al*. (2019), using the data from 2012 to 2018 in Anhui, China, around nine percent of patients with imported human malaria cases developed severe malaria with a mortality rate of 0.6%. Another study in France compared severity between those who used to live in malaria-endemic countries such as migrant workers

from sub-Saharan Africa, with those who originated in non-endemic malaria countries such as European-origin travellers. The result shows that only three percent of those who used to live in malaria-endemic countries develop severe malaria compared to European-origin traveller, 11%. This might be due to the development of long term semi-immunity that arise from repeated exposures to the parasite (Pistone *et al*., 2014).

2.5 Conceptual framework

The conceptual framework in Figure 2.3 below shows the factors associated with imported human malaria cases. All the factors can be grouped into four domains: human, parasite, environment, and vector domains. In this study, four variables were not being included due to the limitation of secondary data, which is annotated with asterisks. The human domain is comprised of sociodemographic factors such as age, sex, nationality, country source of infection, and occupation. The parasite domain consists of the type of parasite, gametocytes, and severity status, while the environment domain consists of locality status. The outcome of this study is the classification of malaria cases into imported or locally acquired malaria.

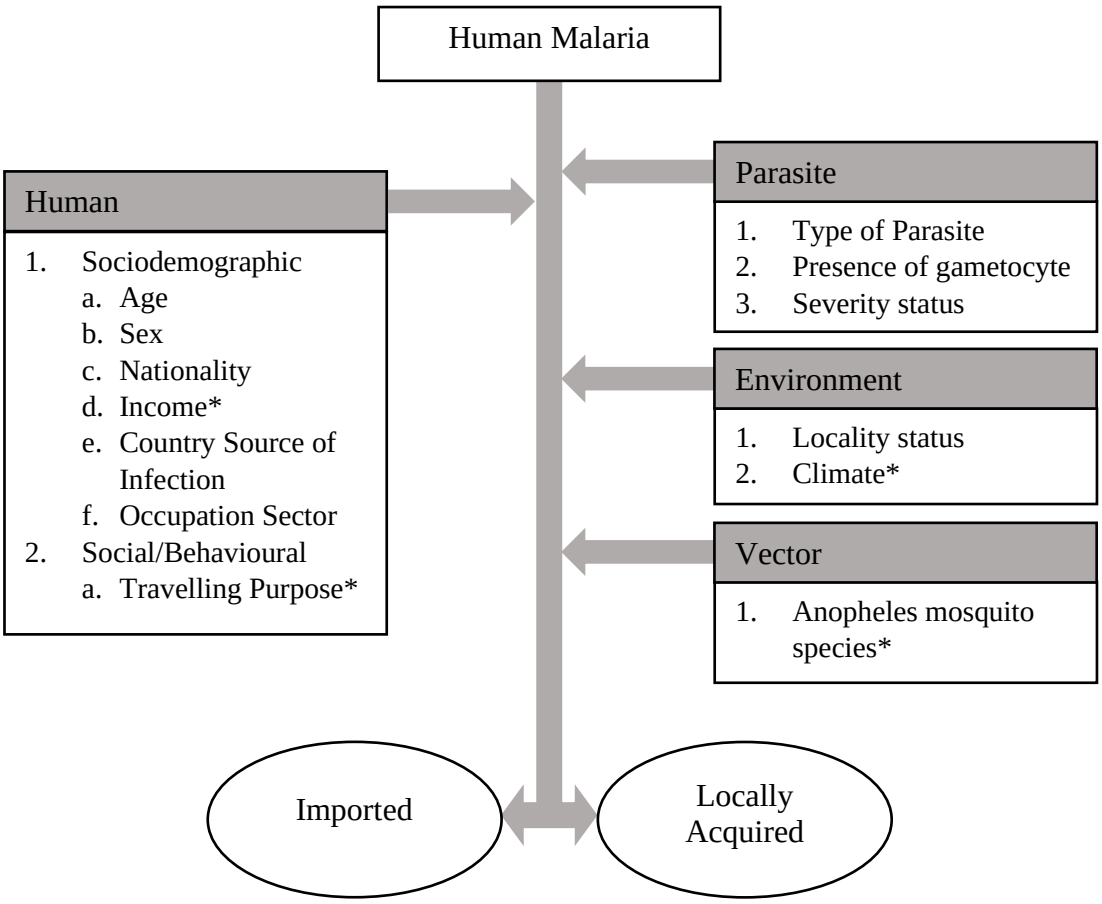


Figure 2.3: Conceptual framework of factors associated with imported human malaria cases

CHAPTER 3 : METHODOLOGY

3.1 Research design

This study was a retrospective record review using secondary surveillance data taken from Vekpro Online from 2015 till 2019.

3.2 Study location

This study was done in Peninsular Malaysia. The data collection was done by the Disease Control Division of the Ministry of Health Malaysia. Peninsular Malaysia covers an area of 132,265 km^2 or nearly 40% of the country's total land area. Kelantan, Terengganu, Pahang, Perlis, Kedah, Penang, Perak, Selangor, Negeri Sembilan, Malacca, Johor, Selangor, Kuala Lumpur, and Putrajaya are its 11 states and two federal territories. Thailand, Indonesia, and Singapore are the neighbouring countries. The majority race is Bumiputra (67.4%), followed by Chinese (24.6%) and Indians (7.3%). Islam is the predominant religion in the country (61.3%), followed by Buddha (19.8%), Christian (9.2%) and Hindu (6.3%) (Department of Statistics Malaysia, 2011).

3.3 Study duration

The study involved surveillance data taken from January 2015 till December 2019.

3.4 Study population

Reference population: Human malaria cases in Peninsular Malaysia

Source population: Human malaria cases in Peninsular Malaysia registered in Vekpro Online from January 2015 till December 2019.

3.5 Subject criteria

3.5.1 Inclusion criteria

a. Human malaria cases in Peninsular Malaysia from 2015 till 2019 reported in vekpro online
b. Diagnosis of malaria confirmed by BFMP and verified by PCR

3.5.2 Exclusion criteria

a. Relapse cases
b. Cases with missing 30% or more variables

3.6 Sample size calculation

For Objective 1, no sample size calculation is needed since it is descriptive statistics.

For objective 2, the sample size was calculated using two proportion formula in PS software (dichotomous). Conventionally, the power of the study was set at 80% with α=0.05. The sample size was calculated, including an additional 10% possibility of data entry error.

Table 3.1: Summary of sample size calculation for some of the factors associated with imported human malaria cases

Variables	**P0**	**P1**	**m**	**n1**	**n2**	**Total + 10%**	**Reference**
Sex (Female)	0.61	0.80	1	89	89	541	Lai *et al.*, 2019
Nationality (Foreigners)	0.17	0.30	2	121	242	399	Hussin *et al*., 2020
Occupation sector (non-agricultural)	0.38	0.5	2	200	400	660	Barber *et al.*, 2013
Type of parasite (non *p. vivax*)	0.29	0.40	2	217	434	716	Hussin *et al*., 2020

P0 = Proportion of imported human malaria in non-exposed subjects

P1 = Expected proportion of imported human malaria in exposed subjects

m = number of non-exposed subjects per exposed subject

$\alpha = 0.05$

Power = 80%

Based on the above calculations, the biggest sample size for this study is 716.

3.7 Sampling method

A simple random sampling was done among human malaria cases in Peninsular Malaysia registered in Vekpro Online between January 2015 till December 2019, who fulfil subject criteria to obtain the study sample.

3.8 Subject recruitment and informed consent

This study involves secondary data collection only. Informed consent is not required in this study.

3.9 Research tools

Vekpro Online is the online reporting system was developed by Ministry of Health Malaysia in order to aid in the management of vector-borne and other infectious disease outbreaks. It is centrally managed by the Ministry of Health Malaysia's Division of Disease Control. Its objective was to track the occurrence of infectious diseases using standardised data for the management of the disease. All the data in the Vekpro online is entered by Assistant Environmental Health Officer and verified by Medical Officer of Health at district level.

A proforma checklist, as in Appendix A, is used to extract data from Vekpro Online. The variables required are age, sex, nationality, country source of infection, occupation sector, type of parasite, presence of gametocytes, severity classification, locality status and malaria classification.

3.10 Operational definitions

In this study, the operational definitions used are listed below. All of them are based on WHO Malaria Terminology Document (WHO, 2016).

i. Imported malaria

Malaria case or infection in which the infection was acquired outside Malaysia.

ii. Locally acquired malaria

A case acquired in Malaysia by mosquito-borne transmission. Locally acquired cases can be indigenous, introduced, relapsing or recrudescent.

iii. Introduced malaria

A case contracted in Malaysia, with strong epidemiological evidence linking it directly to a known imported case (first-generation local transmission)

iv. Indigenous malaria

A case contracted in Malaysia with no evidence of importation and no direct link to transmission from an imported case

v. Relapse malaria

Malaria case attributed to activation of hypnozoites of *P. vivax* or *P. ovale* acquired previously

vi. Malaria elimination

Reduction to zero incidence of indigenous cases of all human malaria parasite in Malaysia as a result of deliberate activities.

3.11 Data collection

This study involves secondary data collection based on surveillance data collected from Vekpro Online under the management of the Disease Control Division, Ministry of Health Malaysia. All human malaria cases registered in Vekpro Online in Peninsular Malaysia from 2015 till 2019 were retrieved and recorded in a password-protected

proforma. The variables needed in this study were extracted in Microsoft Excel format, and the data were cleaned and imported into IBM SPSS version 26 for analysis.

Data extracted were from Vekpro Online were:

i. Sociodemographic data;
    1. Age
    2. Sex
    3. Nationality
    4. Country source of infection
    5. Occupation

ii. Environmental information:
    1. Type of locality

iii. Malaria parasites
    1. Type of malaria parasites
    2. Presence of gametocyte
    3. Severity

iv. Malaria classification

3.12 Data analysis

IBM SPSS version 26 was used to analyse the data. Data were validated, analysed, and cleaned. A preliminary data description was performed in order to identify any missing values. Errors in the data set were identified and corrected.

All data were categorised. Age was initially collected as a numerical variable in years but was later classified as less than 18 years old, 18 to 59 years old, and 60 years and older, representing minors, working-age adults, and the elderly. According to the

WHO categorisation, country sources of infection were classified into Western Pacific, South-East Asia, Africa, the Americas, and Eastern Mediterranean (WHO, 2021a). Occupations were regrouped according to the United Nations' classification of occupation sectors (2008). Indigenous and introduced human malaria was grouped as locally acquired human malaria according to WHO Malaria Terminology Document (WHO, 2016).

For objective 1, all data were tabulated for descriptive statistics. Descriptive statistics were used to summarise the sociodemographic, parasite and environmental factors for imported and locally transmitted human malaria cases in Peninsular Malaysia, 2015-2019. Categorical data were presented as frequency (n) and percentage (%).

For objective 2, the associated factors for imported human malaria cases in Peninsular Malaysia from 2015 till 2019 were analysed using simple and multiple logistic regression. The risk estimated were presented as crude odds ratio (crude OR). Several variables were regrouped to have more powered analysis. Nationality was regrouped into Malaysian and Foreigner. Occupation sectors were regrouped into Agricultural and Non-Agricultural. The type of parasites was regrouped into *P. vivax* and Non-*P. Vivax*. The output was a binary variable coded "0" for human malaria acquired locally and "1" for human malaria imported. Factors with a *p*-value of 0.25 from univariate analysis or variables of clinical significance were included in the preliminary final model for multiple logistic regression analysis to evaluate the factor related to imported human malaria infection in Peninsular Malaysia. This *p*-value was set to be greater than the level of significance to allow for the inclusion of more significant variables in the model.

After evaluating the model using forward and backward LR, a preliminary main effect model was developed. The criteria for choosing the model's important variable are statistical significance, parsimony, and biological plausibility. Multicollinearity was

determined in linear regression using VIF and tolerance. A tolerance of greater than 0.1 and a VIF of less than 10 suggest that the independent variables are not multicollinear. Since the sample is large and there is no biological explanation between the variables, the interaction between the variables is not the main interest of this study. The model's fitness was determined using a classification table and the area beneath the receiver operation characteristic (ROC) curve. If the classification table showed greater than 70% accuracy and the ROC area under the curve was greater than 0.7 with a *p*-value less than 0.05, the model was fit. The final model is supplied with the adjusted odds ratio (AdjOR) and 95% confidence interval (CI), as well as the *p*-value. With a two-tailed hypothesis, the level of significance was fixed at a *p*-value less than 0.05.

3.13 Ethical consideration

The study has received approval by Jawatankuasa Etika Penyelidikan Manusia of Universiti Sains Malaysia (JEPeM Code: USM/JEPeM/20110579) and National Medical Research Registry, Ministry of Health (NMRR ID: NMRR-20-2837-57262) prior to data collection. Permission for data access was obtained from the Ministry of Health, Malaysia.

3.13.1 Subject vulnerability

This study does not involve vulnerable populations as this study used secondary data.

3.13.2 Declaration of the absence of conflict of interest

The authors declare that there is no conflict of interest in any form.