PROPORTION AND FACTORS ASSOCIATED WITH SEVERE ZOONOTIC MALARIA INFECTION IN PAHANG FROM 2011 UNTIL 2022

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UNIVERSITI SAINS MALAYSIA

2024

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by

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Research Project Report submitted in partial fulfilment of the requirements for the degree of Master of Public Health

JUNE 2024

ACKNOWLEDGEMENTS

بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْمِ

I would like to express my deepest gratitude to my supervisor, Dr Noor Aman A. Hamid, for his exceptional dedication and unwavering support through the completion of my dissertation. His commitment to academic excellence was both captivating and inspiring.

I am also deeply thankful to my co-researchers, Dr. Wan Rosmawati Binti Wan Ismail from Pahang State Health Department. Additionally, my sincere appreciation goes out to all the lecturers in the Department of Community Medicine at the School of Medical Sciences, Universiti Sains Malaysia. I am grateful to my encouraging classmates in the Master of Public Health 2023/2024 program for their unwavering support and inspiration throughout this journey.

Finally, I want to thank my loving and understanding wife, Sharifah Norsyahidah, as well as my children, Tengku Muhd Hasif and Tengku Nurhana Sofea. I am also deeply grateful to my kind-hearted parents, Tengku Mokhtar Zainal and Nor Malaysia Rose and in-laws for their patience, resilience, and enduring support. Their prayers and encouragement have been instrumental in my success.

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LIST OF SYMBOLS

%	Percent
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
±	Plus or minus
n	Sample size
μL	Microliter
p	p-value (probability value in hypothesis testing)
df	Degrees of freedom

LIST OF ABBREVIATIONS

ACD	Active Case Detection
ACTs	Artemisinin-based Combination Therapies
AOR	Adjusted Odds Ratio
ARDS	Acute Respiratory Distress Syndrome
CDCIS	Communicable Disease Control Information System
CI	Confidence Interval
CFR	Case Fatality Rate
G6PD	Glucose-6-Phosphate Dehydrogenase
IRS	Indoor Residual Spraying
ITNs	Insecticide-treated Nets
MBS	Mass Blood Surveys
MOH	Ministry of Health
nPCR	Nested Polymerase Chain Reaction
OR	Odds Ratio
PCD	Passive Case Detection
PCR	Polymerase Chain Reaction
ROC	Receiver Operating Characteristic
SD	Standard Deviation

ABSTRAK

FAKTOR-FAKTOR YANG BERKAITAN DENGAN JANGKITAN MALARIA ZOONOTIK YANG TERUK DI PAHANG DARI 2011 HINGGA 2022

***Pengenalan*:** Malaria terus menjadi cabaran kesihatan awam global, dengan malaria zoonotik yang disebabkan oleh parasit Plasmodium knowlesi menimbulkan ancaman yang signifikan di Asia Tenggara, terutamanya di Malaysia. Malaria zoonotik yang teruk, yang dicirikan oleh komplikasi yang mengancam nyawa, adalah kebimbangan utama. Kajian ini bertujuan untuk menentukan kadar dan faktor-faktor yang berkaitan dengan jangkitan malaria zoonotik yang teruk di Pahang, Malaysia, dari tahun 2011 hingga 2022.

***Metodologi*:** Reka bentuk kajian keratan rentas telah digunakan dengan menggunakan data sekunder dari pangkalan data malaria VEKPRO. Kajian ini melibatkan 487 kes malaria zoonotik yang didaftarkan di Pahang antara tahun 2011 dan 2022. Statistik deskriptif digunakan untuk menentukan kadar kes malaria zoonotik yang teruk, manakala regresi logistik berganda digunakan untuk mengenal pasti faktor-faktor yang berkaitan dengan jangkitan yang teruk.

***Keputusan*:** Daripada 487 kes yang dianalisis, 97 (19.9%) adalah jangkitan malaria zoonotik yang teruk, manakala 390 (80.1%) adalah kes tidak teruk. Kajian ini mengenal pasti beberapa faktor utama yang berkaitan dengan jangkitan malaria zoonotik yang teruk: umur, pekerjaan, dan kelewatan dalam diagnosis. Individu berusia lebih 60 tahun mempunyai kebarangkalian yang lebih tinggi untuk jangkitan teruk (OR Terlaras: 18.49, 95% CI: 2.17–157.59) berbanding mereka yang berusia kurang daripada 15 tahun. Pekerjaan yang berkaitan dengan hutan menunjukkan risiko yang lebih tinggi (OR Terlaras: 2.21, 95% CI: 1.32–3.71) berbanding pekerjaan yang tidak berkaitan dengan hutan. Kelewatan dalam diagnosis melebihi 4 hari dikaitkan dengan peningkatan risiko yang ketara untuk jangkitan teruk (OR Terlaras: 5.18, 95% CI: 2.65–10.14).

***Kesimpulan*:** Kadar tinggi kes malaria zoonotik yang teruk di Pahang menunjukkan kebimbangan kesihatan awam yang signifikan. Faktor risiko kritikal seperti umur yang lebih tua, pekerjaan berkaitan hutan, dan kelewatan dalam diagnosis memerlukan intervensi kesihatan awam yang disasarkan. Meningkatkan kemudahan diagnostik dan memastikan akses penjagaan kesihatan yang pantas, terutamanya di kawasan luar bandar, adalah penting untuk mengurangkan malaria zoonotik yang teruk. Walaupun terdapat batasan data sekunder dan pengecualian beberapa pembolehubah, kajian ini menyediakan maklumat berharga untuk memaklumkan strategi kesihatan awam dalam menangani malaria zoonotik yang teruk.

***Kata kunci*:** Malaria zoonotik, *Plasmodium knowlesi*, jangkitan teruk, Pahang, Malaysia, faktor risiko.

ABSTRACT

FACTORS ASSOCIATED WITH SEVERE ZOONOTIC MALARIA INFECTION IN PAHANG FROM 2011 UNTIL 2022

Introduction**:** Malaria remains a global public health challenge, with zoonotic malaria caused by the Plasmodium knowlesi parasite posing a significant threat in Southeast Asia, particularly in Malaysia. Severe zoonotic malaria, a disease stage characterized by life-threatening complications, is a major concern. This study aims to determine the proportion and factors associated with severe zoonotic malaria infection in Pahang, Malaysia, from 2011 to 2022.

Methodology**:** This cross-sectional study utilized secondary data from the VEKPRO malaria database, including 487 zoonotic malaria cases reported in Pahang between 2011 and 2022. Descriptive statistics were employed to determine the proportion of severe cases of zoonotic malaria, while multiple logistic regression identified factors associated with severe infection of zoonotic malaria.

Results**:** Among the 487 cases analyzed, 97 (19.9%) were classified as severe zoonotic malaria infections, and 390 (80.1%) were uncomplicated. Key factors significantly associated with severe infection included age, occupation, and delay in diagnosis. Individuals over 60 years old had a significantly higher risk of severe infection (Adjusted OR: 18.49, 95% CI: 2.17–157.59) compared to those under 15. Forest-related occupations were also a significant risk factor (Adjusted OR: 2.21, 95% CI: 1.32–3.71) compared to non-forest-related jobs. Additionally, a diagnosis delay of more than 4 days substantially increased the risk of severe zoonotic malaria (Adjusted OR: 5.18, 95% CI: 2.65–10.14).

Conclusions**:** The high proportion of severe zoonotic malaria cases in Pahang underscores significant public health concerns. Critical risk factors such as older age, forest-related occupations, and delayed diagnosis require targeted public health interventions. Enhancing diagnostic facilities and ensuring prompt healthcare access, especially in rural areas, are crucial to reducing the severity of zoonotic malaria.

***Keywords*:** Zoonotic malaria, Plasmodium knowlesi, severe zoonotic malaria infection, risk factors.

CHAPTER 1

INTRODUCTION

1.1 **Introduction**

1.1.1 Malaria

Malaria is a significant public health problem worldwide, as around 3.3 billion individuals, which accounts for over 40% of the world's population, are susceptible to acquiring the disease. The latest World Malaria Report (2023) indicates that there were around 249 million cases of malaria globally in 2022, with Africa being the region where most cases were reported. The WHO Western Pacific Region experienced a significant 23% surge in malaria cases between 2021 and 2022, resulting in a total of 1.9 million cases. Papua New Guinea accounted for over 90% of all cases in the region in 2022 (WHO, 2023a).

Countries like Malaysia, Republic of Korea, and China were aiming to eliminate malaria by 2020 under the Regional Action Framework for Malaria Control and Elimination in the Western Pacific (2016-2020) (WHO, 2019). Malaysia has made significant progress in malaria prevention and management since the Malaria Eradication Programme was implemented in 1961 in Sabah and Sarawak, and in 1967 in Peninsular Malaysia (Ho, 1985a). In 2011, Malaysia achieved a malaria case rate of less than one per 1,000 people, indicating that it had entered the elimination phase of malaria control (WHO, 2014). This was followed by the National Malaria Elimination Strategic Plan, established in 2011, with the goal of achieving "malaria-free" status by 2020. This status refers to the elimination of human indigenous malaria species such as P. falciparum, P. malariae, P. ovale, and P. vivax (Chin *et al.*, 2020). In 2018, Malaysia has consistently reported zero cases of human malaria, a trend that has continued for five consecutive years until 2022. However, the celebrated success was short lived as a new concern has emerged: a rise in zoonotic malaria cases (WHO, 2023a).

1.1.2 Zoonotic Malaria

The parasite P. knowlesi, which causes zoonotic malaria, has become an imminent threat to human health, particularly in Southeast Asia. The very first recorded incident of spontaneously acquired P. knowlesi malaria took place in 1965, involving an American tourist who had visited Peninsular Malaysia (Chin *et al.*, 1965). Following this, a traveller from United State of America who had previously visited the region was also involved in a second case of possible infection (Magaudda et al, 1969). Zoonotic malaria attracted significant attention in 2004 due to the occurrence of widespread outbreaks in the Kapit Division of Sarawak, Malaysian Borneo (Singh *et al.*, 2004). Since then, nearly all nations in Southeast Asia have reported cases of P. knowlesi malaria (Cox-Singh et al, 2008). The rise in tourism and international travel has made the region a focal point for the global spread of P. knowlesi malaria (Lee *et al.*, 2022).

The long-tailed macaques (M. fascicularis) and pig-tailed macaques (M. nemestrina) are the main carriers of simian malaria in Malaysia. They act as natural reservoirs for P. knowlesi (Sam *et al.*, 2022). P. knowlesi is mostly found in Southeast Asia, an area where both the macaque hosts and Anopheles mosquito vectors are abundant (Singh et al, 2013). The vectors, which inhabit forests, exhibit a preference for moist surroundings and reproduce in temporary water sources such as puddles, ground holes, stream edges, and wheel tracks (Naserrudin et al, 2022). Among these vectors, the Anopheles balabacensis mosquito is particularly notable, preferring secondary forest areas surrounded by mountainous terrain and agricultural estates that include oil palm and rubber plantations (Manin *et al.*, 2016). These environments create an ideal setting for zoonotic spillover, where the close interaction between macaques, mosquitoes, and humans becomes more frequent (Manin *et al.*, 2016). The majority of zoonotic malaria cases have been linked to human activities in jungle or forest fringe areas. Here, the parasite circulates in a complex cycle involving Anopheles mosquito vectors and macaque reservoirs (Daneshvar et al., 2009; Singh et al, 2013). Individuals who engage in forest-related activities such as logging, farming, or camping in these locations are more likely to contract the disease. The abundance of vegetation and the presence of water sources provide ideal conditions for mosquitoes to reproduce, thereby facilitating the transmission cycle. Furthermore, the expansion of agricultural frontiers and deforestation have brought humans into closer contact with these natural reservoirs and vectors. For instance, as oil palm plantations encroach further into forested areas, the interactions between humans and infected macaques increase, facilitating the transmission of P. knowlesi malaria (Manin *et al.*, 2016).

The prevalence of zoonotic malaria has increased significantly over the last decade, affecting public health in Southeast Asia. The prevalence of Zoonotic Malaria in Malaysia has been recorded at 71%, the highest among Southeast Asian countries (Cox-Singh *et al.*, 2008). A recent study found that the prevalence of P. knowlesi in Malaysian Borneo was 55.6% and currently is the leading cause of malaria in Malaysia, surpassing other Plasmodium species previously associated with human malaria (Lai *et al.*, 2021). While other country in Southeast Asia, the prevalence of zoonotic malaria was at 1% for Thailand and the rest of other country like Indonesia, Myanmar, Philippines, Singapore, Laos, and Cambodia at below 1% (Zaw et al., 2019).

The growing incidence of P. knowlesi malaria is significant, as it can cause severe or even fatal illness. P. knowlesi, unlike other types of malaria, can cause significant parasitaemia, which can lead to complications such as acute respiratory distress, renal failure, and cerebral malaria (Daneshvar *et al.*, 2009). The extremely rapid replication cycle of P. knowlesi, which occurrs every 24 hours, poses a significant threat due to its ability to rapidly advance from mild symptoms to severe zoonotic malaria infection (Cox-Singh et al., 2008; Naserrudin et al., 2022).

1.1.3 Severe Zoonotic Malaria

Severe zoonotic malaria is characterized by the presence of one or more clinical symptoms, laboratory findings, or complications. It typically presents with symptoms such as coma (cerebral malaria), metabolic acidosis, severe anemia, hypoglycemia, acute renal failure, or acute pulmonary edema (WHO, 2010; MOH, 2014). Accurate diagnosis is critical in the management of zoonotic malaria, as misdiagnosis can lead to inappropriate treatment and severe outcomes. Historically, Plasmodium knowlesi infections have often been misdiagnosed as Plasmodium malariae due to similarities in their morphological features when viewed under a microscope (Seilmaier et al., 2014; William et al., 2014; Rajahram et al., 2019). This misidentification has significant clinical implications because P. knowlesi replicates every 24 hours, leading to rapid increases in parasitemia and potentially severe and life-threatening disease (Kotepui et al., 2020; Phang et al., 2020; Naserrudin et al., 2022).

Cox-Singh et al., (2008) conducted a comprehensive study, analyzing blood samples from patients across various regions in Malaysia, and found that P. knowlesi actually caused a substantial proportion of infections initially identified as P. malariae. The study also reviewed four suspected fatal cases, confirming that these deaths were due to P. knowlesi malaria, with patients exhibiting hyperparasitemia and severe hepatorenal dysfunction. The patient received an inappropriate treatment regimen based on the incorrect diagnosis, which resulted in severe illness and ultimately death. Between 2010 and 2011, misdiagnosis of Plasmodium knowlesi was responsible for almost half of malaria deaths in Sabah (Rajahram *et al.*, 2012).

To address the issue of misdiagnosis, the introduction of sensitive and specific diagnostic methods, such as nested polymerase chain reaction (PCR), has been pivotal. PCR allows for the precise identification of P. knowlesi DNA in blood samples, distinguishing it from other Plasmodium species. This advancement in diagnostic technology has significantly improved the accuracy of malaria diagnoses, enabling appropriate and timely treatment for patients (Zaw et al., 2014).

In 2011, the state of Sabah in Malaysia, faced an alarming peak in zoonotic malaria prevalence, with rates soaring as high as 39% (William et al., 2011; Barber et al., 2017). The prevalence appeared to be on the decline, but recent data from 2018 to 2022 suggested alarming issues. Studies conducted over these years revealed a significant and concerning rise in severe zoonotic malaria cases, with prevalence rates increased from 6% to a staggering 23.7% . These alarming statistics highlight the urgent need for action at all levels of healthcare management. From policy development to implementation, comprehensive strategies are required to effectively address and manage the issue of zoonotic malaria.

1.2 **Problem Statement and Rationale of Study**

In recent years, zoonotic malaria has seen an alarming increase in prevalence. In 2020, Malaysia reported that zoonotic malaria accounted for a significant 92% of malaria cases in Malaysia, with Sabah and Sarawak being the most affected states

(MOH, 2021a). This increase in zoonotic malaria cases has now surpassed the number of human malaria cases, indicating a steady escalation in the disease burden over time.

While the WHO Global malaria Elimination Programme has been successful in eliminating indigenous human malaria, the focus of the program has been on human malaria while neglecting zoonotic malaria (Ooi *et al.*, 2022). This has resulted in an unfocussed and poorly organised effort in zoonotic malaria prevention thus resulting in the rapid increase in its prevalence. A worrying feature of zoonotic malaria is the higher risk and rapid development of severe malaria when compared to human malaria, thus making it more fatal (Grigg *et al.*, 2017).

While a considerable amount of research has been conducted in Sabah and Sarawak, there is a noticeable lack of studies from Peninsular Malaysia. Considering the unique socioeconomic and cultural context of the population in Peninsular Malaysia, it is crucial to explore the risks associated with severe zoonotic malaria using data from Peninsular Malaysia.

The insights gained from such research will be instrumental in enhancing the National Strategic Plan for the Malaria Elimination Program. It will provide valuable data to inform policy decisions and intervention strategies, ultimately contributing to the battle against this escalating health concern.

1.3 Research Questions

1. What is the proportion of severe zoonotic malaria infection in Pahang from 2011 until 2022?
2. What are the associated factors of severe zoonotic malaria infection in Pahang from 2011 until 2022?

1.4 **Research Objectives**

1.4.1 General objective

To determine the proportion and factors associated with Severe Zoonotic Malaria Infection in Pahang from 2011-2022.

1.4.2 Specific objectives

1. To determine the proportion of severe zoonotic malaria infection in Pahang from 2011 until 2022
2. To determine the factors associated with severe zoonotic malaria infection in Pahang from 2011 until 2022.

1.5 **Research Hypothesis**

H1: There are significant associations between sociodemographic factors, behaviour and environmental factors, health and medical history factors, and biomarkers with severe Zoonotic Malaria infection in Pahang.

CHAPTER 2

LITERATURE REVIEW

2.1 Severe Zoonotic Malaria

The clinical picture of zoonotic malaria infection varies widely, ranging from mild, uncomplicated cases to severe, potentially life-threatening conditions. The differences in symptoms, treatment, and management for uncomplicated and severe cases are significant. Uncomplicated zoonotic malaria infection is characterized by milder symptoms that do not involve severe complications or organ failure (Plewes et al., 2018). Typical symptoms include fever, chills, headache, and myalgia (Mahittikorn *et al.*, 2021). These cases are generally self-limiting with appropriate treatment. In contrast, severe zoonotic infection involves significant complications and can be life-threatening (Kotepui *et al.*, 2020). It is defined by criteria such as severe anaemia, respiratory distress, jaundice, renal failure, shock, or other signs of organ dysfunction. These cases require immediate medical attention and intensive treatment (MOH, 2014; WHO, 2014).

The incubation period for zoonotic malaria infection—the time from the bite of an infected mosquito to the appearance of symptoms—typically ranges from 9 to 12 days (Anstey *et al.*, 2021). However, this period can vary depending on the individual's immune status and the intensity of parasite exposure (Froeschl *et al.*, 2018).

In uncomplicated infections, fever is often the most prominent symptom, typically cyclical every 24 hours due to the rapid erythrocytic cycle of Plasmodium knowlesi parasite (Froeschl *et al.*, 2018). Patients may also experience intense chills, severe headaches, muscle pain, and gastrointestinal symptoms such as nausea and vomiting. In severe cases, patients can develop significant anemia due to the rapid destruction of red blood cells. Pulmonary complications, including acute respiratory distress syndrome (ARDS), are common, and patients may also experience jaundice due to liver involvement, kidney failure, and shock due to severe infection and multi-organ failure (Cowman *et al.*, 2016; Froeschl *et al.*, 2018; Kotepui *et al.*, 2020).

The pathophysiology of Plasmodium knowlesi infection involves the parasites invading red blood cells, multiplying, and causing the cells to burst, releasing new parasites to infect more red blood cells. This cycle leads to the characteristic fever spikes and can result in severe anemia and other complications. After being bitten by an infected Anopheles mosquito, sporozoites enter the bloodstream and migrate to the liver, where they mature and replicate. Mature schizonts then rupture, releasing merozoites into the bloodstream that invade red blood cells. The erythrocytic cycle continues with repeated cycles of invasion, replication, and rupture of red blood cells (White et al., 2018; Anstey et al., 2021). The severity of the infection depends on various factors, including the parasite load, the host's immune response, and the presence of comorbidities (Ooi *et al.,* 2022). Severe cases are often associated with higher parasite densities and more significant immune responses that can lead to complications like ARDS and renal failure (William *et al.*, 2011). In severe cases, patients can exhibit hypoglycaemia, metabolic acidosis, and hyperparasitemia, which are critical factors contributing to the high morbidity and mortality rates (Kotepui *et al.*, 2020).

The Anopheles mosquito is the primary vector for Plasmodium knowlesi. The mosquito becomes infected when it takes a blood meal from an infected macaque monkey or human. The parasites develop within the mosquito over a period of about 10 to 14 days (Shearer *et al.*, 2016). During subsequent blood meals, the mosquito transmits the parasites to a new host. Anopheles mosquitoes breed in stagnant water, such as ponds, puddles, and rice fields. They are most active during dusk and dawn, which is when most bites occur (Manin *et al.*, 2016). Understanding the mosquito’s lifecycle, breeding habits, and feeding patterns helps in designing effective control strategies. Vector control measures, such as insecticide-treated nets, indoor residual spraying, and environmental management to eliminate breeding sites, are vital in reducing transmission (Shearer *et al.*, 2016). The mosquito's lifecycle consists of four stages: egg, larva, pupa, and adult. Female Anopheles mosquitoes lay eggs on water surfaces, where they hatch into larvae. The larvae undergo several molts before becoming pupae, and finally emerge as adult mosquitoes ready to transmit the malaria parasite (Christiansen-Jucht *et al.*, 2015).

Macaque monkeys, particularly the long-tailed and pig-tailed macaques, are the natural reservoir hosts for Plasmodium knowlesi. The parasite completes part of its lifecycle in these primates, which then serve as a source of infection for mosquitoes. Macaques typically inhabit forested areas, which include primary and secondary forests, as well as forest fringes (Shearer *et al.*, 2016). Human activities such as agriculture, logging, and urbanization bring people into closer contact with these habitats, increasing the risk of zoonotic transmission (Akter *et al.*, 2015; Sam *et al.*, 2022). Macaques often forage for food in areas where humans are present, such as farms and plantations, further enhancing the risk of human-macaque interactions. Understanding these dynamics is essential for developing targeted interventions to break the transmission cycle. These monkeys live in social groups and have a wide-ranging diet, which often brings them into proximity with human settlements, thus facilitating the spillover of Plasmodium knowlesi from wildlife to humans (Jeyaprakasam *et al.*, 2020).

2.2 Malaria Elimination Program

Malaysia's commitment to malaria elimination has been significant and comprehensive, focusing on effective surveillance, vector control, and community engagement. The journey began with the Malaria Eradication Programme in the 1960s, which laid the groundwork for future initiatives. Significant strides in malaria prevention and control have been made since the Malaria Eradication Programme was introduced in 1961 in Sabah and Sarawak, and in 1967 in Peninsular Malaysia (Ho et al., 1985). By 2011, the country recorded less than 1 case per 1,000 people, entering the elimination phase of malaria control. The National Malaria Elimination Strategic Plan (2011–2020) was introduced in the same year, targeting “malaria-free” status by 2020 (WHO, 2014). This status refers to the elimination of human indigenous malaria species, such as P. falciparum, P. malariae, P. ovale, and P. vivax. Over the decades, Malaysia has evolved its strategies, culminating in the successful achievement of zero locally acquired human malaria cases by 2018 (WHO, 2019).

The programme employs a comprehensive approach, encompassing vector control, case management, surveillance, and community engagement. Vector control measures include insecticide-treated nets (ITNs), indoor residual spraying (IRS), and environmental management. Case management focuses on early diagnosis and prompt treatment using artemisinin-based combination therapies (ACTs). Surveillance involves passive case detection (PCD), active case detection (ACD), and mass blood surveys (MBS). Community engagement entails collaboration with local communities, NGOs, and international partners to raise awareness about malaria prevention and control. These strategies led to Malaysia reporting zero indigenous cases of human malaria for the first time in 2018, maintaining this status for four consecutive years (MOH, 2021b).

From 2000 to 2021, Malaysia recorded an 87.3% reduction in human malaria incidence, from 0.55 per 1,000 population to 0.03 per 1,000 population. Malaria mortality also recorded 87% reduction, from 0.15 per 100,000 population to 0.04 per 100,000 population. The last imported human malaria death was in 2017, and between 2018 and 2021, all malaria deaths were related to zoonotic malaria. Despite the progress, challenges persist, such as the emergence of drug-resistant malaria parasites and the persistent transmission of zoonotic malaria. The close interaction between humans and macaques in forested areas complicates efforts to eliminate this species. Additionally, the increasing movement of migrant workers and refugees poses a risk for malaria reintroduction and transmission. Zoonotic malaria, which accounted for 82% of total malaria cases in Malaysia in 2019, poses significant challenges due to a lack of effective vector control strategies and control of the macaque population (WHO, 2023b).

To address these challenges, the newly developed National Strategic Framework for Prevention of Malaria Re-Establishment (2021-2025) aims to secure WHO malaria elimination certification by 2025. This goal will be achieved through strengthened surveillance with molecular and genetic tools for tracking drug resistance and parasite populations, enhancing detection and monitoring of zoonotic and human malaria. Vector control will involve developing new tools tailored to the unique ecology of zoonotic malaria, such as insecticide-treated clothing, spatial repellents, and habitat modification to reduce mosquito breeding sites. Targeted interventions will address the transmission of zoonotic malaria from macaques to humans, including research on reducing macaque populations near settlements and exploring innovative approaches to interrupt the parasite’s life cycle. Education campaigns will promote personal protective measures like insecticide-treated nets and repellents, especially for those working in or near forested areas (White et al., 2018; Koh et al., 2019). By integrating these strategies, Malaysia aims to create a resilient and adaptive malaria control program, ultimately achieving WHO certification for malaria elimination by 2025.

2.3 Prevalence of Severe Zoonotic Malaria

Zoonotic malaria, caused by a plasmodium knowlesi parasite that primarily infects macaques, has emerged as a significant cause of severe malaria in humans, particularly in Southeast Asia. The global burden of severe zoonotic malaria is not fully quantified, but the disease poses a substantial health risk in areas where human activities overlap with macaque habitats. In Southeast Asia, Malaysia is the predominant source of zoonotic malaria cases. According to the World Malaria Report 2022, Malaysia contributed 90.5% of the cases in 2022. Following Malaysia, Thailand accounted for 3.1% of the cases, while Indonesia reported 0.1%.

In Thailand, Seilmaier et al. (2014) reported that the prevalence of zoonotic malaria cases was 1%. However, the prevalence of severe and fatal cases is considered lower, as indicated by Jongwutiwes et al. (2011). In Indonesia, a few studies, such as those by Gunawan et al. (2022) and Bin Said et al. (2022), have mentioned that the prevalence of zoonotic malaria cases was below 1% in Kalimantan, with a recent increase in incidence noted. However, studies specifically addressing the prevalence or proportion of severe cases are limited.

In Malaysia, particularly in East Malaysia (Sabah and Sarawak), several studies have documented the prevalence of severe zoonotic malaria. In these states, severe cases constitute a significant proportion of the overall malaria burden. For instance, Grigg et al. (2018) reported a prevalence of 6%. Data from 2011 indicated particularly high prevalence rates in Sabah, with severe zoonotic malaria cases ranging from 32-39%, as reported by William et al. (2011) and Barber et al. (2017). More recent studies by Ooi et al. (2022) documented a prevalence of 23.7% in Sarawak. Additionally, a systematic review by Kotepui et al. (2020) noted that the pooled prevalence of severe zoonotic malaria cases in Sabah and Sarawak is around 19%. In Peninsular Malaysia, the proportion of severe zoonotic malaria cases is less prevalent compared to East Malaysia. Studies by Yusof et al. (2014) and (Phang *et al.*, 2020) have shown the widespread distribution of zoonotic malaria infection in regions like Kelantan, Pahang, and Perak. However, there are limited studies specifically addressing the proportion of severe zoonotic malaria cases in these regions.

2.4 **Factors associated with severe zoonotic malaria**

2.4.1 Sociodemographic

There are multiple factors that have been shown to be associated with severe zoonotic malaria infection. Factors can be broadly classified into sociodemographic factors, behaviour factors, environmental factors, laboratory factors, and health and medical history factors. Age has a significant impact on the severity of zoonotic malaria infection, influencing disease outcomes in both children and adults. Research has consistently highlighted the impact of age on the clinical presentation and severity of zoonotic malaria infections. Grigg et al. (2018) conducted a comprehensive study from 2012 to 2016, assessing patients of all ages with molecularly-confirmed zoonotic malaria infection in three district hospitals in Sabah, Malaysia. The study included 481 zoonotic plasmodium knowlesi malaria cases, of which 44 were children aged ≤12 years. The findings from the study concluded that age was independently associated with severe zoonotic malaria infection.

Older adults, however, are at a significantly higher risk of severe zoonotic malaria infection. Several studies have confirmed this increased susceptibility. Barber et al. (2013) reported that in Sabah, severe zoonotic malaria occurred in 47% of patients aged ≥50 years, compared to 15% of those aged <50 years. Similarly, Rajahram et al. (2016) found that older patients, with a median age of 61 years, had a strong correlation between age and parasitaemia, which pointed to an increased risk for severe zoonotic malaria infection. Ooi et al. (2022) further supported this correlation by noting that individuals aged 60 years and older had the highest odds of developing severe zoonotic malaria compared to other age groups, with an adjusted odds ratio (AOR) of 2.48. Additionally, Yang et al. (2020) highlighted that older age in zoonotic malaria patients is associated with higher parasitemia and a greater risk of severe disease.

Systematic reviews and meta-analyses have also emphasised the association between age and severity. Kotepui et al. (2020) demonstrated that patients with severe zoonotic malaria infections had higher ages, leucocyte counts, and parasitemia levels than those with uncomplicated infections. Further insights from Barber et al. (2017) suggest that in populations with low immunity to malaria, the risk of severe zoonotic malaria increases with age. Their study on Malaysian patients aged ≥12 years found that parasite biomass, endothelial activation, and microvascular dysfunction were associated with severe disease in zoonotic malaria. The association between each of these processes and aging may account for the greater severity of zoonotic malaria observed in older adults in low-endemic regions.

Sex is also a significant factor in determining the severity of zoonotic malaria infection, with varying risks observed between males and females. Multiple studies have highlighted these differences and their implications for disease severity. Phang et al. (2020) noted that the incidence of zoonotic malaria infection was generally higher among males compared to females in most states of Peninsular Malaysia. This increased risk in males is likely due to occupational exposure, as many men engage in activities such as working in forests that increase their likelihood of mosquito bites. Ooi et al. (2022) supported this finding, indicating that male adults have a higher risk of contracting zoonotic malaria infection than females, primarily due to their occupational exposure. Grigg et al. (2017) corroborated these findings, reporting that adult men working in agricultural areas were at the highest risk of zoonotic malaria infection. The study found that men had a four-fold greater risk of developing severe zoonotic malaria infection (adjusted odds ratio 4.20).

Contrary to the general trend observed in males, some studies indicate a higher severity of zoonotic malaria infection in females under certain conditions. Rajahram et al. (2016) reported that, although males had a higher incidence of infection, the case fatality rate (CFR) was significantly higher in females. The overall CFR for 2010–2017 was 2.5/1000: 6.0/1000 for women and 1.7/1000 for men. Independent risk factors for death included female sex (odds ratio, 2.6) and age more than 45 years (odds ratio, 4.7). According to a study by William et al. (2011), the percentage of female patients in severe cases of zoonotic malaria was 36%, which was much higher than the percentage of female patients in uncomplicated zoonotic malaria, which was 8.8%. The study demonstrated that 8 out of 11 female patients showed a severe form of the condition, suggesting that females had a greater degree of severity. Rajahram et al. (2016) also indicated that older female patients with zoonotic malaria had a higher risk of severe outcomes. Of the seven patients who died in their study, four were female, suggesting a trend towards higher fatality rates in females, particularly among older adults.

Ethnicity has a significant impact on the severity of zoonotic malaria infection, with certain ethnic groups exhibiting higher risks and different levels of disease severity. Research has highlighted these differences, revealing the complex impact of this zoonotic malaria infection across diverse communities. Malays in Peninsular Malaysia had the highest prevalence of zoonotic malaria cases, accounting for 58.7% of cases from 2011 to 2018 Phang et al. (2020). This study attributed the greater burden among Malays to their proximity to forested areas and involvement in forest-related occupations. On the other hand, studies revealed that Orang Asli in Peninsular Malaysia exhibits a low risk of zoonotic malaria infection. According to Phang et al. (2020), the prevalence of zoonotic malaria infection among Orang Asli in Peninsular Malaysia was 10%. However, Dian et al. (2023) found a significantly lower prevalence of zoonotic malaria infection among six settlements of Orang Asli in Kelantan, Malaysia. All detected zoonotic malaria infections were submicroscopic, making them undetectable by standard microscopy and necessitating the use of sensitive molecular techniques such as nested PCR (nPCR) for identification. Traditional diagnostic methods might underestimate the true prevalence of the disease. The low reported prevalence could be due to the limited sensitivity of microscopy, which missed cases of low-density infections that nPCR could detect. This suggests that the actual infection rates may be higher than those detected through traditional diagnostic methods. In Sarawak, the Iban community recorded the highest percentage of zoonotic malaria cases, yet it was the Bidayuh patients who were more likely to develop severe zoonotic malaria infections, indicating significant disparities even within indigenous groups (Ooi *et al.*, 2022).

The geographical distribution of zoonotic malaria infection shows significant variation between urban and rural areas, influencing both the severity and prevalence of the disease. Rural areas, particularly those near forested regions, consistently report higher incidences of zoonotic malaria infections. This increased risk is due to the proximity to macaque monkeys' natural habitats, which are the primary reservoirs for the parasite, and the forest-dwelling mosquitoes that transmit the infection. Research indicates that exposure to denser vector populations and reservoirs in rural and remote areas elevates the risk of zoonotic malaria infection (Braima et al., 2013; William et al., 2014; Akter et al., 2015; Rajahram et al., 2016; Brock et al., 2019). A study by Ooi et al. (2022) highlighted that residing in remote areas significantly increases the risk of severe zoonotic malaria infection. This is likely due to limited transportation infrastructure, leading to delays in diagnosis and treatment, which exacerbates the severity of the disease. In contrast, a study by Dian et al. (2021), which analyzed trends in zoonotic malaria cases in urban Kuala Lumpur from 2005 to 2020, found that cases of zoonotic malaria have notably increased over the past 16 years. Most positive cases in Kuala Lumpur were among foreigners working in the city.

In terms of nationality factors, a study by Phang et al. (2020) noted that foreigners accounted for 16.4% of zoonotic malaria cases in Peninsular Malaysia. This finding suggests that a significant proportion of zoonotic malaria infections occur among non-local, possibly due to their working and living conditions, which often involve greater exposure to vectors and reservoirs of the parasite. Dian et al. (2021) further emphasized this point by showing that in urban Kuala Lumpur, nearly half (47.8%) of malaria cases were found among foreigners. Most of these cases were among male workers from Southeast Asian countries, highlighting the increased risk of zoonotic malaria infection among expatriate and migrant worker populations in urban settings.

In terms of occupation, people involved in forest-related occupations, such as logging, farming, and plantation work, are at a higher risk of contracting severe zoonotic malaria (Chin *et al.*, 2020). In Peninsular Malaysia, these jobs accounted for nearly 54% of the total zoonotic malaria cases (Phang *et al.*, 2020). This is primarily due to the frequent close contact with forest environments that these jobs require, significantly increasing exposure to the disease. Individuals working in agriculture face the highest risk. For instance, studies have found that adult men in agricultural areas have a four-fold greater risk of developing severe zoonotic malaria infections compared to those not engaged in such work (Grigg *et al.*, 2017). Additionally, those engaged in agricultural activities are 1.63 times more likely to contract zoonotic malaria (Fornace *et al.*, 2018). Similarly, deforestation and logging activities in Sarawak have driven macaques closer to human habitats, increasing human exposure to zoonotic malaria (Lai *et al.*, 2021). Older individuals and those with high parasitemia levels, often seen in forest-related occupations, are at greater risk for severe malaria (Rajahram *et al.*, 2016). For instance, compared to those in non-forest-related jobs, those in these occupations had a median parasite density of 1800 parasites/l, indicating that forest workers are not only more susceptible to infection but also more likely to develop severe disease due to higher parasite loads (Ooi *et al.*, 2022). A study conducted in Brunei revealed that males in forest-related occupations, such as border patrol officers, armed forces personnel, security staff, and forestry officers, account for a significant proportion of work-related zoonotic malaria infections (Koh et al., 2019).

2.4.2 Health and medical history

In Malaysia, 99% of Zoonotic Malaria infection was detected by passive case detection. Which means individuals is diagnosed when they had the infection and seek medical treatment (Phang *et al.*, 2020; Ooi *et al.*, 2022). Similarly, a study was done in Aceh Besar District, Indonesia found that majority of the zoonotic malaria infection was detected by passive case detection (Herdiana *et al.*, 2016). There is no association seen between individual who had previous malaria infection and the severity of cases (Barber et al., 2017). Pregnant female individual who contracts the infection face a higher risk of severity and maternal or foetal complication might arise (WHO, 2014; Rajahram *et al.*, 2019). Another study in Sabah, Malaysia mention that zoonotic malaria infection was rare during pregnancy. However, it was associated with severe maternal and pregnancy outcomes if occur (Barber *et al.*, 2015). Gluose-6-phosphate dehydrogenase deficiency confers some protection against Zoonotic Malaria infection thru cell wall fragility which releases the parasite before its maturity (Grigg *et al.*, 2017; Ooi *et al.*, 2022). Patient with comorbidity has been shown to be associated with severity of the infection. Rajahram et al. (2019) found that 50% of patient who died from complication of zoonotic malaria had cardiovascular comorbidities prior to contracting the disease. This shows that comorbidities especially cardiovascular disease was associated with severe disease and death (Anstey *et al.*, 2021). Additionally, a meta-analysis study done by Mahittikorn et al. (2021) found that co-infection with human immunodeficiency virus (HIV) increases the severity and mortality rates of both diseases.

Delayed diagnosis is a critical factor associated with the severity of zoonotic malaria infection. Timely diagnosis and prompt treatment are essential in managing malaria effectively, and delays can lead to increased disease severity and complications. Delayed diagnosis in malaria can result in the rapid progression of the disease to severe forms, particularly in zoonotic malaria infections, which can progress more quickly than other types of malaria. The rapid erythrocytic cycle of Plasmodium knowlesi can lead to high parasitemia levels, resulting in severe complications such as acute kidney injury, respiratory distress, and hyperparasitemia (William *et al.*, 2011; Froeschl *et al.*, 2018; Rajahram *et al.*, 2019). A systematic review by Bastaki et al. (2018) defined different types of diagnostic delays in non-endemic malaria countries. The study highlighted that delays of four or more days are common. These delays include patient delays, health system delays, and diagnostic delays. The study found that patient delays accounted for a significant proportion of the overall diagnostic delay, which can exacerbate the severity of malaria infection. Specifically, median diagnosis delays of four or more days were prevalent, largely due to patient delay, where individuals did not seek medical attention promptly after the onset of symptoms. Additionally, misdiagnosis and delayed diagnosis are strongly associated with higher mortality in zoonotic malaria cases. Routine diagnostic techniques can sometimes fail to distinguish between different Plasmodium species, leading to misdiagnosis. Wesolowski et al. (2015) emphasised the need for accurate and timely parasitological diagnostics, advocating for the use of molecular diagnostic techniques to improve the accuracy of malaria diagnosis and help in the timely initiation of appropriate treatment. Rajahram et al. (2013) described a case where delayed diagnosis of zoonotic malaria infection led to severe complications, including acute respiratory distress syndrome, acute kidney injury, and metabolic acidosis, resulting in death. The study highlighted the importance of early diagnosis and appropriate treatment to prevent severe outcomes. In this case, the patient presented with non-specific symptoms, which delayed the recognition of malaria, underscoring the need for heightened clinical suspicion in endemic areas, even for atypical presentations.

Delays in administering appropriate therapy significantly correlate with severe outcomes in zoonotic malaria cases. A systematic review by Rajahram et al. (2019) of all malaria deaths in Sabah, Malaysia, from 2015 to 2017 found that delays in starting intravenous treatment were identified in 36% of fatal cases, showing a direct link between delayed treatment and increased mortality. The study highlighted that independent risk factors for death included delayed diagnosis, particularly in females and older adults, indicating the urgent need for rapid diagnostic protocols and immediate therapeutic interventions. Additionally, Anstey et al. (2021) also noted that delayed diagnosis is a risk factor for severe disease and death in zoonotic malaria infections. In this study, the authors highlight that delayed diagnosis of zoonotic malaria can lead to severe disease, as it allows for increased parasitaemia and extended immune activation, which contribute to pathological mechanisms such as thrombocytopenia, endothelial dysfunction, microvascular sludging, and subsequent organ damage.

2.4.3 Behavioural and environmental factors

Behavioural and environmental factors play a crucial role in the transmission and severity of zoonotic malaria infections. These factors often determine the level of exposure to the vectors and reservoirs of the disease, influencing the risk and severity of infection. Various studies have highlighted the significance of behavioural factors such as occupational activities and living conditions. For instance, Grigg et al. (2017) conducted a population-based case-control study in Sabah, Malaysia, and found that adult men working in agricultural areas were at the highest risk of zoonotic malaria. The study identified that plantation work (aOR 3.50) and sleeping outside (aOR 3.61) were strongly associated with increased risk of severe zoonotic malaria infection. Additionally, factors like being aware of the presence of monkeys and having open eaves or gaps in walls further increased the risk (Grigg *et al.*, 2017). Similarly, Fornace et al. (2018) in their comprehensive surveys in endemic areas, identified that zoonotic malaria exposure was significantly associated with agricultural work (odds ratio [OR] 1.63) and higher levels of forest cover and clearing around houses (OR 2.40 and OR 2.14, respectively). The study emphasized that exposure patterns differ markedly from non-zoonotic malaria, with higher levels of exposure among women and children in the community. Behavioral factors such as contact with macaques, traveling, and the habit of sleeping outside were found to significantly increase the risk of zoonotic malaria infection. Conversely, the use of insecticides and living in houses at ground level or higher altitudes were associated with lower risk of infection (Grigg *et al.*, 2017; Fornace *et al.*, 2018, 2019).

Environmental factors such as deforestation and land use changes also contribute significantly to the transmission dynamics of P. knowlesi. Lai et al. (2021) indicated that deforestation and logging activities in Sarawak, Malaysia, have led to increased human-macaque interactions, thus raising the incidence of zoonotic malaria. The study noted that areas undergoing intensive land use changes reported higher cases of zoonotic malaria due to the displacement of macaques from their natural habitats into areas closer to human settlements. Furthermore, forest-related activities such as bird watching, hiking, camping, and plantation-related fields have been identified as higher risk activities for zoonotic malaria infection (Grigg *et al.*, 2017; Fornace *et al.*, 2018; Ooi *et al.*, 2022).

2.4.4 Biomarkers

Research has focused on various biomarkers and predictors of severity in zoonotic malaria infections. Interleukin-6 (IL-6) is one of the key biomarkers studied in severe malaria cases. IL-6 is a pro-inflammatory cytokine that plays a significant role in the immune response. Barber et al. (2017) highlighted that elevated levels of IL-6 are associated with severe zoonotic malaria infection. The study indicated that IL-6 levels correlated with parasite biomass and endothelial activation, suggesting its potential as a predictor of severe disease. Patients with severe complications, such as acute kidney injury and respiratory distress, have high IL-6 levels linked to systemic inflammation. Another important biomarker is angiopoietin-2 (Ang-2), which is involved in endothelial activation and vascular permeability. Anstey et al. (2021) noted that Ang-2 levels were significantly elevated in patients with a severe zoonotic malaria infection compared to those with a non-severe disease. Researchers found Ang-2 and IL-6 to be independent risk factors for severe zoonotic malaria. The study emphasized that endothelial dysfunction, shown by high Ang-2 levels, plays a role in the development of severe zoonotic malaria by affecting the integrity of blood vessels, which causes problems like capillary leakage and organ dysfunction. Additionally, thrombocytopenia, or low platelet count, is nearly universal in severe zoonotic malaria cases (Nakaviroj et al., 2015; Anstey et al., 2021; Gunawan et al., 2022). Grigg et al. (2018) reported that thrombocytopenia is a consistent feature in patients with severe disease, often preceding other clinical symptoms. The study proposed the use of platelet counts and other biomarkers to identify patients at risk of developing severe zoonotic malaria early in the clinical course.

Other than that, high parasite density is a well-established predictor of severe disease (Kotepui *et al.*, 2015; Nakaviroj *et al.*, 2015; Mahittikorn *et al.*, 2021; Ooi *et*