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RESEARCH ARTICLE

# Trend and pattern of melioidosis seropositivity among suspected patients in Malaysia 2015 – 2019

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**ABSTRACT**

Melioidosis is a disease of public health importance associated with high case-fatality rates in animals and humans caused by *Burkholderia pseudomallei* (*B. pseudomallei*), a gram-negative bacterium that lives in tropical soil environments. This study determined the seropositivity for melioidosis among patients admitted to healthcare centres in Malaysia, from 2015 to 2019 and identified factors related to it. A total of 26,665 serum samples of suspected melioidosis patients from Malaysia hospitals were tested for IgM against *B. pseudomallei*. About 16.4% of total samples were seropositive and majority of them were 55 years old and above. However, younger people aged less than 15 years old were the most susceptible to the infection (AOR 3.04, $p < 0.001$, 95% CI: 2.73, 3.39). Melioidosis infection was the highest in Sarawak (15.1%) while Perlis was the least exposed to the infection (0.9%). Further analyses showed that patients with chronic lung disease (adjusted OR: 4.03, $p < 0.001$, 95% CI: 2.77, 5.86) were more susceptible to melioidosis infection. In conclusion, although serology testing is not a gold standard test in diagnosing melioidosis, it has been used as a tool in treatment monitoring and disease surveillance among patients and at-risk community in the endemic hot-spots regions.

**Keywords:** Melioidosis; *Burkholderia pseudomallei*; seropositive; Malaysia.

## INTRODUCTION

Melioidosis is an infectious disease caused by *B. pseudomallei*, a gram-negative bacillus found primarily in soil and typically transmitted through direct contact with soil (Cheng & Currie, 2005). The disease can be transmitted from man to man, from animal to animal, and rarely from animal to man. It is endemic in tropical regions worldwide, particularly in Thailand, Malaysia, and northern Australia (Wiersinga *et al*., 2018). In Malaysia, this case was first documented back in 1913 (Stanton & Fletcher, 1925) and more cases continued to be recorded in both human and animal species from both peninsular and eastern Malaysia (Strauss *et al*., 1969; Puthucheary, 2009). There were nearly 2,000 culture-positive cases of melioidosis with an incidence rate of 4.4 cases per 100,000 yearly in north-eastern Thailand. *B. pseudomallei* widely distributed in soil especially in rice paddy fields. The existence of less virulent strain *Burkholderia thailandensis* may also contribute to the high rate of seropositive cases in Thailand (Cheng & Currie, 2005). In Singapore, about 550 cases of melioidosis were reported, one-fifth of which resulted in death in the past 10 years. The reported annual incidence rate of melioidosis was 1.1 per 100,000 population (Sim *et al*., 2018). Cases of melioidosis infection have also been documented in other parts of the world such as Central America, Taiwan, China, Africa, and Middle East countries (Dance, 2000; Raja *et al*., 2005). Paediatric melioidosis is less common than the adult disease, accounted about 5-15% of all melioidosis cases in Malaysia (Mohan *et al*., 2017). Approximately 0.6-16% of children reported having *B. pseudomallei* infection in North Australia (Raja *et al*., 2005; McLeod *et al*., 2015). While most data on paediatric melioidosis to date have originated from limited, retrospective studies, there have been notable variations in disease manifestations between different endemic melioidosis regions. Melioidosis also present in a variety of animal species however goats, sheep and pigs are most susceptible to the disease (Choy *et al*., 2000). This disease has a significant economic burden due to its effect on animal production, with a prevalence of about 13.6% in Malaysia (Musa *et al*., 2016).

Being an opportunistic pathogen, it mainly affects a person with underlying predisposing conditions. Person with risk factors like diabetes, thalassemia, and chronic renal

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disorders had high mortality rates relative to healthy people (Suputtamongkol *et al*., 1999; Fong *et al*., 2015; Zueter *et al*., 2016). More than half of cases diagnosed with melioidosis were reported to have underlying diseases, as about 53% in Thailand, 76% in Malaysia, and 88% in Australia. Diabetes mellitus was the main predisposing condition in Malaysia, Northeast Thailand, and Singapore, with up to 60% of patients with pre-existing or newly diagnosed type 2 diabetes (Puthucheary, 2009). Although the exact type of immune dysfunctions that predisposed to melioidosis is poorly understood, the disease is usually associated with immune deficits such as phagocytic defects, decreased humoral and cellular responses, and decreased development of cytokines (Cheng & Currie, 2005). It was also thought that insulin deficiency would directly contribute to the connection between melioidosis and diabetes (Woods *et al*., 1993). Other predisposing factors were renal failure, retroviral infections, malignancy, steroid treatment, obesity, occupational exposure, trauma, and parenteral misuse in Thailand and also Malaysia (Wiersinga *et al*., 2018).

Clinical presentations of melioidosis include fever, anorexia, lymphadenopathy, abscess formation, pneumonia, septicaemia, or sepsis (Puthucheary, 2009; Kingsley *et al*., 2016; Lim *et al*., 2016). Most patients who were admitted to the hospital had severe disease, and the case-fatality rate remains high despite recent significant advances in antibiotic treatment regimens. Patients recovering from acute melioidosis need an additional 10–20 weeks of oral maintenance therapy (Dance, 2014). Relapse of infection occurs in 10% of patients completing a full course of antibiotic treatment and is more common if patients do not complete the course of oral therapy. Clinical presentations of relapse are as severe as the initial infection, and the mortality associated with relapse is about 30% (Suputtamongkol *et al*., 1999).

This study undertakes to analyse seropositivity of melioidosis over five years period from 2015 to 2019 and to examine factors associated with *B. pseudomallei* seropositivity in Malaysia.

## MATERIALS AND METHODS

***Study design***

Institute of Medical Research (IMR) is the reference centre for melioidosis serology tests in Malaysia. A total of 26,665 serum samples were received from patients with suspected melioidosis from public and private hospitals all over Malaysia from the year 2015 to 2019.

***Serological tests***

Blood specimens from the patients were collected in plain sterile tubes. Sera were tested for immunoglobulin M (IgM) against *B. pseudomallei* by indirect immunofluorescent antibody technique (IFAT) in 2015-2016, and 2017 onwards using Enzyme-linked Immunosorbent Assay (ELISA).

***Indirect immunofluorescent antibody technique (IFAT)***

The assay was performed as described in the previous study (Ashdown, 1981). Briefly, the antigen was air-dried and heat-fixed onto the Teflon coated slides. Each test serum was then serially diluted two-fold in PBS at dilution of 1:10 to 1:160 and allowed to incubate with the antigen at 37°C for 30 minutes in a moist box. Following three washes with PBS (pH 7.4), fluorescein-labelled antihuman immunoglobulin (MFOI, Wellcome Research Laboratories) was added, allowed to incubate for a further 10 minutes, washed three times with PBS, then dried and mounted in buffered glycerol. Positive and negative sera controls were included for each test. A Zeiss large universal microscope with an epifluorescence condenser III RS was used to read the result from degrees of negative to 3+. Titres of ≥ 1:160 were considered seropositive.

***Enzyme-linked Immunosorbent Assay (ELISA)***

ELISA was performed in 96-well flat-bottom immune plates tested for IgM against *B. pseudomallei* as per described by Hii *et al*. (2017). The serum was considered as positive if the titre ≥ 1:320.

*Statistical Analysis*

All statistical analyses were performed using SPSS version 21.0 for Windows. Univariate logistic regression analysis was performed to determine the independent associations between gender, ethnicity, age group, regions, underlying diseases, clinical manifestations, previous exposure, and seropositivity for melioidosis. All variables with a *p*-value < 0.25 were included in multivariate logistic regression. Data with *p*-value < 0.05 is considered significant. All fourteen states in Malaysia were classified according to regions which consisted of central region (Kuala Lumpur, Putrajaya, Selangor, and Negeri Sembilan), southern (Malacca and Johor), northern (Perlis, Penang, Kedah, and Perak), eastern (Terengganu, Kelantan, and Pahang), Sabah (including Labuan) and Sarawak. Ethnic group classification consisted of Malay, Chinese, Indian, Orang Asli, Bumiputra Sarawak, Bumiputra Sabah, and others.

## RESULTS

In Figure 1, total seropositivity cases had shown an increasing trend from 2.0% (2015) to 6.4% (2017), however, in 2018 the seropositive cases have shown a downtrend pattern from 2.3% and further declined to 1.2% in 2019.

Throughout the 5-year duration, Sarawak had the most seropositive cases with 663 cases (15.1%), followed by Pahang (608 cases, 13.9%) and Kuala Lumpur (494 cases, 11.3%) (Figure 2). Sarawak also had the highest seropositive case with a high titre of ≥1:1280 about 28.8% compared to other states in Malaysia as shown in Table 1. Patients from East Malaysia (Sarawak and Sabah) had a higher likelihood of getting melioidosis infection compared to other states and regions in Malaysia (AOR 1.36, *p* = 0.001, 95% CI: 1.13, 1.64, and AOR 1.37, *p* = 0.01, 95% CI: 1.07, 1.75).

Age group was the most significant factor associated with seropositivity for melioidosis, with patients aged less than 15 years old being the most likely at risk (AOR 3.04, *p* < 0.001, 95% CI: 2.73, 3.39) compared to other age groups (Table 3). Between the two genders, female patients had a slightly higher odds of being seropositive compared to males (AOR 1.08, *p* = 0.03, 95% CI: 1.01, 1.16). The majority of seropositive cases were Malays about 15.7% of total seropositive cases but according to multivariate logistic regression analysis, race is not a significant risk for melioidosis infection as *p* > 0.05.

Patients with underlying diabetes mellitus had the highest total seropositive cases 13.2% (658 cases) compared to other underlying diseases however further analysis showed that it did not predispose to melioidosis infection (AOR 0.98, *p* = 0.72, 95% CI: 0.88, 1.10). Patients with chronic lung diseases and hepatitis were at major risk of getting an infection as both had adjusted OR more than 4. About 20.5% (8 cases) were patients with underlying retroviral diseases

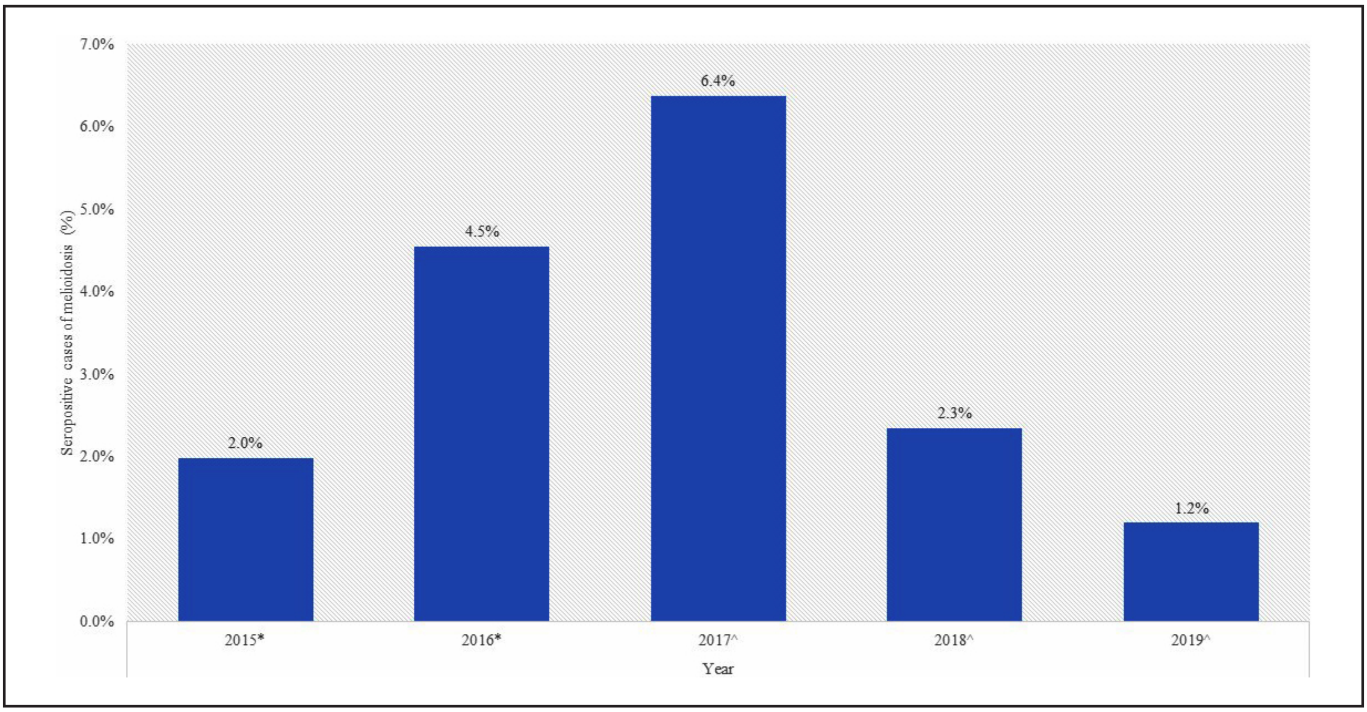


**Figure 1.** Percentage of seropositive cases of melioidosis among suspected patients in Malaysia (2015-2019).
*IFAT technique was used for detection of IgM melioidosis.
^ELISA technique was used for detection of IgM melioidosis.

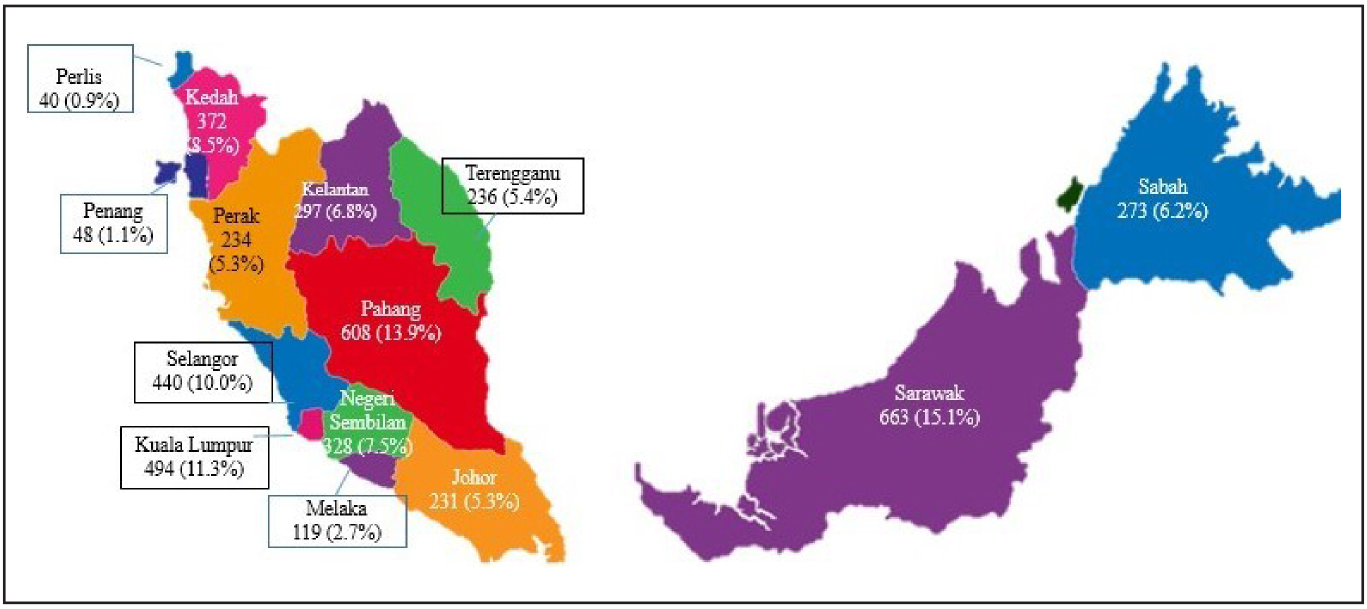


**Figure 2.** Total cases and percentages of seropositive melioidosis in Malaysia (2015-2019).

had seropositive cases while 20% (2 cases) seropositive cases were detected in cancer (Table 2). About 18.8% (1776 cases) of seropositive patients presented with fever (AOR: 1.37, $p < 0.001$, 95% CI: 1.27, 1.48) followed by patients with respiratory symptoms 14.5% (e.g., cough, shortness of breath, chest pain and sputum production). Around 16.7% (9 cases) patients throughout 5-year study were presented with aneurysm, however it was not statistically significant (crude OR: 1.02, $p = 0.96$, 95% CI: 0.50, 2.08). History of swimming in the river had a significant impact on melioidosis infection (AOR: 2.95, $p < 0.001$, 95% CI: 2.19, 3.98) compared to having direct contact with soil which was not much significant (OR: 0.78, $p = 0.89$, 95% CI 0.76, 1.37).

## DISCUSSION

Our data was analysed based on the serodiagnosis of suspected melioidosis patients. Melioidosis, being an infectious disease with common clinical presentations as other infectious diseases. Thus, a high index of suspicion is needed to establish a diagnosis in patients with risk factors in endemic regions. Delay in diagnosis may lead to late

**Table 1.** Seropositive cases based on titres according to states in Malaysia (2017-2019) (using ELISA technique)

| States | Titres | | |
|---|---|---|---|
| | 1:320 n (%) | 1:640 n (%) | ≥ 1:1280 n (%) |
| Johor | 147 (6.4) | 54 (5.3) | 7 (1.7) |
| Kedah | 170 (7.4) | 92 (9.1) | 40 (9.9) |
| Kelantan | 158 (6.9) | 65 (6.4) | 22 (5.5) |
| Kuala Lumpur | 283 (12.3) | 116 (11.4) | 37 (9.2) |
| Melaka | 70 (3.0) | 19 (1.9) | 17 (4.2) |
| Negeri Sembilan | 167 (7.3) | 70 (6.9) | 21 (5.2) |
| Pahang | 317 (13.8) | 136 (13.4) | 52 (12.9) |
| Penang | 20 (0.9) | 10 (1.0) | 9 (2.2) |
| Perak | 131 (5.7) | 44 (4.3) | 17 (4.2) |
| Perlis | 20 (0.9) | 15 (1.5) | 2 (0.5) |
| Sabah | 147 (6.4) | 83 (8.2) | 18 (4.5) |
| Sarawak | 289 (12.6) | 161 (15.9) | 116 (28.8) |
| Selangor | 248 (10.8) | 88 (8.7) | 34 (8.4) |
| Terengganu | 134 (5.8) | 62 (6.1) | 11(2.7) |

*1:160 is the only positive titre in IFAT technique. Thus, positive data from IFAT are not included.

management and increase risks of fatality. Until now, isolation of *B. pseudomallei* from patients' samples remains the gold standard for confirmation of melioidosis (Puthucheary, 2009). However, it takes up to a few days to get a positive culture result. Thus, serology tests may assist in providing a complementary laboratory diagnosis to isolation.

Melioidosis seropositive cases showed an increasing pattern from 2.0% in 2015 to 4.5% in 2016. The year 2017 have shown a fluctuate of positive cases to 6.4% as the IFAT technique was substituted to ELISA technique. A correlation study had not been carried out between the two techniques, however a previous study reported that the later has a higher sensitivity in IgM detection compared to the IFAT (Vadivelu *et al.*, 1995). In 2018, cases of seropositive melioidosis have shown a declining pattern with only 2.3% and further reduced to 1.2% in 2019. We believed that the reduction in seropositive cases were due to higher awareness on the disease among the community and the high-risk groups, which leads to better prevention of infection and complications.

**Table 2.** Univariate logistic regression of total of melioidosis seropositive cases and factors associated with seropositivity of melioidosis among suspected patients

| | | Total of seropositive cases N (%) | Odds ratio | 95% CI | *p*-value |
|---|---|---|---|---|---|
| Age group (years old) | Less than 15 | 782(28.1) | 3.04 | (2.73-3.39) | <0.001 |
| | 15-24 | 462(27.5) | 2.99 | (2.63-3.40) | <0.001 |
| | 25-34 | 675(24.0) | 2.59 | (2.32-2.89) | <0.001 |
| | 35-44 | 682(18.9) | 1.98 | (1.78-2.20) | <0.001 |
| | 45-54 | 679(13.4) | 1.32 | (1.19-1.47) | <0.001 |
| | 55 and more | 1103(10.3) | *reference* | | |
| Gender | Male | 2674(16.2) | *reference* | | |
| | Female | 1709(16.8) | 1.05 | (0.98-1.12) | 0.16 |
| Region | Central | 1262(14.5) | *reference* | | |
| | Southern | 350(13.3) | 0.91 | (0.80-1.03) | 0.13 |
| | Northern | 694(16.5) | 1.17 | (1.06-1.30) | 0.002 |
| | Eastern | 1141(16.5) | 1.17 | (1.07-1.28) | <0.001 |
| | Sabah | 273(21.4) | 1.61 | (1.39-1.86) | <0.001 |
| | Sarawak | 663(22.8) | 1.75 | (1.57-1.94) | <0.001 |
| Race | Malay | 2718(15.7) | 1.27 | (1.12-1.43) | <0.001 |
| | Chinese | 333(12.8) | *reference* | | |
| | Indian | 305(15.3) | 1.23 | (1.04-1.45) | 0.02 |
| | Orang Asli | 96(20.7) | 1.78 | (1.38-2.29) | <0.001 |
| | Bumiputra Sarawak | 524(23.1) | 2.04 | (1.76-2.38) | <0.001 |
| | Bumiputra Sabah | 215(21.8) | 1.90 | (1.57-2.30) | <0.001 |
| | Others | 192(18.3) | 1.52 | (1.25-1.85) | <0.001 |
| Underlying Diseases | Diabetes mellitus | 658(13.2) | 0.73 | (0.67-0.80) | <0.001 |
| | Hypertension | 371(11.1) | 0.60 | (0.54-0.67) | <0.001 |
| | Chronic Kidney Disease | 76(10.0) | 0.56 | (0.44-0.71) | <0.001 |
| | Thalassemia | 21(20.8) | 1.34 | (0.83-2.16) | 0.24 |
| | Chronic lung disease | 52(41.3) | 3.60 | (2.52-5.15) | <0.001 |
| | Hepatitis | 26(46.4) | 4.43 | (2.62-7.49) | <0.001 |
| | Retroviral disease | 8(20.5) | 1.31 | (0.60-2.86) | 0.49 |
| | Malignancy | 2(20.0) | 1.27 | (0.27-5.99) | 0.76 |
| Clinical manifestations | Fever | 1776(18.8) | 1.30 | (1.22-1.39) | <0.001 |
| | Abscess | 299(12.6) | 0.71 | (0.63-0.81) | <0.001 |
| | Respiratory symptoms | 735(14.5) | 0.83 | (0.76-0.91) | <0.001 |
| | GIT symptoms | 677(17.6) | 1.10 | (1.01-1.21) | 0.03 |
| | CNS symptoms | 35(24.3) | 1.64 | (1.12-2.40) | 0.01 |
| | Lymphadenopathy | 122(27.6) | 1.97 | (1.59-2.43) | <0.001 |
| | Ocular symptoms | 161(20.2) | 1.30 | (1.09-1.55) | 0.04 |
| | Aneurysm | 9(16.7) | 1.02 | (0.50-2.08) | 0.96 |
| | Jaundice | 58(21.0) | 1.36 | (1.01-1.82) | 0.04 |
| | Sepsis | 107(12.6) | 0.72 | (0.59-0.89) | 0.002 |
| Previous exposure | History of swimming in the river | 91(46.0) | 4.39 | (3.32-5.82) | <0.001 |
| | History of soil contact | 53 (16.7) | 1.02 | (0.76-1.37) | 0.89 |

GIT, gastrointestinal. CNS, central nervous system.

**Table 3.** Multiple variable logistic regression of total of melioidosis seropositive cases and factors associated with seropositivity of melioidosis among suspected patients

| | | Adjusted Odd Ratio± | 95% CI | *p*-value |
|---|---|---|---|---|
| Age group (years old) | Less than 15 | 3.04 | (2.73-3.39) | <0.001 |
| | 15-24 | 2.99 | (2.63-3.40) | <0.001 |
| | 25-34 | 2.59 | (2.32-2.89) | <0.001 |
| | 35-44 | 1.98 | (1.78-2.20) | <0.001 |
| | 45-54 | 1.32 | (1.19-1.47) | <0.001 |
| Gender | Female | 1.08 | (1.01-1.16) | 0.03 |
| Region | Northern | 1.18 | (1.07-1.31) | 0.002 |
| | Sabah | 1.37 | (1.07-1.75) | 0.01 |
| | Sarawak | 1.36 | (1.13-1.64) | 0.001 |
| Underlying diseases | Hypertension | 0.72 | (0.63-0.84) | <0.001 |
| | Chronic lung disease | 4.03 | (2.77-5.86) | <0.001 |
| | Hepatitis | 4.40 | (2.55-7.62) | <0.001 |
| Clinical manifestations | Fever | 1.37 | (1.27-1.48) | <0.001 |
| | Abscess | 0.74 | (0.65-0.85) | <0.001 |
| | Respiratory symptoms | 0.76 | (0.69-0.83) | <0.001 |
| | Lymphadenopathy | 1.49 | (1.19-1.86) | <0.001 |
| Previous exposure | History of swimming in the river | 2.95 | (2.19-3.98) | <0.001 |

±Hosmer-Lemeshow goodness-of-fit test (p= 0.04).
GIT, gastrointestinal.
CNS, central nervous system.

Our report showed that female patients had higher risk for melioidosis infection compared to the opposite sex. However, there was not much difference between the two genders as the adjusted odds ratio was around 1.08. This finding was similar to other study (Hii *et al*., 2016) which also found that there was a higher exposure among female than male, (Pagalavan, 2005; Suputtamongkol *et al*., 1999) and predominantly present in patients aged 40 to 60 years old (Suputtamongkol *et al*., 1994). According to our study, younger patients from the age group of less than 15 years old were most susceptible to melioidosis as reported in a previous study (Hii *et al*., 2016). The reason could be due to lack of protective antibody towards circulating *B. pseudomallei* and the immature immune system in children and therefore they could be easily infected by *B. pseudomallei* (Puthucheary, 2009).

In this study, we also noted that Sarawak state bore the highest melioidosis seropositive cases in Malaysia. In addition, Sarawak hold the most cases with the highest titre (i.e., ≥ 1:1280) compared to other states. Based on multivariate logistic regression analysis, the Sarawakians are at higher risk of getting an infection (AOR: 1.36, $p$ = 0.001, 95% CI: 1.13, 1.64). These findings correlated with previous report by (Mohan *et al*., 2017). The reason for Sarawak being one of the highly endemic states for melioidosis remain unknown. The most likely explanation to this is possibly be due to difference in environment and human activities compared to other states. The majority of people living in Sarawak are involved with the environmental related occupation i.e., farming, forestry and fishing (Mohan *et al*., 2017).

As for the analysis of the underlying diseases associated with seropositive cases, surprisingly the multivariate logistic regression analysis on risk factors of seropositive cases showed that there was no association between diabetes mellitus with melioidosis infection (AOR: 0.98, $p$ = 0.72, 95% CI: 0.88, 1.10) although most of previous studies have shown that there was a significantly important relation between diabetes mellitus and (Cheng & Currie, 2005; Wiersinga *et al*., 2018). Based on Health and Morbidity report released in 2015 (National Health and Morbidity Survey 2015, 2016), the prevalence of people with undiagnosed diabetes mellitus in Malaysia was 9.2% of the total population. There was a high possibility that those positive cases remain undiagnosed of diabetes mellitus or there was incomplete medical history being recorded in the laboratory form. Thus, a complete and thorough medical history should be investigated and recorded in the future to avoid missing risk factors and enable accurate risk stratification analysis to be carried out.

On the other hand, our study showed that patients with underlying chronic lung diseases such as chronic obstructive airway disease and bronchial asthma had a major risk of getting melioidosis infection similarly as reported previously (Wiersinga *et al*., 2018). Common clinical manifestations in melioidosis were patients with respiratory symptoms such as pneumonia was the most frequent presentation (Cheng *et al*., 2013; Kingsley *et al*., 2016; Nathan *et al*., 2018). However, our study showed that seropositive patients commonly presented with fever similar to a previous report (Puthucheary, 2009). Patients with a history of fever alone without an obvious primary site of infection should be investigated further including septic screening and radiological imaging.

In this study, interestingly we found that history of swimming in the river has shown to have a higher risk to contract melioidosis infection compared to history of exposure to the soil. This is slightly contradicted to previous findings that showed that history of contact with soil is one of the most important risk factors for melioidosis infection (Lim *et al*., 2016). Here, we speculated that those with a positive history of swimming in the river were also exposed to soil as they usually barefooted during the activities.

The advantage of this study is that, it may provide a glimpse of light on the seropositivity rate and its associated factors among suspected melioidosis patients in Malaysia.

This information may provide basic guidance or suggestions on the potential intervention that can be taken by the disease control department to plan and highlight prevention and control strategies in certain areas and groups of populations, by enhancing the knowledges, attitude and practice. On the other hand, the drawback of this study was the findings were made based on two different serology techniques without any correlation study performed. Furthermore, this study was totally based on IgM sero-positivity as there was no positive culture data available for association study.

Melioidosis is a tropical infectious disease with highly diverse disease manifestations which is also known as the great mimicker. This often complicates the diagnosis and delays proper treatment. Although it is known that isolation of *B. pseudomallei* is the gold standard diagnosis for melioidosis, serology diagnosis may complement culture test and assists clinician in treatment monitoring. Furthermore, serodiagnosis of melioidosis remain an important tool for disease surveillance and prevention. Nevertheless, melioidosis serology results should always be interpreted with caution in the endemic regions since majority of the population in the regions have adaptive antibodies developed towards melioidosis. Continuous health promotion and awareness programs especially among the high-risk groups should be strengthen and continued in order to ensure better disease control and prevention for melioidosis.

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**Conflicts of interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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