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ORIGINAL ARTICLE

Associated Factors of *Plasmodium Knowlesi* Malaria among Registered Malaria Cases in Terengganu, Malaysia 2011- September 2019 via Vekpro Online Database

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ABSTRACT

Introduction: *Plasmodium knowlesi* (*P. knowlesi*), zoonotic malaria cases had been increasing in trend in Terengganu, Malaysia. This study aimed to determine the associated factors of *P. knowlesi* malaria among registered malaria cases in Terengganu from 2011 to September 2019 via the Vekpro Online Database. **Methods:** A cross-sectional study was conducted using registered malaria cases in Terengganu from 2011 to September 2019 extracted from the Vekpro Online database and secondary weather data from the Malaysian Meteorological Department. A total sample of 247 malaria cases [(*P. knowlesi* (n=187), *P. falciparum* (n=23), *P. vivax* (n=37), *P. malariae* (n=4), mixed infection (n=3)] were analysed. The cases were grouped into *P. knowlesi* group (*P. knowlesi, P. malariae*, mixed infection) and non-*P. knowlesi* group (*P. falciparum*, *P. vivax*) for comparison of their risk factors. Multiple Logistic Regression analysis was used to identify the associated factors. **Results:** *Plasmodium knowlesi* group contributed to 187 out of 247 (75.7%) of all malaria cases. Four factors were found to be significantly associated with *P. knowlesi* group in the final multivariable logistic regression analysis. Age [AOR=1.04 (95% CI: 1.01,1.08)], Malay race [AOR=39.43 (95% CI: 13.99,111.18)], agriculture and forestry worker [AOR=14.95 (95% CI: 4.10,54.51)] and Passive Case Detection [AOR=11.70 (95% CI: 2.60,52.63)] were the significant associated factors. **Conclusions:** Identified significant associated factors and characteristics of the high-risk group for *P. knowlesi* infection can help medical and health front liners in Terengganu in early diagnosis and prompt treatment of the cases. The factors also will help in the planning and development of health interventions in targeting *P. knowlesi* infection.

KEYWORDS: ***Plasmodium knowlesi*, zoonosis, malaria, Terengganu**

INTRODUCTION

The emerging zoonotic malaria species, *P. knowlesi* has become a new additional challenge to malaria control in Malaysia. *Plasmodium knowlesi* is usually transmitted between non-human primate hosts by *Anopheles* mosquitoes. However, in a background where the parasite, vector, primate host, and humans converge, it can cause spillover infection to humans in which animal to human disease transmission occurs [1]. Pig-tailed macaques (*Macaca nemestrina*), long-tailed macaques (*Macaca fascicularis*), and banded leaf monkey (*Presbytis melalophus*) are the natural hosts of P. knowlesi [2-4].

As human malaria cases in Malaysia have been declining in recent years, zoonotic malaria cases, *P. knowlesi* has shown an increase in trend. According to the Ministry of Health data, the incidence of *P. knowlesi* was 854 in 2011 and 4131 in 2018. The number of malaria deaths in Malaysia has also shifted from contributed mainly by human malaria to zoonotic malaria, *P. knowlesi*. In 2010, there were 33 malaria deaths in which 29 (87.9%) were due to human malaria, and 4 (12.1%) were due to *P. knowlesi*. On the contrary, in 2018, the number of malaria deaths were 12 cases in which were all contributed by *P. knowlesi*.

To date, *P. knowlesi* infections in humans occur only as a result of animal-to-human transmission. There is no proof that natural human-to-human transmission has occurred, although it has been confirmed in a laboratory setting [5]. However, the possibility that *P. knowlesi* being transmitted naturally from human to human does exist due to parasite adaptation and change with time [6, 7]. Hence it is very crucial to recognize associated factors for *P. knowlesi* infections among humans.

Grigg, Cox, et al. (2017) found that those aged 15 years old or older, male, involved in plantation work, history of sleeping outside or traveling were independently associated with increased risk of symptomatic *P. knowlesi* infection. The study also compared *P. knowlesi* infection with other *Plasmodium spp* infections. They found that being 15 years old or older, clearing vegetation, farming occupation, and having long grass around the house increased the risk for *P. knowlesi* infection but not for other *Plasmodium* spp. infection [8]. Another study in Sabah, Malaysia, found a significantly higher median age and a higher proportion of males *in P. knowlesi* cases when compared to *P. vivax* or *P. falciparum* cases [9]. However, a study using microscopically positive malaria blood samples confirmed by nested PCR from all Malaysian states found otherwise. There was no significant association between age and gender with *P. knowlesi* cases when compared to other *Plasmodium* spp. infections [10].

Environmental factors such as monthly rainfall and seasonality were positively correlated with notifications of *P. knowlesi,* whereby, the correlation between the notifications of *P. knowlesi* occurs two to four months after rainfall and from May to August [11]. The average monthly rainfall and humidity are significantly associated with the incidence rate ratio of *P. knowlesi* with a lag time of three months in univariate analysis [12].

Terengganu is a state located on the east side of Peninsular Malaysia which consists of eight districts. In Terengganu, there has been an increase in *P. knowlesi* cases in recent years. In 2015, there were only six cases, and the number of cases kept on increasing to 38 cases in 2018. The increase in the number of cases should alert and prompt the State Health Department as well as the Ministry of Health to enhance their *P. knowlesi* prevention activities in Terengganu based on their specific background. Although several studies on the risk factors for *P. knowlesi* had been done previously, almost all studies related to risk factors were concentrated in Borneo, East Malaysia. The primary vectors in Borneo are from a different complex of the Leucosphyrus group of *Anopheles (An.)* mosquitoes from peninsular Malaysia. *Anopheles cracens*, from the Dirus Complex, is the primary vector of *P. knowlesi* in Peninsular Malaysia whereas *An. balabacensis* and *An. latens* from the Leucosphyrus Complex are the primary vectors of *P. knowlesi* in Sabah and Sarawak, respectively.

Moreover, a study showed that there is likely a different genetic variant of *P. knowlesi* for every location reporting *P. knowlesi* human infections [13]. By knowing these, we can postulate that there might be some variation in the associated factors of *P. knowlesi* infections in Terengganu compared to Malaysia Borneo.

However, to date, there is no published data on the associated factors of *P. knowlesi* infections in Terengganu or neighboring states. Therefore, the specific aims of the study were to determine the factors associated with *P. knowlesi* by comparing registered *P. knowlesi* cases with non-*P. knowlesi* cases in Terengganu via Vekpro Online database and its relationship with rainfall density, relative humidity, and temperature gathered from Malaysian Meteorological Department.

MATERIALS AND METHODS

This study was a cross-sectional study conducted using two sets of secondary surveillance data. The first data set was, data on registered malaria cases in Terengganu from 2011 to September 2019 retrieved from Vekpro Online Database and the second data set was weather data retrieved from the Malaysian Meteorological Department.

Four domains of independent variable namely sociodemographic, occupational, disease profile, and environmental were compared between the *P. knowlesi* group and non-*P. knowlesi* group. Most of the data available in this database were based on microscopy reports. Hence, due to the near similarity of *P. knowlesi* and *P. malariae* under microscopy [14], the two species

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and mixed infections that contain *P. knowlesi* as one of the parasites were grouped as *P. knowlesi* group. Other species were categorized as non-*P. knowlesi*. The sample size was calculated using OpenEpi Version 3.01 sample size calculator. The proportion method and anticipated frequency of 98% [12] were used. With an attrition rate of 20%, a total of 32 samples should be sufficient from the calculation. However, all registered malaria cases from 2011 to September 2019 were used by using universal sampling. The exclusion criteria for the study were any duplicated cases or cases with the absence of more than 50% of the study variables.

Vekpro Online is an online surveillance database designed for the surveillance of vector-borne diseases in Malaysia such as Malaria, Filariasis, Chikungunya, Typhus, and Zika. Details of cases that fulfilled the criteria for registration of these diseases are entered into this database by the investigating officers. This database is used after mandatory notifications of suspected cases are made through another online data called CDCIS e-Notifikasi. This study used the original variables and a few new composite variables which were created based on the data available in the database. The original variables in the Vekpro Online database that were utilized in this study were age, gender, nationality, race, place of resident, district, case detection method, severity, and parasite density count. For the method of detection variable, the recorded options in the data were Active Case Detection (ACD), Passive Case Detection (PCD), and Mass Blood Survey (MBS), however, MBS was categorized into ACD based on WHO guideline [15]. For severity variables, the options were uncomplicated, asymptomatic, and severe. Here uncomplicated and asymptomatic were categorized into non-severe. Employment status variable was a new composite variable created by using the recorded type of occupation for every malaria case where those recorded as not working, housewife, and student were categorized as non-employed whereas the remained were categorized as employed. Agriculture and forestry worker were also a new composite variable categorized by using reference from the Malaysian Standard Classification of Occupation (MASCO).

The data set obtained from the Malaysian Meteorological Department were data on monthly mean temperature, monthly mean relative humidity, and monthly rainfall density for each meteorological station in Terengganu from 2011 to 2019. There was a total of 9 meteorological stations in Terengganu, three of them were called principles station where continuous maintenance of measuring machines was done, and the other six were auxiliary stations where maintenance was done periodically. For this study, data from three principal stations namely Gong Kedak, Kuala Terengganu, and Kerteh were extrapolated to calculate the average monthly temperature, average monthly relative humidity, and average monthly rainfall density for Terengganu state from 2011 to 2019. The monthly average for each variable was then matched to each case by notification but with a lag time of three months as used in prior research [12]. The seasonality variable was created by pairing the month of notification for malaria cases with the monsoon season in Malaysia namely Southwest Monsoon (May to September), Northwest Monsoon (November to March), and Inter-monsoon (April and October).

Both data were received in Microsoft Excel format. Data were compiled together, cleaned, and screened through for any missing data. For any missing data from the Vekpro Online database, the data were requested from the respective District Health Office in Terengganu. If the data were still not available, there were dealt with pairwise deletion method during the analysis part. Missing data for data from the Malaysian Meteorological Department were also dealt with pairwise deletion in the analysis part.

After missing data were dealt with, they were exported and analysed with IBM SPSS version 23. Data were coded or recoded, and new composite variables were created before the data analysis. Descriptive statistics were used to describe four main independent groups of variables according to group *P. knowlesi* or *non-P. knowlesi*. The normality of distributions was tested using Shapiro-Wilk test. They were described using frequencies and percentages for categorical variables and median and Interquartile range (IQR) for numerical variables. Simple Logistic Regression was used for univariate analysis. Factors with significant findings in univariate analysis were recruited into multivariable analysis where Multiple Logistic Regression was used. Backward and Forward LR methods were tested, and the method that yields the

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most significant factors in the final multivariable regression analysis was chosen. For all test, a p-value of less than 0.05 was considered significant with alpha of 0.05. No correlation analysis was done in this study. The significant factors in the final model were checked for Multicollinearity, Interaction and 'Linearity in Logit' assumptions. The model predictability was assessed using Hosmer and Lemeshow test, Cox and Snell test, Nagelkerke R square test, Receiver Operator Characteristics (ROC) Curve, and Cook's Influence Test.

RESULTS

i) Sample and Missing Data

Out of 248 registered cases, we analyze 247 and removed one duplicated case. There were seven variables with missing data initially in which the variable with the highest percentage was parasite density count with 15.7% of missing data. After the missing data were sought from the respective District Health Office, only five variables were left with missing data, and the variable with the highest missing data was still parasite density count with 13.0% (Figure 1).

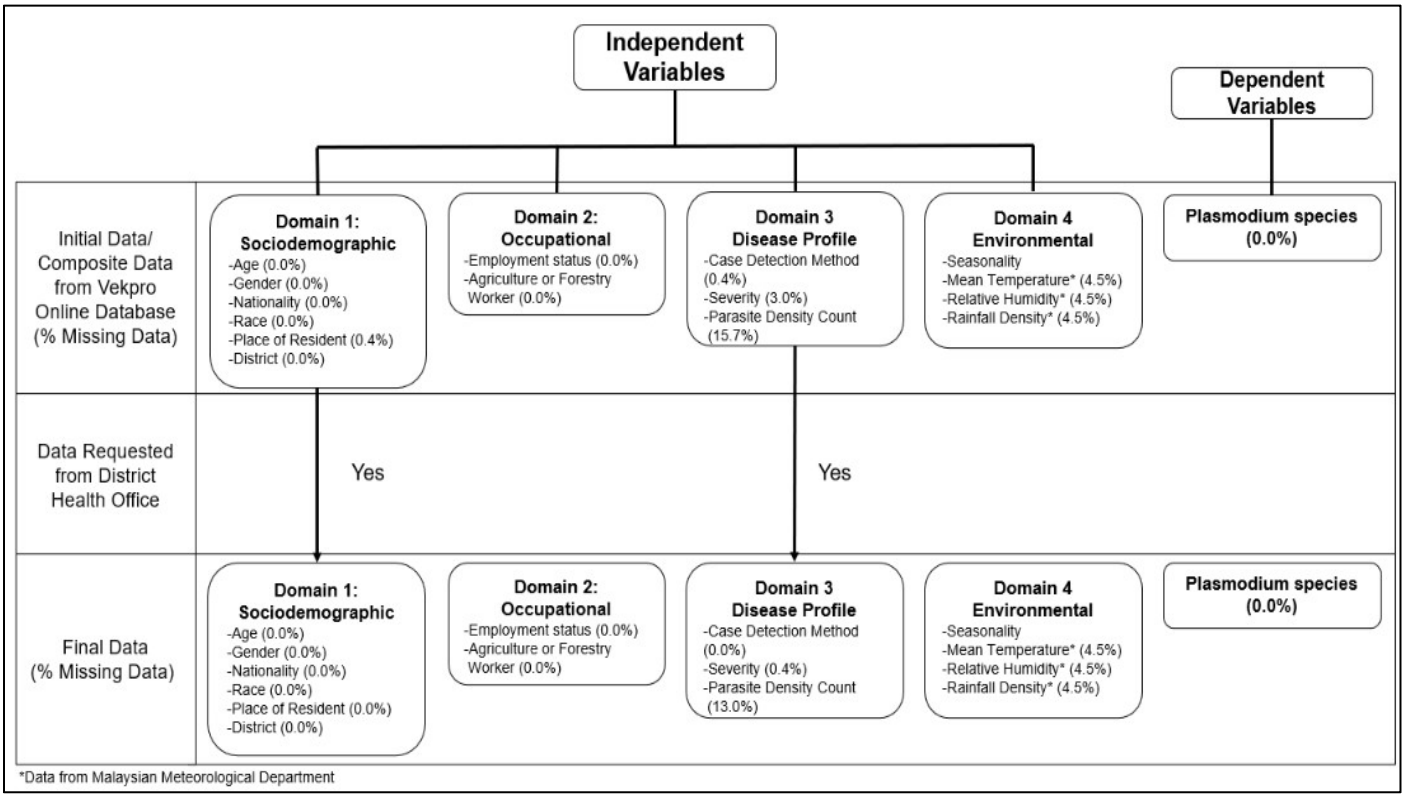

Figure 1 Percentage of missing data before and after data were requested from the District Health Office

ii) Prevalence of Malaria Cases

Most of the registered malaria cases in the Vekpro Online database were *P. knowlesi* (72.9%) followed by *P. vivax* (15.0%) and *P. falciparum* (9.3%). *P. malariae,* which was combined with *P. knowlesi* as an outcome group, only contributed to 1.6% of the cases. After combined, the *P. knowlesi* group made up 75.7% of malarial cases in Terengganu from 2011 to September 2019 (Table 1).

iii) Distribution of Malaria Cases

The median age, 35.0 years old (IQR 28.0-45.0) for *P. knowlesi* group was older, compared to 26.5 years old (IQR 21.0-32.0) for non-*P. knowlesi* group (p<0.001). Most *P. knowlesi* group cases (42.8%) originated from Hulu Terengganu district whereas most non-*P. knowlesi* (38.3%) were from Kemaman district. The majority of the *P. knowlesi* infections were more among Malaysian, Malay, agriculture, and forestry worker and those detected through PCD. Both groups had an almost

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similar distribution in terms of proportions for gender, residential area, employment status, severity, and they were both occurred predominantly during the Southeast Monsoon season. The median parasite density count was higher in *P. knowlesi* group as compared to non- *P. knowlesi* with 4347 /uL blood (IQR 1500-14372) and 4040 /uL blood (IQR 360-10205) respectively. However, the differences were not significant (p=0.561). For environmental variables, *P. knowlesi* generally had a higher median for average monthly relative humidity and average monthly rainfall density but a lower median for the average monthly temperature than non-*P. knowlesi* (Table 2).

Table 1 Types of Malaria and Regrouping into Dependent Variables

Types of Malaria	**Total (n=247)** Frequency,n(%)	**Dependent Variable** Frequency, n (%)
P. knowlesi	180 (72.9)	*P. knowlesi* 187 (75.7)
Mixed *(P. knowlesi + P. vivax)*	3 (1.2)	
P. malariae	4 (1.6)	
P. falciparum	23 (9.3)	Non-*P. knowlesi* 60 (24.3)
P. vivax	37 (15.0)	

*Percentage in columns

Table 2 Distribution sociodemographic profile between types of malaria

Variables	**N**	**TYPES OF MALARIA** *P. knowlesi* Frequency, n (%)	**Non-*P.knowlesi*** Frequency, n (%)	**Total** Frequency,n(%)
1. Sociodemographic				
Age (years)	247	35.0 (28.0-45.0)*	26.5 (21.0-32.0)*	32.0 (26.0-42.0)*
Gender	247			
Male		149 (79.7)	57 (95.0)	206 (83.4)
Female		38 (20.3)	3 (5.0)	41 (16.6)
District	247			
Hulu Terengganu		80 (42.8)	17 (28.3)	97 (39.3)
Kemaman		39 (20.9)	23 (38.3)	62 (25.1)
Dungun		29 (15.5)	7 (11.7)	36 (14.6)
Setiu		22 (11.8)	0 (0.0)	22 (8.9)
Besut		10 (5.3)	3 (5.0)	13 (5.3)
Kuala Terengganu		3 (1.6)	5 (8.3)	8 (3.2)
Marang		3 (1.6)	4 (6.7)	7 (2.8)
Kuala Nerus		1 (0.5)	1 (1.7)	2 (0.8)
Nationality	247			
Malaysian		167 (89.3)	16 (26.7)	183 (74.1)
Non-malaysian		20 (10.7)	44 (73.3)	64 (25.9)

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Race	247			
Malay		156 (83.4)	7 (11.7)	163 (66.0)
Non-Malay		31 (16.6)	53 (88.3)	84 (34.0)
Residential Area	247			
Rural		170 (90.9)	49 (81.7)	219 (88.7)
Urban		17 (9.1)	11 (18.3)	28 (11.3)
2. Occupational				
Employment Status	247			
Yes		148 (79.1)	43 (71.7)	191 (77.3)
No		39 (20.9)	17 (28.3)	56 (22.7)
Agricultural and forestry and worker	247			
Yes		80 (42.8)	11 (18.3)	91 (36.8)
No		107 (57.2)	49 (81.7)	156 (63.2)
3. Disease Profile				
Case Detection Methods	247			
ACD		5 (2.7)	22 (36.7)	27 (10.9)
PCD		182 (97.3)	38 (63.3)	220 (89.1)
Severity	246			
Severe		24 (12.8)	4 (6.8)	28 (11.3)
Non-Severe		163 (87.2)	55 (91.7)	218 (88.3)
Parasite Density Count (/uL blood)	215	4347 (1500-14372)*	4040 (360-10205)*	4320 (1038-14000)*
4. Environmental				
Seasonality	247			
Southwest Monsoon		81 (43.3)	43 (71.7)	124 (50.2)
Northeast Monsoon		74 (39.6)	10 (16.7)	84 (34.0)
Inter-monsoon		32 (17.1)	7 (11.7)	39 (15.8)
Average Monthly Temperature (°C)**	236	27.3 (26.6-27.9)*	27.8 (26.5-28.1)*	27.4 (26.6-28.1)*
Average Monthly Relative Humidity (%)**	236	83.7 (81.8-85.1)*	82.1 (81.3-83.7)*	83.5 (81.4-84.7)*
Average Monthly Rainfall Density(mm)**	236	196.6 (129.5-285.9)*	133.0 (67.5-269.2)*	183.5 (123.6-280.1)*

*Median (IQR)
**Weather Data with 3 months lag from notification date

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iv) Associated Factors for *P. knowlesi*

In univariate analysis, seven variables were found to be significant (Table 3). The variables were age (p<0.001), gender (p=0.011), nationality (p<0.001), race (p<0.001), agriculture and forestry worker (p<0.001), method of detection (p<0.001) and average monthly relative humidity (p=0.037). However, when these variables were recruited to the final Multivariable Regression Analysis, only age (p=0.028), race (p<0.001), agriculture and forestry worker (p<0.001), and method of detection (p=0.001) were found to be significantly associated with *P. knowlesi* (Table 4).

Table 3 Univariate Analysis for Possible Associated Factors of *P. Knowlesi* malaria in the study (Simple Logistic Regression)

Variable	B (SE)	Wald(df)	Crude OR (95% CI)	p-value
Age (Years)	0.06 (0.02)	18.46 (1)	1.07 (1.04,1.10)	<0.001*
Gender				
Female			1	Ref.
Male	-1.58 (0.62)	6.49 (1)	0.21 (0.06,0.70)	0.011*
Nationality				
Non-Malaysian			1	Ref.
Malaysian	3.13 (0.38)	69.55 (1)	22.96 (10.99,47.96)	<0.001*
Race				
Non-Malay			1	Ref.
Malay	3.64 (0.45)	66.13 (1)	38.10 (15.85,91.62)	<0.001*
Residential Area				
Urban			1	Ref.
Rural	0.81 (0.42)	3.72 (1)	2.25 (0.99,5.11)	0.054
Employment				
No			1	Ref.
Yes	0.41 (0.34)	1.44 (1)	1.50 (0.77,2.91)	0.231
Agricultural and forestry worker				
No			1	Ref.
Yes	1.20 (0.37)	10.87 (1)	3.33 (1.63,6.81)	<0.001*
Severity				
Severe			1	Ref.
Non-Severe	-0.71 (0.56)	1.57 (1)	0.49 (0.16,1.49)	0.210
Method of Detection				
ACD			1	Ref.
PCD	3.05 (0.53)	33.51 (1)	21.07 (7.51,59.15)	<0.001*
Seasonality				
Intermonsoon		14.11 (2)	1	Ref
Southwest Monsoon	-0.89 (0.46)	3.75 (1)	0.41 (0.17,1.01)	0.053
Northeast Monsoon	0.48 (0.54)	0.81 (1)	1.62 (0.57,4.63)	0.369
Density of Parasite	<0.01 (<0.01)	0.34 (1)	1 .00 (1.00,1.00)	0.561
Average Monthly Temperature**	0.34 (0.19)	3.15 (1)	0.71 (0.49,1.04)	0.076
Average Monthly Relative Humidity**	0.13 (0.06)	4.36 (1)	1.13 (1.01,1.28)	0.037*
Average Monthly Rainfall Density**	<0.01 (<0.01)	2.73 (1)	1.00 (1.00,1.00)	0.98

*p-value <0.05 is indicated as significant
**Data with 3 months lag from notification date

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Table 4 Associated Factors of P. Knowlesi malaria among All Registered Malaria Cases in Terengganu, Malaysia

Variable	B (SE)	Wald(df)	Adjusted OR (95% CI)	p-value*
Age (Years)**	0.04 (0.02)	4.85 (1)	1.04 (1.01,1.08)	0.028
Race				
Non-Malay			1	Ref.
Malay	3.68 (0.53)	48.29 (1)	39.43 (13.99,111.18)	<0.001
Agricultural and forestry worker				
No			1	Ref.
Yes	2.71 (0.66)	16.80 (1)	14.95 (4.10,54.51)	<0.001
Method of Detection***				
ACD			1	Ref.
PCD	2.46 (0.77)	10.27 (1)	11.70 (2.60,52.63)	0.001

*Test used: Multivariable Logistic Regression Analysis (Method Backward LR; R2:0.670; B Constant: -4.824; Model Assumptions are met; No Interaction between Independent Variables, No Multicollinearity)

** Linearity in Logit Assumptions for Age (Continuous Independent Variable) is met.

***ACD: Active Case Detection, PCD: Passive Case Detection

DISCUSSION

The proportion of *P. knowlesi* (75.7%) cases from 2011 to September 2019 in this study was slightly lower compared to the recent study in Sabah, which was 80%,88%, and 98% out of all malaria cases for 2015, 2016, and 2017 respectively. However, the secondary analysis revealed that the proportion of *P. knowlesi* cases in Terengganu in the second half of the study (2015 to September 2019) was 85.7%, which was higher than the overall proportion. Better diagnostic capacity in detecting *P. knowlesi* increased awareness to *P. knowlesi*, loss of relative immunity due to decreasing human malaria cases, and the proximity of infected vectors or natural reservoir hosts with humans due to changes in human land use could contribute to the increase in the cases [5].

The higher median age of *P. knowlesi* group compared to the other species and the male domination in both *P. knowlesi* and non-*P. knowlesi* groups found in this study were consistent with other studies [8, 9]. These findings may relate to greater forest exposure among these groups, with farmers and plantation workers over-represented. Male was also more susceptible to any malaria infections due to their traditional role of being the main breadwinners [16].

The three districts found to have the highest proportions of the *P. knowlesi* group cases in this study were Hulu Terengganu (42.8%), Kemaman (20.9%), and Dungun (15.5%). Interestingly, data revealed that these three districts also had the highest area of land used for forest reserve and agriculture in Terengganu, Malaysia [17]. Hence, this supports the finding that *P. knowlesi* exposure risk increases with agricultural expansion, the increase in land area used for agriculture, and forest fragmentation [18].

Increasing age was one of the associated factors for *P. knowlesi*, and this finding is supported by a study in Sabah where the study found that increasing age (per 10 years) increased the odds of getting *P. knowlesi* [18]. It could also be supported by the evidence showing most of the farmers in Malaysia were more than 50 years old [19, 20]. Age was also independently associated with severe knowlesi malaria [21]. Thus, recognizing the older age is essential for suspicion of diagnosis and determining the treatment regime for *P. knowlesi.*

Our study showed Malay race was significantly associated with *P. knowlesi.* A meeting report from WHO in 2017 revealed that zoonotic malaria in Malaysia, for 2016, mainly affects the Bumiputera Sarawak (43%) and Bumiputera Sabah (33%) in Malaysia [1]. Studies showed that those from the main ethnicities in Sabah and Sarawak were more likely to be affected by *P. knowlesi*. A study in Sabah revealed that most *P. knowlesi* cases were from Rungus, Dusun, and Murut ethnics [22]. Another study in Kapit, Sarawak revealed that Iban ethnicity had the highest number of *P. knowlesi* cases [23]. Data from the Terengganu Economic Planning Unit, on the other hand, stated that Malays constitute 94% of the population in 2016 [24].

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All these findings indicate that *P. knowlesi* tends to affect the local, which is very likely given the zoonotic nature of transmission of *P. knowlesi* where those who were more regular to the local environmental settings pose more risk of encountering vectors and natural host of *P. knowlesi.*

Agriculture and forestry worker were found to be highly significant in this study, which was supported by previous studies [8, 18, 25]. Given that *Anopheles cracens*, the primary vector for *P. knowlesi* in peninsular Malaysia is found in hilly and mountainous areas containing primary or secondary evergreen and deciduous forests, bamboo, and fruit and rubber plantations [26], this kind of workers will require extra attention for *P. knowlesi* prevention activities. This finding signifies the need for multisectoral collaboration in combating *P. knowlesi*. Stakeholders such as the Forestry Department, Agriculture Department, and companies must be led by the Health Department to empower *P. knowlesi* prevention strategies among their workers.

Passive Case Detection also had shown the association with *P. knowlesi*. The short incubation period of *P. knowlesi* (~12days) compared to other malaria species [27] and no natural human-to-human transmission explains the reason for most *P. knowlesi* cases detected through PCD compared to ACD. The short incubation periods will mean that infected individuals will most likely seek treatment for their symptoms first before being detected by screening activity in the field. Moreover, since there is no natural human-to-human transmission in *P. knowlesi,* the possibility of the case close contacts to be also screened positive through ACD activity after the notification received is very low, unlike other *Plasmodium* spp. with human-to-human transmission. However, this finding also indicates that there is room for improvement in current prevention activities. Community empowerment, like the appointment of public health intelligence among high-risk groups that serve to immediately notify the respective health office when any of their members presented with symptoms, will be very helpful. ACD team will then be mobilized to perform Blood Film Malaria Parasite screening. Such a program in Malaysia like 'Malaria Ambassador' should be intensified so that *P. knowlesi* cases can be diagnosed and treated earlier.

Factors like gender and residential area were found not to be associated with *P. knowlesi* probably due to the methodology of this study which compares *P. knowlesi* group with non-*P. knowlesi group*. Other studies that compare *P. knowlesi* or all *Plasmodium spp* with the malaria-free group had found significant associations with male gender and rural areas [8, 18, 28]. Severity was also not found to be significant, where another study found the association between *P. knowlesi* and severity [29]. However, after examining the obtained data, some mistakes can be detected in the severity classification. Management Guidelines of Malaria in Malaysia 2013 has clearly defined severe cases for every *Plasmodium spp* [30]. The presence of hyperparasitemia (>20,000/µL blood for *P. knowlesi* or > 100,000/ µL blood for other *Plasmodium spp*.) is one of the laboratory findings that should classify a case as having severe malaria. However, some of the cases in the data did not classify those who had hyperparasitemia as severe. Hence, this signifies the need for training for those who oversee entering the data into the Vekpro Online database. A second verification of data from someone with a clinical background will be useful as currently, the data are entered mainly by an assistant health officer who has no proper clinical background. Besides that, different locations of *P. knowlesi* human infections are known to have different genetic variants of *P. knowlesi* [13] and certain parasite genotypes are known to contribute to *P. knowlesi* virulence [31]. These might also explain the differences in findings in this study and other studies. Further study at the genetic level on *P. knowlesi* in Terengganu is needed to prove it.

Regarding the environmental variables, only average monthly relative humidity was found to be significant in univariate analysis but not in the final multivariable regression analysis. This finding was similar to a study in Sabah where rainfall density and relative humidity were both associated with an increased incidence rate ratio of *P. knowlesi* with a lag time of three months; however, they were not independent predictors in the final multivariate model [12]. A study demonstrated seasonal variation for notifications of *P. knowlesi* from 2007 to 2013, with

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notifications peaking from May to August [11]. However, our study did not demonstrate any seasonal variation. The average monthly temperature, average monthly relative humidity, and average monthly rainfall density were not significant, probably due to the inaccuracy of the long-distance measurement between the station and place of occurrence, as suggested by another researcher [32]. Hence, for future study, a meteorological station builds exclusively at *P. knowlesi* prone location might be needed to study the effects of weather on *P. knowlesi* incidence, as it was important in studying zoonotic diseases [33-35].

There were several limitations of this study. First, the use of secondary data limits the variables that can be studied. Important factors like high-risk behaviors among cases, previous exposure or sighting of macaques, polymerase chain reaction (PCR) results, and geographical characteristics such as forest coverage or deforestation were important variables that cannot be studied due to the unavailability of the variables in the database. The lack of PCR results may not truly reflect *P. knowlesi* due to microscopic similarities. The same explanation also means that this research was not able to identify all cases of malaria in Terengganu because some cases may be submicroscopic or asymptomatic. Other than that, a comparison in this study was made to other malaria species, which can have similar risk factors. For a better understanding of the risk factors for *P. knowlesi* in Terengganu, a comparison with those who are free from malaria needs to be done. The inability to use weather data that accurately represents the area of malaria occurrence also limits the study. Lastly, the findings of this study were not suitable to be generalized to the whole of Malaysia, and it is more appropriate to be applied to Terengganu state only. However, the recommendations given may be useful at any level, especially for those related to the planning of *P. knowlesi* prevention activities and the Vekpro Online database.

CONCLUSION

Identified significant associated factors and characteristics of the high-risk group for *P. knowlesi* infection can help medical and health front liners in Terengganu to suspect and diagnose and promptly treated the cases. The factors also will help in the planning and development of health interventions in targeting *P. knowlesi* infection.

The Management Guidelines of Malaria Malaysia 2013 put intravenous (IV) Artesunate as the first-line treatment for all cases of severe malaria. As *P. knowlesi* had lower parasite density cut-off values to be classified as hyperparasitemia and severe malaria compared to other *Plasmodium* spp., most *P. knowlesi* cases required early IV artesunate administration. Therefore, current study findings can guide clinicians to suspect *P. knowlesi* better, thus allowing early referral and early use of IV artesunate that had been proved to be able to decrease the case-fatality rate [36]. Besides, for public health practitioners, this study can provide evidence on malaria intervention programs that should be targeted and changed given the near elimination of human malaria and increasing zoonotic malaria cases.

Conflict of Interest

Authors declare none.

Ethical Considerations

This research has been approved by the Medical and Research Ethics Committe (MREC) NMRR-19-3842-52281(IIR) as well as from the Research Ethics Committee of the Universiti Teknologi Mara Malaysia REC/02/2020 (MR/42).

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Authors Contributions

Author FHH and ZI made substantial contribution to the development conception and design of the study, acquisition, analysis and interpretation of data, and drafting the article. Author ZIA and NI made substantial contribution to the revising the acquisition, analysis and interpretation of data, and drafting the article. Author ASB made substantial contribution to the analysis and interpretation of data. Author NAR made substantial contribution to data collection, interpretation of data and revising the article critically for important intellectual content. All the authors read and approved the final version.

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