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SPATIOTEMPORAL ANALYSIS OF *Plasmodium knowlesi* INFECTION IN PAHANG, 2011-2022: ALIGNING WITH SUSTAINABLE DEVELOPMENT GOAL TARGET 3.3 FOR ENDING MALARIA EPIDEMIC

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Abstract: *Plasmodium knowlesi* is a zoonotic disease prevalent in Southeast Asia. Malaysia had the highest incidence, with 88.9% of total cases in Southeast Asia from 2004 to 2016. This poses a significant challenge to eliminating malaria and achieving Sustainable Development Goal (SDG) Target 3.3. This study aims to describe the trend, map the spatial pattern, and identify hotspots of *P. knowlesi* cases in Pahang for 2011-2022. A cross-sectional study used reported *P. knowlesi* cases within the Pahang e-Vekpro system. Using R software version 4.2.3, density analysis was conducted using Kernel Density Estimation (KDE). Spatial autocorrelation was assessed using Global Moran’s I and Local Indicators of Spatial Association (LISA). A total of 967 cases were registered during the period. A serial analysis shows the patterns of *P. knowlesi* density, with cases concentrated in the Lipis and Jerantut districts during the early years of the study. In the later years, the distribution became spread across various districts. Spatial autocorrelation was demonstrated for 2012-2013 and identified hotspots at Tembeling, Cheka, Kechau, Telang, and Gua sub-districts, all within the Lipis district. The spatiotemporal distribution of *P. knowlesi* malaria showed dynamic changes, indicating the disease’s evolving spatial pattern. Prevention efforts should focus on high-risk areas through surveillance, vector control, and community education.

Keywords: Malaysia, SDG, spatial distribution, vector-borne disease, zoonotic malaria.

Introduction

Plasmodium knowlesi is a zoonotic disease reported in all Southeast Asia countries except Timor Leste (Jeyaprakasam *et al*., 2020). In this region, the distribution of forest-dwelling mosquitoes that belong to the *Anopheles leucosphyrus* group largely overlaps that of long-tailed and pig-tailed macaques, contributing to the transmission of *P. knowlesi* (Singh & Daneshvar, 2013). Out of 5,351 cases reported between 2004 and 2016 in South East Asia, 4,757 (88.9%) cases occurred in Malaysia, 465 (8.7%) in Indonesia, and 37 (0.7%) in Thailand (Zaw & Lin, 2019). Furthermore, the return of travellers infected with *P. knowlesi* from Southeast Asia and subsequent transmission to their respective countries represents a significant global public health concern (Millar & Cox-Singh, 2015; Jeyaprakasam *et al*., 2020). *P. knowlesi* is widely distributed in Malaysia (Cox-Singh *et al*., 2008), accounting for 90% of all types of malaria cases in the country (Ahmed & Quan, 2019). Between 2017 and 2021, a total of 17,125 cases and 48 deaths were reported in Malaysia (WHO, 2022). While in 2021 alone, 3,575 cases were reported, resulting in 13 deaths.

Malaysia announced its commitment to eliminating malaria. The execution of the policy was outlined in the National Strategic Plan for Elimination of Malaria 2010-2020 (MOH & WHO, 2015). However, since 2008, the number of zoonotic malaria cases has increased gradually compared with indigenous or imported malaria. The transition in malaria epidemiology from indigenous malaria to zoonotic malaria has significantly impacted the elimination progress during the last half-century (Chin *et al*., 2020). The high prevalence of *P. knowlesi* can also hinder the country's target to achieve Sustainable Development Goals (SDGs) Target 3.3, where epidemics of malaria need to be ended alongside other diseases like Acquired Immunodeficiency Syndrome (AIDS) and tuberculosis.

The sustainability of *P. knowlesi* transmission is due to the movement of macaques and spillover into human populations (WHO Western Pacific Region, 2017). Furthermore, many environmental factors contribute to the increase in *P. knowlesi* malaria incidence, with increased acquisition risk from deforestation and changes in human land use (Anstey & Grigg, 2019). Note that deforestation causes the migration of the long-tailed macaques from forested areas to farms and semi-urban areas. This may have triggered mosquitoes to follow the host and adapt to forest fringes and farm areas (Jeyaprakasam *et al*., 2020).

This study addresses the current trend of rising *P. knowlesi* transmission, eventually contributing to achieving our country's Target 3.3 of the SDGs. There is still a lack of studies on the spatiotemporal distribution of *P. knowlesi* in East Malaysia, as previous studies have mainly focused on transmission in Malaysian Borneo (Phang *et al*., 2020). In contrast, the Expert Consultation on Malaria Elimination Strategies Report listed that research prioritisation is needed on distribution, hotspots, maps of cases, vectors, and macaques of *P. knowlesi* (WHO Western Pacific Region, 2017). Furthermore, Pahang was among the top three states in Peninsular Malaysia with the highest number of *P. knowlesi* cases, reporting 666 cases between 2011 and 2018. This, alongside Kelantan (812 cases) and Perak (514 cases) highlights the significant burden of *P. knowlesi* in Pahang (Phang *et al*., 2020). This research can contribute to the interventions for malaria, which should be locally specific. The disease needs to be mapped on many different layers, from the country level, district, sub-district, and down to the village level, for efficient control measures, mainly to relocate resources and field interventions.

Thus, applying spatiotemporal analysis allows us to comprehend the transmissions in various geographic places (Ooi *et al*., 2021). In addition, using spatiotemporal analysis to monitor hotspot districts *is* beneficial because disease transmission may expand to other communities due to the result of the cross-district movement of macaque populations that may carry malaria parasites or the long-distance spread of infectious vectors (Phang *et al*., 2020). Thus, this study aims to map the spatial pattern and identify the hotspots of *P. knowlesi* cases in Pahang for 2011-2022.

Methodology

Study Design and Study Population

This cross-sectional study used retrospective secondary data review, involving 12 years of quantitative data from 2011 to 2022. This study was conducted in Pahang, the largest state in Peninsular Malaysia and is located in the eastern part of the Peninsular (Figure 1). The total area of Pahang is 35,965 km^2 and is divided into 11 administrative districts.

The population of Pahang in 2020 was approximately 1.59 million individuals. The population density of Pahang is 44 individuals per square kilometer. Regarding ethnicity, Pahang comprises 75% Malay, 6.1% other Bumiputera, 14.7% Chinese, India 3.7%, and 0.4% others (Department of Statistics Malaysia, 2022a). The total forest coverage is 2,044,146.36 hectares or 56.8% of Pahang's total land (Pahang Forestry Department, 2022). In 2021, the working population in Pahang was documented to be 705,400 individuals. Out of the overall figure, an estimated 63.9% were mainly in the service sector. This was followed by the agricultural sector, which accounted for 19.1%, manufacturing at 9.3%, construction at 7.0%, and mining at 0.7% (Department of Statistics Malaysia, 2022b).

Figure 1: The map of Pahang, displaying the district boundaries
Sources: Skowron Volponi *et al*., 2017; Jalil *et al*., 2022

Operational Definitions

P. knowlesi cases refer to registered cases in the e-Vekpro that are laboratory-confirmed through Blood Film for Malaria Parasite (BFMP) or Polymerase Chain Reaction (PCR). Urban and rural areas are based on the criteria outlined in the 2010 Population and Housing Census, with urban areas defined as gazetted areas (including their adjoining built-up areas) and a collective population of at least 10,000 individuals. Meanwhile, areas with a population of less than 10,000 are classified as rural areas (Department of Statistics Malaysia, 2020).

Study Criteria and Sample Size

P. knowlesi malaria cases in Pahang from 2011 to 2022 were included in this study, which consisted of all confirmed *P. knowlesi* cases registered in the Pahang Vekpro Online Database between January 2011 and December 2022. Exclusion criteria comprise the duplicate entry of *P. knowlesi* cases within the same incubation period, cases with a source of infection outside of Pahang and cases with incomplete addresses that cannot be verified further.

The sample calculation was done using the single proportion formula. The proportion referred to the incidence rate of 0.302/1,000 population (Hussin *et al*., 2020) and the precision is 0.0001. Conventionally, the power of the study was set at 80% with $\alpha = 0.05$. Therefore, the calculated population sample required for this study was 126,735 after adjusting for 10% missing data. However, no sampling method was applied since the Vekpro Online Database

System registry already accounts for the whole Pahang population at risk (N = 1,531,600). All eligible cases were included in the analysis after applying exclusion criteria.

Data Collection

This study collected data from the Vekpro Online Database System (e-Vekpro). It is a national system used to register, collect, and monitor vector-borne diseases and their management in Malaysia, which has been operating since 2011. The dataset comprises a comprehensive list of malaria cases in a tabulated format and aggregated according to epidemiological weeks. Permission to use state-level data for Pahang was granted on 9th March 2023, from the Pahang State Health Department.

For this study, variables obtained from e-Vekpro are the demographic characteristics, which include age, gender, ethnicity, and occupation. The location of *P. knowlesi* cases was obtained in coordinate format. Pahang's sub-district-level population density from 2011 to 2022 was obtained from the Key Findings report of the Population and Housing Census of Malaysia 2020 for the state of Pahang (Department of Statistics Malaysia, 2022a).

Statistical Analysis

In this study, spatial analysis for incidence rate mapping was done using R Studio version 4.2.3. A set of thematic maps was constructed using the tmap package in the software (Tennekes, 2018; Comber & Brunsdon, 2018) to illustrate the incidence of *P. knowlesi* malaria at the sub-district level from 2011 to 2022. Using the same version of R Studio, the spatstat package was utilised to create a Kernel Density Estimation (KDE) on point data representing *P. knowlesi* malaria cases. The plug-in method (bw.ppl), a type of adaptive bandwidth was employed to ensure the most suitable bandwidth choice for the KDE. Additionally, contour lines were overlaid on the KDE plot to provide a more detailed representation of the spatial distribution of malaria cases.

Global Moran's I and Local Indicators of Spatial Association (LISA) were utilised to assess spatial autocorrelation between neighbouring sub-districts. Initially, spatial autocorrelation was tested using Moran's I statistic. Consequently, the findings were further analysed through LISA. This was done using the spdep package in R software (Bivand *et al.*, 2005). The map was created using the tmap package by displaying Pahang's geographical boundaries as light grey polygons. Correspondingly, the results of the LISA statistics are overlaid on the map and different colours are used to represent the LISA quadrants. The legend indicates the meanings of the colours, corresponding to different type of spatial clusters.

The spatial patterns of *P. knowlesi* malaria cases in Pahang are illustrated on the LISA cluster maps, highlighting regions with high and low values. Spatial clusters (high-high and low-low) indicate areas with values similar to those of neighbouring areas. They are represented in cluster maps in red (hotspots) and blue (cold spots), respectively. Outliers (low-high and high-low) indicate areas with values different from neighbouring areas, which are represented in pink and light blue cluster maps, respectively.

Results

967 confirmed *P. knowlesi* malaria cases were registered in Pahang, with a mean age of 36.9 years (SD = 15.83). The majority of the cases were male (83.7%). The ethnicity distribution of the cases is predominantly Malays (58.3%), followed by foreigners (20.6%), and indigenous people (13.3%). The young adult group, aged between 20 and 39, constituted 50% of the total cases. Detailed demography of *P. knowlesi* malaria cases in Pahang from 2011 to 2022 is presented in Table 1.

A serial analysis of KDE shows the distinct patterns of *P. knowlesi* density in Pahang (Figure 2). A colour gradient is used in the KDE map and yellow indicates regions with the highest concentration of *P. knowlesi* cases. At the same time, a gradual shift toward blue signifies

Table 1: Demography of *Plasmodium knowlesi* malaria cases in Pahang from 2011 to 2022 (n = 967)

Variables	Frequency (%)	Mean (SD)
Gender		
Male	809 (83.7)	
Female	158 (16.3)	
Age (years)		36.9 (15.83)
0-9	20 (2.1)	
10-19	87 (9.0)	
20-29	251 (26.0)	
30-39	232 (24.0)	
40-49	162 (16.7)	
50-59	120 (12.4)	
≥ 60	95 (9.8)	
Ethnicity		
Malay	564 (58.3)	
Chinese	51 (5.3)	
Indian	19 (2.0)	
Indigenous people	129 (13.3)	
Bumiputera Sabah/Sarawak	5 (0.5)	
Foreigners	199 (20.6)	

SD = Standard Deviation

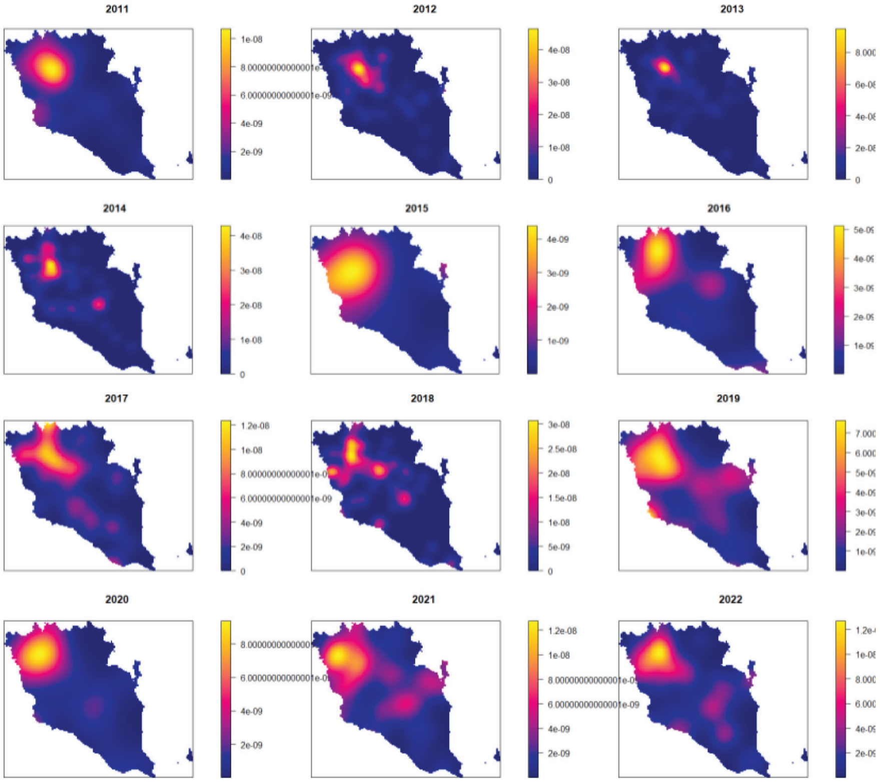

Figure 2: Yearly Kernel density estimation of *Plasmodium knowlesi* malaria in Pahang from 2011 to 2022

areas with lower concentrations. Along with the neighbouring districts of Raub and Jerantut, the KDE map demonstrates that the Lipis district has a substantial density concentration of *P. knowlesi* malaria cases. The others, including Kuantan and Temerloh, which are considered urban areas, exhibit lower density concentration. While in the initial years (2011, 2012, and 2013), case density was predominantly concentrated in the Lipis and Jerantut districts. However, in the later years (2021 and 2022), the distribution of cases became evenly spread across various districts in Pahang.

This study employed Global Moran's I, which provides indications of spatial autocorrelation of *P. knowlesi* malaria in Pahang. The Global Moran's I analysis (Table 2) shows autocorrelation results for 2012 and 2013. A positive Moran's I value of 0.43, higher than the predicted value of -0.06 was seen in 2012, suggesting a positive spatial correlation for *P. knowlesi* malaria cases in that year. Similarly, a positive Moran's I value of 0.29 was observed in 2013, higher than the predicted value of -0.04. In addition, the *p*-values were less than 0.05 in both years, showing statistical significance.

The findings from Global Moran's I were further analysed through LISA statistics. In 2012, three rural Tembeling, Cheka, and Kechau sub-districts were identified as hotspot regions. While Gua, Penjom, Budu, Pedah, Teras, Semantan, Mentakab, Perak, Burau, Chenor, Luit, Penyor, Keratong, and Rompin sub-districts were recognised as cold spots. During 2013, the hotspot areas expanded to include the rural sub-districts of Telang and Gua. While Penjom, Sega, Pedah, Jenderak, Semantan, Sabai, Mentakab, Sanggang, Pelangai, and Penyor were identified as cold spot areas (Figure 3).

It is important to note that in both years, all the identified hotspot areas are located under the district of Lipis, indicating a concentrated occurrence of *P. knowlesi* malaria cases within that district. This finding is supported by consistent results from serial KDE analyses conducted over years of research, which show higher frequency or concentration of *P. knowlesi* malaria cases in these hotspot areas over time. This convergence of evidence significantly strengthens the case for the existence of hotspot areas.

Table 2: Global Moran's I analysis for *Plasmodium knowlesi* malaria in Pahang from 2011 to 2022

Year	Global Moran's I Index	Expected Global Moran's I Index	Standard Deviation	*p*-value
2011	-0.03	-0.08	0.22	0.414
2012	0.43	-0.06	2.39	0.008
2013	0.29	-0.04	1.92	0.027
2014	-0.12	-0.04	-0.57	0.715
2015	-0.11	-0.08	-0.21	0.582
2016	-0.09	-0.13	0.08	0.467
2017	0.09	-0.05	0.77	0.221
2018	-0.01	-0.05	0.24	0.406
2019	-0.20	-0.06	-0.74	0.769
2020	0.05	-0.09	0.52	0.302
2021	-0.02	-0.05	0.19	0.425
2022	-0.01	-0.06	0.23	0.411

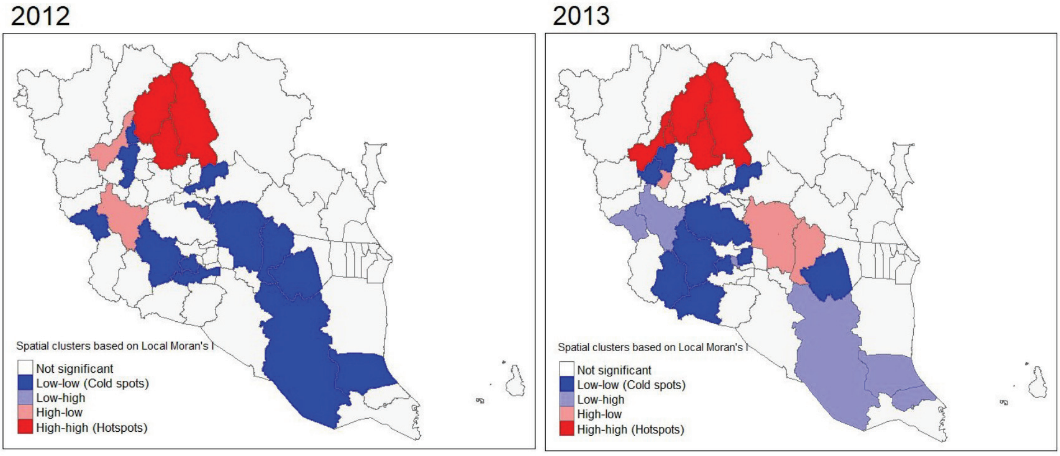

Figure 3: LISA mapping of *Plasmodium knowlesi* in Pahang for 2012 and 2013

Discussion

The study demonstrates that most *P. knowlesi* cases in Pahang are male (83.7%). The finding is consistent with other studies conducted in other states in Peninsular Malaysia (Phang *et al*., 2020), Johor (Pramasivan *et al*., 2021), and Sarawak (Yunos *et al*., 2022). The young adult group, aged between 20 and 39 constituted 50% of the total cases. This is the trend in Southeast Asia, where it is observed that young adult males face a greater risk of contracting diseases from forest-dwelling vectors due to their occupational exposure (Landier *et al*., 2018). The predominant occurrence of *P. knowlesi* cases among the Malay ethnicity is consistent with Phang *et al.* (2020) findings while the prevalence among indigenous people is slightly lower than reported by the same study and Hussin *et al*. (2020).

The spatial analysis results suggest the presence of geographic density and correlation of *P. knowlesi* malaria in Pahang. These are consistent with prior research demonstrating malaria transmission within specific spatial and temporal clusters (Yoep *et al*., 2015; Yunos *et al*., 2022). Moreover, the areas identified as hotspots may suggest the existence of specific conditions that contribute to the transmission of *P. knowlesi*. These conditions include an increased prevalence of the natural host (macaques) (Yusof *et al*., 2016), Anopheles vectors (Vythilingam *et al*., 2018), and the presence of favourable environmental conditions (Anstey & Grigg, 2019; Byrne *et al*., 2021). A study by Jiram *et al*. (2012) revealed that *Anopheles cracens* was the predominant species abundantly found in the Lipis forest.

These areas tend to offer ample water resources, vegetation, and favourable climatic conditions that facilitate the proliferation of mosquito communities. The forested locations provide ideal breeding environments for mosquitoes. Moreover, the risk is increased by occupational exposures linked to local economic activities such as tourism, logging, and agriculture, bringing people and infected macaques closer. These activities, often carried out in or near forested areas, further elevate the potential for *P. knowlesi* transmission (Fornace *et al*., 2016).

Furthermore, these areas exhibit a higher macaque density than locations closer to human settlements. A high population of infected macaques increases the likelihood of human-macaque interactions, thereby increasing the risk of *P. knowlesi* transmission. Moreover, where human activity is limited, the deep forest areas offer uninterrupted ecological conditions for macaques and the mosquito vectors responsible for transmitting the parasite. This explains the situation in the Lipis district, where a significantly high incidence of *P. knowlesi* infection is observed among the indigenous population. Note that 44.2% (57 out of 129) of all reported cases among indigenous people in Pahang occurred in the Lipis district, where they engage in daily activities such as villagers, farmers, and gatherers in the forested areas.

In addition, the availability of healthcare services in forested areas is limited by the lack of immediate diagnostic and medical facilities (Kader Maideen *et al*., 2022). The insufficient infrastructure presents a barrier to the prompt identification and effective management of *P. knowlesi* malaria cases. Delays in diagnosing and treating these cases may worsen the conditions and contribute to transmitting such cases in the Lipis district, particularly within the indigenous area.

On the other hand, the districts of Jerantut and Raub, despite experiencing a higher level of development compared to the Lipis district, exhibit relatively high incidences of *P. knowlesi*, which can be attributed to their proximity to forested areas inhabited by macaque populations. The risk of transmission is raised by proximity to forested areas and greater mobility of people due to economic activities such as tourism, logging, and agriculture, which bring infected macaques and people into closer contact. Jerantut district encompasses a significant portion of the Pahang National Park, attracting a considerable influx of domestic and international tourists, which may have contributed to increased cases among visitors returning from the park. The findings of our study align with Grigg *et al*. (2017), indicating that the spatial distribution of *P. knowlesi* infections is associated with ecological changes that have affected the interface between human populations and vegetation.

The interaction of ecological, social, and infrastructural factors affects the outcomes of spatial analysis. The disease is more likely to be transmitted because of the large macaque populations and their forest settings. In contrast, *Anopheles* mosquitoes are supported by ecological suitability for their proliferation. Despite urbanisation and development in some areas, the risk of transmission is raised by proximity to forested areas and greater mobility due to economic activities such as tourism, logging, and agriculture, which bring infected macaques and people into closer contact. This finding aligns with previous research highlighting the role of deforestation and environmental changes as key factors contributing to *P. knowlesi* transmission (Moyes *et al*., 2016; Fornace *et al*., 2016).

The results, particularly concerning density analysis of KDE and autocorrelation analysis of Global Moran's I and LISA statistics indicate hotspot areas within Pahang with a higher incidence of *P. knowlesi* malaria. The LISA hotspot regions are located within the Lipis district's confines, including the Tembeling, Cheka, Kechau, Telang, and Gua sub-districts. This finding is supported by consistent results from serial KDE analyses, which show increased density in the same region over most of the years from 2011 to 2022.

Furthermore, neighbouring districts exhibit increased density in some cases, implying that *P. knowlesi* cases spread outside the Lipis area. This suggests the possibility of additional hotspot areas in other districts. The findings, which identify persistent *P. knowlesi* malaria hotspots in the Lipis district and its sub-districts, align with existing research on the spatial distribution of this zoonotic malaria. For instance, a study in Johor, Malaysia identified similar hotspot regions, highlighting the role of vector distribution in *P. knowlesi* transmission (Pramasivan *et al*., 2021).

It should be noted that the research relies on information obtainable from the e-Vekpro. The reported cases of *P. knowlesi* infection may be underestimated, given the existence of evidence indicating that individuals infected with this pathogen may exhibit no symptoms (Mohammad *et al*., 2022). The study did not explore the interconnections between climate variables and *P. knowlesi* malaria cases. Rainfall, for instance, can have implications for mosquito breeding habitats. In contrast, extremes in the temperature range reflect on malaria transmission, in which higher temperatures cause the extrinsic incubation period of malaria vectors to shorten, resulting in earlier transmission by mosquitoes (Githeko *et al*., 2000; Patz & Olson, 2006).

Conclusions

The distribution of *P. knowlesi* cases in Pahang has changed, with early hotspots in Lipis and Jerantut and a more widespread pattern in later years. Furthermore, rural areas show higher case densities than urban areas, indicating environmental and socio-economic influences on transmission. This pattern emphasises the dynamic nature of *P. knowlesi* transmission and the importance of continuous monitoring to identify emerging hotspots and mitigate disease spread.

The findings of the spatial analysis of *P. knowlesi* in Pahang suggest that the public health authority should prioritise targeted interventions in high-risk areas that have been identified. Potential strategies to address the issue may encompass implementing enhanced surveillance and monitoring for at-risk populations, strengthening vector control measures, and organising community education initiatives to raise awareness of the disease and its preventative measures.

Furthermore, to enhance the spatial analysis of *P. knowlesi* in Pahang for future research, it is recommended to incorporate environmental factors such as land use, forest coverage, and climatic conditions. This holistic approach can provide a more comprehensive understanding of disease transmission dynamics.

Acknowledgements

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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