Forest fragmentation drives zoonotic malaria prevalence in non-human primate hosts

Authorship information

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ABSTRACT

Land conversion is understood to increase the risk of emergent zoonotic diseases. In simians and humans, infection risk has been linked to fragmented habitats. However, the role of fragmentation on disease dynamics in wildlife hosts is rarely quantified at macroecological scales due to the lack of systematic surveys. In Southeast Asia, non-human primates (NHPs) host *Plasmodium knowlesi,* a prominent zoonotic malaria. We examine reported primate *P. knowlesi* to investigate how landscape impacts parasite prevalence. Firstly, we conducted a meta-analysis of NHP *P. knowlesi* prevalence. Overall prevalence was 9.6% (CI95% 6.3–13.4), with considerable regional heterogeneity (I^2=96.6%; CI95% 95.7–97.3) and high estimates in Borneo (52.4%, CI95% 22.8–81.3). Higher prevalence in NHPs shows clear spatial overlap with human infection foci. Secondly, environmental covariates were assembled from remote sensing data and statistical models were fitted to prevalence at multiple spatial scales. We demonstrate a strong relationship between forest fragmentation (20km, p<0.0001) and *P. knowlesi* in NHPs, suggesting that zoonotic malaria prevalence is maximised at intermediate levels of habitat complexity. Findings indicate a previously hypothesised trade-off between epidemiological and ecological mechanisms determining *P. knowlesi* infection in wildlife reservoirs, and that parasite prevalence in NHPs may be a key driver of human spillover risk.

Keywords

Disease ecology; forest fragmentation; land use change; malaria; *Plasmodium knowlesi*; zoonoses

INTRODUCTION

Background

Zoonotic infectious disease emergence results from the spillover of pathogens into human populations, typically from a reservoir in wildlife hosts. Anthropogenic land use and land cover changes (LULCC) have now been widely linked to infectious disease outbreaks[1–3]. Such practices, including deforestation, logging, clearing for cash-crop plantations or conversion of intact forest into arable land, are accelerating across tropical forests of Southeast Asia[4,5]. Forest fragmentation is a key aspect of landscape modification, whereby large contiguous areas of forest are broken into a mosaic of smaller isolated patches. This disturbs the ecological structure by increasing the density of forest fringes or 'edges', dynamic habitat at the transitional boundaries between natural ecosystems and human-modified landscapes[6]. Such ecological interfaces are thought to facilitate parasite spillover in multi-host systems, including vector-borne diseases such as Zika[7], Babesiosis and Lyme disease[8], *Trypanosoma cruzi*[9], Yellow Fever[10] and zoonotic malaria[3,11]. Mechanisms that underly the association between habitat fragmentation and spillover risk from wildlife hosts are complex and occur over multiple spatial scales[3]. In part, increased edge habitat can facilitate spatial overlap and increase cross-species contact rates between wildlife, vectors and humans[12]. At the same time, habitat complexity can have detrimental impact on wildlife population viability, with reduced host species occupancy in highly fragmented habitats[13]. Disentangling these underlying mechanisms is essential to inform ecological strategies for disease surveillance and mitigation[5].

Simian *P. knowlesi* is a public health threat of increasing importance across Southeast Asia, following the identification of a prominent infection foci in Borneo in 2004[14]. *P. knowlesi* is a zoonosis, with a sylvatic cycle circulating in non-human primates (NHPs). Human cases are thought to occur only from spillover events[15–17]. Human transmission requires bites from infective mosquitos, primarily anopheline mosquitos of the Leucosphyrus Complex (*Anopheles balabacensis, An. latens, An. introlactus)* and Dirus Complex (*An. dirus, An. cracens)*[18–20]. Natural hosts for *P. knowlesi* are typically Long-tailed macaques (*Macaca fascicularis*) and Southern Pig-tailed macaques (*M. nemestrina)*[18], both occurring widely across Southeast Asia. Currently, distribution of *P. knowlesi* cases is thought to be restricted to the predicted ranges of known vector and host species[21], though recent studies have also identified other NHPs found to be harbouring *P. knowlesi.* This includes Stump-tailed macaques *(M. arctoides),* which are now considered to be another natural reservoir[22].

Progress towards malaria elimination in Malaysia has been stymied by a recent rise in human incidence of *P. knowlesi* malaria[23]. Accounting for increases in surveillance and diagnostic improvements, it is now recognised as the most common cause of clinical malaria in Malaysia[24]. LULCC are thought to be driving this emergence of *Plasmodium knowlesi* infections[5]. While sporadic cases have been reported across Southeast Asia, including in Indonesia[25], the Philippines[26], Vietnam[27], Brunei[28] and Myanmar[29], the majority of *P. knowlesi* cases are found in East Malaysia (Borneo) with hotspots in the states of Sabah and Sarawak[30], areas that have seen extensive deforestation and landscape modification. In Sabah, human prevalence of *P. knowlesi* infection has recently been shown to be specifically associated with recent loss of intact forest, agricultural activities and fragmentation across multiple localised spatial scales[3,31,32].

Prevalence of the pathogen in reservoir hosts is considered to be one of three crucial factors determining the force of infection in zoonotic spillover events[33]. Despite this, very little is known of the impact of rapid LULCC on the distribution of *P. knowlesi* in NHPs. Literature on the impacts of fragmentation on primates tends to focus on primate density and abundance[34,35]. What is known is that effects of LULCC on primate-pathogen dynamics are highly variable and context-specific. Macaques have been known to preferentially rely on

fringe habitat, which may be enhanced in response to forest fragmentation and facilitate exposure to vectors[12,15]. Although sylvatic vectors remain unknown, the *Anopheles leucospryphus* group, the only vector group implicated in *P. knowlesi* transmission, is widely associated with secondary, disturbed forest[20,36,37]. Changes to land composition can also create the biosocial conditions for higher rates of parasitism in primates. Under conditions of limited resources and reduction in viable habitat, conspecific primate density may increase as troops compete for available space. In turn, this can favour transmission via intra-species contact or allow the exchange of pathogens between troops dwelling in intact forest versus edge habitat[15,38]. Habitat use may also become more intensive, preventing parasite avoidance behaviours[39]. Land use change is known to favour more adaptable, synanthropic species such as *M. fascicularis*[40]. Concurrent loss of biodiversity may result in loss of the 'dilution effect', with a reduced range of susceptible hosts and a concentration of parasite burden in more competent primate species[2]. In light of the spillover risk posed by wildlife reservoirs of *P. knowlesi,* clarifying any relationships between environmental factors and parasitaemia in key host species may contribute to a more comprehensive understanding of *P. knowlesi* transmission patterns.

Earth Observation (EO) data provides novel opportunities to investigate epidemiological patterns of diseases which are linked to environmental drivers[41]. In relation to *P. knowlesi,* utility of fine-scale remote-sensing data has been demonstrated: examples include satellite-derived data used to examine household–level exposure risk in relation to proximate land configuration[32], UAV-imagery used to link real–time deforestation to macaque host behavioural change[15], and remote-sensing data used to interrogate risk factors for vector breeding sites[42]. Macroecological studies that utilise geospatial data are often confounded by issues of matching temporal and spatial scales, as well as by the quality and accuracy of available georeferencing, which should be accounted for when examining the role of environmental factors in modulating disease outcomes. Even accurate georeferenced datapoints are unlikely to entirely reflect surrounding habitat within the macaque home range. Furthermore, ecological processes occur and interact over a range of distances, or 'spatial scales'[2,3,31]. This applies to determinants of vector-borne disease ecology, from larval breeding microclimate to host foraging behaviour. As multiple influential variables are rarely captured by a single scale[43], data-driven methods can be applied to examine risk factors over multiple scales and identify covariates at their most influential extent[42].

We hypothesise that higher prevalence of *P. knowlesi* in primate host species is driven by LULCC, contributing to the strong associations described between LULCC and human *P. knowlesi* risk. Despite concerns about transmission of simian malarias in Southeast Asia, there is limited data on regional infection dynamics within NHPs. In conceptual frameworks and transmission models, it is often assumed that *P. knowlesi* infections in NHPs are chronic and ubiquitous[30,44]. No studies have systematically assessed the extent and quality of available data on *P. knowlesi* in NHPs. Independent studies investigating *P. knowlesi* in primates are typically constrained by small sample sizes and confined geographic areas, limiting inference that can be made about relationships between infection dynamics and landscape characteristics. Systematic tools developed for epidemiological studies of disease prevalence in human populations are rarely applied to the study of wildlife disease prevalence; however, such tools can be used to capture the scale and contrast required in macroecological studies to quantify disease burdens regionally. Furthermore, while recent research has shown the impact of deforestation on the distribution of macaques in the context of *P. knowlesi* [15,18], associations between LULCC and variation in the prevalence of simian *Plasmodium* spp. in primates have not been explored. This study is the first to systematically assess *P. knowlesi* prevalence in NHPs at a regional scale, and across a wide range of habitats. We aimed to 1) systematically assemble a georeferenced dataset of *P. knowlesi* in NHPs; 2) evaluate variation in NHP *P. knowlesi* prevalence by geographic region; and 3) assess environmental and spatial risk factors for *P. knowlesi* prevalence in NHPs across Southeast Asia.

METHODS

(a) Study site

This study focused on simian malarias across Southeast Asia, within 28°30'00.0"N, 92°12'00.0"E and 11°00'00.0"S, 141°00'00.0"E. Climate mainly corresponds to the equatorial tropical zone, with high temperatures and high humidity.

(b) Data assembly

A systematic literature review was conducted in Medline, Embase and Web of Science under the CoCoPop framework (Condition, Context, Population)[16,45] to identify articles reporting prevalence of naturally-acquired *P. knowlesi* in NHPs. Key authors were contacted to request unpublished or supplementary datasets identified from articles and reference lists. All studies were screened for data on NHPs with a confirmed *P. knowlesi* diagnosis or absence data (zero counts of *P. knowlesi* with appropriate diagnostic methods). Exclusion criteria included (a) studies exclusively relying on microscopy[46] (b) laboratory, animal model or experimental infection studies (c) data from outside of Southeast Asia. No limit was set on the temporal range. Duplicate records reporting results from the same surveys were removed, with one record per survey retained. Critical appraisal of the studies was conducted using the Joanna Briggs Institute (JBI) checklist for prevalence studies[45] (see Supplementary Information (SI) for details and criteria). A flowchart of the selection process is illustrated in Figure S3, with a full list of articles included provided in Table S2.

Primary outcome was defined as *P. knowlesi* prevalence (*p,* proportion positive for *P. knowlesi* infection from *n* sampled NHPs). For each independent primate study, the following variables were extracted: year of data collection, primate species sampled, primate status (wild/captive), diagnostic test (PCR/sequencing) and target gene(s), sampling method (routine/purposive), number of *P. knowlesi* positive samples, number of *Plasmodium* spp. positive samples, total number of primates tested and geographical information. For the majority of samples identified, study site was geolocated to a geographic area. Geolocation was assigned at the lowest available level of administrative polygon (district/state/country) by cross-referencing reported sampling location with GADM (v3.6) administrative boundaries. If specific location was given, GPS coordinates were assigned via Google Maps. For the purpose of data visualisation, centroids were extracted per polygon and point coordinates were plotted in QGIS (3.10.14) and R (4.1.0) software.

(c) Meta-analysis of NHP prevalence

Using methodology standardly used for analysis of human disease prevalence in individual participant datasets (IDP)[47,48], a meta-analysis of *P. knowlesi* prevalence (number positive out of the number sampled) was conducted on primate malaria survey data. Data were disaggregated by geographic location and primate species, to illustrate variance in prevalence by sampling site[48]. One-stage meta-analysis is considered appropriate for studies where the outcome may be infrequent, so data was included in a single model under the 'DerSimonian and Laird' variance estimator [45]. For studies where prevalence estimates tend towards 0% or 100%, variance tends towards 0. To stabilise the variance and accommodate for the inclusion of a high number of zero prevalence records, the Freeman-Tukey double arcsine method was applied in the transformation of prevalence[45]. Sensitivity analysis for the back-transformation of estimates were conducted using both the logit transformation and untransformed proportions (see SI for details). Overall heterogeneity of prevalence records was assessed using the I^2 statistic[49]. Sub-group analysis was conducted according to geographic region, with the heterogeneity of reported prevalence within regional sub-groups assessed using prediction intervals derived from the τ^2 statistic. Small-study effects, including selection and publication biases, were assessed by examining funnel plots and imputing 'missing' estimates using the trim-and-fill method[50]. Full rationale and details of small-study effect assessments can be found in Supplementary Information.

(d) Remote sensing data

For samples geolocated to administrative polygons, there is considerable geographic uncertainty in the exact study location and subsequent uncertainty related to the spatial and environmental determinants (SI, Table S7). To account for this, for data that could only be derived within an administrative boundary, 10 random sampling points were generated within each polygon and environmental covariates were extracted for each site, as described below (see SI, Table S8). For study sites with exact GPS coordinates, precise environmental data was multiplied 10-fold. This mimics a weighted regression to adjust for spatial uncertainty. Data point iterations with identical or higher degrees of environmental similarity lend more weight to the final model, and larger polygons with more variable covariate measurements, and as such less certainty in ecological conditions, have a lesser representation in the model. Selection of random points was validated by visual inspection of the stability of model coefficients with the inclusion of an increasing number of points. Number of points was selected conservatively at the point where coefficients stabilised (n=10), minimising the risk of retaining spurious covariates in model selection due to artificially inflated sample sizes.

Environmental covariates were extracted at each sampling point from satellite-derived remote-sensing datasets (Table 1). Gridded UN-adjusted human population estimates were assembled at 1km resolution from WorldPop[51]. A threshold for high population density was set at 300 persons/km^2 pixel according to recent urban classifications[52]. Elevation data was obtained from NASA SRTM 90m Digital Elevation Database v4.1 (CGIAR-CSI)[53] with a spatial resolution of 1km x 1km. Contemporaneous tree cover was derived from Hanson's Global Forest Watch (30m)[54]. Tree cover was classified as ≥50% crown density, and then matched to primate data by sample site geolocation and by year of sample collection to account for rapid forest loss (SI, Figure S8). Where a broad timeframe of sampling was provided (≥3 years), median year was used. Full details can be found in Supplementary Information (Table S11–12, Figure S9).

Table 1. *Spatial and temporal resolution and sources for environmental covariates. PARA=Perimeter: Area ratio of forest class.*

Covariate	Spatial res.	Temporal res.	Source
Human population (p/km^2)	1km	2012	WorldPop[51]
Elevation (m)	1km	2003	SRTM 90m Digital Elevation v4.1[53]
Forest cover (%)	30km	Annual	Hansen's Global Forest Watch[54]
Fragmentation (PARA>0)	30km	Annual	Hansen's Global Forest Watch[54]

In a disease ecology context, habitat fragmentation is expected to correlate with disease transmission parameters[6,38]. Definitions often lack precision and can be considered with respect to 'separation effects' (division and isolation of patches) and 'geometric effects' (changes to ratios of perimeter and core habitat)[55]. Perimeter: area ratio (PARA, ratio of patch perimeter length to patch surface area) of given land class is a key metric for habitat conversion, where a higher PARA provides a measure of boundary complexity and can indicate a more fragmented landscape[56]. Mean PARA was extracted from forest cover within circular buffers. This provides a measure of forest edge density in a given area (PARA>0) and has been shown to provide a good index of fragmentation and discrimination of spatial aggregation across areas where habitat abundance is high[57] (SI, Figure S10).

For every georeferenced sampling point, mean values for all selected covariates were extracted within buffer radii at 5km, 10km and 20km (SI, Figure S11). Buffer areas were selected to investigate multiple spatial scales over which associations between risk factors and *P. knowlesi* prevalence might occur. A minimum radius of 5km was chosen to approximate the maximum ranging distance for *M. fascicularis*[58], with wider radii (10–20km)

included to account for the geographic uncertainties in areal data. Continuous fixed-effect covariates were scaled and mean-centred to standardize the data.

(e) Analysis of environmental risk factors

P. knowlesi prevalence data and covariate data were then used to fit separate models to explore the relationships between land cover and NHP malaria prevalence. Generalised linear mixed-effect regression models (GLMM) were fitted using a binomial distribution with a logit link. To account for within-study correlation, a unique identifier for publication was included as a random intercept in all models. Spearman's rank tests were conducted to examine correlation in independent variables at multiple spatial scales, plotted as a correlation matrix (Supplementary Figure S12).

Selection of explanatory variables was achieved using a 'two stage' stepwise approach. A quadratic term for the fragmentation index 'PARA' was included to account for possible nonlinearity. Multicollinearity among independent predictors at multiple scales was examined according to calculation of variance inflation factor (VIF). The VIF of each predictor variable was examined following a stepwise procedure, starting with a saturated model and sequentially excluding the variable with the highest VIF score from the model. Stepwise selection continued in this manner until the entire subset of explanatory variables in the global model satisfied a moderately conservative threshold of VIF ≤ 6[59]. Qualifying variables obtained were then assessed for model inclusion using a forward step-wise strategy, building up from an intercept-only model. Model parsimony was examined according to reduction in Akaike's Information Criterion (AIC) value. Fully-adjusted OR and marginal effect curves for associations between environmental covariates and *P. knowlesi* prevalence were derived from the final multivariable GLMM.

(f) Ethics

Ethics approval was not required for this research, following assessment by the LSHTM Research Governance & Integrity Office (LSHTM MSc Ethics Ref: 25429).

RESULTS

(a) Descriptive analysis

18 articles were retained in in the study[17,22,60–75], containing 123 unique primate survey records to form the dataset for analyses (see SI for details of JBI Critical Assessment, Table S5)[45]. Year of sampling ranges from 2004–2019. No primatological studies were identified from Vietnam, Brunei or Timor-Leste. Full characteristics of the articles and individual study methodologies are reported in Supplementary Information (Table S2).

Within the 123 records identified, survey data had been collected from a total of 92 unique sites. Spatial resolution of the survey sites varied from GPS point coordinates to GID0 (country) classification (Supplementary Table S7). Geographic distribution of sampling is illustrated in Figure 1. Overall, records report on a total of 4931 primates, with the majority sampled from Peninsular Malaysia (49.5%, n=2442/4931). Primates sampled were primarily Long-tailed macaques (*Macaca fascicularis*) (88.6%, n=4368/4931) and *M. nemestrina* (n=495/4931)[17,65,74,75] (Table S3).

Reported prevalence of *P. knowlesi* in NHPs ranged from 0%–100%. Only 73 of the identified records (59.3%, n=73/123) reported a positive diagnosis, with the remaining 50 sites finding no molecular evidence of *P. knowlesi* infection (40.7%). A full breakdown of *P. knowlesi* infection rates according to reported primate characteristics can be found in SI, Table S4.

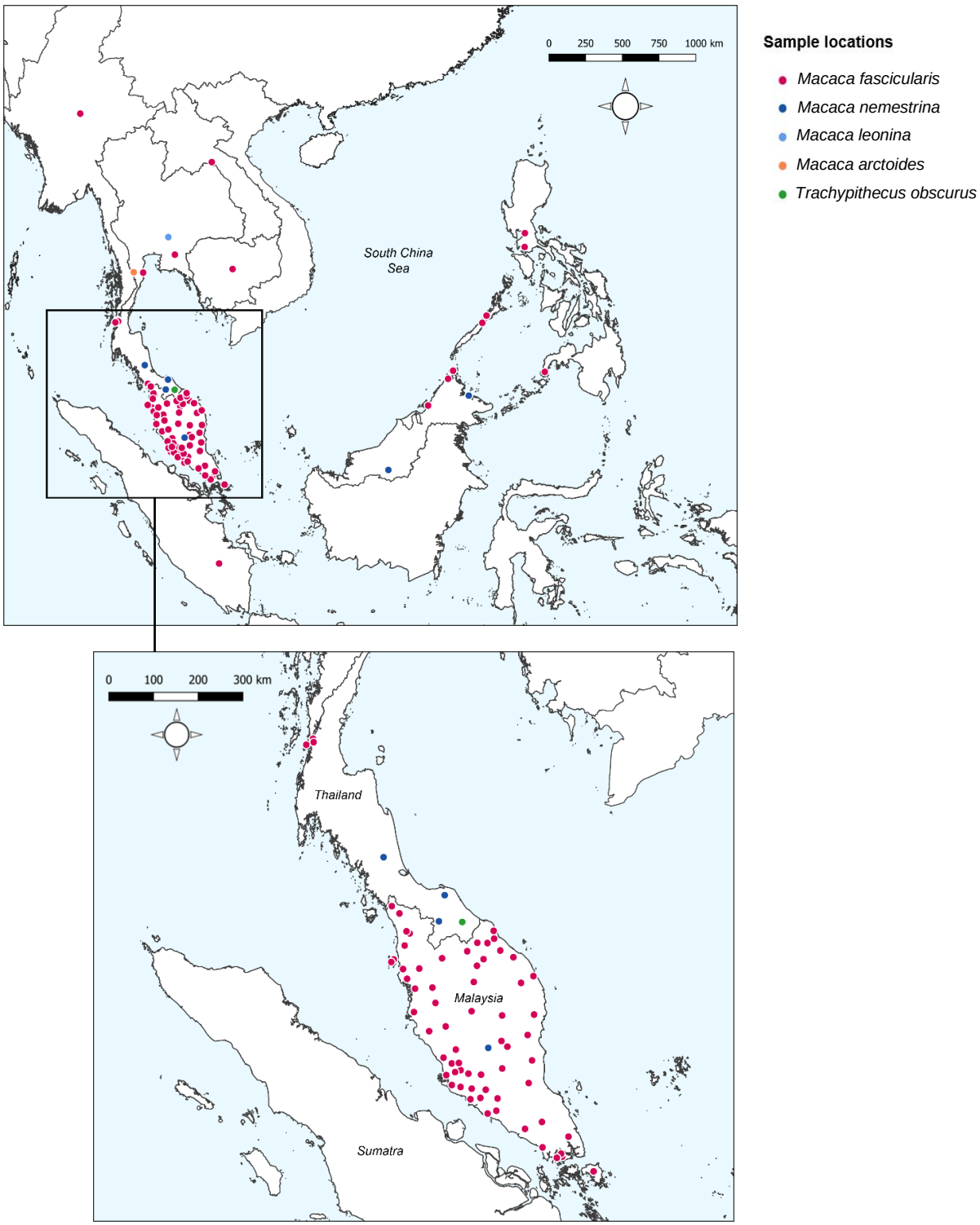

Figure 1. *Sampling sites and primate species across Southeast Asia. N=123.*

(b) Meta-analysis of prevalence

Meta-analyses were first conducted with data aggregated by study and geographic region (*k*=22, accounting for articles reporting on multiple countries)[69] (SI, Figure S5). For the meta-analysis using data disaggregated by survey site and primate species, overall pooled estimate for *P. knowlesi* prevalence was 9.6% (CI95% 6.3–13.4), with high heterogeneity across all prevalence records (I^2=91.5%; CI95% 90.4–92.6). In the sub-group analysis by region, pooled prevalence estimates are notably low for Laos (2.3%, CI95% 0.0–9.5) and the

Philippines (3.5%, CI95% 0.0–21.8), moderate in Peninsular Malaysia (9.7%, CI95% 6.6–13.3) and elevated in Malaysian Borneo (52.4%, CI95% 22.8–81.3) and Singapore (24.9%, CI95% 7.1–47.8) (Figure 2). Wide prediction intervals indicate high heterogeneity of estimates within geographic regions, consistent with high expected variability across individual study sites. Forest plots of prevalence estimates can be found in Supplementary Figures S6–7.

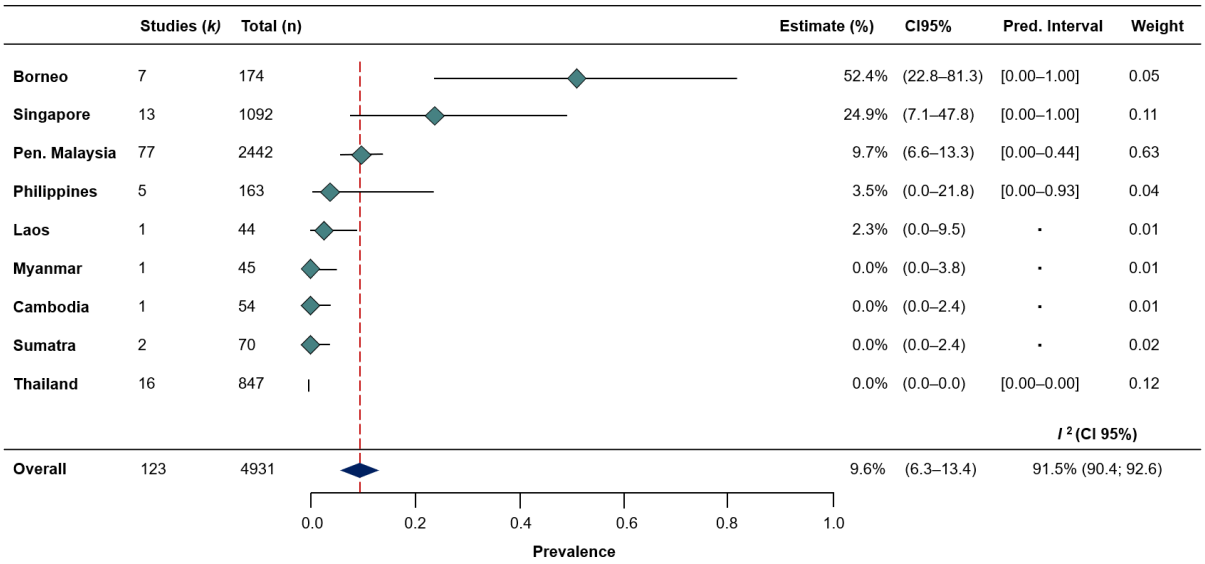

Figure 2. *Pooled estimates for* P. knowlesi *prevalence (%) in all NHP species tested in Southeast Asia. Random-effects analysis on data disaggregated by sampling site, sub-grouped by region. N=123.*

(c) Risk factor analysis

Only 48.8% (n=60/123 records) of data reported survey year, meaning there was insufficient data to assess temporal patterns. Forest cover ranged from negligible to near total cover (100%) within buffer radii (Table S12). Covariate data extracted is illustrated in Supplementary Information (Figure S8–9). Host species was grouped as '*Macaca fascicularis'* or 'Other' to account for sample counts of <10 for several species. Following a forward stepwise selection strategy, all covariates were retained in the final model. Forest cover and PARA were included at both 5km and 20km.

When adjusted for all other covariates in the model, we identified strong evidence of a negative quadratic effect between perimeter: area ratio (PARA) and higher prevalence of *P. knowlesi*, with the strongest associations at intermediate levels of boundary complexity within a 20km radius (p<0.0001). Evidence was also found for an association with likelihood of *P. knowlesi* in NHPs per SD increase in forest cover over 5km (aOR=1.69, CI95% 1.23–2.31; p=0.0012) and over 20km (aOR=4.98, CI95% 3.14–7.89; p<0.0001), and with *M. fascicularis* relative to all other non-human primate species (aOR=2.69, CI95% 2.13–3.41; p<0.0001). Additional complexity did not provide a more optimal model fit and effect modification was not pursued. Final adjusted OR for the multivariable model can be visualised in Figure 3a, with the marginal effect curve for perimeter: area ratio at 20km in Figure 3b.

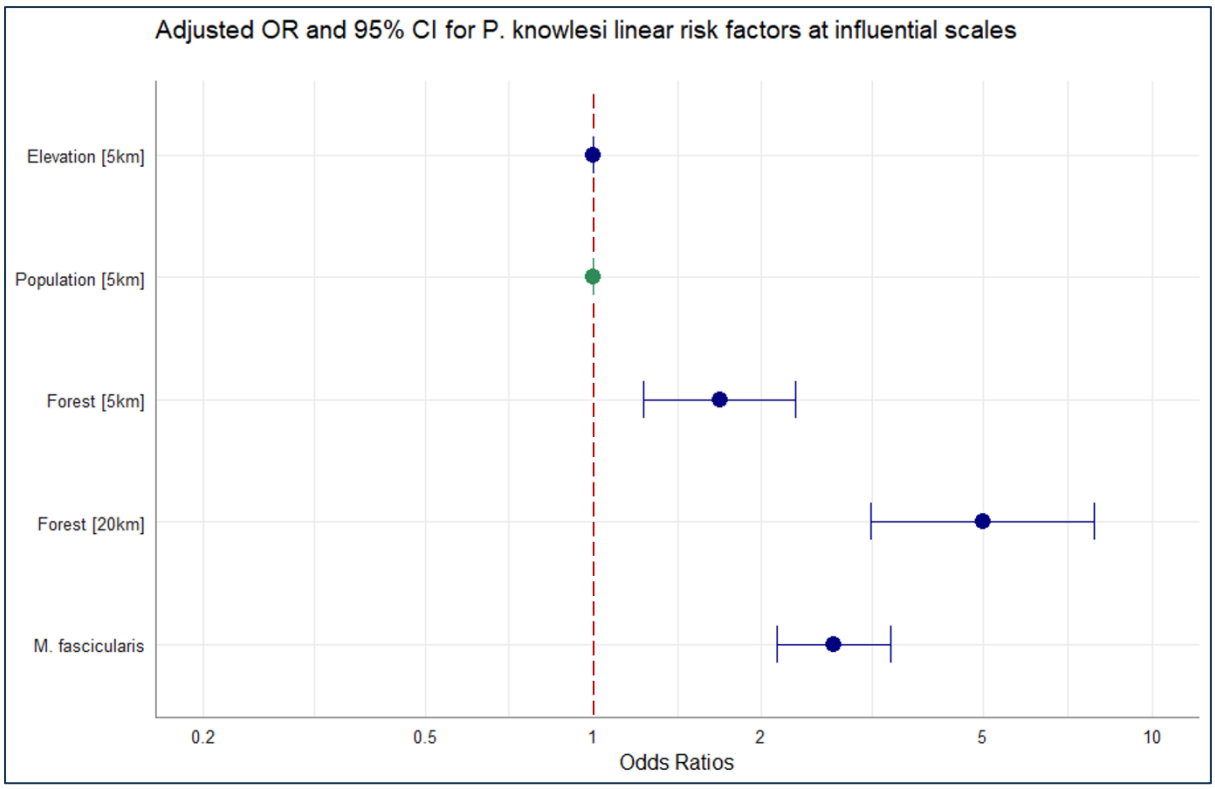

Figure 3a. *Multivariable regression results. Spatial scale denoted in square bracket. Forest =% forest cover. Adjusted OR (dots) and CI95% (whiskers) for factors associated with* P. knowlesi *in NHPs at significant spatial scales. N=1230.*

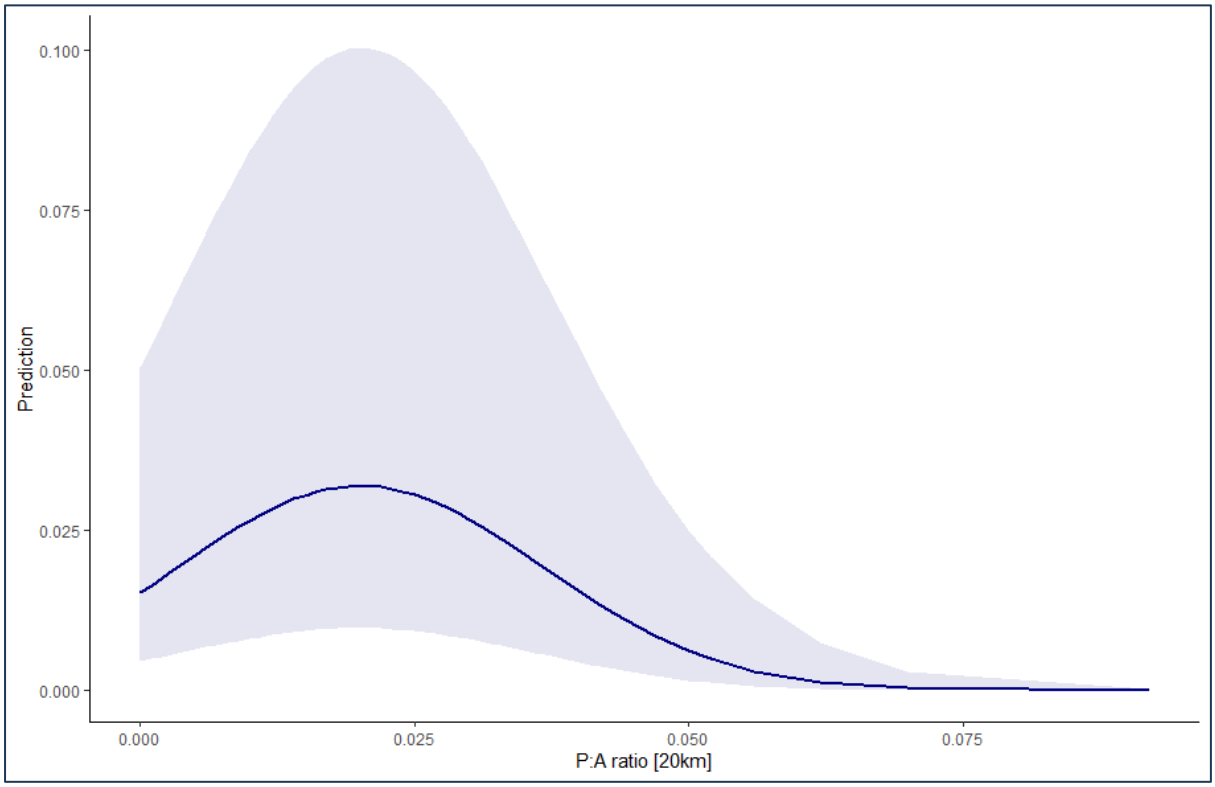

Figure 3b. *Multivariable regression results. Marginal effect curve (line) and CI95% (ribbon) for effect of increasing PARA on* P. knowlesi *prevalence at 20km. N=1230.*

DISCUSSION

LULCC are widely linked to spillover of zoonotic pathogens from sylvatic reservoirs into human populations, and pathogen prevalence in wildlife host species is key in driving the force of infection in spillover events. Our initial analyses found that for *Plasmodium knowlesi,* there is substantial spatial heterogeneity and prevalence in non-human primates (NHPs) varies markedly between regions of Southeast Asia[69]. Consistent with our hypothesis that parasite density in primate hosts would be higher in areas experiencing habitat disturbance, we identified strong links between *P. knowlesi* in NHPs and measures of contemporaneous forest fragmentation. The most compelling result is that the strongest associations are found at intermediate levels of fragmentation, indicating that there may be an optimum level of habitat complexity that potentiates *P. knowlesi* in NHPs. To our knowledge, this is the first systematic study to find evidence of landscape changes influencing the distribution of *P. knowlesi* prevalence in NHPs. Results offer evidence that *P. knowlesi* infection rates in NHPs are linked to changes in land use across broad spatial scales, and that prevalence of *P. knowlesi* in reservoir species may be driving spillover risk across Southeast Asia. These findings could provide insight to improving surveillance of *P. knowlesi* and to the development of ecologically targeted interventions.

While previous studies have estimated that *P. knowlesi* infection would be chronic in all macaques, or as high as 50–90% for modelling *P. knowlesi* transmission in Malaysia[44], this data strongly suggests that this is not the case. Overall prevalence of *P. knowlesi* infection in all NHPs is markedly lower than usual estimates, emphasising the importance of accounting for 'negative' data in estimations of prevalence. Considerable heterogeneity was identified between and within regional estimates for *P. knowlesi* across Southeast Asia, which likely reflects genuine differences according to distinct climates and distinct habitats[76]. Malaysian Borneo has an estimated prevalence over five-fold higher than West Malaysia. Crucially, such extreme prevalence estimates for NHPs in Borneo align with the known hotspot for human incidence of *P. knowlesi*[24]. In Peninsular Malaysia, estimated prevalence is far lower than anticipated. Cases of human *P. knowlesi* do occur in West Malaysia, though transmission has been found to exhibit spatial clustering[77] which may correspond to pockets of high risk within the wider context of lower prevalence of *P. knowlesi* in macaques. Regional trends in *P. knowlesi* will mask differences in infection rates between sample locations, driven by more localised factors. Multiple studies reported finding *P. knowlesi* infections in wild macaques to be low or absent in peri-domestic or urbanised areas, attributed to the absence of vector species typically found in forest fringes[78–80]. This pattern is seen in reports from Peninsular Malaysia[66,68] , Singapore[63,64] and Thailand[22,75]. The high heterogeneity of reports here suggests that the picture is even more complex. *P. knowlesi* infections may even vary between troops within a single study site, as was seen in the Philippines[62]. Fine-scale interactions are unlikely to be captured by the scale of this study.

Ecological processes determining *P. knowlesi* infection are influenced by dynamic factors over multiple spatial scales[43]. We utilised a data-driven methodology to select variables at distances that capture maximum impact on *P. knowlesi* prevalence[32,42], with surface elevation and human population density most influential at localised scales (5km) and forest cover and forest fragmentation showing strongest influence within 20km radii. Contrary to previous studies on risk factors for human incidence of *P. knowlesi*[31,32], geographical elevation and human population density were not found to be associated with *P. knowlesi* in NHPs. In Southeast Asia, species composition can vary substantially across tropical ecotones. Mosquitos of the Leucosphyrus Complex and Dirus Complex, primary vectors of *P. knowlesi*, each exhibit specific ecological niches in respective geographic areas. The extent of Southeast Asia is also likely to encompass a wide range of putative vectors across different landscapes, such as those of the Minimus Complex in northern regions[81] or the recently incriminated *An.-collessi* and *An.-roperi* from the Umbrosus Group[82]. Mean values within buffer distances may also mask finer-scale variation for a given area. As such,

different ranges of permissive altitudes for vectors and host species niches may explain the lack of observed relationship between elevation and human density and *P. knowlesi* in NHPs.

A key finding is the link between high prevalence of *P. knowlesi* in primate host species and forest fragmentation. Other studies have linked habitat fragmentation to increased generalist parasite density in primates. In Uganda, a higher prevalence and infection risk of protozoal parasites was observed in wild populations of red colobus primates (*Procolobus rufomitratus*) inhabiting fragmented forests compared to those in undisturbed habitat[83]. Interestingly, we observed a bell-shaped curve in the relationship between fragmentation and *P. knowlesi* prevalence, indicating an 'ideal' amount of forest complexity that encourages prevalence in primate hosts. For *P. knowlesi,* creation of edge habitat is thought to favour vectors of the Leucosphyrus Complex[1,37]. *Anopheles* spp. presence can be predicted by indices of fragmentation in Sabah, Borneo, with changing land use creating more suitable micro-climate for larval habitats[42], and an increased abundance of *An. balabacensis* found in forest fringes[20,37]. Increasing complexity of forest boundaries results in increased density of edge habitat, with conceivably higher density of vectors in forest fringes. Therefore, preferential use of fringe habitat and high exposure to vectors in forest fringes may contribute to higher conspecific transmission of *P. knowlesi* between primates in increasingly fragmented habitats. Conversely, more sizable forest fragments may be necessary to provide sufficient resourcing for macaques and to maintain transmission cycles[20,80]. Individual-based disease models combined with movement ecology approaches have shown that for species with larger perceptual ranges, the effects of habitat fragmentation are nonlinear and parasite transmission is less supported by the most highly fragmented areas[84]. In Sabah, individual macaques were shown to increase ranging behaviour and movement in response to deforestation[15]. This interplay between disease ecology and metapopulation theory may explain why moderate levels of habitat complexity pose a greater risk for simian *P. knowlesi* than maximally fragmented landscapes, at which point the smaller forest patches are insufficient to support macaque troops and have lower population occupancy. Likewise, this may relate to the finding that in Borneo, lower fragmentation indices (larger forest patches) were associated with *P. knowlesi* spillover in Borneo[32].

Our finding that prevalence of *P. knowlesi* in macaques is higher in fragmented habitat also lends clarity to evidence that forest fragmentation is a risk factor for human exposure to *P. knowlesi* in Malaysian Borneo[3,32]. In Brazil, re-emergence of Yellow Fever Virus in both simians and humans has been linked to areas with highly fragmented forest [10]. Recent mechanistic models have demonstrated that the effect of landscape changes on risk of disease spillover into human populations is also nonlinear, with the greatest probability of disease emergence at intermediate levels of habitat loss. Edge density is thought to peak at moderate levels of land conversion[6], which alters respective population structure and facilitates inter-species contact between forest interiors and human matrices[12]. Studies have also posited that vector species could adapt to match changes in macaque-human host availability[85]. Changes in relative host density, vector density and wildlife parasite prevalence in nascent forest fringes may enhance the spillover potential of this disease system into human populations in fragmented habitats. Overall, this finding offers an insight to understanding the mechanisms that underpin the increased force of infection of *P. knowlesi* that is associated with LULCC.

There are limitations to consider in the interpretation of these findings. 'Small-study effects' were observed in the dataset, suggestive of a bias toward positive effect estimates[48]. This may be a result of data disaggregation and small samples creating artefactually higher estimates or may reflect true bias in data collection toward areas known to be endemic for *P. knowlesi* and convenience sampling of macaques. Systematic sampling in key locations would be required to address these gaps. Variability in study designs and data reporting

effected geospatial accuracy. Steps were taken to account for spatial bias by extracting covariates at randomly generated pseudo-sampling points. Whilst uncertainty cannot be eliminated, we demonstrate a robust methodology to accommodate for geographical uncertainty in ecological studies. Assumptions have been made that sample site equates to habitat, which may not reflect actual habitat use. Effects of fragmentation are likely to be dependent on land conversion type, species composition and surrounding matrix habitat. Results are based on broad regional ecological trends and may not be generalisable at individual levels, or to all putative host species in all geographic contexts[69]. Use of perimeter: area ratio (PARA) as a fragmentation index was justified given high forest coverage[57], though Edge Density (ED) or normalised Landscape Shape Index (nLSI) might be more appropriate in future analyses to account for variation in forest abundance. Composite fragmentation index (CFI) is another metric based on pixel-classification methods of Vogt et al. that explicitly accounts for the ratio of forest "core", "margin" and "patch" areas. Since specific land configurations have been linked to *P. knowlesi* exposure in Borneo[32], broad forest classifications used here may mask important differences in *P. knowlesi* between land classes. As it was not possible to include contemporary land cover classification in this analysis, future studies would benefit from looking at forest type (e.g. primary or agroforest).

Strong links have been identified between land use changes and ecosystem perturbation that favours the transmission of vector-borne diseases[2]. Prevalence of *P. knowlesi* in macaques is likely to be a crucial determinant of human infection risk, and more representative estimates of *P. knowlesi* prevalence derived here might better inform regionally-specific transmission risk models. This study characterises risk factors for heightened prevalence of *P. knowlesi* in NHPs. Findings provide evidence in support of the premise that *P. knowlesi* in primate hosts is partly driven by LULCC across Southeast Asia. While the full complexity is not captured by the covariates used, it is clear that *P. knowlesi* infection in NHPs is not restricted to densely forested areas. This study also demonstrates the utility of systematic meta-analysis tools and remote-sensing datasets in the investigation of macroecological disease trends, in conjunction with methods to standardise a spatially heterogeneous dataset and data-driven selection of spatial scales. Gaps identified in data reporting should inform more systematic and localised primatological surveys to disentangle precise mechanisms. Notwithstanding limitations, this study highlights the marked spatial heterogeneity and role of landscape complexity in *P. knowlesi* infection rates in NHPs. Given the clear intersection between human epidemiology and wildlife ecology, it is essential that infection dynamics within wildlife reservoirs are considered in future public health interventions.

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