# Deforestation as a driver of emerging viral spillover events at the human-animal-environment interface in Malaysian Borneo

Grace Ikuko Hood BSVc MPH (PhD candidate), Kimberly Fornace PhD (PhD advisor), Miles Carroll PhD (PhD advisor)

# **Project Narrative**

# I. Abstract

Malaysian Borneo has undergone extensive deforestation over the past 40 years [1], significantly altering the epidemiology of zoonotic diseases in both humans and non-human primates [2, 3]. The Stability of Altered Forest Ecosystems (SAFE) project in Malaysian Borneo is one of the world's largest ecological studies, and examines the effect of a planned conversion of old growth forest to oil palm plantations on ecosystems. Our project aims to utilise the ecological gradient of the SAFE project to describe the prevalence of zoonotic viruses in macaques across different landscapes, using multiplexed serology and pathogen-agnostic metagenomic sequencing. By combining serological and molecular measures of disease prevalence with earth observation and biodiversity data we aim to produce a One Health investigation into the drivers of viral emergence at the human-animal-environment interface.

# II. Background & Introduction

Tropical regions with high levels of biodiversity, urban expansion and rapid ecological change are considered most at risk for spillover: the cross-species transmission of pathogens. Land-use changes such as deforestation drive spillover by disturbing host ecology, simultaneously increasing both human-animal interaction and pathogen excretion from the reservoir [4-6].

Malaysia has one of the highest rates of landscape change globally, with 34% of the land area of Borneo deforested over the past 40 years [1]. These rapid landscape changes have been implicated in the significantly altered epidemiology of zoonotic diseases, such as the 50-fold increase in zoonotic malaria over the past 20 years [2, 3], and human outbreaks of chikungunya and Zika virus alongside detection in non-human primates (NHPs) [7, 8]. NHPs serve as an important disease reservoir, and villagers report significant contact (21% report weekly sightings), highlighting this as an important interface [9].

# III. Project goals

The overall objective of my doctoral research is to investigate spillover events and their drivers, by combining sero-epidemiological and molecular data with earth observation data. Thus far, my work has focussed on quantifying the seroprevalence of high-consequence zoonotic viruses in wildlife hunters in Forested Guinea, Republic of Guinea from 2017 – 2023, and the role of deforestation.

Having completed the fieldwork and laboratory studies to complete these aims, I hope to use the Dr. Gregory D. Bossart Memorial scholarship to fund a project applying similar methodologies to another high-risk interface. Here, I hope to investigate the prevalence of key zoonotic pathogens in macaques (*Macaca fascicularis* and *M. nemestrina*) living in proximity to humans, across different landscapes in Malaysian Borneo. The proposed project will build upon the Stability of Altered Forest Ecosystems (SAFE) project, which is a large-scale land-use study examining the effect of human landscape modification. The SAFE experimental design allows for the comparison of conserved primary forest (1020 km<sup>2</sup>) to an experimentally fragmented landscape (100 km<sup>2</sup>) of logged forest, fragmented forest, and oil palm plantations to elucidate the impact of landcover on various ecological and social factors. This includes an ethics-approved and funded longitudinal study investigating the impact of land-use on macaque populations and the transmission of zoonotic malaria

### Dr. Gregory D. Bossart Memorial One Health Scholarship application

and arboviruses. Using detailed landcover data and biodiversity metrics, five sites from distinct habitats were identified based on their proximity to humans. From late 2024, macaques will be cross-sectionally trapped at these locations and tested for malaria and arboviruses.

Here, a surveillance gap exists, especially for other zoonoses, including the high-consequence filoviruses, which includes Reston virus, which was isolated in macaques in the Philippines [10]. The Sabah Wildlife department has highlighted their limited diagnostic capacity for novel pathogens as a challenge, and identified the need for non-invasive sampling techniques as a priority.

The Bossart scholarship would be used to fund the further analysis of the macaque samples to detect molecular and serological evidence of pathogens, focussing on high-consequence viral families, and pathogen-agnostic sequencing methods for non-invasive samples.

This project aims to:

- 1. Describe the seroprevalence of zoonotic viruses in macaques relative to land type.
- 2. Describe infection in macaque faeces through next-generation sequencing (NGS).
- 3. Investigate the impact of landcover, forest type and biodiversity on pathogen distribution.

#### IV. Methods

Macaques will be trapped by teams led by a wildlife veterinarian at the Sabah Wildlife Department. Serum will be screened by multiplexed bead-based immunoassay for filoviruses and paramyxoviruses. Faeces will be sequenced by Nanopore NGS. High-resolution biodiversity and landcover data will be provided by the existing study, and include satellite-based remote-sensing data, drone surveys and passive acoustic monitoring devices. Molecular and serological results will be examined along an ecological gradient to examine the effects of landcover on pathogen distribution, using hierarchical Bayesian modelling approaches.

#### V. One Health framework

By leveraging the combination of field, laboratory and computational studies we aim to provide transdisciplinary insight into landscape immunity, "the ecological conditions that reduce the risk of pathogen spillover from reservoir hosts" [5]. We hope for such data to inform sustainable nature-based One Health solutions which acknowledge the importance of biodiversity as both a conservation and biosecurity priority [5].

#### VI. Importance & contribution

This project provides a unique and time-sensitive opportunity. Given the rapidly changing ecology and epidemiology of Malaysian Borneo, establishing a serological and virological baseline in this important reservoir species is imperative. These findings will be provided to local public health officials and policy makers, providing important context during the ongoing construction of Nusantara, the planned capital city being established in neighbouring Indonesian Borneo.

By nesting this project within SAFE, we access highly detailed landcover data and biodiversity metrics to stratify our analysis, exploit a unique opportunity to cost-effectively maximise sample use and pilot the utility and feasibility of on-site NGS in non-invasive samples for surveillance; filling a key needs gap identified by the Sabah Wildlife Department.

As per the One Health High-Level Expert panel recommendations [11], access and inclusivity were central considerations, and local stakeholders will be actively engaged throughout in community feedback workshops.

#### VII. Budget

Item	Quantity	Unit cost (USD)	Total (USD)	Rationale
MinION Mk1B (Mk1B, Flow Cells, Wash Kit, Control expansion Kit, Direct RNA sequencing Kit)	1	2700	2700	
Native barcoding Kit 24 V14	1	800	800	
Laptop	1	1500	1500	Required for MinION with high- efficiency M3 silicon-chip [12].
Total			5000	

#### Word count: 999 (excluding references)

#### References

- 1. Gaveau, D.L., et al., *Rapid conversions and avoided deforestation: examining four decades of industrial plantation expansion in Borneo.* Sci Rep, 2016. **6**: p. 32017.
- Cooper, D.J., et al., Plasmodium knowlesi Malaria in Sabah, Malaysia, 2015-2017: Ongoing Increase in Incidence Despite Near-elimination of the Human-only Plasmodium Species. Clin Infect Dis, 2020. 70(3): p. 361-367.
- 3. William, T., et al., *Increasing incidence of Plasmodium knowlesi malaria following control of P. falciparum and P. vivax Malaria in Sabah, Malaysia.* PLoS Negl Trop Dis, 2013. **7**(1): p. e2026.
- 4. Eby, P., et al., *Pathogen spillover driven by rapid changes in bat ecology*. Nature, 2022: p. 1-3.
- 5. Plowright, R.K., et al., *Land use-induced spillover: a call to action to safeguard environmental, animal, and human health.* Lancet Planet Health, 2021. **5**(4): p. e237-e245.
- 6. Kessler, M.K., et al., *Changing resource landscapes and spillover of henipaviruses*. Ann N Y Acad Sci, 2018. **1429**(1): p. 78-99.
- 7. Sam, I.C., et al., *Chikungunya Virus in Macaques, Malaysia*. Emerg Infect Dis, 2015. **21**(9): p. 1683-5.
- 8. Wolfe, N.D., et al., *Sylvatic transmission of arboviruses among Bornean orangutans*. Am J Trop Med Hyg, 2001. **64**(5-6): p. 310-6.
- 9. Klim, H., et al., *Quantifying human-animal contact rates in Malaysian Borneo: Influence of agricultural landscapes on contact with potential zoonotic disease reservoirs.* Frontiers in Epidemiology, 2023. **2**.
- 10. Miranda, M.E.G. and N.L.J. Miranda, *Reston ebolavirus in humans and animals in the Philippines: a review*. The Journal of infectious diseases, 2011. **204**(suppl\_3): p. S757-S760.
- 11. Mettenleiter, T.C., et al., *The One Health High-Level Expert Panel (OHHLEP)*. One Health Outlook, 2023. **5**(1): p. 18.
- 12. Oxford Nanopore Technology, *Oxford Nanopore meets Apple's M3 silicon chip, hailing a new era of distributed genome sequencing.* 2023, ONT: Oxford.