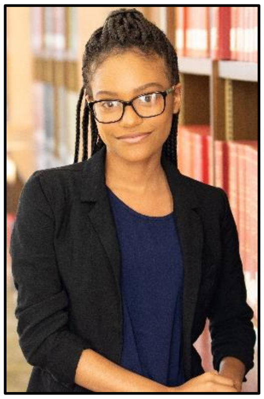

Understanding Hendra Virus Spillover from Pteropus Using Satellite Imagery of Critical Habitat

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Abstract

Hendra virus (HeV) is an emerging infectious disease that is endemic on the continent of Australia. The virus naturally and asymptomatically exists in Australian fruit bats (*Pteropus*) and causes severe or even fatal disease in horses and humans. Geographic overlap between these bats and horses leads to spillover of HeV. The frequency of contact is likely correlated with the decline in the abundance of Eucalypt trees, the bats' primary winter food source, which forces the bats to forage in more urbanized areas. This study explores the changes in Eucalypt population over space and time as a driver in HeV spillover. We use high-resolution satellite imagery to identify species that comprise critical foraging habitat. These findings can facilitate the targeted preservation of critical foraging habitat for *Pteropus* as a means of disease management.

Introduction

Changes in land use have had a historical impact on the emergence of novel infectious diseases to the human population. The rapid clearing of vegetation on the continent contributes significantly to Australia's ecology. In recent years, there has been a common cause-effect relationship between deforestation and zoonotic spillover events by bats. The University of New South Wales Newsroom reports that, "More than 20 million trees are cleared every year in Queensland alone." (1) We hypothesize that deforestation, specifically of Eucalyptus trees, is an underlying cause of Hendra virus (HeV) spillover in Australia. Deforestation in Australia was largely focused on the agriculturally suitable soil of the continent's eastern coast; primarily in Queensland (Qld), New South Wales (NSW), and Victoria (2). Currently, Hendra virus is a significant public health concern in Qld and NSW.

Despite the increasing concern regarding Hendra virus in Australia, the environmental dynamics of the epidemiological triangle (host, pathogen, and environment) for Hendra virus are not fully understood. A zoonotic spillover event occurs when the reservoir host, which is the species that naturally carries the pathogen, successfully transmits the pathogen to a susceptible individual of another species. A pathogen will be successfully transmitted if the alignment of factors between the host, pathogen, and environment permit the necessary conditions for the pathogen to transmit and persist (3).

Gaining a better understanding of these relationships, particularly the environmental factors, is critical to current efforts to manage emerging infectious disease.

Hendra virus first spilled over in 1994 in Australia and is currently endemic in fruit bats on the continent, predominantly along the eastern coast. Hendra virus is a single-stranded RNA virus belonging to the Henipavirus genus, which naturally exists in Australian fruit bats of the genus *Pteropus* (4). Viruses from bats have been the causative agent in multiple spillovers and infectious disease outbreaks including SARS, Marburg virus, Nipah virus, Ebola virus, and Hendra virus (4). Such viruses have been found to naturally and asymptomatically exist within bats, the reservoir host, but can cause severe or fatal disease in humans and other species.

Spillover of Hendra virus from Australian fruit bats to humans follows a pattern of transmission. The virus is first shed by the bats in feces, urine, or saliva and can then infect a susceptible horse, which serves as an intermediate host, or bridge species, amplifying the virus and facilitating further transmission to other susceptible horses, humans, and other species. Hendra virus cannot be directly transmitted from bats to humans. As the geographic overlap between bats and horses increases, the occurrence of a spillover event becomes more probable. While bats forage near horses, feeding on foods of lower nutritional quality, the virus is more likely to be transmitted.

The current hypothesis is that Hendra virus spillover events occur as a direct result of nutritional stress in *Pteropus* (1). Australian fruit bats rely on nectar from flowering eucalypt trees as a primary winter food source. Changes in human land use and deforestation have led to a decline in the eucalypt tree population and eucalypt tree species flower unreliably (1). Furthermore, changes in human land use and deforestation have led to a decline in the Eucalypt tree population. In response, *Pteropus* bats have been driven to relocate to more urbanized areas where food is more readily available but of lower nutritional quality (2). Resultantly, the bats become nutritionally stressed and may potentially excrete larger amounts of virus, which increases the probability of spillover occurrence. Recent studies have aimed to quantify the effect of environmental factors that impact Eucalypt phenology on bat migration patterns and spillover occurrence with reasonable but limited success (1). Further research is crucial to developing a deeper understanding of Hendra virus and reducing its impact on global health.

We use satellite imagery to identify areas of critical foraging habitat for Australian fruit bats. This study aims to identify Eucalypt tree species based on their unique spectral signature at high resolution and in mixed vegetation forests. In prior studies, satellite imagery aided in performing retrospective analyses of human population fluxes (3) and changes in vegetation (4) with high levels of accuracy. We compared the satellite imagery to ground-truthed data collected in Australia, to create models that accurately identify the location of eucalypt trees. These findings help to prioritize areas for conservation and/or restoration of critical foraging habitat for Australian fruit bats in future management efforts of Hendra virus.

Materials & Methods

Study Region

We used vegetation maps created by the Australian Government: Department of Environment and Energy's "National Vegetation Information System" (NVIS) GIS file (5) to identify eucalypt populations along the eastern coast of Australia. The map provided a rough estimate of areas of land in Australia that were covered by eucalypt tree species. Our study area was confined to areas within the Australian states of Queensland (Qld) and New South Wales (NSW) because both states contain sites of HeV spillover. Our study area was further narrowed into areas of interest (AOIs) based on the following criteria: a protected land area where eucalypt trees are known to exist (Site A: Pelion Forest Reserve, Qld), an area where eucalypt trees and flying fox species have been studied prior to the first HeV spillover event in 1994 (Site B: Bateman's Bay, NSW), and where the human and bat populations are highly likely to overlap due to recent human population growth (Site C: Gold Coast, Qld).

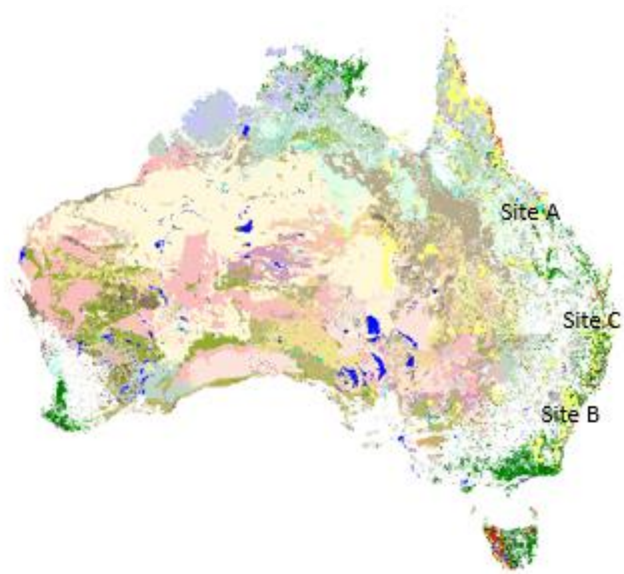

Figure 1: Overlaid vegetation map & protected land maps as viewed in ArcGIS. Selected study sites shown. Areas of forest primarily made of eucalypt tree species are depicted in dark green.

Selection of Site A considered land areas that are protected by the law as nature reserves or forest reserves. We overlaid the vegetation map and protected land map to identify this site (Figure 1). Such reserves are significant to our study as they are areas of land that are not disturbed by the surrounding human population. Nature and forest reserves allow us to assess changes in the eucalypt population mainly due to environmental effects. We identified the nature and forest reserves significant to the study area using the "2016 Collaborative Australian Protected Areas Database" (CAPAD) GIS file provided by the same government source (5).

Ground-truthed data for Site B provided information on the locations and blooming events of eucalypt trees within the area in 2012 (Figure 1). However, our satellite imagery for this study was from 2016 – 2018. Further analysis of our satellite imagery will be done to verify our results using more recent ground-truthed data. This information was used to test the accuracy of satellite imagery in detecting eucalypt trees. Site C was selected due to the current increases in the human population and deforestation within the area (Figure 1). This site allows us to assess changes in eucalypt tree abundance as a direct effect of human pressure for land.

Vegetation Analysis

We utilized ESRI's ArcGIS ArcMap (6) mapping program to analyze vegetation maps of the study's geographic area using the 2018 Vegetation map (5). The maps show the locations of various vegetation groups across the continent. We calculated the areas of land covered by eucalypt trees in Australia using ArcMap. This provided an estimate for how much land-cover we would expect to observe in the satellite imagery of our study sites. Land-cover for all assessed vegetation outside of protected land areas were considered as results of environmental conditions, deforestation, and other human-related factors. We assessed available ground-truthed data in the study sites which provided accurate accounts of changes in the Eucalypt population over time. We also calculated the total area of protected land in Qld and NSW from the 2016 CAPAD map (5) using ArcMap.

Imagery Selection

Bounding boxes allowed us to gather imagery of an exact land area based on the decimal degree location of the corners of the bounding box. Table 1 provides the upper left (UL) and lower right (LR) boundaries for all study sites. For each study site, we acquired satellite imagery for the years 2016, 2017, and 2018. The selection of the satellite imagery took multiple factors into account. We only selected images that covered 100% of the area within our bounding box. We also only selected imagery that had minimal, if any, cloud cover. Additionally, the imagery used in the study was 4-band multispectral imagery which allowed us to use mathematical algorithms on the reflectance of red light, blue light, green light, and near infrared light. Satellite imagery used for this study (Table 2) was acquired from the satellite company *Planet*, which provides multispectral (4 band) satellite imagery captured at 3-5m resolution (7). When necessary, we selected multiple images for a specific day to ensure 100% coverage of the area within our bounding boxes.

Table 1: Bounding boxes within study sites

Study Site	Upper Left Boundary	Lower Right Boundary
Site A: Pelion Forest Reserve, Qld	21.02°S, 148.62°E	21.09°S, 148.70°E
Site B: Bateman's Bay, NSW	35.67°S, 150.17°E	35.77°S, 150.22°E
Site C: Gold Coast, Qld	27.95°S, 153.24°E	28.13°S, 153.43°E

Table 2: Detailed description of the satellite imagery used in this study. All images used for the specified site and year were captured by the same sensor. Image details are listed in Coordinated Universal Time (UTC)

Year of Imagery	Site A	Site B	Site C
2016	July 23, 2016 22:42 UTC	August 25, 2016 23:10 UTC	July 19, 2016 23:02 UTC
2017	July 21, 2017 23:31 UTC	May 15, 2017 23:12 UTC	June 7, 2017 23:11 UTC
2018	July 5, 2018 23:45 UTC	May 8, 2018 23:27:49 UTC	July 16, 2018 23:19:03 UTC

<u>Satellite-Based Analysis</u>

Analysis of all satellite imagery was performed using ArcGIS. We calculated approximate chlorophyll levels for the vegetation within the study region using Normalized Difference Vegetation Index (NDVI). NDVI uses red light and near infrared light reflectance as a proxy for chlorophyll to measure vegetation growth and health using the formula $(R_{NIR}-R_{Red})/(R_{NIR}+R_{Red})$. This measurement assigns a number to the amount of "greenness" observed in the imagery on a scale of -1.0 to 1.0. The scale allows us to more clearly distinguish vegetation from other types of land cover captured in the image such as road, bodies of water, and man-made structures. We then used the Eucalypt Chlorophyll a Reflectance Ratio (ECARR) and Eucalypt Chlorophyll b Reflectance Ratio (ECBRR) to distinguish eucalypt trees from other vegetation species (8). We calculated the ECARR and ECBRR using the formulas developed by Datt, $ECARR=0.0161[R_{672}/(R_{5503}xR_{708})]^{0.7784}$ and $ECBRR=0.0337[R_{672}/R_{550}]^{1.8695}$ (8). These algorithms recognize the unique spectral signature of eucalypt trees based on their reflectance peaks at green and near infrared light for chlorophyll a and chlorophyll b. Applying these algorithms to multispectral satellite imagery distinguishes probable locations of eucalypt trees from other vegetation.

Results

The Australian mainland has a total land area of 765.9 million hectare (ha) (9). The 2018 vegetation map revealed that of this total land area, approximately 654.4 million ha is covered by vegetation. In 2018, approximately 196.28 million ha of Australian land was covered by eucalypt trees (5) accounting for 25.6% of the continent's vegetative landcover (Figure 2).

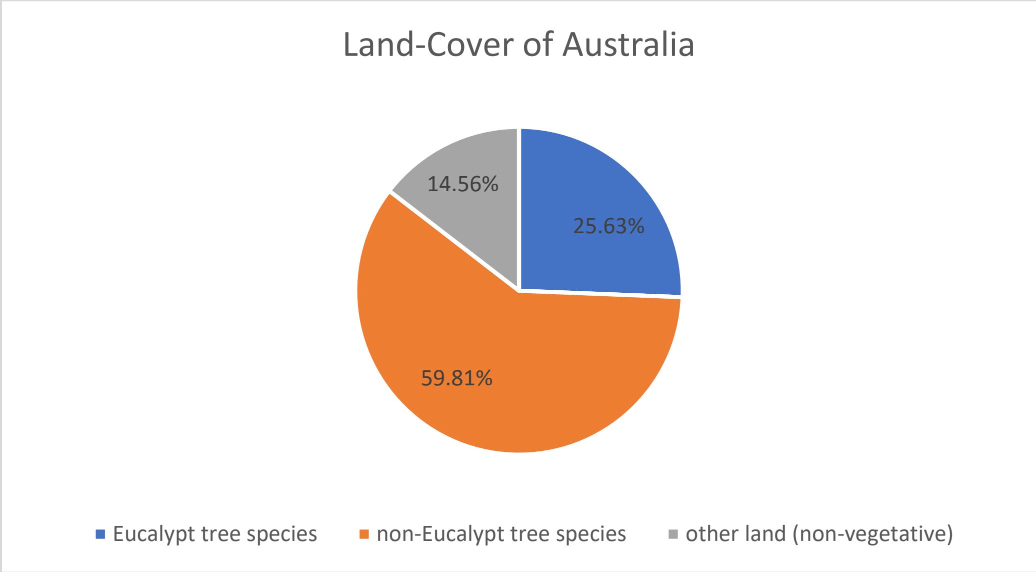

Figure 2: The percentage of Australia's total land area that is covered by eucalypt trees, non-eucalypt vegetation, or non-vegetative land areas (i.e. human habitat, bodies of water, cleared or barren land)

Furthermore, calculations from the 2016 CAPAD (5) map revealed that approximately 154.4 million ha of Australia's total land area (765.9 million ha) is protected by government agencies. Of this protected land, 7.78 million ha (5%) and 15.96 million ha (10%) are located within NSW and Qld respectively.

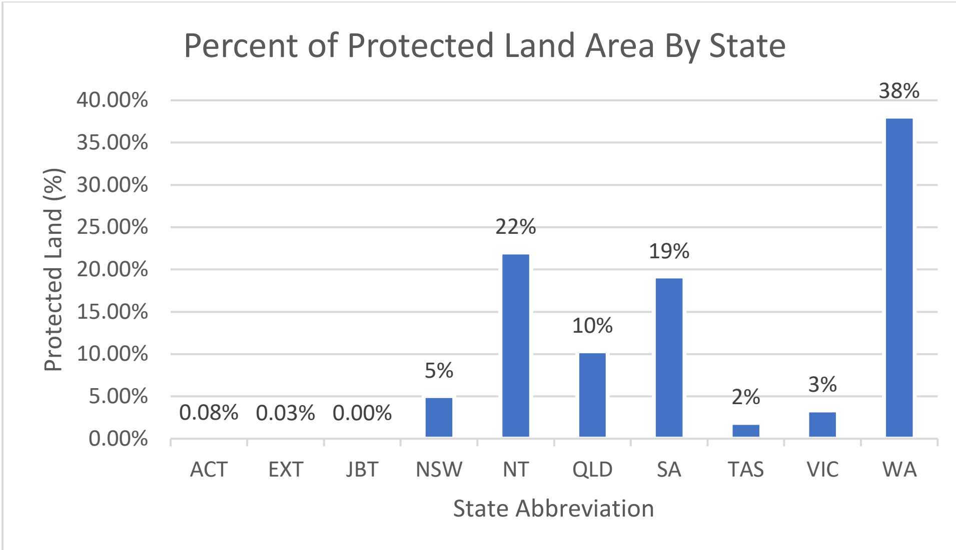

Figure 3: The percentage of protected land per state as a portion of the total protected land area in Australia.

Within Site A and Site C we found evidence of eucalypt trees. However, in Site B we could not identify any eucalypt trees. Within Site A (Pelion Forest Reserve, Qld) we observed eucalypt trees consistently from 2016-2018. This indicates that when in areas of protected land and under environmental conditions these eucalypt tree populations are growing. This area can serve as a significant 'control' for quantifying changes in eucalypt abundance against deforestation rates in other areas of Australia.

Our findings for Site B (Bateman's Bay, NSW) were unexpected. We did not find any evidence of eucalypt trees in this area although we are certain that they are there. Since we know definitively from our 2012 ground-truthed data that eucalypt trees were located within Site B, further investigation into our results must be done using up-to-date ground-truthed data for verification of eucalypt presence or loss. We may have to adjust our bounding boxes or adjust the sensitivity of the ECARR and ECBRR algorithms for this site. We plan to use our ground-truthed data for this region to verify if there are indeed eucalypt trees in the area we observed using satellite-imagery. If the trees are on this area, then we must adjust the algorithms' sensitivity to be able to visualize them in the imagery. If our ground-truthed shows that there are no eucalypts within our bounding box area, we will then select another area within Bateman's Bay where we know the trees are located to assess using satellite-imagery.

Within Site C (Gold Coast, Qld), we analyzed satellite-imagery from 2017 and 2018 we identified the presence of eucalypt tree species. We observed a decline in eucalypt tree abundance in this area from June 2017 to July 2018. In recent years, Gold Coast has been an area of rapid population growth and land use change. The decline in eucalypt abundance is likely due to increasing land pressure and deforestation in the area. However, we will need ground-truthed data or higher-resolution satellite imagery to confirm this hypothesis.

Overall, these results indicate that we can identify eucalypt trees in mixed forests using high-resolution satellite imagery. We can use reflectance ratios to distinguish likely Eucalypt trees and can provide valuable information on changes in Eucalypt abundance.

Discussion

Understanding HeV spillover in Australia required an understanding of the complex ecological systems that naturally exist in the continent and how human interactions with the land influences these systems. Eucalypt tree species were shown to be located predominantly on the eastern coast of Australia when assessing the 2018 vegetation map. The rapid rates of deforestation along Australia's eastern coast play a significant role in the occurrence of HeV spillover. Deforestation along the eastern borders of Queensland and New South Wales is likely a factor for the increased frequency in Hendra virus spillover occurrence within the past decade. Deforestation leads to loss of habitat and winter food (eucalypt tree nectar) for flying foxes and results in HeV spillover events. Understanding and managing HeV spillover requires and understanding of the environmental and ecological consequences of human changes in land use.

The ability to identify eucalypt trees in mixed forests using high-resolution satellite imagery is significant to conservational efforts. Accurate identification of eucalypt trees using satellite-imagery allows us to assess large areas of land in the past and present. The use of satellite imagery, as opposed to data collected from a field team, allows for a quicker assessment of the land. This allows for the quantification of changes in eucalypt tree abundance within our study regions. These measurements will allow us to see how the abundance of eucalypts is changing between study sites. We can also assess the rates of deforestation in these areas to see if they correlate with the observed loss of eucalypt trees.

Further research must be done for the targeting of specific land areas for eucalypt conservation and identification of areas where eucalypt trees have been lost for restoration. Identifying a single grove of eucalypt trees at such high resolution allows for greater accuracy in determining target land areas for conservation and restoration. Our continued research will aim to identify eucalypt trees at higher resolution in addition to analyzing canopy changes for occurrences of flowering events and areas of Eucalypt population decline and loss over space and time. These findings are significant for conservation efforts because a single eucalypt grove can sustain hundreds to thousands of bats throughout the winter. Conservation efforts must happen in a timely manner since it takes 2 decades for eucalypt trees to mature and flying fox populations are currently losing flight capability due to the lack of eucalypt nectar to feed on.

Knowing the current and previous locations of groves of eucalypt trees can aid in minimizing HeV spillover. Deforestation policy changes must be implemented in Australia to decrease the rate at which native vegetation along the eastern coast is cleared. Since flying foxes are pollinators, they have a significant role in the area's ecology. Bat habitat must be sustained in the forests for flying foxes if management efforts for HeV are going to be successful.

Conclusion

HeV spillover is a significant public health issue in Australia. Spillover of HeV has been increasing in frequency in the past decade. Reduction of flying fox migration into human habitat for low quality substitute foods is a key component to reducing the frequency of HeV spillover. This can be achieved by slowing the rate at which forest are cleared in Australia and protecting native trees that serve as flying fox foraging areas. However, the pressure for land by humans and the need for forested habitat by flying foxes continues to thwart conservation efforts. Assessing areas of critical habitat for flying foxes using satellite imagery can produce effective efforts towards reducing HeV spillover. Identifying eucalypt trees within mixed forests using high-resolution satellite imagery provides the necessary data to make conservation efforts effective and occur in a timely manner.

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