An Update on Nipah Virus: Past Lessons, Current Efforts, & Future Goals

by

Larisa Garza Chapa

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This essay is submitted

by

Larisa Garza Chapa

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and approved by

Essay Advisor: Jeremy Martinson, DPhil, Assistant Professor, Infectious Diseases and Microbiology, Human Genetics, School of Public Health, University of Pittsburgh

Essay Reader: Cynthia Salter, PhD, MPH, Assistant Professor, Behavioral and Community Health Sciences, School of Public Health, University of Pittsburgh

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Larisa Garza Chapa, MPH

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Abstract

Nipah virus (NiV) is an emerging infectious disease first identified in 1998 in Malaysia. Over the course of the quarter century since the disease emerged, multiple identified spillovers have occurred in Malaysia, India, and Bangladesh, with case fatality rates ranging from 40% to 100%. While technologies and surveillance have improved since the identification of Nipah virus, it is notable that the characterizations and locations of the outbreaks have broadened. *Pteropus* bats are the reservoir host of NiV; the disease commonly presents as myalgia and fever, progressing to fatal encephalitis. Monoclonal antibodies and the use of mRNA vaccines are currently being explored, but there is no approved NiV vaccine or specified treatment for infected patients. It is important from a public health perspective to understand the potential in this emerging infectious disease, as preventative measures should be undertaken on local, state, federal, and global scales to identify, diagnose, and prevent the spread of NiV through the use of multiple interdisciplinary collaborative preventative and outbreak control efforts.

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Preface

I would like to take this time to thank my Essay chair Dr. Jeremy Martinson and first reader, Dr. Cynthia Salter for their encouragement, feedback, and time in reading this essay. I would like to thank my fellow Pitt Public Health students for their support and advice throughout my academic career and in my personal life and especially for their encouragement this semester as we worked on our essays and theses.

I would like to thank my family: grandparents, tias, tios, and cousins for their unending love and support. I would like to thank my parents and my siblings for continuing to inspire and cheer me on. Finally, I would like to thank my cat for refraining from attacking my ankles while I worked on this essay.

1.0 Introduction

In a world that becomes increasingly interconnected year by year, as the global population continues to rise, humans have encountered dangerous pathogens with a growing occurrence. Our recent and ongoing bout with SARS-CoV-2 is proof of the ability of emerging infectious diseases to quickly spread across borders, overwhelm underprepared healthcare systems, and infect millions of people. As these zoonotic emerging infectious diseases pass from animals to humans with increasing frequency, it is imperative to identify and research dangerous pathogens while preparing to combat outbreaks through preparation and surveillance (Uday et al, 2022). Nipah virus is one such disease, emerging from Malaysia in 1998 and continuing to cause outbreaks with high fatality rates ranging from 40% to 100% with each occurrence (Cheliout Da Silva et al, 2021).

1.1 Paramyxoviruses

Nipah virus (NiV) and its close cousin Hendra virus (HeV) are both members of the family Paramyxoviridae. This family of negative strand RNA viruses is made of seven genera that includes measles virus, mumps, human respiratory syncytial virus, and human parainfluenza virus. Hendra and Nipah virus form their own genus under Henipavirus, which now includes several other members: Cedar henipavirus, a non-pathogenic member that was isolated from fruit bat urine samples in Australia, Ghanaian bat henipavirus (GhV) identified from fecal samples of straw-colored fruit bats (*Eidolon helvum*), and Mojiang henipavirus (MojV), found in yellow-breasted rats (*Rattus flavipectus)* in China (Wu et al, 2014). HeV and NiV are both unique as they are the

only two zoonotic Paramyxoviruses and are highly pathogenic, although viral isolates from GhV and MojV have not been recovered, leaving their pathogenic potential unknown (Georgiev et al, 2009; Cheliout Da Silva et al, 2021).

1.2 Hendra & Nipah Virus Classifications

The National Institute of Allergy and Infectious Diseases (NIAID) classifies both NiV and HeV as Category C agents for their bioterrorism potential (Georgiev et al, 2009). The NIAID publishes a pathogen priority list, grouping pathogens based on their threat to national security and public health. The pathogens are ranked into three categories (A,B, and C) based on factors like ease of person to person transmission, high morbidity and mortality rates, and capabilities to cause mass panic and social disruption. Category C agents are those that are dangerous based on the possibility of ease of production and availability and could have high morbidity and mortality rates if they were specifically engineered for mass dissemination and weaponization. Both HeV and NiV are Biological Safety Level-4 (BSL-4) select agents, the highest classification of biosafety for labs around the world (Cheliout Da Silva et al, 2021).

2.0 History of Nipah Virus

2.1 Starting the Genus: Initial Spillover of Hendra

Hendra virus was first observed in September of 1994 in Hendra, Australia. This outbreak first appeared in horses, presenting as an acute respiratory illness. As a result, 14 horses died, as well as a horse trainer that had come into close contact with bodily fluids; the second human case survived (Georgiev et al, 2009). After further investigation, it was found that bats had been roosting in fruit trees on the ranch, where it was surmised the horses had been exposed to HeV. This virus was distinct from other Paramyxoviridae viruses, and along with NiV, founded the Henipavirus genus. Repeated outbreaks of HeV have occurred throughout Australia, resulting in seven total human cases with four deaths.

2.2 First Nipah Outbreak: Malaysia 1998

The first known outbreak of Nipah virus started in September 1998 among the pigs and pig farmers in Perak state, Malaysia. A second outbreak occurred in another Malaysian state, Negeri Sembilan, in December 1998 (Soman Pillai et al, 2020). Initially mistaken for Japanese encephalitis virus, immunizations and mosquito vector control measures were implemented with little success; human cases continued to appear, presenting with encephalitis, and many pigs died of severe respiratory difficulties. After Nipah virus was isolated and confirmed as a new emerging infectious disease in March 1999, the outbreak was contained by May 1999 after a total of 265

cases and 105 deaths, a case fatality rate (CFR) of 39.6%. However, in February 1999 NiV had spread to Singapore through the importing of NiV positive pigs. This resulted in 11 cases among slaughterhouse workers and one death (Soman Pillai et al, 2020). As a precaution, importing of pigs from Malaysia was banned, resulting in the mass culling of 1.2 million pigs (Georgiev et al, 2009).

Investigation into the source of the outbreak found that NiV had been passed from *Pteropus* bats, commonly known as fruit bats, or flying foxes, to pigs, and subsequently spreading through the swine population (Centers for Disease Control and Prevention, 2020). It is suspected that the transmission route was either half-eaten fruit nibbled by bats or bat waste that had fallen into pigsties (Georgiev et al, 2009). All human cases were the result of close contact with infected swine; no evidence was found of human-to-human transmission (Madera et al, 2022). This outbreak resulted in the identification of the Malaysia strain NiV-M of Nipah virus.

2.3 Bangladesh Outbreaks: The Nipah Belt

The first outbreak in Bangladesh occurred in the Meherpur district in April 2001 with 13 diagnosed human cases. Since then, spillovers of NiV have occurred on a near annual basis across twenty-three districts, some repeatedly. Approximately 261 confirmed cases with 199 deaths (a 76.2% CFR) were reported between 2001 to 2015 (Soman Pillai et al, 2020). These repeated outbreaks in western Bangladesh have been described as the “Nipah Belt” (Donaldson & Lucey, 2018).

The transmission route in Bangladesh differs from the NiV outbreak in Malaysia; spillovers occur directly from bats to humans, most commonly via the consumption of NiV-contaminated

raw date palm sap (Cheliout Da Silva et al, 2021). These outbreaks result in subsequent human-to-human transmission, causing fatal encephalitis (Madera et al, 2022). Surveillance efforts in recent years have reported that Nipah virus RNA in *Pteropus* bats exists throughout much of Bangladesh, even beyond the Nipah Belt, implying that outbreaks of NiV may have gone unrecognized (Donaldson & Lucey, 2018). These outbreaks resulted in the identification of the Bangladesh strain NiV-B of Nipah virus.

2.4 Early 2000s India Outbreaks

The first identified outbreak in India was reported between January and February 2001 in the Siliguri district of West Bengal, adjacent to Bangladesh. Information about the true index patient is not available, but a total of 66 cases were retroactively identified from laboratory testing via ELISA assay, testing positive for NiV specific IgM and IgG antibodies (Soman Pillai et al, 2020). Out of confirmed patients, 45 deaths occurred, resulting in a 68% CFR (Uday et al, 2022). Febrile illness and neurological symptoms were documented, consistent with the outbreaks in Bangladesh. However, nosocomial spread accounted for most of the human-to-human transmission. A second identified outbreak occurred in West Bengal, this time in the Nadia district in 2007 (Gokhale et al, 2022). Five NiV cases were reported; all died within 10 days of infection (100% CFR).

Part of the difficulty in identifying NiV as the causative agent of these outbreaks was that India at the time had low laboratory capacity; specimens taken required testing at the Center for Disease Control and Prevention (CDC) in the United States (Sadanadan et al, 2018). This delay due to sample transportation across the world slowed laboratory confirmation.

India would not see another Nipah virus outbreak until 2018, when an outbreak in Kerala's Kozhikode and Malappuram districts occurred (Uday et al, 2022). This 3rd outbreak would be the first of three in Kerala, a state located on the southwestern side of the country, distant from the Bangladesh border. These outbreaks will be discussed later.

3.0 Phylogeny of Nipah Virus

A study conducted by Lo Presti et al in 2016 used 29 nucleocapsid (N) gene sequences available to conduct a time-scaled phylogenetic analysis. According to the findings, NiV originated in 1947 in the southeastern Asiatic region. The two main clades of Nipah virus, NiV-B and NiV-M had been introduced separately at different times, 1995 and 1985 respectively. A recent study of an NiV N gene sequence from India suggests that a new genotype, NiV-I, may have independently evolved in Southern India, although further research is needed to confirm this (Paul et al, 2018).

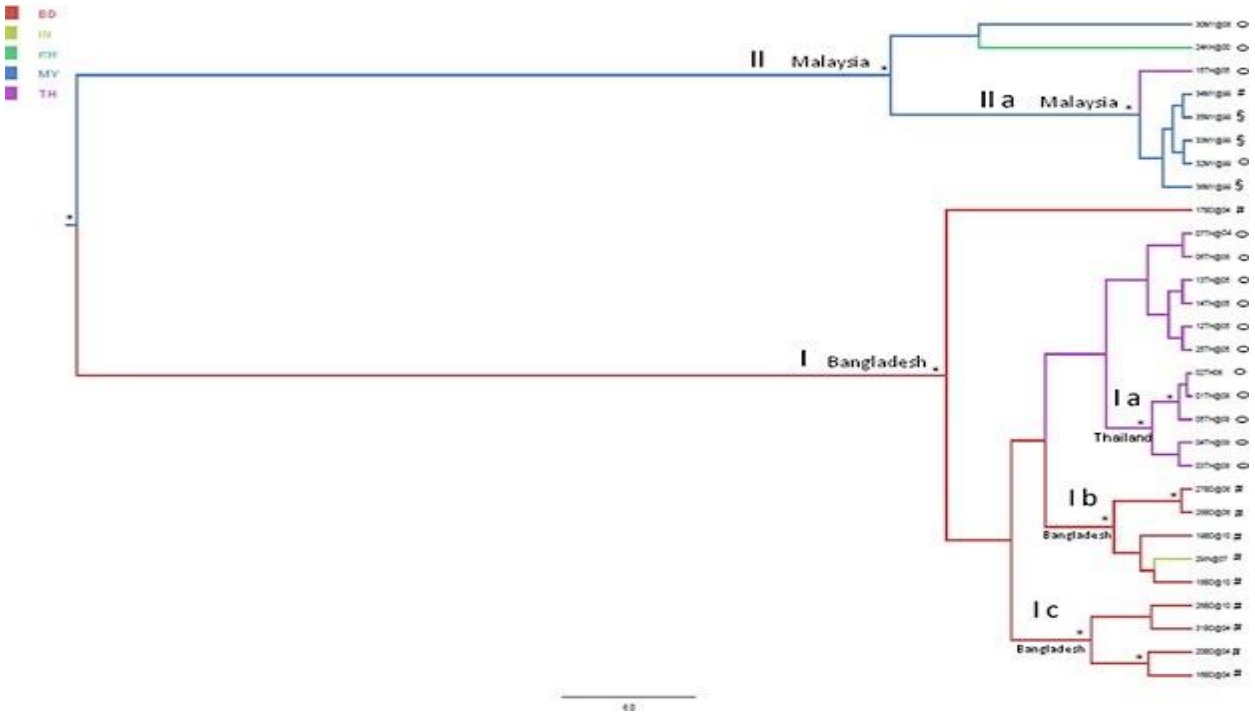

Figure 1. Bayesian phylogeographic tree of 29 NiV N gene sequences (Lo Presti et al, 2016). The branches are colored on the basis of the most probable state location of the descendent nodes. Red from Bangladesh, Yellow from India, Green from Cambodia, Blue from Malaysia and Purple from Thailand. The

asterisk(*) along the branches represents significant statistical support for the clade subtending that branch (posterior probability>0.98). Locations are indicated in different colors. The most probable location of the main nodes was highlighted. Different symbols next to the tip are shown in the tree, depending on the isolation host: # indicates Homo sapiens; ° indicates *Pteropus*; § indicates pig. (Lo Presti et al, 2016)

4.0 Microbiology of Nipah Virus

4.1 Structure

NiV is an enveloped, non-segmented, negative sense, single stranded RNA virus, with a genome approximately 18.2 kb long, significantly larger than other paramyxoviruses (Soman Pillai et al, 2020). The genome encodes six major structural proteins: the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) and RNA polymerase (L), arranged in the order of 3'-N-P-M-F-G-L-5' (Aditi & Shariff, 2019). The N, P, and L proteins, along with the viral RNA form the ribonucleoprotein complex that regulates transcription and viral RNA synthesis (Soman Pillai et al, 2020). The P gene encodes for the non-structural proteins C, V, and W (Aditi & Shariff, 2019).

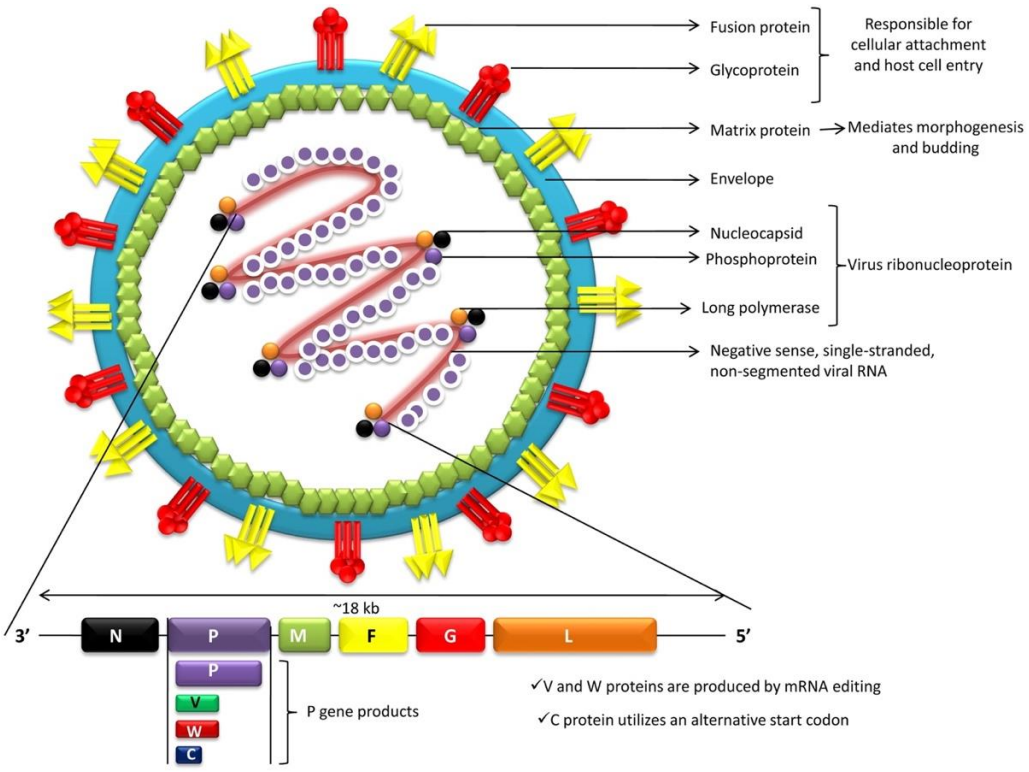

Figure 2. Image of NiV structure (Singh et al, 2019).

4.2 Viral Entry & Fusion

Viral entry is mediated first through the tetrameric attachment glycoprotein (G) and trimeric fusion glycoprotein (F). While the G glycoprotein is fully functional at the cell level, the F glycoprotein is initially "expressed as a F_0 inactive precursor, then recycled and cleaved by the endosomal protease cathepsin L to generate a biologically active protein composed of two subunits, F_1 and F_2, covalently linked by a disulfide bond and subsequently trafficked back to the plasma membrane binding to the host cellular ephrin-B2 and -B3 receptors" (Cheliout Da Silva et al, 2021). The G glycoprotein binds to the ephrin-B2 and -B3 receptors, triggering an irreversible conformational change in the F glycoprotein that induces the viral-cell membrane fusion, allowing

for the entry of virion (Soman Pillai et al, 2020). This fusion is the source of syncytia (multinucleated giant cells), a cytopathic effect observed in paramyxovirus infections (Cheliout Da Silva et al, 2021).

The use of ephrin-B2 and B-3 as entry receptors helps account for the wide breadth of potential hosts including humans, domestic animals, and wildlife seen in NiV infections (Georgiev et al, 2009). Ephrin receptors are highly conserved across mammalian species; the ephrin receptors of bats and pigs possess a 95% to 96% similarity. These receptors mediate cell-cell adhesion and attraction mechanisms and play a crucial role in the migration of neuron precursors during embryogenesis (Cheliout Da Silva et al, 2021). As such, ephrin-B2 receptors are expressed on endothelium and smooth muscles, in the lungs, and in elevated levels on the brain, explaining the hallmark symptoms seen in NiV infections discussed later (Aditi & Shariff, 2019).

5.0 Reservoir of Nipah Virus: *Pteropus* Bats

As discussed before, the bats of genus *Pteropus*, commonly known as fruit bats or flying foxes, have been identified as the natural reservoir of Nipah virus. Bats of the *Chiroptera* order form the second largest group of mammalian species after rodents and have been documented as the natural hosts for many diverse viruses, including filoviruses, coronaviruses, and paramyxoviruses (Hu et al, 2015). However, despite being a reservoir for these emerging zoonoses they commonly are asymptomatic, not appearing to experience disease from the pathogens they harbor (Brook et al, 2020).

The uniqueness of the bat immune system is responsible for this ability to support viruses as long-term persistent infections as opposed to immunizing pathologies, affecting virus dynamics. Most mammal immune systems possess an interferon pathway that activates in response to viral infection. Bats are unique in that their interferon pathway is constantly activated, balanced by a suite of anti-inflammatory traits (such as loss of pro-inflammatory genes) that protect them from the harmful effects of long-term inflammation that other mammals would experience. A study done by Brook et al, 2020 challenged the black flying fox species bat cell line (*Pteropus Alecto*) with various zoonotic viruses to observe the interaction between the interferon pathway and viral dynamics. They found this antiviral state protected *P. Alecto* live cells from mortality in culture, but in turn pressured viruses to mutate in response to establish rapid cell-to-cell virus rates. The findings indicated that viruses that evolved in bat reservoirs would likely "generate extreme virulence upon spillover to hosts lacking similar immune capacities to bats" (Brook et al, 2020). Nipah virus possesses a high mutation rate that allows it to infect new hosts and bypass spillover host immune systems, and NiV neutralizing antibodies have been found in the bat populations of

Ghana and Madagascar, far from Bangladesh (Kulkarni et al, 2009). An additional factor in potential for virus spread is that *Pteropus* bats follow a nomadic lifestyle, migrating and merging in roosts, where viruses then have a chance at spreading into new bat populations.

While they harbor viruses, *Pteropus* bats are essential members of many ecosystems. As their common name suggests, *Pteropus* bats feed on fruits and nectar in their ranges. They play a key role in forest regeneration and plant diversity as they retain seeds in their gut for several hours, move in long distance foraging paths, and fly over forest clearings that are avoided by other forest animals (Hahn et al, 2014). Almost 300 plant species rely on *Pteropus* bats for seed dispersal and pollination, and these plants in turn are the source of a wide range of products including food, medicine, and timber (Soman Pillai et al, 2020).

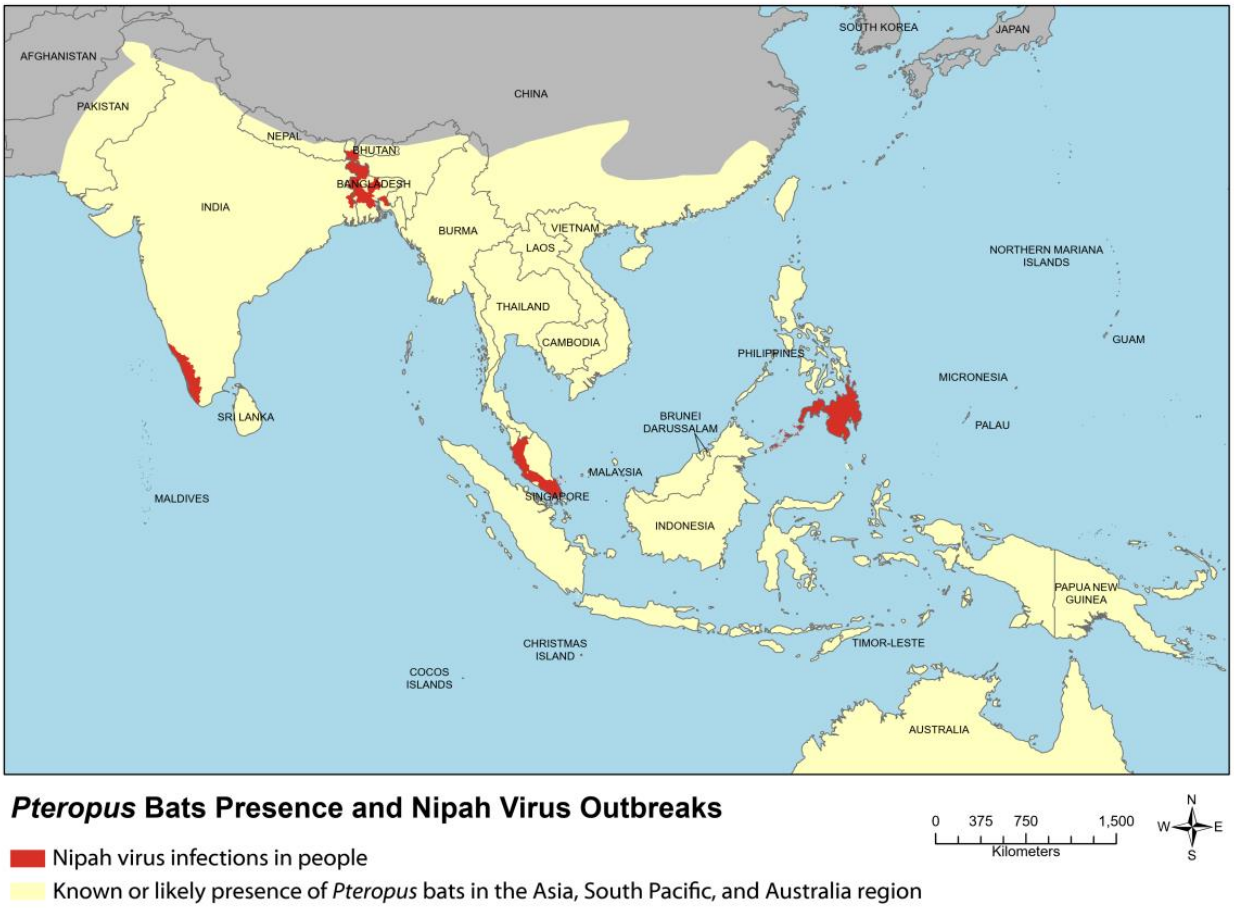

Figure 3. Nipah virus outbreak map & *Pteropus* bat distribution (Centers for Disease Control and Prevention, 2023).

Habitat loss is associated with spillovers of numerous zoonotic diseases, and Nipah virus is no exception (Mallapaty, 2022). As humans encroach on their habitat, *Pteropus* bats have been forced to adapt, commonly foraging in farms and orchards in close vicinity of human settlements, resulting in their human-granted status as pests (Soman Pillai et al, 2020). A study on the flying fox population in Australia found that Hendra virus spillovers occurred after years when bats experienced food stress (Mallapaty, 2022). This food stress, in turn, was linked to the El Niño climate effect that occurs in the Pacific Ocean and is associated with droughts in Eastern Australia,

where HeV outbreaks occur. It was determined that if bats had certain winter trees, they could rely on the fruits produced, and there were no spillover events; however, there was hardly any winter habitat left. Other studies have confirmed that when bats experience stress due to food scarcity, they are more likely to shed viruses, resulting in transmission to novel hosts, as is the case for Nipah virus.

6.0 Transmission of NiV

Nipah virus is spread via direct contact with infected *Pteropus* bats or pigs, or through direct contact with their bodily fluids (Centers for Disease Control and Prevention, 2020). It can also be transmitted via consumption of contaminated foods; ingestion of contaminated raw date palm sap (or occasionally homemade liquor made from the sap) has led to Bangladesh NiV outbreaks during the winter months when the sweet treat is frequently consumed (Donaldson & Lucey, 2018).

While person-to-person transmission was not noted in the Malaysia outbreak, this method is commonly reported in the Bangladesh and India outbreaks (Centers for Disease Control and Prevention, 2020). NiV spreads through close person-to-person contact through nasal or respiratory droplets, urine, or blood. The spread of Nipah virus via handling of NiV-positive corpses has also been recorded. Transmission commonly occurs in the families and caregivers of NiV-infected patients and in healthcare settings. In terms of potential differences of pathogenicity and symptom severity at the index case exist between the two strains, there is not enough information yet due to the unpredictable, and therefore incompletely documented, nature of the spillover events.

Nipah virus enters the susceptible host via the oro-nasal route, where it undergoes replication. Due to the disease's nature, human tissue samples have only been studied at the terminal stage, so the initial site of replication is unconfirmed. However, due to high concentrations of NiV antigen found in the lymphoid and respiratory tissues, it is likely these are the sites of initial replication (Aditi & Shariff, 2019). Once NiV has spread throughout the body, illness follows.

7.0 Symptoms of NiV

The incubation period of NiV varies from 4 to 21 days, differing between strains (Aditi & Shariff, 2019). The Malaysian NiV-M strain has an incubation period ranging from 4 days to 2 months, whereas the Bangladesh NiV-B strain incubation period lies between 6 to 14 days (Soman Pillai et al, 2020).

The incubation period is followed by early symptoms such as fever, headache, and myalgia, which then commonly progress to high fever, altered sensorium, and disorientation (Aditi & Shariff, 2019). Acute encephalitis develops within a week, which results in altered mental status, behavioral changes, and sometimes seizures (Soman Pillai et al, 2020). Additionally, there are clinical feature differences between the strains. Patients infected by NiV-B suffer from severe cases of acute respiratory distress syndrome presenting as cough, respiratory distress, and atypical pneumonia (Aditi & Shariff, 2019). NiV-M infected patients did not suffer from significant respiratory involvement. NiV-B outbreaks in India and Bangladesh have a higher average mortality rate (70%) than NiV-M outbreaks (40%).

Risk factors for poor NiV outcomes include old age, comorbidities, and thrombocytopenia, as well as histories of seizures (Aditi & Shariff, 2019). Furthermore, 20% of NiV survivors of NiV-associated encephalitis suffer from neurological sequalae, including fatigue, depression, behavioral changes, and seizure disorders (Uday et al, 2022). Several cases of relapsing or late onset-NiV encephalitis occurring months or even years after infection have been described (Georgiev et al, 2009).

8.0 Treatment of NiV

Focus for treatment of NiV positive patients is supportive: maintaining breathing and circulation, fluids, and electrolyte balance. In patients with severe pneumonia and acute respiratory failure, invasive mechanical ventilation is used (Aditi & Shariff, 2019). Supportive therapy with antivirals such as Ribavirin has been distributed in several NiV outbreaks over the years, but studies completed in animal models have not proved its efficacy (Georgiev et al, 2009).

For people who have had a possible NiV exposure, there is a potential prevention method. Viral structural glycoproteins are the target of the host humoral immune response; the neutralizing antibodies produced are key to providing vaccine-induced protection. Unfortunately, this process can take time, during which viral pathogens like NiV establish themselves within a host. However, monoclonal antibodies (mAbs) can be used to "jump-start" the humoral immune response, providing early preventative protection against an exposure to a pathogen, preventing NiV from establishing a presence within the host. Monoclonal antibodies against both NiV and HeV have been identified; the human mAbs m102.4 was proven to provide protection to ferrets and African green monkeys from lethal challenges (Dang et al, 2019). After 2010, a m102.4 antibody vaccine has been approved and distributed several times for compassionate use for individuals with significant NiV/HeV exposure risks in India, Australia, and the USA (Soman Pillai et al, 2020). Continuing research into monoclonal antibodies has suggested administering mixes of mAbs that target multiple antigenic sites on the F and G NiV glycoproteins, limiting the emergence of mutants and widening the neutralization range (Dang et al, 2019).

9.0 Warning Signs: Recent Outbreaks in India

9.1 2018 Kerala Outbreak

The third known NiV outbreak in India began 2 May 2018 when a 27-year-old man, a reputed animal lover, was admitted with fever and myalgia to a hospital in the Kozhikode district of the state of Kerala (Sadanadan et al, 2018). Despite initial treatment, his condition worsened to vomiting, high grade fever, and altered sensorium. He was then transferred to another hospital, where he succumbed to febrile illness. On 17 May 2018, his brother visited Baby Memorial Hospital in Kozhikode, Kerala with a fever. Again, despite treatment, he passed the next day. However, his doctors, along with Kerala's additional director of public health, noticed the similarities between the brothers' symptoms: both were consistent with encephalitis (Sadanadan et al, 2018). As a precaution, samples taken from the second case were sent to Manipal Centre for Virus Research (MCVR), a Grade I Virus research and Diagnostic Laboratory of the Indian Council of Medical Research (ICMR) located in Manipal, Karnataka on 18 May 2018. Next-generation sequencing (NGS) analysis came back with the verdict: it was Nipah virus, similar to the Bangladesh strain responsible for the Western Bengal outbreaks of the 2000s. The ICMR's National Institute of Virology (NIV) in Pune, Maharashtra quickly confirmed the finding with their Biosafety Level-4 facility on 20 May 2018. By that time, human-to-human transmission had begun (Gokhale et al, 2022).

As discussed before, Kerala is located along the coast of southwestern India, far away from the earlier northeastern outbreaks of West Bengal. While there was no clinical evidence available to prove it, the index patient is suspected to have contracted NiV from handling an NiV infected

baby bat, as the timing of the outbreak falls within the bat breeding season (Soman Pillai et al, 2020). It is assumed that his brother came into close contact with him, resulting in his subsequent infection and admittance to the hospital. After this point, spread in this outbreak was exclusively nosocomial. An additional 21 NiV-positive cases were identified; 18 died, resulting in a 91% CFR. While the case fatality rate was consistent with previous outbreaks, the number of cases could have been worse. Kozhikode is the largest urban area of Kerala, and the outbreak could have quickly spread. Rapid testing allowed for the state and central governments to quickly react.

After the MCVR and ICMR/NIV laboratories confirmed the presence of NiV, the Union Health Minister deployed a multidisciplinary team to review and respond to the NiV situation in Kerala (Singhai et al, 2021). This team was headed by the director of the National Center of Disease Control (NCDC) and included members from "the NIV in Pune, the All India Institute of Medical Sciences, the Department of Animal Husbandry, Dairying & Fisheries, and the Division of Emergency Medical Relief, with the Ram Manohar Lohia Hospital in New Delhi providing technical support" (Sadanadan et al, 2018). This team worked with the Government of Kerala's Department of Health and Family Welfare local health authorities to rapidly initiate public health response measures such as active disease detection, contact tracing and observation. In the healthcare facilities they implemented guidance on infection prevention and control, patient isolation, PPE training and use by healthcare workers, and procedures for the safe disposal of human remains. Ribavirin, an antiviral that has been used in previous outbreaks, was imported from Malaysia within days, and the Kerala state government received limited monoclonal antibody m102.4 supplies for post exposure prophylaxis use. Surveillance teams sent out from NIV Pune collected and tested *Pteropus* bat samples in the area; 19.2% of the samples tested via RT-PCR

were positive for NiV RNA. These coordinated actions helped contain the outbreak, preventing further NiV spread.

The other major factor in limiting this outbreak was the ability of MCVR and NIV Pune to quickly identify NiV. When the 2000s West Bengal outbreaks occurred, India did not have any labs capable of testing the samples; this time, none of the samples were transported to the USA CDC for confirmation (Sadanadan et al, 2018). International collaboration, described below, assisted with this ability.

The Global Health Security Agenda, built on WHO (World Health Organization) International Health Regulations, provides guidance for countries to "assess and manage serious health threats that have the potential to spread across borders" (Sadanadan et al, 2018). In India, focus was placed on the 'Core 4' public health functions: increasing real-time surveillance of potential public health threats, strengthening laboratory systems within the country, training health workers in key epidemiological capacities, and "establishing emergency operations centers with rapid response teams capable of activating a coordinated emergency response" (Sadanadan et al, 2018). Through this global health partnership, in August 2017, the US CDC provided in-country wet lab training to participants from MCVR and NIV, aiming to increase diagnostic capacity. This allowed the MCVR and NIV scientists to not only detect NiV without needing to send the samples out of country, but also developed their diagnostic skills that allowed a turnaround time of only 12 hours. The quick diagnostic testing via next-generation sequencing permitted doctors to anticipate clinical presentations, such as respiratory distress seen in previous India-based and Bangladesh-based outbreaks. The rapid response by the state and central governments limited the severity of the outbreak by establishing an emergency response protocol, limiting the severity of the outbreak.

The 2018 Kerala NiV outbreak was declared over in June 2018, and in its wake the Institute of Advanced Virology in Kerala was established in February 2019 (Uday et al, 2022). The aim of this institute was to eventually provide Kerala with a BSL-3+ laboratory capable of NiV testing locally. However, just three months later, another Nipah virus spillover would challenge Kerala's outbreak response.

9.2 2019 Kerala Outbreak

On 30 May 2019, a 23-year-old man from the Ernakulam district of Kerala was referred to a private hospital, where he was admitted with a fever and altered sensorium. Samples of his cerebrospinal fluid (CSF) were sent to NIV Pune for testing (Singhai et al, 2021). The results were positive, and the index patient was moved to an isolation facility where he was monitored (Soman Pillai et al, 2020).

Government response was swift. The neighboring districts of Ernakulam, Thrissur, and Idukki were placed under surveillance for potential NiV cases. Monoclonal antibodies (mAbs m102.4) were again obtained from the Australian government for compassionate use in any high-risk NiV exposures. At the same time, the state government officials were able to identify 330 contacts of the index patient, monitoring them for any NiV-like symptoms. Samples taken from 13 suspected NiV cases were sent to NIV Pune for testing. All results were NiV negative, and the index patient recovered. This first test of the Kerala outbreak response guidelines established in the 2018 outbreak was seen as a success, although the exact cause of the spillover has not been traced (Gokhale et al, 2022).

9.3 2021 Kerala Outbreak

The latest and 3rd identified outbreak in Kerala (5th in India) occurred 29 August 2021. The index case, a 12-year-old boy, developed a low-grade fever and was taken to a local health care facility (World Health Organization, 2021). As the boy's condition worsened, he was transferred to several other hospitals, and were admitted to a hospital in Kozhikode district in Kerala on 1 September 2021. RT-PCR and ELISA testing done on plasma, serum, and cerebrospinal fluid samples on 4 September 2021 in NIV Pune were confirmed positive for NiV. Close contacts were identified and monitored for NiV symptoms, but no other positive cases were identified. Tragically, the 12-year-old died on 5 September; his remains received a safe burial and cremation.

As touched on previously , it is notable that these three latest occurrences of NiV outbreaks in India have emerged in the south-western state of Kerala, which is geographically distant from the "Nipah Belt" and West Bengal-Bangladesh border (Gokhale et al, 2022). As the threat of NiV continues to grow and expand beyond the Nipah Belt, calls for action in addressing this virus have been issued, covering a wide range of significant factors.

9.4 Difficulties in Combating Syndemic Outbreaks

In 2021, Nipah was not the only potential epidemic combated in Kerala. The state had faced an earlier Zika virus outbreak in July 2021 and an outbreak of Kala-azar, also known as black fever, later in September 2021. Of course, the region also dealt with the coronavirus that caused a pandemic, now familiar to all as COVID-19.

These outbreaks are termed “syndemic”, where two or more epidemics interact synergistically and contribute to “the clustering of excess disease burden in a population which is more than their sum” (Uday et al, 2022). Syndemic outbreaks place harsh pressure on government, healthcare, and public health institutions to handle diseases that differ in etiopathogenesis but whose symptoms and signs present across a shared spectrum.

The success of addressing syndemic outbreaks also depends on the location of the outbreaks. India, which spends 3% of its gross domestic product (GDP) on healthcare, faced a second COVID-19 outbreak during May 2021, during which Kerala was hit particularly hard as it attempted to handle multiple epidemics. Kerala suffered chronic shortages of medicine, oxygen supplies, and ventilators. While Kerala has the highest human development index of Indian states, it has experienced a shift from public to private healthcare, and decreased funding from expensive medical treatments during the pandemic, leading to a decline in the availability of healthcare infrastructure and resources (Uday et al, 2022). Climate change and flooding along dense forests have increased the levels of vector-borne infectious diseases, and a migrant crisis that has grown over the past few decades continues to hang over Kerala.

While Kerala was able to handle the latest NiV outbreak, the emergence of COVID-19 in conjunction with other potential epidemic-causing diseases will continue to complicate future outbreaks, and Kerala must prepare for that likelihood. Acclimating the population to vaccines should be prioritized (Uday et al, 2022). The COVID-19 vaccine distribution in Kerala began in January 2021, but only 44% of the population was fully vaccinated at year’s end. Although there is no approved human NiV vaccine available yet, recent developments have been promising.

9.5 Vaccine Development

While vaccines have been in development against NiV since its discovery, the development and use of mRNA vaccines used to combat the COVID-19 pandemic has provided a promising answer to the NiV vaccination question (National Institute of Allergy and Infectious Diseases, 2022). ModernaTX Inc, the Vaccine Research Center, and National Institute of Allergy and Infectious Diseases have developed a novel mRNA vaccine. MRNA-1215 “encodes for the secreted prefusion stabilized F component covalently linked to G monomer (pre-F/G) of Malaysian strain NiV, resulting in a post-expression trimerization” (NIH U.S. National Library of Medicine, 2022). Clinical trials consisting of a two-dose vaccine regiment are currently ongoing at the National Institute of Health (NIH) Clinical Center in Bethesda, Maryland. The estimated completion date for the study is June 2024.

9.6 Calls for One Health Approach

One Health is a unifying approach that accounts for the interactions between human, domestic and wild animals, and the environment to understand the health impacts each have individually and together on emerging infectious diseases (Uday et al, 2022). In the case of NiV, this essay has described how bats will shed viruses in times of stress. Deforestation in the Southeast Asia region is an environmental factor crucial to understanding this pressure. Industrialization, urbanization, grazing and farming management have been responsible for forest fragmentation (Soman Pillai et al, 2020). Climate factors play a role in Nipah virus spillovers; the first NiV outbreak in Malaysia occurred after a drought due to the El Niño climate pattern, and similar

droughts following El Niño were reported from Kerala in 2016. While the patterns of *Pteropus* foraging patterns require further study, it is theorized that these droughts reduced the availability of natural fruit sources, forcing bats to feed on fruit trees in gardens and orchards near human habitats. Climate change caused by humans could cause The El Niño climate pattern to intensify, potentially leading to further droughts and further NiV outbreaks.

While research on *Pteropus* migration patterns and habitat requirements in India and other Southeastern Asia countries is limited, the One Heath interaction between bats, humans, and the environment has been examined in Bangladesh (Hahn et al, 2014; Gokhale et al, 2022). A study on *Pteropus giganteus* habitat requirements in Bangladesh was conducted from December 2011 to February 2012, which mapped *Pteropus giganteus* roosting sites that were in proximity to local villages (Hahn et al, 2014). These villages were randomly selected from across the country, consisting of locations that had experienced previous outbreaks (known as 'spillover villages') and those that had not, acting as control sites. The controls were selected from a random sample of geographic points and then linked to a nearby village. Speaking with residents of each village, researchers were directed to known roosting sites that had been active in the past five years. Counts of bats were taken, along with the size and type of tree used for the roost. The distances from the villages to the roosts and to the closest perennial water body were calculated using ArcGIS 9.3 and Geospatial Environment. Measures of climate patterns, land cover and forest fragmentation were recorded.

The results found *Pteropus giganteus* roosts were in highly fragmented forest areas, as *Pteropus* bats are generalists, feeding on a wide variety of plant species. Most roosting trees had been occupied for over a decade, and were often located near large ponds, natural food resources, and homestead gardens of nearby human settlements. The roost fidelity of *Pteropus giganteus* bats

was high, although other studies observed *Pteropus* fidelity to a "home range rather than a single roost" (Hahn et al, 2014). In addition, the results confirmed that the majority of the spillover events did occur within the Nipah Belt region, which were predicted to be highly suitable for *Pteropus giganteus* roosting. However, there were similar highly suitable roosting areas identified in regions that previously had not reported Nipah outbreaks, outside of the Nipah Belt. Surveillance bias may be responsible for this, as citizens and healthcare workers that reside outside the Belt may not be as familiar with the signs and symptoms of NiV (Hahn et al, 2014). Similar techniques of roost mapping could be used in Australia, India, and other countries in Southeast Asia. The One Health approach should continue to be undertaken to anticipate locations of potential spillover risks, informing land management practices, public health messaging, and bat conservation efforts to reduce the likelihood of NiV outbreaks.

9.7 Calls for Improving Healthcare Worker Knowledge & Healthcare Facilities

Because of the high transmission rate of Nipah virus, frontline healthcare workers (HCW) face a particularly high-risk of infection (Uday et al, 2022). A cross-sectional study conducted a month after the 2018 Kerala outbreak at a medical college hospital in Kerala found that among healthcare personnel,"65.7% was found to have good and 34.3% had poor awareness regarding Nipah infection" (Kumar et al, 2019). These results were satisfactory at the time, considering the distance between Kerala and West Bengal.

Another study performed in June through July 2019 at K.S. Hedge Medical Academy in the city of Deralakatte, Karnataka state, India (Himes et al, 2022). While not in Kerala, the distance of this study's medical facility to the Karnataka-Kerala state border is 5.6 miles, meaning it is

entirely feasible for a Nipah outbreak to occur in Karnataka. After interviewing healthcare workers (HCWs), including physicians, nurses, and allied health professionals, it was found that while most providers were willing to care for infected patients, only a "minority of providers stated that they [had] changed their practice to manage" Nipah virus, and less than half knew about any standard operating procedures (SOPs) for NiV at their workplace. These findings highlight the need for healthcare facilities to establish guidelines to address potential care of infected patients in areas adjacent to high-risk areas and to train healthcare personnel in those guidelines. SOPs must cover contact tracing, infection control and prevention practices in each unit and ambulatory services, as well as management of biomedical waste and procedures for handling NiV-positive corpses to address routes of potential transmission (Uday et al, 2022).

Additionally, in both the Varghese et al and Himes et al studies, HCWs indicated that the major source of information on Nipah virus was from news media sources and the Internet, not from continuing education within the medical system. These findings highlight the need for reliable communication between the public health departments and hospitals of all types, as well as intra-hospital communication on NiV. Strategies to bridge these knowledge gaps must be tailored to diverse groups of HCWs "based on their specific needs, experience, and training" (Himes et al, 2022). It is important to emphasize that this education should extend to all healthcare associated workers, including nonmedical staff, as they can serve as unofficial educators of the wider community (Kumar et al, 2019).

Further research should be conducted on HCW knowledge in government, academic, and private healthcare facilities throughout Kerala and its neighboring states. This knowledge can inform further education training for recognition of NiV symptoms to identify outbreaks early.

9.8 Calls for Improving Communication with the Public

As with any public health policy, program development is only the initial step. Success of the plan depends on how the public responds to the new guidelines. Community outreach programs are essential for communicating the program's aims in a way that allows the public to understand the project's importance and address concerns that may arise.

During NiV outbreaks, it is crucial that this communication is effective. Calls from Indian and international medical and public health officials have been issued for the development of government-promoted community outreach programs alongside advertisements and radio broadcasts to increase the public's awareness of the risks and signs of NiV (Aborode et al, 2022; Uday et al, 2022). Community healthcare workers should do this work.

Communication pathways with the public need to be effective, but it is essential that these are reciprocal as well. If interventions are deployed without considering if they are culturally competent, they are likely to fail, as evidenced by the response of the public to a large NiV 2004 outbreak in the Faridpur district of Bangladesh. During this outbreak, the residents became suspicious of local government hospital care and of HCWs because treatment at these facilities did not prevent the deaths of infected patients, which led to panic in the community (Parveen et al, 2016). Later, during a 2010 NiV outbreak in the same district, the local health authority used announcements over loudspeakers to proclaim that drinking raw date palm sap could cause illness and directed the populace to stop drinking it. Instead of following the guidance, residents continued to drink raw date palm sap, and the outbreak elongated.

In an effort to improve communication and compliance with prevention guidance during the 2010 NiV outbreak, the outbreak response team used the International Crisis and Emergency Risk Communication (CERC) model during the 2010 NiV outbreak with the goal to "develop a

strong rapport with residents of the outbreak community to permit the investigation and as a basis for communicating prevention messages" (Parveen et al, 2016). The international CERC model is a culture-centered approach, where community engagement is used to understand the outbreak community, thereby building trust and identifying resident perceptions of the crisis, which are then used to build educational messaging.

Their first step was to develop rapport through participatory approaches, such as "home visits, interviews, group discussions and engaging the insider volunteer team" (Parveen et al, 2016). This work fostered an environment where residents were able to express their perception of the outbreak and of hospital care to the team. The team also interviewed the religious leaders of the area to learn about the religious burial practices. They found that residents did at first seek medical treatment at the hospitals when the illness presented as a "normal fever;" however, when treatment failed to save the infected persons, residents suspected that doctors were purposefully responsible for the deaths due to malpractice or even through "vaccines in the back," fostering distrust. Healthcare professionals were partially at fault for these perceptions; they did not explain the disease or the treatments, or that samples were being taken for diagnostic testing. Local health care officials had also come to the community and made house-to-house visits to inform residents about the NiV outbreak, but they did not answer questions about the disease. These communication failures led to widespread mistrust of hospital care and led community members to blame the illness on supernatural reasons.

The next step undertaken by the outbreak team using the CERC model was to communicate the NiV prevention messaging using the culture-centered approach. Members of the outbreak team and of the volunteer teams arranged several group meetings, and made house-to-house visits, this time personally inviting community members to attend and explaining that any questions would

be answered at the meeting. Once at the meetings, the outbreak team explained the cause of NiV was a disease-causing illness, similar to cholera (a disease this community understood). They then showed infrared camera photos of bats feeding on raw date palm sap, contaminating it with NiV and causing illness, and explained the person-to-person transmission route. Instead of advocating for abstaining from drinking raw date palm sap, the team emphasized the use of bamboo skirts to keep the bats out of the raw date palm sap (see Fig. 4), and answered further questions from the community. In follow-up interviews the citizens commented how they appreciated the approach taken and said they would make behavioral changes to reduce the chance of NiV infection.

Figure 4. Pteropus bats feeding on date palm sap collection & bamboo skirt covering (Park et al, 2018).

By working with the community to consider the specific context of an outbreak, the team was able to establish communication with the public that was then used to inform their messaging approach (Parveen et al, 2016). This technique can be expanded upon for future or recurrent

outbreaks, by stationing a community health worker to maintain the rapport, and by developing a set of communication materials that could be informed by participation of local residents who have experienced previous outbreaks. Culture-centered messaging should be used by local healthcare officials in other high-risk countries to prepare for and manage future NiV outbreaks.

9.9 Calls for Diagnostic Trainings & Improved Facilities

As seen in the difference of outcomes between the early 2000s NiV outbreaks and the 2018 to 2021 outbreaks in India, the ability to quickly identify pathogens via diagnostic testing is key to containing and addressing epidemics. It is especially critical in the case of NiV, where the case fatality rate falls between 40% to 100% and person-to-person transmission via bodily fluids requires use of PPE and infection control measures to contain.

Samples collected and used for diagnosis from infected cases include “nasal swab, throat swab, urine, blood and CSF” in humans and “lung, spleen, and kidneys” from deceased animals (Soman Pillai et al, 2020). Several methods can confirm the presence of NiV or NiV antibodies. Real-time reverse-transcription polymerase chain reaction (RT-PCR) is the preferred method for its extreme sensitivity. The conserved segments of the NiV genome (N, M, and P) are commonly used as the targets of this method, but it is expensive.

Next-generation sequencing is also used to identify viral strains, and is also costly (Soman Pillai et al, 2020). ELISA, a serological assay, detects NiV antigens and antibodies in serum samples. A combination of these diagnostic tests is often followed: ELISA followed by RT-PCR. While these types of tests are available, there are constraints on which types of laboratories can handle NiV samples.

Due to its highly pathogenic nature, NiV diagnostic testing can only be done in enhanced BSL-3 (otherwise called BSL-3+) and BSL-4 facilities (Soman Pillai et al, 2020). Biosafety is extremely important, as NiV is transmitted via bodily fluids. Specialized laboratory facilities are built to follow the biosafety requirements and must meet security standards and staff capabilities to be granted each biosafety level designation.

Training personnel to meet BSL-4 requirements and perform accurate diagnostic testing for NiV, along with the investment into specialized facilities, are limiting factors in rapid pathogen identification (Soman Pillai et al, 2020). In the 2000s West Bengal did not possess the facilities or personnel capable of identifying Nipah virus; in contrast, in 2018 India was able to send the samples to NIV Pune, although notably Kerala did not have these capabilities. The Advanced Institute of Virology was established in 2019 in Kerala with the aim to gain the designation of BSL-4 at this facility, but as of April 2023 it only meets BSL-2 requirements. The rapid turnaround time of 12 hours was a critical factor in providing the NiV confirmation that allowed the state and central governments to start their outbreak management. However, sending the samples still relies on the availability of the BSL-4 laboratory to perform the diagnostic tests (Uday et al, 2022). As such, calls for more BSL-3+ and BSL-4 laboratories and advanced training in diagnostic testing methods for lab personnel have been made by researchers in India; these same sentiments are echoed by international health researchers to establish these trainings and institutions in countries with areas at high-risk of spillovers.

9.10 Calls for Improved Surveillance

Improving the understanding of NiV infection and transmission in *Pteropus* bat populations is critical for developing effective risk prevention and management strategies to avoid future NiV spillover events (Breed et al, 2011). NiV surveillance performed in 2015 by the ICMR and NIV Pune in northeast India found NiV in *Pteropus medius* bat populations (Gokhale et al, 2022). Another example of this active surveillance was conducted from January to November 2019 (Gokhale et al, 2022). They surveyed bats across five states, Kerala and four neighbors (Telangana, Karnataka, Tamil Nadu, and Odisha) and a union territory (Puducherry). Blood samples, throat swabs, and fecal swabs were taken from the bats and tested using RT-PCR and ELISA. While they found a high anti-NiV IgG prevalence of 20% in the *Pteropus* bats tested, they did not detect Nipah virus in the surveyed bats. The bats with anti-NiV antibodies came from Karnataka, Kerala, Tamil Nadu, and Puducherry (Gokhale et al, 2022)

A study undertaken between January 2006 and July 2012 gathered data on "host ecology, molecular epidemiology, serological dynamics, and viral genetics to characterize spatiotemporal patterns of NiV dynamics" in *Pteropus medius* bats of Bangladesh (Epstein et al, 2020). They found that while NiV was present in the majority of bats surveilled, Nipah outbreaks in Bangladesh depended on several factors: "1) multiyear fluctuations in transmission intensity among bats driven by immunity and colony size/density-dependent transmission; 2) relatively localized bat movements creating spatially variable transmission dynamics; 3) occasional shedding by previously infected bats due to recrudescence; 4) highly seasonal contact between bats and humans via consumption of raw date-palm sap" (Epstein et al, 2020). They also suggest that because NiV outbreaks were sporadic and rare, there was a high likelihood of underreporting due to misdiagnosis as other infections of the region, such as malaria and Japanese encephalitis. However,

because this study was undertaken over a decade ago, their assurance of the rarity of NiV spillovers does not ring as true now. Still, surveillance projects of *Pteropus* bats must be continued and expanded to improve knowledge of Nipah virus dynamics.

Recall that NiV possesses a broad host range due to the use of ephrin-B2 and -B3 receptors by NiV to enter host cells (Cheliout Da Silva et al, 2021). Other recently identified henipaviruses such as Mojang virus have been found in rodent hosts. As such, surveillance of domestic and wild mammals near high-risk areas is necessary to determine the presence of NiV, as we do not yet possess clear answers if other transmission routes exist that use intermediary hosts can infect humans, just as the NiV-M strain spreads to humans via pigs.

Finally, epidemiological surveillance within human populations should be undertaken. The fruit bats live near villages throughout India and Bangladesh; therefore, clinical samples taken from people residing in these high-risk areas should be routinely taken and screened (Uday et al, 2022). Such a program can utilize the pre-existing government infrastructure like India's National Rural Health Mission (NRHM) and National Vector-Borne Disease Control Program (NVBDCP) (Uday et al, 2022) to guide deployment of community workers and establishing supply chains to take such samples. These plans should include creating rapport with the community to establish trust and provide culturally competent guidance according to local traditions. These actions, along with surveillance in bats and other mammals of the region will aid in curbing spillovers of Nipah virus and other zoonotic diseases.

9.11 Expanding Prevention Efforts in Changing High Risk Regions

Because of the wide range of *Pteropus* bats (shown in Fig. 3), continuing deforestation, and increasing human encroachment into former dense forest areas, other nations in Asia, the Middle East, and Africa must prepare for potential NiV spillovers (Donaldson & Lucey, 2018). Without active surveillance, our lack of understanding in regard to NiV distribution will continue to place humans at unknown risk of infection. For example, in Thailand, NiV was found in bats; however, no human infections have been documented there. The same cannot be said for the Philippines.

A suspected NiV outbreak occurred in Mindanao, the second-largest island in the Philippines, in 2014. A total of 17 people met the case definition of Nipah virus (or a very closely related henipavirus). Unlike previous outbreaks, this one involved the butchering and consumption of horse meat (in 7 cases), and two of the cases were due to person-to-person transmission. “Five had provided care to other patients either in the hospital, during transportation to the hospital, or in the home. At least 10 horses died, including nine that had an acute neurological illness” (Donaldson & Lucey, 2018). NiV antibodies were confirmed in patient samples via ELISA assay and a CSF sample was found to have a single sequence read of the P gene of Nipah virus, although a complete NiV sequence was not recovered from this outbreak. These spillover events emphasize how crucial it is for other countries to identify high-risk regions for spillovers, develop surveillance practices, monitor potential outbreaks, develop infrastructure for diagnostic laboratories, strengthen and educate healthcare systems, and establish clear pathways for emergency communication to the public.

10.0 Conclusion: Next Steps Across the World

Nipah virus is an incredibly deadly pathogen that causes fever, myalgia, and acute encephalitis. *Pteropus* bats are the NiV reservoir host; NiV spillovers have been reported in Malaysia, Bangladesh, and India, with a suspected NiV outbreak in the Philippines. Transmission occurs directly from bats (NiV-B strain) or from bats to pigs (NiV-M strain) into human hosts. There is no vaccine approved for humans against Nipah virus.

Due to expanding epidemiological patterns in southeast Asia, there is an increasing potential of severe urban outbreaks in the future (Donaldson & Lucey, 2018). Steps to increase preparedness for future NiV outbreaks must include "heightened surveillance, coordinated rapid diagnostic testing, training exercises including the use of and sufficient supplies of personal protective equipment (PPE)" alongside the establishment of SOPs in healthcare facilities and direction of funding to research for developing therapies and vaccines.

Long-term surveillance in particular is a powerful tool for preventing outbreaks, allowing scientists to identify new variants and possible spillover hosts and help prevent future outbreaks (Aborode et al, 2022). Nipah virus experts from India and Bangladesh should be engaged to "close gap knowledge" in neighboring countries as they develop preventative programs (Donaldson & Lucey, 2018). Stakeholders in high-risk areas should work with policy makers to push for further NiV research, and "social, financial, and political commitment" to developing public health interventions should be leveraged to build culture-centered responses to future NiV outbreaks (Uday et al, 2022).

In terms of public health implications, on a local to global scale, future Nipah virus outbreaks must be addressed in a well-rounded multi-pronged interdisciplinary approach. No

single area of improvement will be solely responsible for the ability to diagnose, contain, and control the outbreak of Nipah virus. Further study using the One Health approach should continue to research *Pteropus* bat behaviors and environmental factors that lead to higher risks of spillovers. NiV antibody and antigen surveillance in humans and animals must be undertaken to identify areas at high risk of spillover events; community-based communication and surveillance will help establish the public's trust in healthcare workers and develop culturally competent prevention materials. For hospitals and clinics, improving medical and non-medical healthcare worker knowledge of Nipah virus, will quicken sampling and response to arrest nosocomial spread, as well as direct treatment based on NiV strain differences. Developing BSL-4 capable laboratories near high-risk sites will lessen pressure on the diagnostic system as a whole, allowing for quick identification. Finally, continuing efforts to craft and test a NiV vaccine must be followed by the targeted distribution of vaccination campaigns in high-risk areas. The frequency of Nipah virus spillover events will undoubtedly continue to increase; it is up to the public health networks to determine how well we are able to recognize and combat the next outbreak, wherever it may begin.

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