

Paper title: Indian Flying Foxes as hosts of the Nipah virus in the Indian subcontinent.

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About the Student:

Chaitra Barkam is a student studying in Candor International School. This paper was written by her as a part of the fall Scholarly program: LS190 Introduction to College-Level Research in Biology. This course was instructed by Professor Ron Mumme from Allegheny College and covered topics like Darwinian Science, Evolutionary Biology and Natural Selection. Due to the student's interest in Epidemiology and Evolution, she chose a geographically relevant topic about the Nipah virus and chose to explore bats as common hosts for this virus and many other zoonotic viruses. She enjoys learning Biology and likes spending time with animals, hence she chose a topic that merges the 2 disciplines and also acknowledges the human-animal conflict.

Abstract:

The Nipah Virus (NiV) is on WHO's list of most deadly zoonotic diseases. Known to originate from bats, the virus has been traced to Malaysia in the late 20th century. Over the past 20 years, outbreaks have been recorded in Malaysia, Bangladesh and India. In 2018, a large Nipah outbreak took over the state of Kerala in India and was suspected to start from local colonies of fruit bats. There was no official vaccine or standardized treatment protocol and the mortality rate for the NiV outbreaks ranged from 70-98%, making it highly deadly. As the virus is known to be originated from bats, this academic paper explores the virulence of the Nipah virus along with bats as evolutionary hosts of Nipah. The common fruit bat species *Pteropus medius* is associated with this virus and displays a range of immune responses that may be attributed to hosting many viruses without suffering high mortality rates like humans. These evolutionary trade-offs and other behaviours of this bat species are further explored in this paper.

Paper:

Introduction

Serologic evidence has shown that bats are reservoirs of many viruses. 66 viruses occur naturally in bats, including SARS coronavirus that was extrapolated back to bats after the 2003 SARS epidemic. Zoonotic viruses usually happen from animals to humans directly or through an intermediate vector. When there is an outbreak of a virus in a particular colony, the chances of a spillover into humans increases (Calisher et al. 2016).

One of the potent emerging diseases in humans is the Nipah virus (NiV) has become prevalent in the Indian subcontinent in recent years. The Nipah virus, family paramyxoviridae, is closely related to the Hendra virus. (Yob et al. 2001). The virus was observed in multiple outbreaks in Asian countries like Malaysia, Bangladesh, and India. In India, the 2018 Kerala outbreak caused panic amongst the people due to the lack of a vaccine and the high mortality rate of the virus. In this subcontinent, the *Pteropus medius* (formerly *P.giganteus* bat) has been identified as a primary host of this virus. *Pteropus* bats are widely distributed across Bangladesh and India (Yadav et al., 2012). My aim is to review the various evolutionary aspects of bats along with the Nipah virus outbreaks in India to try to provide reasons for why bats are such significant virus reservoirs.

Bat characteristics and adaptations

Bats are known to fly in search of food for large distances every day, and when they are in flight their metabolic rate is extremely high. The body temperature is also increased to high levels (O'Shea et al., 2014). Bats also have varying flight paths across a large range of regions. These flight paths have been associated with the incidence of viruses. Migrating birds can exchange viruses with native and non-native species and bring them back to their country of origin (Calisher et al, 2016).

Bats have long life spans ranging from 20-30 years, which is unusual for a mammal of such a low body mass. Smaller organisms have higher metabolic rates. Due to having an extremely high metabolic rate in flight, the proportion of increased metabolic rate in response to fighting a virus is significantly lower (O'Shea et al., 2014). This could be a significant adaptation that bats have against disease-causing microbes. Bats have antibodies and immunoglobulin proteins in their bodies just like other mammals, but the gene regulation and mediation of inflammatory response are different (Calisher et al, 2006). This can be attributed to the absence of certain immune response genes like NRP3 or the metabolic pathway changes due to trade-offs between high body temperature and infection (Mandl et al., 2018).

Their flight pattern and physiological attributes such as wing shape evolved in synchronization with the altered immune system (O'Shea et al., 2014), hence the correlation with the adaptive radiation in response to changing environments could have significantly impacted the way bats are tolerant to hosting viruses as well.

The Nipah virus and bats

The Nipah virus was first identified in Malaysia in 1998 and was suspected to be transmitted from pigs. More than 1 million pigs killed were to cull the spread. Epidemiologists investigated areas around the farm for potential sources of the Nipah virus that was plaguing Malaysia in 1998 and 1999. The virus was named Nipah or NiV in the *paramyxoviridae* family also commonly known as *hepinaviruses* (Yob et al., 2001). After finding bat colonies around the farming establishments they sampled the sera of 324 bats across 14 species. They measured the prevalence of antibodies using titers and the species with the highest percentage (30%) out of 5 species that contained antibodies was the Island Flying Fox (*Pteropus hypomelanus*) (Yob et al., 2001)

The Nipah virus is a relatively new virus that can almost directly be associated with the emergence of farming and cultivation breaching deeper into wild habitats. This means a periodic outbreak of Nipah amongst bats would increase the chances of spilling over into humans or other species (Calisher et al, 2006). After 2000, there hasn't been a single Nipah outbreak in Malaysia. The virus did emerge in India and Bangladesh though, but in this case, no intermediate vector was responsible for the transmission. Serologic evidence proves that the virus can be traced back exactly to Indian Flying Foxes; *P.medius* (Calisher et al., 2006). Compared to 30% of antibody percentage in the titer for the Island Flying Fox, Indian Flying-foxes were discovered to have over 50% Nipah antibodies explaining the higher frequencies of outbreaks in the past 20 years (J. H. Epstein et al., 2006).

Nipah makes more bats get sick compared to other viruses, as bats aren't fully tolerant of it. Only 1% of healthy bats tested NiV positive while sick bats were 7% more likely to test positive (Yob et al., 2001). Yadav et al in 2012 found that only 1 healthy male bat was positive in 140 tested species in the whole state of West Bengal.

Siliguri outbreak

66 probable cases of Nipah were reported between January 31st and February 23rd, 2001 in Siliguri in West Bengal (Harit et al., 2006). West Bengal is geographically close to Bangladesh. In India, the disease was recorded in humans without any involvement of pigs or any intermediate vector.

The NiV strain in the Siliguri outbreak was more closely related to the Bangladesh strain than the Malaysian strain. The transmission was through fomites rather than livestock and it was

traced to infected date palm sap that was left in exposed containers in the outskirts of the Siliguri village. (Kulkarni et al., 2013). Because 75 % of cases occurred among hospital staff or visitors, human-to-human transmission was confirmed. (Kulkarni et al., 2013).

In 2007, 5 cases were reported in Nadia, West Bengal; however, the outbreak wasn't as large as the first. Human to human transmission was recorded though (Arankumar et al, 2019).

Kerala outbreak

In 2018, there was an outbreak in Kerala with 23 patients who acquired the virus and 21 deaths. The mortality rate was 91% and the patients who survived had lasting brain damage (Arunkumar et al., 2019)

The first case or the index patient was a 28-year-old in Kozhikode. He was hospitalized with encephalitis and had symptoms like seizures, fever, erratic blood pressure. Two of his family members reported similar symptoms on the same day. Other patients reported symptoms after days of the index patient and sample swabs were sent to the ICMR center in Pune for analysis. Both serological tests and PCR tests were done and of 23 with Nipah symptoms, 18 were positive and 4 were probable (Arunkumar et al., 2019). The outbreak was contained in one cycle as all the patients who were NiV positive were admitted within the incubation period of 5-14 days (Arunkumar et al., 2019)

Around 75% of the cases occurred in staff or the medical facility due to contact with the infection of the patient before the virus was identified as Nipah. After the outbreak, Epidemiologists investigated colonies of bats living in the outskirts of a village in Kozhikode where the index patient lived. The outbreak was during the peak of Indian summer and many fallen fruits were suspected to be contaminated. During this period, there was a ban on all fruit

sales in Kerala along with the import of ripe fruits to other states. The fruits were suspected to act as a fomite contacted by the patient or consumed orally.

In 2019 1 person tested positive for Nipah but was isolated and recovered. Health authorities acted quickly, and no outbreak occurred. In 2021, a 12-year-old boy was infected with Nipah and passed away (WHO, 2021)

Evolution and immune response

Smaller mammals have higher metabolic rates, which are associated with their shorter life spans and lower body mass. Bats are small mammals, but they have exceptional life spans ranging from 20-30 years (O'Shea et al., 2014). Bats outlive both birds and rodents (Mandl et al., 2018). According to a study done by Luis et al in 2013, bats also host more zoonotic viruses than rodents. One of the biggest reasons for this is the social behavior and roosting, which ensures that viruses get transmitted across bat colonies (Luis et al., 2013).

Bats hosting the Nipah virus do get sick as suggested by the Yadav et al short report study in 2012 showing that only 1 out of 140 healthy bats were NiV positive. Sick bats are 7 times more likely to be NiV positive (Yob et al., 2001)

Bat colonies have also shown that delayed senescence and increased individual fitness are an evolutionary advantage to bats. Comparative genomic studies have also found that most bat species bats have most of the mammal immune responses however they lack the PYIHN gene which can detect foreign DNA (Mandl et al., 2018). Usually, this gene regulates the activation of the inflammatory response and it is suggested that the lack of this might be beneficial in preventing excessive immune system response.

The Nipah virus has a short replication period which means that bats shed them quicker. One of the explanations for this can be connected to chronic stress in bats or the high presence of the viruses subduing the NLRP3 or PIYHN activation. Studies have shown that though bat species can tolerate viruses due to immune system mediating genes, excessive doses of viruses like Ebola (similar to Nipah) and Rabies do harm and kill bats (Hayman 2019). This could explain that bats with Nipah can show sickness.

Intermediate levels of viremia and virulence are preferred in the natural selection of fruit bats as the trade-off between acute infection and infection is the result. If the virus replicates faster, the inter-host virulence is higher, but within-host competition increases, and the organism also deteriorates faster. As bats do not often succumb to viral infections like other mammals, this indicates a low virulence which is a trade-off for both the Pteropid and NiV. The trade-off has significant consequences for the fruit bat, however, as it makes them vulnerable to non-viral infections like the fungal white-nose syndrome (O'Shea et al, 2014)

High temperatures favour the immune response of bats. (O'Shea et al., 2014). Due to the longevity of the bats' lifespan and frequent migration patterns, viruses benefit as they can spread within the bat species and between bats and other species (Calisher et al., 2006).

Another important aspect of bats is their flight. Though they have longer lifespans than birds, they still maintain the same mass and wingspan (smallest at around 2 cm). According to researchers, the flight could be one of the biggest evolutionary explanations for how bats host numerous viruses, yet they do not show illness. These researchers hypothesize that the increased immune vigilance has given bats due to the evolution of flight has given them the advantage of not suffering the effect of viruses that significantly impact other mammals. This does not have enough supporting evidence, but the hypothesis is called the "flight or fever" hypothesis (O'Shea et al., 2014).

Another explanation for hosting a multitude of viruses could be torpor, which is a hibernating pattern many temperate bats display to conserve energy but is also suggested to boost their lifespan which makes them better hosts for infectious diseases which need a longer period to infect other organisms (Calisher et al., 2016). This explanation is not very relevant in the topic of the Nipah virus, as all outbreaks were in tropical regions, however the wide temperature range that bats can live at may play a role.

Indigenous species and comparison to international outbreak species

During the years 1998 and 1999 there was a severe Nipah epidemic in Malaysia where patients and pigs suffered critical symptoms and encephalitis. 105 people died and the virus was suspected to originate from Pteropid bats found around the area of the settlement (Kulkarni et al., 2013)

Populations of flying foxes were non-randomly sampled. All cell harvests were checked for Nipah virus antibodies. A total of 324 animals from 14 species of bats were sampled. None of the sera from wild boar, hunting dogs, or rodents tested positive in the enzyme-linking fluorescence assay. Pigs in contact with bats were 40% more likely to test positive than other mammals.

In the bat species, 5 of the 14 bat species tested had Nipah antibodies. The presence of antibodies in *P.hypomelanus* which live on island coasts suggests that the virus can be spread by outbreaks within and between colonies and to remote places through bat travel. Four out of the five bat species that tested positive for NiV antibodies were fruit bat species. One was insectivorous. (Yob et al., 2001)

Human to human transmission was first detected in Bangladesh in 2001. (Kulkarni et al., 2013). According to Yadav et al's field report in 2012, the homogeneity results of the NiV strains in the West Bengal positive bats were a 100% match with the nucleocapsid gene of the strain in the 2001 Siliguri outbreak and 2007 Nadia outbreak along with the Bangladesh outbreaks. The homology with the Cambodian and Malaysian strains was 96% showing that the NiV in Indian and Bangladesh bats may have originated through the interaction of the same migrating colony (Yadav et al., 2012).

The frequency of the outbreaks in India increased in the 21st century compared to Malaysia (which stopped completely). The close distance and similarity in fruit bat species in India and Bangladesh explain why the outbreaks were so similar in viral strain but also the year it occurred.

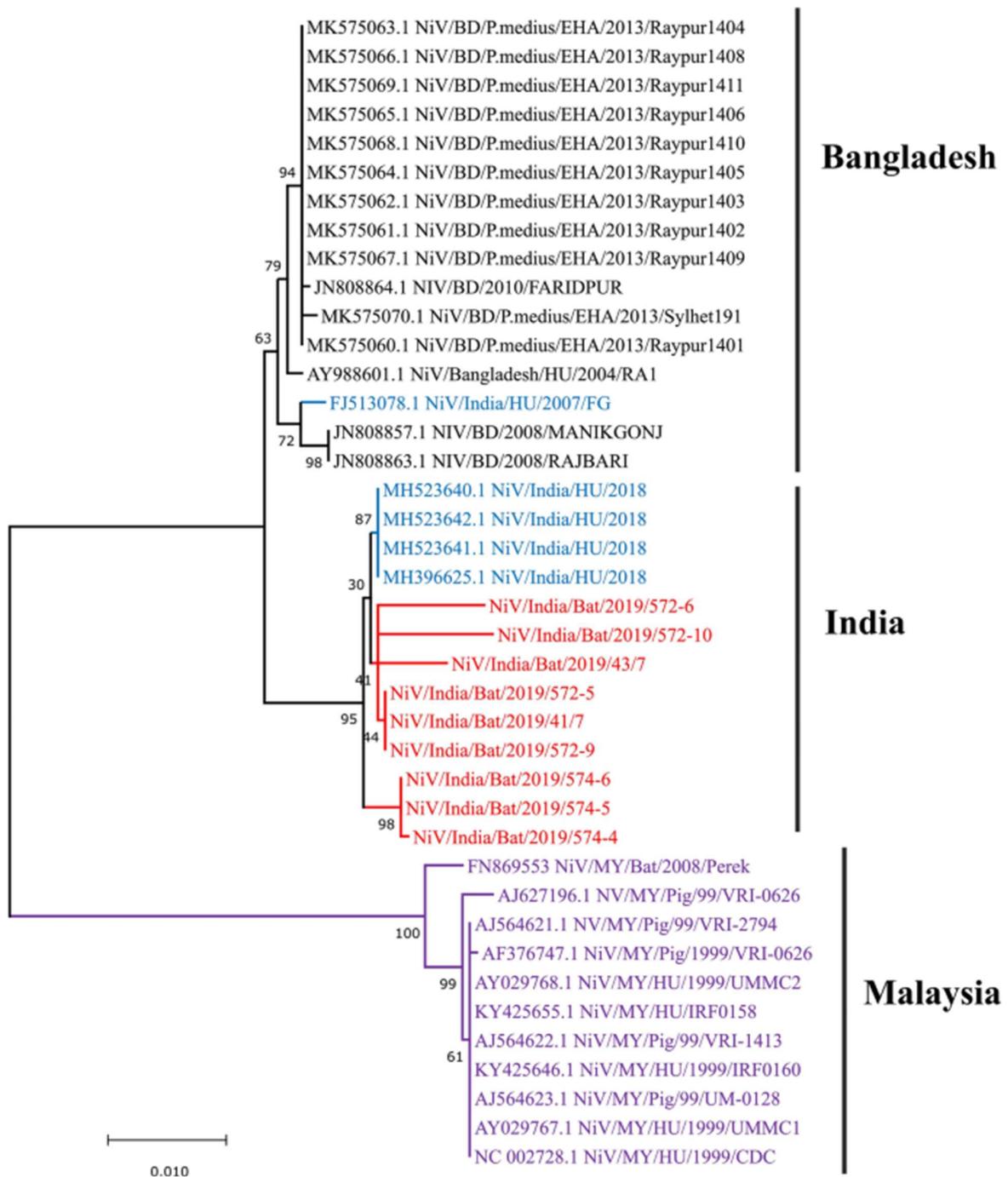


Figure 1: Phylogenetic tree comparing the Nipah virus strains in Malaysia, India, and Bangladesh (Yadav et al., 2021)

This virus and other bat viruses

Many studies have been done using the cyt b gene to observe the neighbor-joining and maximum parsimony phylogenetic trees between *Pteropidae* species. One study by Kho Han Guan and Yuzine Esa in 2006 aimed to determine the phylogenetic relationship of bats in Malaysia, where Nipah was first recorded. The study failed to support the hypothesis of subdivisions of bats in the *Pteropidae* family-like roussetine and cynopterine being closely related (Guan and Esa 2006).

Due to the variation and diversity of subdivisions within bat families, the range of distance commuted along with the physiological adaptations of bats would be different. Other than the Nipah virus in Malaysia, bats did test positive for the Tioman virus and Menangle virus (*rubulavirus* family). These bats were found close to farm settlements, showing that human settlements were a significant area of virus incidence in bat colonies. (Calisher et al, 2006).

Fruit Bats have been discovered to carry many viruses in the past including the ALV (Australian rabies), Ebola virus, Marburg, SARS-CoV, and MERS (Ranjan et al, 2016). What was especially concerning was that the 2006 Calisher study directly correlated increased coronavirus zoonotic diseases to the meat-wildlife market. It was later detected that most Chinese bat species like the horseshoe bat (*Rhinolphidae*) tested positive for some SARS CoV strain antibody, indicating that most bats contained this virus (Calisher et al, 2006). This hindsight on previous research on bats as reservoirs for coronavirus families more than a decade ago posed as a warning for the potency of SARS-CoV viruses, just like the current COVID-19.

Human-animal conflict

In Jared Diamond's book- *Guns, Germs and Steel*, ideas of the development of civilization, human expansion, and limitations are explored. One of the significant themes Diamond touches upon is the severe consequence of large agriculture and animal domestication: zoonotic viruses.

India's fast-growing human population and increasing animal-human interactions have severe consequences. The population also comes with a larger need for basic needs like food and shelter, which results in humans breaching further into the habitat of wild animals. This crossover causes a large amount of spillage of zoonotic infections.

The human encroachment also puts bats in more chronic environmental stress, which causes them to shed viruses more frequently or succumb to acute infection sooner making the whole colony susceptible (If the stress is environmental). The increase of susceptible bats causes an increase in the rate of spread of infection across colonies, even if outbreaks are rare. If more bats acquire the virus, the chance of zoonotic spillover is higher. Altered foraging and behavioral patterns, and virus niche expansion, due to the depletion of their natural habitat, all lead to closer proximity to humans and livestock (Calisher et al., 2006).

Another aspect is climate change. The global increase in the Earth's surface temperature has also influenced the bats' migratory patterns and hibernation patterns. It could also put bats under stress or even favour higher viral transmission between species (Calisher et al., 2016).

The human takeover of the Pteropids' natural habitat along with the changing environment is a significant reason for the increase in zoonotic spillovers, making it a factor that must be carefully monitored to prevent future bat originated diseases.

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