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Perspectives

Nipah virus infection in humans in Kerala, India: Hypothesis of air-borne transmission

Nipah virus disease (NiVD) is a zoonosis that emerged in Malaysia in 1998 in swineherds on a pig farm[1]. Nipah virus (NiV) is enzootic in *Pteropus* bats or flying foxes, such that they remain asymptomatic natural reservoirs of the virus[2]. Since 2001, recurrent NiVD outbreaks have been reported in India and Bangladesh[3,4]. The first outbreak among pig farmers in Malaysia was preceded by respiratory illness and encephalitis in pigs in the Ipoh Perak region[5]. The likely NiV spillover from bats to pigs occurred through contamination of pig feed with bat secretions/excretions present on bat-bitten fruits. Thereafter, many pig farms were affected through pig trade and movement, while the farmers contracted NiV from infected pigs through close contact with respiratory secretions[5,6]. This episode was preceded by the eruption of the Mount Merapi volcano in July 1998, resulting in smoke and ash covering a huge area[7], thereby shifting bat flyways from Indonesian forests to Malaysia.We speculate that bats had NiV, unknown to man until then, explaining the sudden onset of NiVD in Malaysia in September 1998.

Subsequently, bats in Bangladesh and West Bengal were infected with NiV by or before 2000 winter, as evidenced by human NiVD outbreaks. Thereafter, the territory of bat infection seems to have expanded from the Bengal region to Kerala, where infection became enzootic in bats prior to 2018. Currently, fruit bats trapped in Kerala, Karnataka, Tamil Nadu, and Puducherry are known to be infected[8]. In Bangladesh, most NiVD outbreaks occurred during winters in the Central and Northwestern parts. The route of transmission was ingestion of raw date palm sap contaminated with bat urine/saliva[9]. The use of 'bamboo skirts' to cover sap collection pots prevented bats from accessing the sap and reduced NiV spillover to humans[10].

Human-to-human transmission through droplet infection has been reported in almost all early outbreaks in Bangladesh[4]. Of the 248 NiV cases detected between 2001 and 2014, 82 (33%) were suspected to be due to person-to-person transmission. In contrast, the source of infection in the remaining two-thirds was attributed to spillover from bats or remained unknown. The risk of transmission increased with increased duration of exposure of the contacts and with exposure to the body fluids of NiVD case[11]. In Malaysia, transmission from pigs to humans occurred through close contact, either by inhaling droplets/aerosol of oral/nasal secretions or *via* hands to mouth[5,6]. However, in Bangladesh, neither intermediary hosts nor reports of eating fallen, bat-bitten fruits were linked to NiV outbreaks. *Pteropus* bats frequently inhabit areas near human communities in Bangladesh, often roosting in trees in rural areas, where intermittent shedding of NiV in bat urine, saliva, and excreta may expose nearby residents to NiV. This was a potential pointer towards airborne transmission. However, investigations of eight NiVD outbreaks in Bangladesh found no association between living near bat roosts and acquiring NiV infection, indicating that the virus concentration in the environment contaminated by bat emanations may be too low to cause infection[12].

India's first NiVD outbreak occurred in a hospital in Siliguri, West Bengal, during January-February 2001[3]. The source of infection in the primary case was unknown. Nosocomial spread of infection resulted in 65 persons with NiVD, 45 of whom died, for case-fatality of 69 per cent[3]. In Kerala, six outbreaks were reported between 2018 and 2024[13-15], with the number of cases ranging from 1 (Kozhikode, 2019; Ernakulam, 2021; Malappuram, July and September 2024) to 23 (Kozhikode, 2018)[13-15]. The precise mode of spillover of NiV from bats to the 'primary case' in Kerala, as also in outbreaks in West Bengal and several outbreaks in Bangladesh, where transmission was not linked to palm sap consumption, remains unknown. However, subsequent horizontal transmission occurred through close contact and exposure to body secretions of the primary case[9]. Unlike Malaysia and Bangladesh

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outbreaks, recurrent outbreaks in Kerala have not involved an intermediary host or consumption of food items contaminated by bat body fluids. Under these circumstances, identifying the exact route of bat-to-human transmission remains a challenge.

No subclinical infections were reported in Bangladesh[11,] and a serosurvey in Kerala indicated only one per cent seroprevalence in close contacts[16]. These findings suggest that subclinical infections in Bangladesh and Kerala are rare, and spillovers causing unrecognised subclinical infections can be virtually excluded.

In this paper, we propose a hypothesis of air-borne virus transmission as a potential spill-over route in Kerala and possibly elsewhere.

The hypothesis and supporting information

In earlier outbreaks of Nipah in Malaysia, Bangladesh, and Kerala, respiratory transmission was the likely mechanism in most secondary, human-to-human transmission. The virus in oral secretions and other body fluids could be transmitted by inhaling droplets or aerosolised particles. Blood and other body fluids may also form droplets or aerosols that can be inhaled[17]. Transmission *via* fomites could occur through ingestion, inhalation, or perhaps inoculation by fingers into eyes, mouth, or nostrils[18].

In all NiVD outbreaks in Kerala and many outbreaks in Bangladesh, primary cases lacked exposure to bats or their saliva and urine, raising the possibility of another route through which transmission occurred. Inhalation of airborne virus particles as the mechanism of transmission from bats to humans can explain exposure to NiV without contact with bat(s) or bat saliva/urine. Several factors may influence airborne NiV transmission from bats, such as bat distribution and density, viral shedding, aerosol generation, virus survival in the environment, and human exposure to viable virus particles.

Fruit bats (*Pteropus* species) are widely distributed across the Indian subcontinent and typically roost in large colonies on trees. Following each spillover event in Kerala, bats were sampled from affected areas and tested for viral RNA and NiV antibodies. Viral RNA was detected in bats in three of the five outbreaks, with positivity ranging up to 25 per cent among *Pteropus* bats. Overall, 20/647 tested bats had the presence of viral RNA (Table)[14,19-21]. Despite the proximity of human dwellings to bat roosts, NiV RNA was not consistently detected in bats roosting nearest to the primary case's residence. In the 2018 outbreak, viral RNA was detected in bats near primary case's house, within a 5 km radius of the primary case's residence in the 2019 outbreak, and within a 42-55 km radius of the primary case's residence in the 2023 outbreak (Table)[14,19-21] . These findings suggest that proximity to bat roosts alone does not directly correlate with NiV infection risk, consistent with patterns observed in several Bangladesh outbreaks[12].

NiV is shed through oral secretions, urine, and faeces. Studies on the related Hendra virus (HeV) in Australia suggest that urine is the primary medium driving bat-to-bat and potentially bat-to-horse transmission. In Bangladesh, NiV RNA was detected in bat urine up to 52 days after the presumed human exposure event, although viral load had declined over time[22]. Virus shedding from bats is sporadic and influenced by several factors, including age (with juvenile bats shedding more virus), physiological stress (during pregnancy, lactation, and weaning), nutritional deficiencies, habitat disturbances, and co-infections[23]. Viral shedding is intermittent as indicated by the seasonality of the spillover pattern of NiV in Bangladesh, Marburg virus in Uganda, and Hendra virus in Australia[23]. Viral load may be an important determinant of spillover.

Bats generate aerosols through laryngeal movements during vocalisation and respiration[24,25]. Urination from roosting sites may produce aerosols, especially when droplets of bat excreta mix with dust particles[26]. Evidence from other bat-borne viruses supports the plausibility of airborne transmission. For example, aerosolization of the Marburg fever virus in caves has been associated with infection and outbreaks following visits to caves or mines[27]. Limited evidence also suggests that airborne rabies transmission may occur in bat caves with dense bat populations and poor ventilation, although other routes cannot be ruled out[28]. Furthermore, there is speculation about airborne Hendra virus transmission to horses *via* contaminated aerosols from bat secretions[29]. This aerosolization process may facilitate the airborne dissemination of NiV.

Virus survival in the environment is essential for airborne transmission. Experimental studies indicated that HeV remained infectious in *Pteropus alecto* urine (pH ~7) for over four days at 22°C, with a half-life of approximately 19 h. However, at 37°C, HeV was inactivated within one day, with a reduced half-life of

Table. Details of Nipah viral RNA and anti-Nipah antibody positivity in bats in Kerala

Outbreak, yr (month)	Basic profile of the index case	Suspected location of transmission	Results of samples tested from bats
2018 (May)[19]	Young man in his late 20s. Gulf returnee Have a habit of travelling Animal lover	Village: Changaroth Block: Perambra District: Kozhikode	In June 2018, samples from *Pteropus* species were collected near the household of the index case from within 21-30 days of onset of illness in the index case. 52 samples from *Pteropus* species and 12 from *Rosettus* species were collected. A total of 13 throat and rectal swab samples from *Pteropus* spp. tested positive. Three of the positive bats also showed Nipah viral positivity in their organs. Samples of bat-bitten fruits did not show Nipah viral positivity.
2019 (June)[20]	Young man in his early 20s Student	House located at North Paravur, District: Ernakulam He studied in Thodupuzha, District Idukki.	Bats/samples captured from within 5 km radius of the residence of the index case in Ernakulum and his college in Idukki district. All samples were collected between day 5-10 after detection of the index case. The five sites selected were: Vavakkad, Aluva, Thuruthipuram in Ernakulam district and Thodupuzha, Muttam in Idukki district. 141 throat and rectal swabs; 92 viscera and 78 serum samples were collected from *Pteropus* (109) and *Rosettus* (32) species. 3 bats (2 from Idukki and 1 from Ernakulum) had viscera positive for Nipah viral RNA whereas 12 samples tested positive for Nipah IgG antibodies. All positive samples were from *Pteropus* bats.
2021 (August)[14]	A boy of 12 yr Student	Village: Chathamangalam Block: Koduvalli District Kozhikode	Four bat roosting sites were selected in vicinity of the house of the index case. These sites were in Kodiyathur (1 km),Cheruvadi (4 km), Omassery (12 km), and Thamarassery (18 km). 38 *Pteropus medius* and 63 Rousettus leschenaultia bat samples were collected in September 2021. All were negative for viral RNA whereas 8 *P. medius*and 20 *R leschenaultia* from site 1 and 4 tested positive for Nipah IgG
2023 (September)[21]	A middle-aged man in late 40s Gulf returnee Farmer	Village: Changaroth Block: Perambra District: Kozhikode	Sampling was done in February, July and September 2023. A total of 289 samples were collected from bats from Mananthavady & SulthanBathery, Wayanad district and Perambra, Manassery, Kuttiady, Kallad & Thaleekkara, Kozhikode district. All 289 throat and rectal swab samples tested negative. Spleen sample of 1 *P. medius* collected in July from Manassery which is at a 42 km distance from the house of index case and 3 spleen/kidney samples collected in September from Sulthan Bathery which is 55 km away from the 2023 index case's residence, tested positive for Nipah RNA.
2024 (July) (unpublished data)	A boy of 13 yr Student	Village: Chembrasseri Block: Pandikkad District: Malappuram	A total of 52 bats were sampled from four different sites in Malappuram district in July-August 2024. All samples tested negative for Nipah viral RNA. Serum samples of 6 bats 5 km away from the house of the index case tested positive for anti-Nipah IgG antibodies.

three hours. In contrast, NiV incubated in *Pteropus vampyrus* urine (pH 2) was rapidly inactivated within thirty minutes at both 22°C and 37°C. These findings suggest that NiV's environmental stability is limited under most natural conditions[27].

Given the above pieces of evidence, airborne transmission of NiV is theoretically possible if humans are exposed to aerosols containing viable virus or free virus particles. However, NiV's limited environmental stability, particularly in acidic bat urine, may restrict the likelihood of sustained airborne spread. The occurrence of single spillover events across the six NiV outbreaks in Kerala, along with the seemingly random distribution of primary cases, aligns with the hypothesis of rare airborne transmission. Such transmission may occur at an extremely low frequency, but with recurrence. In short, the pattern of NiV spill-over events in Kerala is compatible with rare instances of airborne transmission. It accommodates both recurrence and rarity. No other transmission route has been proposed by anyone.

The way forward

Although the hypothesis of airborne NiV transmission is plausible, laboratory experiments and field investigations are required to confirm or negate this. Studies in the Biosafety Level 4 laboratory may address the virus viability decay rate at different temperatures and humidity. The assumption that stray virus particles may remain viable in the atmosphere must also be tested in the field. Large volumes of air should be sampled by filtration for capturing virus particles. For example, a study conducted in 72 South African High Schools employed high-volume air filtration to detect airborne *Mycobacterium tuberculosis* and analysed samples using digital droplet PCR[30]. Similar air sampling methods for NiV around trees with bat roosts may be explored. Chang *et al*[31] have described several devices used worldwide to trap airborne viruses from the environment and test them by molecular assays or paper-based strips. Such tools could be valuable in investigating the role of the airborne transmission of NiV coupled with artificial intelligence, to detect fluctuations in virus concentrations around bat roosts due to intermittent shedding, with increased shedding during nutritional stress or birthing[29]. The experimental Syrian hamster model clearly suggests the possibility of airborne transmission of NiV[32]. However, laboratory studies are only suggestive in nature and cannot be directly extrapolated to humans. The known animal models for NiV are Syrian hamsters and possibly pigs, as they acted as intermediary hosts in Malaysia. Sentinel surveillance of these animals could also be another way of investigating the possibility of airborne NiV.

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