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Editorial

Detection of possible Nipah virus infection in *Rousettus leschenaultii* and *Pipistrellus Pipistrellus* bats in Maharashtra, India

Bats harbor several high-risk viruses like Ebola, Marburg, Hendra, Nipah virus (NiV), etc. and spillover of these viruses to humans have resulteds in fatal disease outbreaks [1]. NiV is on the top 10 priority list pathogens identified by World Health Organization (WHO).

NiV, a member of the genus *Henipavirus* (family *Paramyxoviridae*) was first identified in Malaysia in 1998–99 during an encephalitis-like outbreak among pigs and pig handlers with a case fatality rate (CFR) of 40% [2]. Till date, India has experienced four episodes of NiV outbreaks with CFR ranging from 65 to 100%. First evidence of NiV infection was reported in Siliguri district, West Bengal in 2001 followed by Nadia district in West Bengal in 2007 [3,4]. The presence of NiV antibodies were detected in Mynaguri and Dubri district of Assam and Cooch Behar area of West Bengal, both places situated close to Bangladesh border during earlier proactive studies [8]. A third outbreak occurred in a geographically distant region of India in Kozhikode district of Kerala state in 2018 with 18 case fatalities, followed by another outbreak in the same state in 2019 [5]. A recent study by Deka and Morshed in 2018 has identified many South East Asian countries including Indian states as potential hotspots for the NiV disease [6].

Pteropus medius bats are the incriminated reservoir for NiV in India as both NiV RNA and antibodies were detected in the samples of these bats collected during NiV outbreaks [7,8]. The studies on other species of bats as potential NiV reservoirs in India is very less. Hence, a cross sectional survey was initiated to study the prevalence of NiV in bats of India by random sampling of *P. medius*, *Rousettus leschenaultii* and *Pipistrellus pipistrellus* bats that have wide prevalence in India. *Pteropus* spp. bats are large fruit eating bats. In contrast to *Pteropus*, *Rousettus* spp. bats are medium sized fruit eating bats and *Pipistrellus* spp. bats are tiny insectivorous bats, mainly found in identical habitats as *Rousettus* spp. bats. During different surveys conducted in the past, *Rousettus* and other species of bats have been screened for NiV but it has been postulated that since virus circulates in a large population of bats and due to low viral loads detection of the same is challenging.

During March 2020, from a cave in Mahabaleshwar, Satara district of Maharashtra state two species of bats ie., *R. leschenaultii* (N = 65) and *P. pipistrellus* (N = 15) were trapped using mist nets. Blood, throat and rectal swab samples were collected onsite from anaesthetized bats strictly adhering to bio-safety protocols. Throat and rectal swab specimens were collected from all the bats while blood could be collected only from 56 *Rousettus* spp. and 4 *Pipistrellus* spp. bats. Necropsy of ten bats of each species was performed at the containment facility of Indian Council of Medical Research-National Institute of Virology (ICMR-NIV), Pune. RNA was extracted from throat swab, rectal swab and organ samples (kidney, liver and spleen) using Magmax RNA extraction kit (Applied Biosystems, USA) as described earlier. NiV-specific TaqMan based real-time reverse transcription-polymerase chain reaction (rRT-PCR) was performed as described earlier [9].

All the available serum samples of bats were heat inactivated at 56 °C for 30 min and anti- NiV IgG antibodies were determined using in-house developed ELISA as well as with reagents received from Center for Disease Control and Prevention, United State of America (CDC, USA) [8]. Briefly, for coating of ELISA plates, inactivated NiV Vero CCL81 whole cell lysate was used. Bat serum (1:100 diluted) was added and after incubation for 1 h at 37 °C bat specific horseradish peroxidase (1:2000 dilution), (Thermo fisher Scientific) was added. Plates were incubated for 1 h at 37 °C. After each incubation, plates were washed 5 time with wash buffer (1XPBST). TMB substrate was added and reaction was stopped after 12–15 min using 1 normal (N) Sulfuric acid. Reading was noted at 450 nm. Nipah bat IgG positive and negative bat serum controls were included in the test. The assay showed 100% and 83.3% sensitivity and specificity when compared to CDC gold standard assay.

Anti-NiV IgG antibodies were detected in 33/56 Rousettus and 01/04 Pipistrellus bat sera samples respectively. Earlier studies by Yob et al. have shown detection of anti-Nipah virus neutralizing antibodies in non-pteropid species [10].

Nucleocapsid gene-based real time RT-PCR detected NiV RNA in different organs as well as throat/rectal samples from both the species of bats (Table 1). Real time RT PCR cycle threshold values ranged from 34 to 38 for the NiV RNA positive specimens. One bat each from *R. leschenaultii* and *P. pipistrellus* species was tested positive for both NiV RNA and anti NiV IgG antibodies. Detection of only NiV RNA in the bats without the presence of anti-NiV antibodies is suggestive of probable recent infection. All the positive samples were processed for the next generation sequencing as well as by amplification of short fragment by RT-PCR and sequencing by methods as described earlier [5]. Due to the low viral load, sequence data could not be retrieved.

Isolation attempts in VeroCCL81 cells using the NiV RNA positive specimens were also not successful probably due to the low viral load. Earlier studies also shows very less virus isolation rates from field surveillance and experimental bat samples of Henipa viruses

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Table 1
Detection of Nipah virus in two species of bats.

Bat species	Detection of NiV RNA using real time RT-PCR (positive samples/total samples)				Detection of anti-NiV IgG antibody in serum samples (positive samples/total samples screened)
	Liver/spleen	Kidney	Throat swab	Rectal swab	
Rousettus leschenaultii.	1/10	2/10	1/65	1/65	33/56
Pipistrellus pipistrellus	2/10	1/10	0/15	0/15	01/04
Total	**3/10**	**3/10**	**1/80**	**1/80**	34/60

Note: Nipah viral RNA positivity was detected in liver/spleen as well as kidney specimens of the same Pipistrellus bat. Pipistrellus bat which was NiV RNA and IgG antibody positive, have >1 tissue type i.e liver/spleen as well as kidney positive for NiV RNA but R. leschenaultia bat that was NiV RNA and IgG antibody positive have one tissue or swab type that was positive for NiV RNA.

indicating limited window period for transmission [11–14]. Due to lack of anti-NiV IgM antibody testing for bats, we could not confirm the presence of IgM antibody in these samples.

This is the first report of possible NiV infection in *R. leschenaultii* bats in India, which was demonstrated by the presence of both NiV RNA and anti-NiV IgG antibodies in bats (Table 1). Boosted regression model by Plowright et al. [12] predicted six species which are not earlier identified as Nipah reservoirs on high likelihood of exposure to Nipah virus. These include *R. luctus*, *Murina cyclotis*, *Taphozous theobaldi*, and *P. Pipistrellus*, which supports the findings of the current study.

However, in our earlier investigations during the last decade, NiV activity could not be detected in *R. leschenaultii*, despite processing several hundred bats including bats from the same location [11] During the earlier surveys, Malsoor virus, Tioman virus and a novel adenovirus have been isolated from *R. leschenaultia.*

Pipistrellus spp. are known reservoirs for rabies virus, bat corona viruses as well as lyssavirus. Serological evidence to NiV or other Henipavirus have been reported in Rousettus species from China and Vietnam earlier [13]. The exposure of *R. leschenaultii* bats to NiV warrants further investigation as roosting and breeding habitats of the *Rousettus* spp. and *Pteropus* spp. vary greatly. More studies in bats and humans are therefore needed to understand the prevalence of the virus in the state. The roost which was sampled was age-old and the virus might have been circulating among the inhabitants at low levels and not detected during earlier studies. Alternatively, a new introduction might have occurred from *P. medius* to *Rousettus* bats through NiV contaminated fruits, as both are frugivorous and share the same fruit trees for food. Presence of NiV RNA in the bat saliva poses a threat to humans.

NiV detection in *P. pipistrellus* bats, an insectivorous species and their role in virus spill-over to humans appears remote. Their positivity might be explained through sharing the same habitat with *R. leschenaultii* bats inside the cave. Experimental studies have shown that close contact or aerosol exposure could transmit NiV infection in Syrian hamsters [14]. However, it is difficult to infer any conclusion as only a few bats were screened during the present study. A systematic approach that includes sampling a large number of *Pipistrellus* and *Rousettus* bats is necessary to determine their role in NiV transmission.

Recurring outbreaks, high case fatality rate, human to human transmission and lack of effective vaccine/antivirals pose a major concern in India as bat roosts are very common in areas where large human populations reside. It is very important to understand the bat ecology, seasonality of NiV and the transmission risks associated with it, for the one health approach. This will in turn help in understanding the wildlife reservoirs as well as the risk of reintroduction of NiV into animal or human populations.

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Conflict of interest

None declared.

Ethical approval

The Institutional Bio-safety and Animal Ethics committee of ICMR-National Institute of Virology (NIV), Pune has approved this study. Requisite permission to trap and sample bats was also obtained from the Principal Chief Conservator of Forests, Maharashtra.

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