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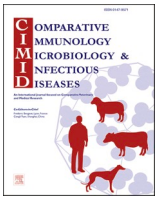

Letter to editor

Serosurvey for Nipah virus in bat population of southern part of India

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ABSTRACT

Nipah virus (NiV) is one of the priority pathogens with pandemic potential. Though the spread is far slower than SARS-CoV-2, case fatality is the biggest concern. Fruit bats belonging to genus *Pteropus* are identified to be the main reservoir of the virus causing sporadic cases and outbreaks in Malaysia, Bangladesh and India. The sudden emergence of Nipah in Kerala, India during 2018–2019 has been astonishing with respect to its introduction in the unaffected areas. With this, active Nipah virus surveillance was conducted among bat populations in Southern part of India *viz.*, Karnataka, Kerala, Tamil Nadu, Telangana, Puducherry and Odisha during January-November 2019. Throat swabs/rectal swabs (n = 573) collected from *Pteropus medius* and *Rousettus leschenaultii* bat species and sera of *Pteropus medius* bats (n = 255) were screened to detect the presence of Nipah viral RNA and anti-Nipah IgG antibodies respectively. Of 255 *P. medius* bats sera samples, 51 bats (20%) captured from Karnataka, Kerala, Tamil Nadu and Puducherry demonstrated presence of anti-Nipah IgG antibodies. However, the presence of virus couldn't be detected in any of the bat specimens. The recent emergence of Nipah virus in Kerala in September 2021 warrants further surveillance of Nipah virus among bat populations from the affected and remaining states of India.

1. Introduction

Nipah virus (NiV) is an emerging *paramyxovirus* capable of causing lethal infection in a number of mammalian species including humans. The first human infection with NiV was identified during an outbreak of severe encephalitis in Malaysia in 1998–1999 [1]. Both animal-to-humans and human-to-human transmission has been documented during different outbreaks. More than 700 human cases of Nipah virus infections were reported from Malaysia, India, Bangladesh, Singapore and Philippines during 1998−2018 [2].

India has witnessed four outbreaks of Nipah virus disease during 2001–2019. The first outbreak of NiV with presentation of febrile illness and neurological symptoms was observed among human population in Siliguri, West Bengal during 2001 with a case fatality rate (CFR) of 74% [3]. Subsequently, an outbreak was reported from Nadia district, West Bengal in 2007 which affected five people and all succumbed to infection (CFR 100%) [4]. ICMR-National Institute of Virology, Pune conducted Nipah surveillance among bat population in North-eastern states of India during 2015. The surveillance revealed ~~the~~ presence of NiV among *P. medius* bats collected from Cooch Bihar district, West Bengal and Dhubri district, Assam [5]. Similarly, Nipah surveillance was carried out amongst pig population of eight districts of Mizoram state in North-East. However, all the pig serum samples tested negative for anti-Nipah IgG antibodies [6].

The recent emergence of NiV was reported from Kerala state in the Western Ghats during 2018–2019 [7,8]. Investigation of 2018 outbreak revealed the exposure of the index case to the bats and subsequent human to human transmission [9]. Further, the source of infection in a single case of NIV infection in Kerala during 2019 was also linked to bats. However, the exact cause of the spillover event remained untraced. Studies in bat populations at the locations associated with the index case confirmed presence of NiV RNA and anti-IgG NiV antibodies [10]. The sequence analyses showed its deviation from the NiV strains from Bangladesh and North-eastern region of India.

Kerala is the southernmost state of India, distinctly located far away from the earlier NiV hot-spots. Knowledge of the distribution and movement patterns of bat species that act as the reservoir hosts of Nipah virus is necessary to identify the regions at risk, possible events of spillover and the role of eco-geographical factors implied in NiV dynamics in bat population. Considering all these factors, the present study was carried out to determine presence of NiV activity in bat populations in southern states and union territories which are geographically close to the new hotspot of NiV and a state (Odisha) bordering West Bengal in the south east.

2. Materials and methods

An approval to carry out the present study was obtained from the Institutional Biosafety Committee and Institutional Animal Ethics Committee of ICMR-NIV, Pune, India. Prior to initiating the work, permission was also obtained for trapping the bats from Principal Chief Conservators of Forest of the respective states/Union Territories. ICMR-NIV, Pune team visited the pre-identified areas of different states/UTs to locate the bat colonies.

P. medius and *R. leschenaultii* bats were captured from pre-identified sites in five states (Telangana, Karnataka, Tamil Nadu Kerala and Odisha) and one union territory (Puducherry) respectively using the methodology described earlier [9]. After species identification of trapped bats, throat and rectal swab specimens were collected in virus transport medium (HiViral™ Transport Medium, HIMEDIA) and stored immediately in dry ice. Blood samples (2–3 ml) were collected from the wing (cephalic) vein and serum was separated. After recovery from

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anesthesia, the bats were released back. Utmost precautions were taken while handling bats and during specimen collections as per defined biosafety protocols and safety practices developed by ICMR-NIV, Pune.

A total of 573 throat swabs/rectal swabs of *P. medius* (n = 541) and *R. leschenaultii* bats (n = 32) and blood samples of *P. medius* bats (n = 255) were collected from Telangana, Kerala, Karnataka, Tamil Nadu, Odisha and Puducherry during January-November 2019 [Table 1]. Throat/rectal swab specimens were screened using Nipah specific real-time reverse transcription–polymerase chain reaction (rRT-PCR) as per the procedure described elsewhere [5]. Serum specimens were heat inactivated at 56°C for 30 min and further tested for the presence of anti-NiV IgG antibodies by indigenously developed Enzyme-Linked Immunosorbent assay (ELISA) as described earlier [10].

3. Results

All the throat swabs/rectal swab specimens of *P. medius* and *R. leschenaultii* bats were found negative for the NiV RNA using NiV

Table 1
Details of bat capturing sites and anti-Nipah IgG positivity in different states and union territory in India.

Sr. No.	Month and year of bat capture	District/State	Site	Species of bats collected (n)	NiV Positive/Total TS/ RS tested by Real Time RT-PCR	NiV IgG Positive /tested by Anti-Nipah IgG ELISA
1	August 2019	Karnataka	Directorate of Health and Family Welfare Services, Anandrao Circle, Gandhi Nagar 580008, Bengaluru GPS 12.981179,77574204	*P. medius* (n = 6)	0/6	NA
			Site 1- Anamanahalli	*P. medius* (n = 56)	0/56	20/56
			Site 2- BPL Industry	*P. medius* (n = 13)	0/13	2/13
			Site 3- Koornagere State Forest	*P. medius* (n = 1)	0/1	0/1
			Site 4 - Anche, Chittana Halli	*P.medius* (n = 6)	0/6	2/6
Total				*P.medius* (n = 82)	**0/82**	**24/76**
2	April 2019	Kozhikode/Kerala	Pallikkunnu, Panthirikkara, Near Changaroth LP& UP School, Perambra Town, Kozhikode (April 2019)	*P. medius* (n = 73); *R. leschenaultii* (n = 10)	0/83	NA
			Kuttiady (Opposite to Kuttiady Bar, Adjacent to Citizens' Club, Kuttiady Town), Kozhikode (April 2019)	*P. medius* (n = 1)	0/1	NA
	November 2019		Site 1 (Perambra) MUP School Road, Changaroth, Perambra, Kozhikode	*P. medius* (n = 25)	0/25	4/24
			Site 2 Kuttiady, Maruthonkera bridge, Kozhikode	*P. medius* (n = 2)	0/2	1/2
			Site 3 Olipram Kadavu road, Kozhikode	*P. medius* (n = 1)	0/1	1/1
	November 2019	Malappuram, Kerala	Site 1 (Karakunnu Vandoor)	*P. medius* (n = 1)	0/1	0/1
			Site 2	*P. medius* (n = 7)	0/7	0/7
		Idukki, Kerala [during June 2019 outbreak]	Site 1 (Near River Thodupuzha, Dist. Idukki, Kerala)	*P. medius* (n = 54)	0/54	4/23
			Site 2 (Muttom, Thodupuzha, Dist. Idukki, Kerala)	*P. medius* (n = 8)	0/8	0/8
			Site 3 (Aluva, Dist. Idukki, Kerala)	*P. medius* (n = 61)	0/61	6/39
			Site 4 (Thuruthipuram, Thekekkara Panchayat)	*P. medius* (n = 4)	0/4	1/4
			Site 5 (Vavakkad, Paravoor, Thekekkara Panchayat)	*P. medius* (n = 14)	0/14	1/4
Total				*P. medius* (n = 251); *R. leschenaultii* (n = 10)	**0/261**	**18/113**
3	July and August 2019	Tamil Nadu	Site 1: Near Shivmandir Thisupudale marudur (Western Ghat)	*P.medius* (n = 55)	0/55	07/26
			Site 2: Courtallam, Parasakthi Women College Tirunevelli, Tamil Nadu	*P. medius* (n = 4)	0/4	NA
			Site 3: SN College, Madhurai, Tamil Nadu	*P. medius* (n = 7)	0/7	NA
Total					**0/66**	**7/26**
4	July 2019	Puducherry	Site 1: GPS 11.929138.79.8177744412 Urban Forest	*P. medius* (n = 23)	0/23	2/21
Total				*P. medius* (n = 23)	**0/23**	**2/21**
5	February and March 19	Sangareddy/ Telangana	Manjira Wildlife Sanctuary, Sangareddy	*P. medius* (n = 8)	0/8	NA
		Rangareddy/ Telangana	Premises near Chilkur Balaji Temple, Chilkur, Moinabad Mandal, Rangareddy	*P. medius* (n = 81); *R. leschenaultii* (n = 4)	0/85	NA
		Jangaon/ Telangana	Premises of the Senior Civil Judge's court, Jangaon,	*P. medius* (n = 5)	0/5	NA
Total				*P. medius* (n = 94); *R. leschenaultii* (n = 4)	**0/98**	**NA**
6	January 2019	Dhenkanal/ Odisha	Site-1 (Village: Birasagar; Taluka: Kamakhyanagar; District: Dhenkanal; State: Odisa)	*P. medius (n = 6)*	**0/6**	**0/1**
			Site-2 (Village: Brahmani Devi temple, Badamgiri, Kushida; Distirict: Dhenkanal; State: Odisa)	*P. medius (n = 35)*	**0/35**	**0/15**
				R. leschenaultii (n = 2)	**0/2**	NA
Total				*P. medius* (n = 41); *R. leschenaultii* (n = 2)	**0/43**	**NA**

*TS-Throat swab, RS-Rectal swab, n-Number, NA-Not available

specific rRT-PCR. However, anti-NiV IgG antibodies were detected in serum samples of *Pteropus* bats collected from Karnataka (24/76), Kerala (18/113), Tamil Nadu (7/26) and Puducherry (2/21). High antibody prevalence of 20% was detected in *Pteropus* bat species. Anti-NiV IgG antibodies couldn't be detected in *Pteropus* serum samples collected from two sites in Dhenkanal, Odisha. Sites of sample collection and anti-NiV IgG positivity in different states and a union territory are depicted in Table 1.

4. Discussion

An earlier report indicated anti-NiV IgG positivity in recaptured fruit bats related to *Pteropus* spp. in Madagascar [11]. The reintroduction of infected bats or the waning or loss of antibodies over time in bats can result in variations in antibody level in a bat population roosting in the same geographical unit. This phenomenon supports our findings of presence of anti-NiV IgG in only a few bat specimens sampled from the colonies in the same geographical area. Similar observations were also reported in Bangladesh, where the presence of anti-NiV IgG with varied seroprevalance was observed in various locations during the six years of study. Besides this, NiV RNA could be detected in a very few bat samples [12,13]. Migratory behavior is comparatively less common in bats of tropical and sub tropical region as compared to the temperate region and is never associated with hibernation. Migration is mainly driven by the food resources [13]. The paucity of information on bat migration patterns in India limits the knowledge on the migratory bat populations.

Phylogenetic analysis of NiV N gene sequences from the new hot spots in Kerala showed grouping into a cluster distinct from the sequences from Malaysia and Bangladesh suggesting the presence of a new genotype independently evolving in Southern India [9]. Besides this, a fatal case of Nipah was recently reported from Kozikode, Kerala during September 2021 for the second time over a period of three years. The evolving epidemiology of NiV infections, varied involvement of other animal species as intermediate hosts and source of infection in human beings in different geographical regions warrants detailed nationwide proactive surveillance for Nipah with "One Health" approach throughout the year in bats and other animals. This would be of immense importance in understanding the dynamics of NiV in the geographical region and might inform about effective strategies for the prevention and control of Nipah.

5. Conclusion

The fruit bats of genus *Pteropus* are identified to be the main reservoir of the nipah virus causing annual outbreaks in Malaysia, Bangladesh and other countries in South-East Asia including India. Three incidences of NiV infections in humans in Southern state of Kerala which is far distant from the known "Nipah belt" in consecutive years with no identified intermediate animal host or confirmed mode of entry into human population warrants the heightened need of constant surveillance of NiV in bats, animals and humans.

Author Contributions

PDY, DTM, MG, AS contributed to study design, data analysis, interpretation and writing and critical review. BM, RB, UPT, SM, DRP, AMS, SD, GC, SG, MH, ATS, PS, RJ, DYP contributed to data collection and interpretation. PDY, MG, AS, AMS, DYP contributed to the critical review and finalization of the paper.

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Conflicts of Interest

The authors do not have any conflict of interest.

References

[1] K.B. Chua, K.J. Goh, K.T. Wong, A. Kamarulzaman, P.S.K. Tan, T.G. Ksiazek, S. R. Zaki, G. Paul, S.K. Lam, C.T. Tan, Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia, Lancet 354 (9186) (1999) 1257–1259.
[2] E.S. Gurley, C.F. Spiropoulou, E. de Wit, Twenty years of Nipah virus research: where do we go from here? J. Infect. Dis. 221 (Supplement_4) (2020) S359–S362.
[3] M.S. Chadha, J.A. Comer, L. Lowe, P.A. Rota, P.E. Rollin, W.J. Bellini, T.G. Ksiazek, A.C. Mishra, Nipah virus-associated encephalitis outbreak, Siliguri, India, Emerg. Infect. Dis. 12 (2) (2006) 235.
[4] V.A. Arankalle, B.T. Bandyopadhyay, A.Y. Ramdasi, R. Jadi, D.R. Patil, M. Rahman, M. Majumdar, P.S. Banerjee, A.K. Hati, R.P. Goswami, D.K. Neogi, Genomic characterization of nipah virus, west bengal, India, Emerg. Infect. Dis. 17 (5) (2011) 907.
[5] P. Yadav, A. Sudeep, M. Gokhale, S. Pawar, A. Shete, D. Patil, V. Kumar, R. Lakra, P. Sarkale, S. Nichol, D. Mourya, Circulation of Nipah virus in Pteropus giganteus bats in northeast region of India, 2015, Indian J. Med. Res. 147 (3) (2018) 318.
[6] D. Mourya, P. Yadav, M. Rout, B. Pattnaik, A. Shete, D. Patil, Absence of Nipah virus antibodies in pigs in Mizoram State, North East India, Indian J. Med. Res. 149 (5) (2019) 677.
[7] G. Arunkumar, R. Chandni, D.T. Mourya, S.K. Singh, R. Sadanandan, P. Sudan, B. Bhargava, Outbreak investigation of Nipah virus disease in Kerala, India, 2018, J. Infect. Dis. 219 (12) (2019) 1867–1878.
[8] R.R. Sahay, P.D. Yadav, N. Gupta, A.M. Shete, C. Radhakrishnan, G. Mohan, N. Menon, T. Bhatnagar, K. Suma, A.V. Kadam, P.T. Ullas, Experiential learnings from the Nipah virus outbreaks in Kerala towards containment of infectious public health emergencies in India, Epidemiol. Infect. (2020) 148.
[9] L. Paul, Nipah virus in Kerala: a deadly Zoonosis, Clin. Microbiol. Infect. 24 (10) (2018) 1113–1114.
[10] A.B. Sudeep, P.D. Yadav, M.D. Gokhale, R. Balasubramanian, N. Gupta, A. Shete, R. Jain, S. Patil, R.R. Sahay, D.A. Nyayanit, S. Gopale, D.T. Mourya, Detection of Nipah virus in Pteropus medius in 2019 outbreak from Ernakulam district, Kerala, India, BMC Infect. Dis. 21 (1) (2021) 1–7.
[11] C.E. Brook, H.C. Ranaivoson, C.C. Broder, A.A. Cunningham, J.M. Héraud, A. J. Peel, L. Gibson, J.L. Wood, C.J. Metcalf, A.P. Dobson, Disentangling serology to elucidate henipa-and filovirus transmission in Madagascar fruit bats, J. Anim. Ecol. 88 (7) (2019) 1001–1016.
[12] J.H. Epstein, S.J. Anthony, A. Islam, A.M. Kilpatrick, S.A. Khan, M.D. Balkey, N. Ross, I. Smith, C. Zambrana-Torrelio, Y. Tao, A. Islam, Nipah virus dynamics in bats and implications for spillover to humans, Proc. Natl. Acad. Sci. USA 117 (46) (2020) 29190–29201.
[13] T.H. Fleming, Bat migration, in: Encyclopedia of Animal Behavior, 2019, 605.

Mangesh Gokhale[a,1], A.B. Sudeep[a,1], Basavaraj Mathapati[a,1], R. Balasubramanian[b], P.T. Ullas[a], Sreelekshmy Mohandas[a], Dilip R. Patil[a], Anita M. Shete[a], Sanjay Gopale[a], Pradeep Sawant[a], Rajlaxmi Jain[a], Manjunath Holeppanavar[a], Annasaheb T. Suryawanshi[a], Ganesh Chopade[a], Sachin Dhaigude[a], Deepak Y. Patil[a], Devendra T. Mourya[a], Pragya D. Yadav[a,*]

[a] *Indian Council of Medical Research-National Institute of Virology, Pune 411021, India*

[b] *Indian Council of Medical Research-National Institute of Virology, Field Unit, Vandanam, Kerala 688005, India*

* Correspondence to: Maximum Containment Facility, Indian Council of Medical Research, National Institute of Virology, Sus Road, Pashan, Pune 411021, India.

E-mail address: yadav.pragya@gov.in (P.D. Yadav).

[1] Equal first author.