



## Correspondence

### Antibody response in symptomatic & asymptomatic Nipah virus cases from Kerala, India

As per the World Health Organization, Research and Development Blueprint List of epidemic threats, Nipah virus (NiV) disease is one of the priority diseases that needs urgent action<sup>1</sup>. Since its detection in 1998, many outbreaks of Nipah have been reported from Malaysia, Singapore, Bangladesh and India<sup>2-7</sup>. It is a serious public health threat for the countries in Southeast Asia. In the last two decades from (2001 to 2021), India has reported five Nipah outbreaks among human population in West Bengal and Kerala<sup>4,7</sup>. In 2018, the sudden emergence of NiV was observed in Kozhikode and Malappuram districts of Kerala<sup>6</sup>. The outbreak had a case-fatality rate of 89 per cent with two cases survived the NiV infection. Subsequently, another NiV outbreak was reported from Ernakulum district, Kerala, during 2019<sup>8</sup>. A single individual was affected with NiV who survived the infection and recovered completely<sup>8</sup>.

Nipah outbreaks have been found to occur in sporadic form with a few cases. Consequently, NiV-specific antibody response has been studied only in Nipah symptomatic survivors. There is no information available on the persistence of the antibodies during follow up among asymptomatic contacts of Nipah-positive cases. Hence, the present study was carried out to evaluate the antibody response among symptomatic survivors of NiV infection and their asymptomatic contacts identified during 2018 and 2019 NiV outbreaks from Kerala, India.

The study was approved by the Institutional Human Ethics Committee of the Indian Council of Medical Research-National Institute of Virology (ICMR-NIV), Pune, Maharashtra, India. The NiV infection survivors (n=3) and their asymptomatic contacts (n=3) of 2018 and 2019 outbreaks were identified, and blood samples were collected at different time intervals. The samples were transported to ICMR-NIV, Pune, under cold chain at 4°C. A total of 28 follow up blood samples were collected from the survivors of NiV outbreak during May 2018 (cases 1

and 2) till 438<sup>th</sup> day post-onset of disease (POD). Five follow up samples from 2019 NiV outbreak (case 3)<sup>8</sup> were collected from 11<sup>th</sup> to 113<sup>th</sup> days POD. Seven follow up samples were collected from asymptomatic contacts (contacts 1, 2 and 3) identified in 2018 NiV outbreak on 49<sup>th</sup> to 476<sup>th</sup> days post-exposure. Serum was separated from these samples and screened for the presence of anti-NiV human immunoglobulin (Ig) M and IgG using an in-house-developed indirect ELISA. Each sample was tested in duplicate, and reproducibility of the assay was checked. Receiver operating characteristic (ROC) curve was used to determine the cut-off of the assay. Samples were considered as positive if average optical density (OD) of negative control was greater than 0.2 and P/N ratio was more than 1.5. Both the anti-NiV IgM and IgG assays demonstrated specificity of 99.28 per cent and sensitivity of 100 per cent compared to the reference test from the US Centers for Disease Control and Prevention (unpublished data).

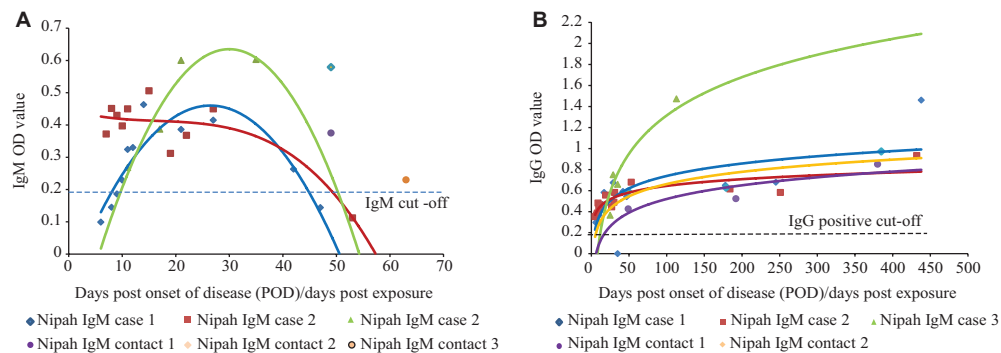
Three symptomatic and two asymptomatic contact cases were tested positive for anti-Nipah IgM and IgG antibodies. However, one asymptomatic contact showed positivity only for anti-NiV IgM antibodies. Anti-NiV IgM was detectable from 5<sup>th</sup> POD to 27<sup>th</sup> POD, while anti-NiV IgG was detected for more than one year among symptomatic NiV cases, respectively. Asymptomatic contacts had detectable levels of anti-NiV IgG from 49<sup>th</sup> POD to approximately 13 months post-exposure (Table). Contact 3 was positive for anti-NiV IgM at days post-exposure (DPE) 63 but did not show the presence of IgM and IgG antibodies in further collections till 300 till 450 days post-exposure. Considering this, Contact 3 was excluded from IgG analysis. The trends of IgM and IgG response in these cases was represented as a scatter plot (Figure 1A and B).

The samples showing IgG positivity among survivors/contacts (cases 1-3; contacts 1 and 2) were

**Table.** Anti-Nipah antibodies among symptomatic survivors of Nipah virus infection and their asymptomatic contacts from Kerala, India

Post-onset of disease/ days post-exposure	Anti-NiV IgM		Anti-NiV IgG	
	Number of samples	Average OD ( $\pm$ SD)	Number of samples	Average OD $\pm$ SD
0-7	3	0.81 $\pm$ 0.24	3	0.352 $\pm$ 0.03
8-14	11	0.33 $\pm$ 0.11	11	0.4 $\pm$ 0.07
15-21	5	0.38 $\pm$ 0.11	5	0.55 $\pm$ 0.15
22-28	3	0.41 $\pm$ 0.041	3	0.56 $\pm$ 0.05
29-35	1	0.6	1	0.65
36-60	5	0.26 $\pm$ 0.189	5	0.59 $\pm$ 0.37
61-180	4	0.36	4	0.62 $\pm$ 0.48
181-365	4	ND	4	0.59 $\pm$ 0.06
366-476	4	ND	4	0.95 $\pm$ 0.27

IgM cut-off: 0.20 and IgG cut-off: 0.35. OD, optical density; ND, not detected; SD, standard deviation; Ig, immunoglobulin; NiV, Nipah virus



**Figure.** ELISA for anti-Nipah virus immunoglobulin M (IgM) and G (IgG) optical density (OD) read at 450 nm for Nipah positive cases - cases 1, 2 and 3 and contacts 1, 2 and 3. (A) Anti-Nipah virus IgM and (B) anti-Nipah virus IgG antibody OD at different post-onset of disease (POD)/days post-exposure. The curves represent IgM and IgG response trends in the individual cases.

tested with plaque reduction neutralization test (PRNT) to determine the neutralizing antibody titre at the Maximum Containment Facility of ICMR-NIV, Pune<sup>9</sup>. NiV-positive human serum sample was used as positive control. Briefly, ten-fold dilution of heat-inactivated (56°C for 1 h) serum samples to a dilution factor of  $10^{-6}$  was mixed with an equal amount of virus suspension ( $10^{-4}$ ) and was incubated for one hour. Subsequently, 200  $\mu$ l of serum-virus mixture was added to each well (in duplicates) of 24-well pre-seeded Vero cell plates and incubated in a CO<sub>2</sub> incubator for one hour. Inoculum was removed, and 3 ml overlay medium (2% CMC+2X MEM+2% FBS) was added to each well followed by incubation at 37°C in a CO<sub>2</sub> incubator for four days. Overlay medium was removed, and cells were washed with 1x PBS (phosphate buffered saline) and stained with amido black stain. The neutralization titre (PRNT50) of the test serum sample is defined as

the reciprocal of the highest test serum dilution, for which the virus infectivity is reduced by 50 per cent when compared with the average plaque count of the challenge virus control<sup>9-12</sup>.

Neutralizing antibody titres of cases 1, 2 and 3 at POD 483, 432 and 113 were 11482, 2291 and 661 whereas those of contacts 1 and 2 at DPE 380 and 385 were 457 and 1145, respectively. The findings suggest the persistence of neutralizing antibody response in symptomatic as well as asymptomatic cases after one-year POD/DPE.

Among the *Paramyxovirus* family, NiV has demonstrated a high zoonotic potential along with high fatality rates<sup>8</sup>. Serology plays an important role in the diagnosis of NiV infection; however, antibody kinetics in Nipah infection is poorly studied. A study conducted by Nikolay *et al*<sup>10</sup> reported the absence of anti-NiV

antibodies in asymptomatic cases. Earlier studies by Ramasundram *et al*<sup>11</sup> revealed anti-NiV IgM and IgG positivity in patients with symptomatic NiV infections till 3-7 months and eight months, respectively. Our study demonstrated the presence of IgM antibodies for more than two months and IgG for more than one year after infection in symptomatic and asymptomatic cases.

Irrespective of the presence of clinical symptoms, we observed comparable IgM and IgG immune response against NiV infection. Both the survivors and asymptomatic contacts showed detectable levels of neutralizing antibody till 14 and 11 months, respectively. Further research is needed to determine long-term immune response or waning immunity.

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