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# Correspondence

## Absence of Nipah virus antibodies in pigs in Mizoram State, North East India

Sir,

Bat-borne viruses are among the important emerging viruses, as these can pose a serious threat to human and animal health. Henipaviruses, coronaviruses, filoviruses and the rabies-causing lyssaviruses are all transmissible from bats to humans. These are often transmitted through an intermediate host, and the resulting human disease is frequently fatal. Bats harbour more zoonotic viruses per species than rodents and are recognized as a significant source of zoonotic agents[1,2]. The emergence of many zoonotic bat-borne viruses in Southeast Asia demonstrated the favourable conditions for such events[3].

Old World fruit bats of the family *Pteropodidae*, particularly species belonging to the genus *Pteropus*, have been considered as natural hosts of a large number of emerging viruses, especially of the family *Paramyxoviridae*[1,4]. Due to their special characteristics, *Pteropus* bats are a perfect reservoir for most of the recently emerging zoonotic pathogens. They often live in large colonies or roosts and travel long distances; thus, they are very effective in transmitting viruses among colony members and disseminating them over a considerable distance[1]. Interactions between *Pteropus* bats, humans and livestock are constantly increasing due to anthropogenic activities, thereby increasing the potential for virus spillover events. Deforestation in tropical areas has destroyed the natural habitats of these fruit bat species, forcing them to live in the vicinity of human settlements. The resulting close contact is responsible for the emergence of highly pathogenic paramyxoviruses, such as Hendra and Nipah virus (NiV), in human populations in Southeast Asia and Australia[4].

In nature, NiV is found in bats which cause severe disease in pigs and humans. Because of high case fatality rates in humans, it is classified as a Risk Group 4 pathogen[4]. Human-to-human transmission of NiV has been documented during earlier Nipah outbreaks in Bangladesh[5,6] and India. In India, *Pteropus giganteus* species of bats are suspected to be the reservoir of NiV. India has witnessed the emergence of NiV in Siliguri district, West Bengal, in 2001 and an outbreak in Nadia district in 2007, both led to fatal outcomes[7-9]. Recently, Nipah outbreak was confirmed in Kozhikode, Kerala State, which claimed lives of 17 people[10].

Although the presence of NiV has been confirmed among bats from northeastern region of India in the past[7-9,11], there is no information available on the presence of NiV among pig population from India. Considering this, the present study was undertaken to determine NiV prevalence among pig population in Mizoram, one of the northeastern States of India.

The Scientific Advisory Committee, the Institutional Biosafety Committee and the National Institute of Virology (NIV) Institutional Animal Ethics Committee approved this study (September 2014-September 2016). The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, New Delhi, were followed. Prior permission for animal sample collection was obtained.

A total of 1113 serum samples collected at random from apparently healthy pigs of Mizoram State were obtained through the Indian Council of Agricultural Research (ICAR)-Directorate of Foot and Mouth Disease, Mukteshwar, India. The samples were collected from eight districts (Aizawl, Champhai, Kolasib, Lawngtlai, Lunglei, Mamit, Saiha and Serchhip) of Mizoram State during 2015. The samples were collected from pigs of different age groups at random without any bias on a particular age group. All the samples were transported in cold-chain to NIV, Pune, India.

Pig serum samples were tested for the presence of anti-NiV IgG-antibodies using the reagents provided

by the Centers for Disease Control and Prevention (CDC), USA. ELISA plates were coated with NiV (genotype M) antigen *Vero-E6* cell slurry or negative control *Vero-E6* cell slurry in phosphate-buffered saline (PBS, *p*H 7.4) overnight at 4°C. The next day, 100 µl of 1:100 diluted serum samples were added and the plates were incubated for one hour at 37°C. The wells were washed and incubated with anti-pig IgG horseradish peroxidase-conjugated secondary antibody (1:8000 dilution; KPL, USA) for one hour at 37°C. The substrate, 2,2´-azino-bis(3)-ethylbenzothiazoline-6-sulphonic acid, was added and the plates were incubated at 37°C for 30 min. The reaction was stopped by adding one per cent sodium dodecyl sulphate. The plates were read at 414 nm. The plates were then washed five times using 10 mM PBS (*p*H 7.4) with 0.1 per cent Tween-20 (Sigma-Aldrich, USA) at the end of each step. All the samples were tested in duplicate. Positive and negative pig serum controls were included in the assay for determining the cut-off point and for quality control of the assay[12].

All the 1113 serum samples from Mizoram State were tested negative for anti-NiV IgG-antibodies. Till now, NiV-specific IgG and NiV RNA have only been detected from one *P. giganteus* bat from Myanaguri, West Bengal, in 2009 and further in *P. giganteus* bat populations of West Bengal and Assam State[7-9,11]. However, during NiV outbreaks in Malaysia, pigs reportedly played a critical role in transmitting the disease to pig handlers by direct contact[13]. It may be because of organized piggeries provided better expansion of viral infection among pigs. Pigs also have been reported to act as an intermediate or amplifying host for human transmission during menangle virus outbreaks[14].

Pig is the most important livestock in the Mizoram State and plays a major role in the livelihood of the small farmers. Approximately 70-90 per cent of the pigs consumed in North East (NE) region are reared at rural households. Pig keeping is considered important in NE States of the country and particularly for the tribal communities[15].

The absence of anti-NiV IgG in pigs in Mizoram State and the presence of NiV in bats suggest the potential role of bat in virus transmission to humans rather than pigs in the Indian scenario. However, further studies are needed as the present data are limited and from only one State of north eastern region of the country. Although India has witnessed three Nipah outbreaks[7,8,16], no information is available on the presence of NiV among pig population from India. Thus, the present data highlights the need for systematic proactive surveillance of NiV in pig population of northeastern region for keeping track on any emergence of NiV, especially in the States bordering Bangladesh.

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***Conflicts of Interest*:** None.

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