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Nipah Virus Antibodies in Bats, the Philippines, 2013–2022

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In 2014, an outbreak of zoonotic Nipah virus (NiV) occurred on Mindanao Island, the Philippines. We investigated the prevalence of NiV in Philippine bats. Because neutralizing antibodies were detected in insectivorous bats on Siargao Island, public health officials should consider that the distribution range of NiV is not limited to Mindanao Island.

Nipah virus (NiV; family *Paramyxoviridae*, genus *Henipavirus*) was first discovered in 1998–1999. Officials in Malaysia and Singapore identified it as a causative virus of severe respiratory disease in pigs and highly fatal encephalitis or respiratory disease in humans (*1*). Subsequently, Bangladesh and India have reported sporadic outbreaks of the virus almost annually (*2*,*3*). Direct bat-to-human transmission is assumed in those outbreaks; however, human-to-human transmission through concentrated contact has also been reported (*3*).

In Southeast Asia, some frugivorous bat species (mainly of the genus *Pteropus*) and several insectivorous bat species (genera *Hipposideros*, *Scotophilus*, and *Rhinolophus*) are reservoirs of the virus, which has led to its widespread transmission (*4*–*6*). In 2014, in Sultan Kudarat Province, which is located in the southern part of Mindanao Island in the Philippines, 10 horses died, and serious infections occurred in 17 humans, mainly in those who had slaughtered horses or consumed horse meat (*7*). The humans who died had acute encephalitis syndrome, a severe influenza-like illness, or meningitis, and the etiology was diagnosed as henipavirus infection on the basis of neutralizing antibody detection in patient serum samples. One patient had a short 71-bp fragment sequence that was 99% homologous to the NiV strain from Malaysia, suggesting that NiV was the etiologic virus (*7*). The likely source of infection in horses is bats, which are a natural host of the virus.

Residual serum samples used in epidemiologic studies of bat-derived viruses conducted before 2019 were reused in this NiV epidemiologic study (*8*). In addition, we conducted new bat trapping at the end of 2022. In each study, we collected specimens from wild bats.

We attempted to detect NiV-neutralizing antibodies by using serum samples collected from bats in 6 regions of the Philippines, spanning from north to south (Figure). We determined the neutralization titer of each serum sample by using a surrogate assay without an infectious NiV, as previously established (*9*). Using vesicular stomatitis virus expressing secreted alkaline phosphatase pseudotyped with G and F proteins of the NiV strain from Malaysia (VSV-NiV-SEAP) (*9*), we determined the titer of the neutralizing antibody. Moreover, we performed detection of NiV RNA with reverse transcription PCR by using consensus primers that widely detect paramyxoviruses (PAR-F1, PAR-F2, and PAR-R) (Appendix, https://wwwnc.cdc.gov/EID/article/31/8/25-0210-App1.pdf) (*10*).

In total, we diluted 326 bat serum samples 80-fold and screened for VSV-NiV-SEAP (Table) (*9*). We subjected 4 serum samples that tested reactive in screening to serial dilution. We determined antibody titers as values of 16, 41, 47, and 141, which are shown as the reciprocal of the serum dilution factor at which SEAP activity was suppressed by ≥75% after VSV-NiV-SEAP entered the cells (*9*). We obtained positive samples from the insectivorous bat *Hipposideros diadema*, which was captured on Siargao Island j(Figure). We used a similar surrogate system to detect neutralizing antibodies against Hendra virus. The same 4 serum samples showed cell entry inhibition rates ranging from 35.2% to 63.1% against VSV pseudotyped with Hendra virus G and F proteins. Those results were weaker than those obtained for VSV-NiV-SEAP in the screening (Appendix Table). However, because of an insufficient volume of serum samples, we could not perform titration by serial dilution. In contrast, we did not detect any neutralizing antibodies in bats from Mindanao Island or elsewhere (Table). Moreover, we did not detect any viral RNA in reverse transcription PCR targeting paramyxoviruses (including NiV and Hendra virus) using RNA extracted from the 252 samples (collected from serum or spleen) (Table).

In this study, we investigated the prevalence of NiV with bat serum samples collected from 6 regions in the Philippines (Figure). We did not detect any antibodies on Mindanao Island, where the henipavirus outbreak occurred, which may be partially because

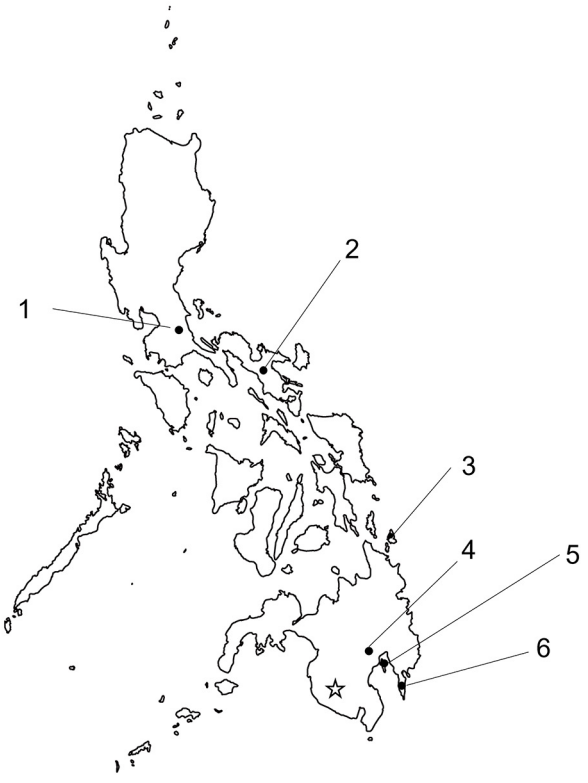

Figure. Locations of 6 bat collection sites for Nipah virus antibodies in bats, the Philippines, 2013–2022. 1, U.P. Laguna Quezon Land Grant, Siniloan, Laguna; 2, Naga, Camarines Sur; 3, Siargao Islands, Surigao del Norte; 4, Baguio District, Davao City, Mindanao; 5, Island Garden City of Samal and Talicud Island, Davao del Norte Province; 6, Lavigan, Governor Generoso, Davao Oriental, Mindanao. Star denotes area where Nipah virus outbreaks were reported in 2014.

Table. Neutralizing antibody titers in serum samples from 13 bat species for Nipah virus antibodies in bats, the Philippines, 2013–2022*

Bat species	No. positive/no. tested using pVSV-SNT						No. positive/no. tested using PaV RT-PCR					
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Cynopterus luzoniensis	0/41	0/25	0/22	NA	NA	NA	0/28	ND	0/17†	NA	NA	NA
Eonycteris spelaea	0/3	NA	0/2	NA	0/13	NA	0/3	NA	0/2†	NA	0/13	NA
Haplonycteris fischeri	0/1	NA	NA	NA	NA	NA	ND	NA	NA	NA	NA	NA
Macroglossus minimus	0/1	NA	0/9	0/1	NA	NA	ND	NA	0/6†	0/1	NA	NA
Ptenochirus jagori	0/63	0/22	0/17	NA	NA	NA	0/63	ND	0/12†	NA	NA	NA
Rousettus amplexicaudatus	0/5	NA	0/3	NA	0/44	0/19	0/5	NA	0/1†	NA	0/46	0/20
Hipposideros coronatus	NA	NA	ND	NA	NA	NA	NA	NA	0/1†	NA	NA	NA
Hipposideros diadema	NA	NA	4/23	NA	NA	NA	NA	NA	0/24†	NA	NA	NA
Hipposideros obscurus	NA	NA	ND	NA	NA	NA	NA	NA	0/9†	NA	NA	NA
Hipposideros pygmaeus	NA	NA	0/1	NA	NA	NA	NA	NA	ND	NA	NA	NA
Rhinolophus arcuatus	NA	NA	0/1	NA	NA	NA	NA	NA	0/1†	NA	NA	NA
Miniopterus eschscholtzii	NA	NA	0/3	NA	NA	NA	NA	NA	ND	NA	NA	NA
Scotophilus kuhlii	NA	0/7	NA	NA	NA	NA	NA	ND	NA	NA	NA	NA

*Site 1, Laguna 2022; site 2, Naga 2019; site 3, Siargao 2019; site 4, Baguio district 2013 (in Davao City); site 5, Samal and Talicud 2013; site 6, Lavigan 2013 (in Governor Generoso Municipality). NA, not applicable; ND, not done; PaV, paramyxovirus; pVSV-SNT, serum neutralizing test using vesicular stomatitis virus pseudovirus expressing the Nipah virus surface proteins (*9*); RT-PCR, reverse transcription PCR.
†RT-PCRs were performed by using RNAs extracted from spleen and not from serum samples.

we could not capture and study the primary reservoir, *Pteropus* bats, which fly and migrate at high altitudes. However, we detected NiV antibodies in 4 samples from 1 insectivorous bat species on Siargao Island (Table), which is geographically close, indicating that the distribution range of NiV is not limited to within Mindanao Island.

Antibodies have been reported from other *Hipposideros* bat species closely related to *H. diadema* (*5*). We also captured a species (*Scotophilus kuhlii*) other than *Pteropus* bats, for which antibodies were similarly detected in bats in previous reports (*5*), but we did not detect any antibodies. In contrast, we could not detect viral RNA in all samples because of the small number of samples. We consider it crucial to obtain more viral genetic information to understand the nature of the virus responsible for the henipavirus epidemic in the Philippines and to take countermeasures. More detailed surveys with larger sample sizes on Mindanao Island and surrounding areas are needed. Surveillance of NiV carriage in bats in the Philippines is necessary to characterize the virus, investigate risk factors for future outbreaks of henipavirus, and implement control measures.

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Wild bats were captured under a permit issued by the Department of Environment and Natural Resources to the University of the Philippines Los Baños for this research purpose (Wildlife Gratuitous permit nos. RXI-2013-06, R13-2019-27, and R5-2019-105). Furthermore, for every scientific expedition undertaken by the authors to capture bats, a permit was issued by the Biodiversity Management Bureau. Each scientific expedition to capture bats was also covered by a permit granted by the local regional office of the Department of Environment and Natural Resources. The procedures for serum and spleen sample collection after euthanasia of the captured bats were carried out based on the guidance of the institutional animal care and use committee of the University of the Philippines Los Baños.

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Crimean-Congo Hemorrhagic Fever Virus Africa 1 Lineage in *Hyalomma dromedarii* Ticks, Algeria, 2023

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We conducted a Crimean-Congo hemorrhagic fever virus (CCHFV) survey of *Hyalomma* spp. ticks collected from camels in southeastern Algeria. Of 138 tick pools, 1 was CCHFV positive; the sequenced strain belonged to the Africa 1 genotype. Healthcare professionals in Algeria should be aware of this detection of a circulating pathogenic CCHFV genotype.

Infection with Crimean Congo hemorrhagic fever virus (CCHFV; *Orthonairovirus hemorrhagiae*; Nairoviridae: Bunyavirale) provokes fever and hemorrhagic manifestations in humans but results in asymptomatic infections in animals (*1*). CCHFV is maintained in nature through wild and domestic animals serving as amplification hosts and ticks as reservoirs. CCHFV is endemic to Africa, the Middle East, Asia, and Europe (*2*). However, knowledge of CCHFV in North Africa is limited to few serologic surveys and molecular characterization in ticks.

In Algeria, Agai virus (*Orthonairovirus parahemorrhagiae*), previously known as AP92-like CCHFV, has been detected in *Hyalomma aegyptium* ticks collected from tortoises (*3*). In addition, 2 seroprevalence studies of CCHFV conducted on dromedary camels (*Camelus dromedarius*) in different regions from southern Algeria showed a high rate of IgG against CCHFV (*2*,*4*). We aimed to detect CCHFV among ticks in southern Algeria, where serologic evidence of the virus was reported among camels.

[1]These authors contributed equally to this article.