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SUPPLEMENT ARTICLE

Seroprevalence of Nipah Virus Infection in Peninsular Malaysia

Ming-Yen Yong,[1] Soo-Ching Lee,[2] Romano Ngui,[2] Yvonne Ai-Lian Lim,[2,3] Maude E Phipps,[4] and Li-Yen Chang[1]

[1]Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [2]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [3]Center for Malaysian Indigenous Studies, University of Malaya, Kuala Lumpur, [4]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Sunway, Malaysia

Nipah virus (NiV) outbreak occurred in Malaysia in 1998. The natural host reservoir for NiV is *Pteropus* bats, which are commonly found throughout Malaysia. Humans become infected when NiV spills over from the reservoir species. In this study, NiV serosurveillance in Peninsular Malaysia, particularly among the indigenous population, was performed. The collected samples were tested for presence of NiV antibodies using a comparative indirect enzyme-linked immunosorbent assay based on the recombinant NiV nucleocapsid (rNiV-N) protein. We found that 10.73% of the participants recruited in this study had antibodies against rNiV-N, suggesting possible exposure to NiV.

Keywords. Nipah virus; serosurveillance; Malaysia; indigenous population; comparative indirect ELISA; nucleocapsid protein.

Nipah virus (NiV) encephalitis outbreak in humans was first reported in Malaysia in 1998–1999 [1]. In Bangladesh, NiV was initially reported in 2001 and since then, NiV outbreaks occur almost annually [2]. NiV outbreaks in India were reported in 2001, 2018, and 2019 [3], and in 2014 an outbreak occurred in the Philippines [4]. *Pteropus* bats have been implicated as the natural reservoir host for NiV, supported by successful isolation of the virus from these bats in several areas in Asia [5, 6]. In addition, seroprevalence studies of bats in Malaysia, Cambodia, and Vietnam have reported NiV prevalence rates ranging from 1% to 20% [7–9]. Ongoing NiV human infections in Bangladesh and India are associated with direct contact with bats or consumption of contaminated date palm sap [2, 3].

Although NiV has not reemerged in Malaysia, NiV spillover events are highly possible, because *Pteropus* bats are abundant and widely distributed across the country, particularly in semiurban and rural areas or forested areas. In Malaysia, specifically Peninsular Malaysia, indigenous communities, also known as Orang Asli, mostly live in these semiurban and rural or forested areas. Transmission of NiV from its natural host reservoir to Orang Asli is possible owing to several factors, including close proximity between villages of indigenous communities and the habitat of *Pteropus* bats, as well as increased contact between Orang Asli and bats during the daily activities of the Orang Asli, such as hunting and gathering forest resources for living [10]. More data on NiV seroprevalence in humans, particularly in countries with previously reported NiV outbreaks, could help in efforts to reduce future NiV health threats by improving disease prevention and management strategies. It would also be beneficial to identify possible associated risk factors for NiV infection in humans. In the current study, we determined the seroprevalence of NiV in 4 indigenous communities in Peninsular Malaysia.

METHODS

The study protocol was approved by the University Malaya Medical Centre Medical Ethics Committee (MEC) (MEC identifiers 638.36 and 934.14). Participants consisted of Orang Asli of the Senoi tribe recruited from 4 communities in Peninsular Malaysia: 41 participants (23.16%) from Sungai Perah village (Semai subtribe) and 53 (29.95%) from Pos Piah village (Temiar subtribe) in Perak, 67 (37.85%) from Kuala Betis village (Temiar subtribe) in Kelantan, and 16 (9.04%) from Kepau Laut village (Mah Meri subtribe) in Selangor. These communities are located 30–75 km from either previously confirmed NiV infections or locations where *Pteropus* bats were determined to have neutralizing antibodies against NiV. Informed consent was obtained from the volunteer participants.

A total of 177 serum samples was collected. Archived human serum samples from the 1998–1999 Malaysia NiV outbreak that had been previously confirmed positive for the presence of immunoglobulin M and immunoglobulin G (IgG) were used as positive control [11, 12]. Serum samples from consenting healthy individuals were used as negative controls. Ethical approval for positive and negative serum samples was obtained from the University Malaya Medical Centre MEC (MEC identifier 20147–410).

All serum samples were tested for presence of NiV IgG using recombinant NiV nucleocapsid (rNiV-N) in a comparative indirect enzyme-linked immunosorbent assay (ELISA), developed

Correspondence: Li-Yen Chang, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia (changliyen@um.edu.my).

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