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# Exposure to Zoonotic West Nile Virus in Long-Tailed Macaques and Bats in Peninsular Malaysia

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**Simple Summary:** The role of wildlife animals, such as macaques and bats, in the spreading and maintenance of deadly zoonotic pathogens in nature are documented in several studies. The present study substantially highlights the first evidence of West Nile Virus (WNV) infection, a mosquito borne virus in the Malaysian macaques and bats. Of the 81 macaques sampled, 24 of the long-tailed macaques were seropositive to WNV, indicating that they were exposed to the virus in the past. The long-tailed macaques were found in the mangrove forests located in the Central, Southern, and West Peninsular Malaysia. Meanwhile, five out of 41 bats (Lesser Short-nosed Fruit Bats, Lesser Sheath-tailed Bats, and Thai Horseshoe Bats) that were found in the caves from Northern Peninsular Malaysia showed susceptibility to WNV. Therefore, a constant bio surveillance of WNV in the wildlife in Malaysia is a proactive attempt. This study was aligned with the Malaysian government's mission under the Malaysia Strategy for Emerging Diseases and Public Health Emergencies (MYSED) II (2017–2021) and the Ministry of Health priorities in order to enhance the regional capability to rapidly and accurately survey, detect, diagnose, and report outbreaks of pathogens and diseases of security concern.

**Abstract:** The role of wildlife such as wild birds, macaques, and bats in the spreading and maintenance of deadly zoonotic pathogens in nature have been well documented in many parts of the world. One such pathogen is the mosquitoes borne virus, namely the West Nile Virus (WNV). Previous research has shown that 1:7 and 1:6 Malaysian wild birds are WNV antibody and RNA positive, respectively, and bats in North America may not be susceptible to the WNV infection. This study was conducted to determine the status of WNV in Malaysian macaques and bats found in mangrove forests and caves, respectively. Archive sera and oropharyngeal swabs from

long-tailed macaques were subjected to the antibody detection using WNV competitive enzyme-linked immunosorbent assay (c-ELISA) and WNV RNA using RT-PCR, respectively, while the archive oropharyngeal and rectal swabs from bats were subjected to RT-PCR without serological analysis due to the unavailability of serum samples. The analysis revealed a WNV seropositivity of 29.63% (24/81) and none of the macaques were positive for WNV RNA. Meanwhile, 12.2% (5/41) of the bats from Pteropodidae, Emballonuridae, and Rhinolophidae families tested positive for WNV RNA. Here, we show a high WNV antibody prevalence in macaques and a moderate WNV RNA in various Malaysian bat species, suggesting that WNV circulates through Malaysian wild animals and Malaysian bat species may be susceptible to the WNV infection.

**Keywords:** west nile virus; arbovirus; zoonotic; macaque; bats; c-ELISA; RT-PCR

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## 1. Introduction

Deadly emerging and re-emerging zoonotic pathogens are transmitted mostly from wildlife reservoirs to humans or other animals during spillover events, with or without a vector intervention. Evidence has shown that some of the medically important mosquito borne illnesses causing West Nile fever, dengue, malaria, chikungunya, zika, and Japanese encephalitis were isolated from the wildlife [1–3]. The West Nile fever is distributed in Africa, USA, Europe, and Western Asia causing febrile illness and encephalitis in humans and animals [4]. The causative agent is an envelope RNA virus known as the West Nile Virus (WNV), which is classified in the genus of *Flavivirus* under the family of Flaviviridae [5]. Despite the role played by wild birds as amplifier hosts of WNV and mosquitoes as vectors, the role of wildlife such as macaques and bats in the WNV transmission cycle remains poorly understood [6].

Several epidemiological studies attested that the macaque species could become infected with WNV [6–8]. Nevertheless, the macaques developed a low level of viraemia based on the experimental study, making them unlikely to perpetuate the virus [8]. Meanwhile, bats have been demonstrated as a competent amplifying host of arthropod borne Flaviviruses transmission, but have not been proven to maintain WNV infections as seen in the North American bats, which were infrequently infected with WNV [9,10]. Due to the nature of wildlife habitats and constant exposure to mosquito bites during blood meals, there is an abundant considerable opportunity for WNV introduction to the wildlife. There is also the possibility of spillover events as well as the sylvatic transmission of the virus from the wildlife to humans due to human activities such as deforestation and urbanization, and also due to other climate-related factors that lead to a loss of wildlife habitat [11,12].

In Malaysia, some studies have reported evidence of exposure of humans, birds, and mosquitos to WNV. A study by Marlina et al. [13] reported a WNV seroprevalence of 1.21% (9/742) in the Orang Asli from several states in Peninsular Malaysia. Additionally, Rais et al. [14] and Ain-Najwa et al. [15] reported a WNV seroprevalence of 4.41% (3/68) in captive birds and 18.71% (29/155) in wild birds, respectively. In 1970, a sub-type of WNV designated as the Kunjin Virus (KUNV), which was originally endemic in Australia was detected in *Culex pseudovishnui* mosquitoes in Sarawak, a Malaysian state of Borneo [16]. More recently, WNV was also detected in pooled samples of *Culex* spp. mosquitoes, which were trapped close to the migratory birds landing areas in Malaysia (under review). These findings collectively demonstrated evidence of WNV exposure with asymptomatic infection in animals and humans in Malaysia [17,18]. Nevertheless, the status of WNV infection in Malaysian macaques and bats remains poorly understood. Therefore, this study was conducted to determine the serological and/or molecular prevalence of WNV in bats and macaques in selected areas of Malaysia.