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Interactions of duck Tembusu virus with *Aedes aegypti* and *Aedes albopictus* mosquitoes: Vector competence and viral mutation

Nichapat Yurayart [a], Patchareeporn Ninvilai [b], Theeraphap Chareonviriyaphap [c], Theerayuth Kaewamatawong [d], Aunyaratana Thontiravong [a,b], Sonthaya Tiawsirisup [a,*]

[a] *Animal Vector-Borne Disease Research Unit, Veterinary Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*
[b] *Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*
[c] *Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand*
[d] *Veterinary Pathology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*

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ABSTRACT

Duck Tembusu virus (DTMUV) is an emerging flavivirus that causes severe disease in avian hosts, while also affecting mammalian hosts; however, information on viral interaction with mosquito vectors for mammalian hosts is limited. Vector competence of *Aedes* (*Ae.*) *aegypti* and *Aedes albopictus* mosquitoes for DTMUV were investigated. Both *Aedes* mosquito species were orally infected with DK/TH/CU-1 strain of Thai DTMUV and isolated DTMUV from BALB/c mouse. Genomes of the viruses isolated from hosts and vectors were analyzed and compared with the positive virus. Findings showed that both *Aedes* mosquito species could serve as vectors for DTMUV with minimum viral titer in blood meal of 10^6 $TCID_{50}$/mL. After taking blood meal with viral titer at 10^7 $TCID_{50}$/mL, vector competence of the mosquitoes was significantly different from the lower titer in both species. Both *Aedes* species did not support development of the isolated viruses from mouse. A point mutation of nucleotide and amino acid was found in all isolated DTMUV from *Ae. aegypti* saliva, while other viruses were similar to the positive virus. Our findings demonstrated that both *Ae. aegypti* and *Ae. albopictus* had potential to transmit the virus and play important roles in the viral transmission cycle in mammalian hosts, while viral mutation occurred in *Ae. aegypti* mosquitoes.

1. Introduction

Duck Tembusu virus (DTMUV) is an emerging vector-borne pathogen that causes severe neurological and reproductive diseases in avian hosts, especially in ducks and chickens. The disease impacts agriculture and economic systems with high morbidity and mortality rates (Su et al., 2011). Since 2010, virus outbreaks have been reported in several Asian countries including Thailand, China, and Malaysia (Thontiravong et al., 2015). DTMUV is a member of the *Flavivirus* genus and classified as a mosquito-borne cluster similar to dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), Zika virus (ZIKV), and yellow fever virus (YFV) (Mukhopadhyay et al., 2005). The viral genome is a positive sense, single-stranded RNA that encodes three structural proteins (capsid (C), pre-membrane (PrM), and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Bollati et al., 2010). The natural vector for DTMUV is *Culex* (*Cx.*) mosquitoes, while another transmission route is direct contact with secretion (Li et al., 2015; Ninvilai et al., 2020; Sanisuriwong et al., 2020; Tang et al., 2015).

DTMUV mainly causes disease in avian hosts but the virus also has potential to infect mammalian hosts, especially mice. Previous research showed that infected mice exhibited severe clinical signs similar to the original host including blindness, hind limb paralysis, disorientation, and death (Li et al., 2013; Yurayart et al., 2020). Morbidity and mortality rates were 100% and 30–80%, respectively (Li et al., 2013; Ti et al., 2016). To the best of our knowledge, viral transmission in the *Aedes* mosquito that prefers mammalian hosts has never been reported. Therefore, interactions among *Aedes* mosquito, avian-origin DTMUV, and mice-propagated DTMUV require investigation.

Changing host or vector by the flavivirus relates to viral genome mutation, especially in genes that play a crucial role in viral entry, replication, and immune evasion including E, NS1, and NS5 genes (Lei

* Corresponding author.
E-mail address: sonthaya.t@chula.ac.th (S. Tiawsirisup).

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