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Short Communication

Prevalence of Phasi Charoen virus in female mosquitoes

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The authors previously characterized a partial nucleic acid sequence for a novel virus, Phasi Charoen virus (PhaV), which was isolated from *Aedes aegypti* larvae in Thailand, and PhaV appears to belong to the family *Bunyaviridae* based on phylogenetic analysis of amino acid sequences. In this study, we examined whether adult female mosquitoes in Thailand are infected by PhaV, and they found that over 20% of adult female mosquitoes were infected by PhaV. These viruses were genetically similar to strains. This result suggested a high prevalence of PhaV in adult female mosquitoes.

Key words: PhaV, bunyavirus, female mosquito, high prevalence.

INTRODUCTION

Arboviruses are biologically transmitted to vertebrate hosts by blood-feeding arthropod vectors, such as mosquitoes, biting flies and ticks. The surveillance of viral infection in mosquitoes depended on amplification by reverse transcription-polymerase chain reaction (RT-PCR) . However, unknown virus is difficult to detect by PCR. The authors recently developed a rapid system for determination of viral nucleic acid sequences (RDV) for detecting unknown viruses (Mizutani et al., 2007; Kihara et al., 2007; Maeda et al., 2008; Yamao et al., 2009), and by this method they identified the novel virus Phasi Charoen virus (PhaV), which was isolated from *Aedes aegypti* larvae collected from the Phasi Charoen district of Thailand using C6/36 cells in previous study (Yamao et al., 2009). PhaV infects and replicates slowly in mosquito C6/36 cells. The previously characterized partial nucleic acid sequence for this virus and PhaV appears to belong to the family *Bunyaviridae* based on phylogenetic analysis of amino acid sequences. Members of the *Bunyaviridae* family are icosahedral enveloped viruses with a tripartite negative-sense RNA genome comprising small, medium and large segments, named according to their molecular sizes (Gonzalez-Scarano et al., 1991). Among the members of *Bunyaviridae* family, the mosquito- transmitted Rift Valley Fever Virus (RVFV) is important as a human pathogen. PhaV was not found to be infectious to mammalian Vero cells, even after 8 days of incubation (Yamao et al., 2009). However, we cannot discount the possibility that PhaV has non-pathogenic properties in mammalian. In addition, we did not investigate data regarding the infectivity of adult female, in which blood was taken from mammal. In this study, we examined whether adult female mosquitoes in Thailand are infected or not by PhaV.

Aedes aegypti specimens (77 adult females) were collected from houses of patients clinically diagnosed with dengue fever from the Bang Khun Thian, Bang Bon,

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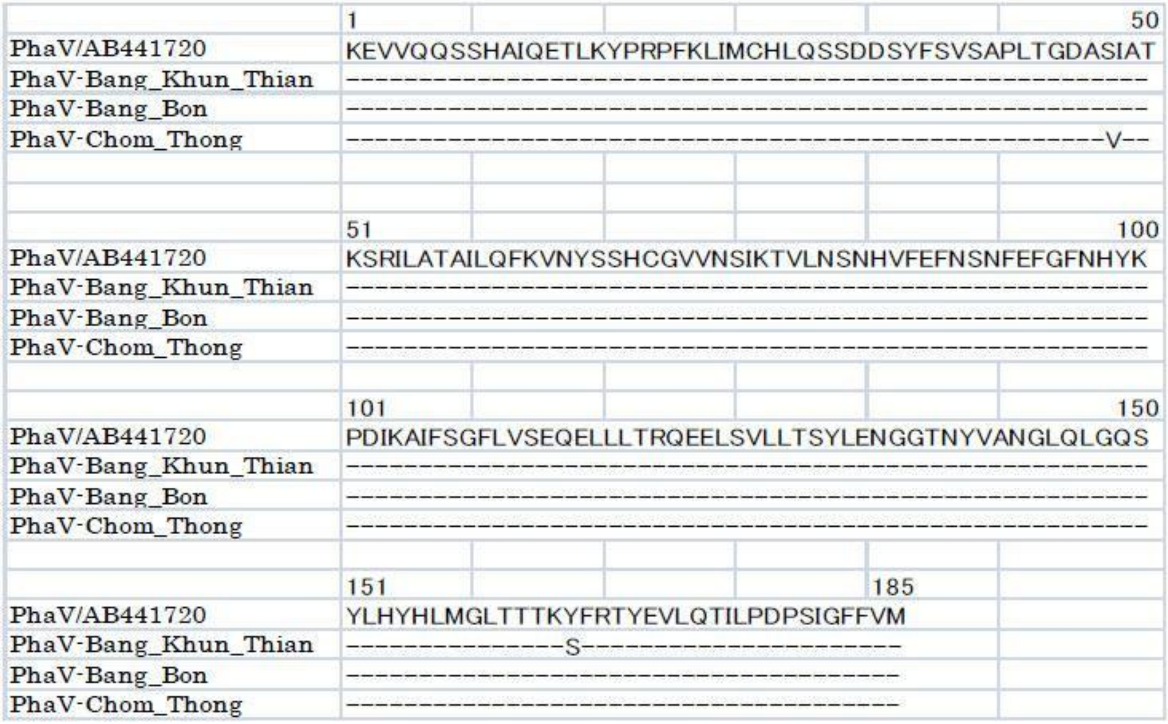

	1	50
PhaV/AB441720	KEVVQQSSHAIQETLKYPRPFKLIMCHLQSSDDSYFSVSAPLTGDASIAT	
PhaV-Bang_Khun_Thian	--	
PhaV-Bang_Bon	--	
PhaV-Chom_Thong	---V--	
	51	100
PhaV/AB441720	KSRILATAILQFKVNYSSHCGVVNSIKTVLNSNHVFEFNSNFEFGFNHYK	
PhaV-Bang_Khun_Thian	--	
PhaV-Bang_Bon	--	
PhaV-Chom_Thong	--	
	101	150
PhaV/AB441720	PDIKAIFSGFLVSEQELLLTRQEELSVLLTSYLENGGTNYVANGLQLGQS	
PhaV-Bang_Khun_Thian	--	
PhaV-Bang_Bon	--	
PhaV-Chom_Thong	--	
	151	185
PhaV/AB441720	YLHYHLMGLTTTKYFRTYEVLQTILPDPSIGFFVM	
PhaV-Bang_Khun_Thian	---------------S-------------------	
PhaV-Bang_Bon	-----------------------------------	
PhaV-Chom_Thong	-----------------------------------	

Figure 1. Alignment of four amino acid sequences of PhaV. AB441720 is amino acid sequences of original PhaV (4), and other three were determined in this study.

and Chom Thong Districts in Bangkok Province, Thailand, during May and June of 2007. Adult individual mosquito was homogenized in 100 µL of modified Eagle's medium supplemented with 2% FBS. Each homogenate was centrifuged at 550 x g for 10 min at 4˚C, and the supernatant was filtered through 0.22 µm Millex-GX filters (Millipore, Billerica, MA, USA). RNA was extracted from 25 µL of homogenates using ISOGEN-LS (Nippon *Gene*, Toyama, Japan) or TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. To investigate infection of viruses, RT-PCR was performed using primers for flavivirus (consensus) (Kuno et al., 1998), DENVs (all serotypes) (Lanciotti et al., 1992), and alphavirus (Eshoo et al., 2007). Only DENV was detected in two mosquitoes. The other viruses were not detected. They examined the infectivity of PhaV in 75 adult female mosquitoes (15 groups; five mosquitoes including per group) except for two DENV positive-mosquitoes. Published primers were used for amplification of the PhaV 735 bp partial large segment (Yamao et al., 2009). PhaV was detected in 15 groups by RT-PCR.

The three PCR products of PhaV from the Bang Khun Thian, Bang Bon, and Chom Thong districts were purified from the agarose gel, and nucleic acid sequences were determined. The nucleic acid sequence data are available in the DDBJ/EMBL/Genbank databases under accession numbers AB541986 to AB541988. Three nucleic and amino acid sequences were compared with the original PhaV sequences (DDBJ/EMBL/GenBank accession number AB441720) as determined by the previous study. The three viruses shared nucleotide identities of 93.9 to 96.3%. Amino acid sequence of Bang Bon virus was identical to the original PhaV sequence (Figure 1). In contrast, the Bang Khun Thian and Chom Thong viruses were one amino acid different from the original PhaV.

In this study, we found that over 20% of female mosquitoes were infected by PhaV and these viruses were genetically similar to strains detected in mosquitoes in nearby Bangkok. DENV RNA was detected in only 2.6% of mosquitoes. The results suggested a high prevalence of PhaV in female mosquitoes. Biological transmission of arboviruses can be vertical, which involves the passage of the virus from an infected female vector to its offspring. In addition, horizontal transmission can be venereal, with a vertically infected male directly infecting a female, or can occur orally, from a vector to a vertebrate host via deposition of saliva during blood feeding. Mosquitoes of the *Aedes* and *Culex* genera are important for both human and animals because they transmit viruses causing serious illness. For example, dengue and chikungunya virus, which were transmitted by *Aedes* genus, were occurred epidemic many times at Southeastern Asia region including Thailand. In Asia, chikungunya virus mainly induces high fever and severe arthralgia, and had limited impacts on public health before its emergence in the Indian Ocean in 2005

(Vazeille et al., 2007). These chikungunya outbreaks spread rapidly and caused several million clinical cases in the Indian Ocean Islands and India (Pialoux et al., 2007). PhaV transmission from female mosquitoes to humans and animals will be examined in a future study.

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