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LETTER TO THE EDITOR

Characterization of recent Getah virus isolates from South Korea

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Getah virus, a member of the genus *Alphavirus* in the family *Togaviridae*, was first isolated in Malaysia in 1955 from *Culex gelidus* mosquitoes (*1*). Getah virus is widely distributed throughout much of southeast Asia and northern Australia and has been frequently isolated from mosquitoes (*2–6*). Getah virus infection, which is characterized by fever, rash, hind limb edema, and lymph node tumescence, appears to be a mild and self-limiting illness in horses (*7–9*). Getah virus is also pathogenic in swine, where in a few cases, fetal deaths were reported after natural and experimental infections of sows (*6, 10, 11)*. Epidemiological studies indicate that the virus is widespread but information on the molecular epidemiology or phylogenetic analyses of Getah virus in Republic of Korea (ROK) has not been reported in peer-reviewed journals. To investigate Getah virus prevalence in mosquitoes and understand the genetic characteristics of Getah virus currently circulating in ROK, we performed molecular screening in mosquitoes collected in horse farms and a rural area.

As part of mosquito-borne disease surveillance, mosquitoes were collected biweekly at 6 localities from June to October 2010 in the ROK. After identification using morphological techniques on a cold table using standard keys (*12, 13)*, culicine mosquitoes were sent under dry ice to the Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA, Anyang, ROK), where they were assayed for Getah virus. Mosquito pools were homogenized and used directly for RNA extraction. The prepared RNA was used as the template for cDNA synthesis using the Maxime™ RT PreMix (Intron Biotechnology, ROK) according to the manufacturer´s protocols. PCR reactions were carried out by previously described methods (*14*). After cloning PCR products into pLUG® Multi TA-cloning vector (Intron Biotechnology, ROK), the sequences of purified clones were analyzed by Macrogen (ROK). Nucleotide sequence homology searches were analyzed by the National Center for Biotechnology Information (NCBI) BLAST network service and aligned using the MegAlign software package (Windows version 7.1; DNA-STAR, USA). The Getah virus partial *nsp1* gene sequences determined in this study have been deposited in Genetic sequence database (GenBank) at the NCBI under IDs: JN410944 to ID: JN410946. To clarify genetic relationships, phylogenetic analyses were performed using the available published sequence data for 9 alphaviruses, 6 non-Korean Getah virus strains and 1 Korean Getah virus strain. The culicine mosquitoes used in this study were distributed into 371 sample pools, of which 2 pools of *Aedes vexans nipponii*, collected on 24 June 2010, were positive for Getah virus RNA [KorL915 (GenBank ID JN410944) and KorS1010 (GenBank ID JN410945)]. Of the alphaviruses sequenced, the Getah virus strains detected

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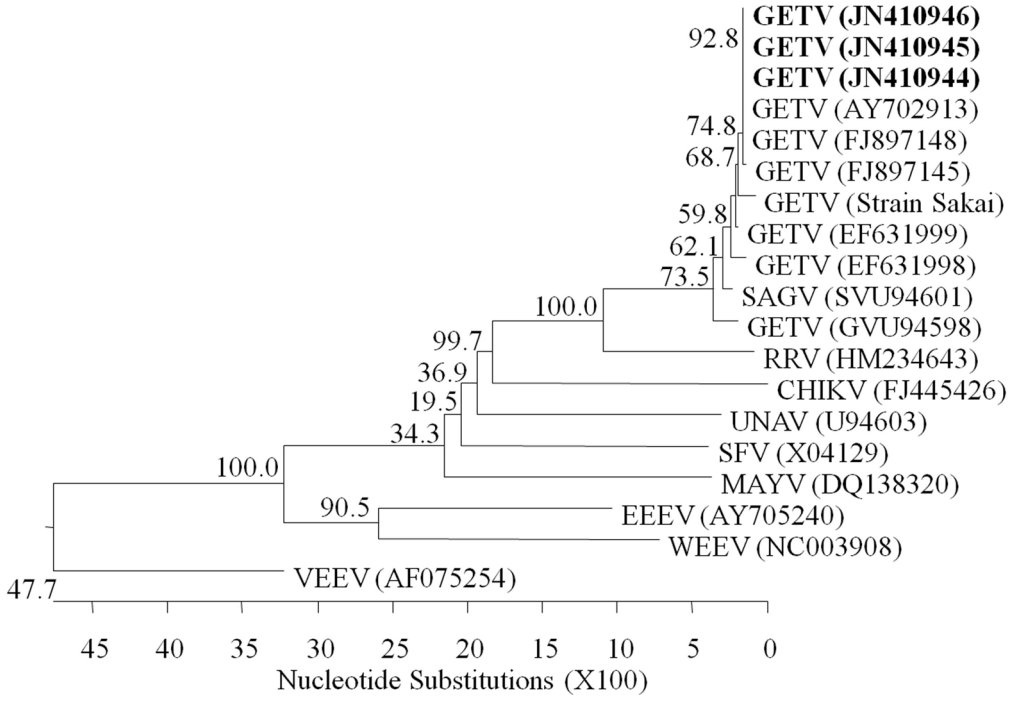
Abbreviations: ROK = Republic of Korea



Figure

A phylogenetic tree illustrating the genetic relationship based on partial *nsp1* gene nucleotide sequences

from the ROK were most closely related to the Sagiyama virus (98.2% nucleotide sequence identity and 100% amino acid sequence identity with the sequenced Sagiyama virus, GenBank ID SVU94601) as seen in Figure. Phylogenetic analysis of the partial *nsp1* gene showed that the Korean Getah virus strains had 58.9–98.2% nucleotide sequence identity and 62.7–100% amino acid sequence identity when compared to other selected alphaviruses.

Nucleotide sequencing confirmed close phylogenetic relationships with reported Getah virus sequences. Overall, the Korean Getah virus strains demonstrated 96.4–100% and 98.4–100% identities at the nucleotide and amino acid levels, respectively, when compared to non-Korean Getah virus strains. The nearest homologies between the non-Korean and Korean Getah viruses were observed with Getah viruses from China and Mongolia, which demonstrated 99.4–100% identities at the nucleotide level, and the derived amino acid sequences were totally conserved. Compared with the nucleotide sequences of previously reported Korean Getah virus strains, the Korean Getah virus strains detected in this study had 100% nucleotide and amino acid sequence identity with each other. This is the first report comparing the Getah virus partial *nsp1* genes derived from *Aedes vexans nipponii* mosquitoes in the ROK with other non-Korean Getah virus strains and selected alphaviruses. This work provides data about phylogenetic analysis showing that the partial *nsp1* gene sequences demonstrated high homology with Korean Getah viruses from pigs and that the amino acid sequences were the same as deduced earlier for the Korean Getah viruses from pigs. These findings also indicate that the highest homology was observed with Getah viruses from China and Mongolia in comparison with the several sequenced Getah virus strains from other geographical regions that are currently available. It is interesting that the strains from 2010 in ROK have 100% nucleotide identity to a 2004 ROK strain (AY702913), an ROK strain of unknown date (Jin-Ju), and a 1964 strain from China (M1). At present, experiments on phylogenetic analysis for whole genome sequences of the Getah virus genes obtained from this study are underway to determine whether any phylogenetic differences or changes occurred compared to other strains isolated in different times and regions. These results provide insight into the genetics of Getah virus strains, which is necessary for understanding their molecular epidemiology and genetic diversity.

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