

Detection of the Insect-Specific Flavivirus Chaoyang in Mosquitoes in the Jewish Autonomous Region of the Far East of Russia

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Rec date: Feb 23, 2016; Acc date: Feb 24, 2016; Pub date: Feb 27, 2016

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Background

Metagenomics research revealed enormous diversity of viruses up to 1030 and their possible role as global drivers of evolution. Using molecular genetic methods, numerous unknown viruses were detected during the entomological surveillance [1]. Among them insect-specific flaviviruses (ISFVs) with significant divergence from all known flaviviruses could represent an ancient primordial form of flaviviruses with replication restricted to insects and unable, or slightly adapted, to infect vertebrate cells. Their global distribution points out an evident underestimation of their real number, their putative interactions with the pathogenic flaviviruses, and their probable influence on the bionomics of arthropod hosts. Our goal was to estimate the flaviviral genetic diversity in mosquitoes in the Far East of Russia.

Methods

Unknown flaviviruses were detected in *Aedes vexans* mosquito pools by means of reverse transcription with subsequent PCR with universal flavivirus-specific primers [2] corresponding to their NS5 gene fragment. New-born laboratory mice and mammalian tissue cultures (Vero E6 and porcine embryo kidney cells) were infected with the clarified mosquitoes pools. Nucleotide sequences of reverse transcription-PCR products were determined using BigDye 3.1 Terminator Cycle Sequencing Kit and DNA analyzer ABI 3500 (Applied Biosystems, USA). Phylogenetic analysis of the nucleotide sequences was performed using MEGA 6.06.

Results

In July of 2013 by means of RT-PCR flavivirus-specific RNA was detected in 4 of 24 pools of mosquitoes *Aedes vexans* (16.7%), collected in the Jewish Autonomous region (latitude: 48°33'15.57" longitude: 134°50'16.48") of the Far East of Russia. Results of RT-PCR detection of the tick-borne encephalitis virus (TBEV) and the West Nile virus, formerly revealed in the Far East of Russia, were negative. Subsequent attempts to isolate the corresponding strains in mammalian tissue cultures (Vero E6 and porcine embryo kidney cells) and in the laboratory 1-2 days old ICR mice after intracerebral and subcutaneous inoculations were unsuccessful. RT-PCR products from two samples of flavivirus isolates from mosquito pools 6-JAR and 8-JAR were sequenced.

Phylogenetic analysis revealed their close relationship with the Chaoyang virus strains ROK144 (JQ068102) isolated in South Korea in 2003 and the strains Deming (FJ883471) and HLD115 (JQ308185)

isolated in China in 2008 and 2010, respectively [3]. The site of the mosquitoes collection in the Jewish Autonomous region belongs to the Malokhingansky endemic region of the TBEV. Recently, three TBEV strains were isolated from *Aedes vexans* mosquitoes [strains Malyshevo (GenBank accession number KJ744034) and Lazo MP36 (GenBank KT001073) in Khabarovsk territory [4] neighboring to Jewish Autonomous region in the Far East of Russia; the third TBEV strain Sakhalin 6-11 (KF826916) was isolated from mosquitoes in Sakhalin island in the Far East of Russia [5]. Phylogenetic analysis of the nucleotide sequences of the RT-PCR products of the flavivirus NS5 gene fragment allowed us to reveal two different flavivirus species in mosquitoes *Aedes vexans* in the Far East of Russia.

Conclusions

Two isolates of the insect-specific flavivirus Chaoyang 6-JAR and 8-JAR were detected in 24 pools of mosquitoes *Aedes vexans* collected in the Jewish Autonomous region of the Far East of Russia in 2013. All our attempts to isolate the corresponding strains in mammalian tissue cultures and in the laboratory mice were unsuccessful. According to phylogenetic analysis both Chaoyang isolates from Russia were closely related to the strains isolated in South Korea and China and diverged from the pathogenic TBEV isolates from the same species of mosquitoes in the Far East of Russia.

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