Virology 523 (2018) 35–40

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Discovery and high prevalence of Phasi Charoen-like virus in field-captured *Aedes aegypti* in South China

Xiaomin Zhang[a,b], Suibin Huang[a,c], Tao Jin[d,e], Peng Lin[d,f], Yalan Huang[a], Chunli Wu[a], Bo Peng[a], Lan Wei[a], Hin Chu[g], Miao Wang[a], Zhirong Jia[a], Shaohua Zhang[a], Jianbin Xie[a], Jinquan Cheng[a], Chengsong Wan[b], Renli Zhang[a,*]

[a] Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, China
[b] School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, China
[c] School of Public Health, Shanxi Medical University, Taiyuan 030001, China
[d] BGI-Qingdao, BGI-Shenzhen, Qingdao 266555, China
[e] China National GeneBank, BGI-Shenzhen, Shenzhen 518120, China
[f] BGI Education Center, University of Chinese Academy of Sciences, Shenzhen 518083, China
[g] Department of Microbiology, the University of HongKong, HongKong, China

ARTICLE INFO

Keywords:
Phasi Charoen-like virus
Bunyavirus
Insect-specific virus
Aedes aegypti

ABSTRACT

Arboviruses have caused significant global health concerns during the past decade. In this regard, continuous viral surveillance is essential to timely identify emerging arboviruses and other novel viruses. Here, a novel isolate of Phasi Charoen-like virus (PCLV Zhanjiang01) was identified from field-captured *Aedes aegypti* mosquitoes in Zhanjiang by next generation sequencing. Phylogenetic analysis suggested that PCLV Zhanjiang01 belonged to the genus *Phasivirus* in the family *Phenuiviridae.* The presence of PCLV in three batches of *Aedes aegypti* confirmed its high prevalence in nature. Further detection of PCLV in progenies and adult males suggested vertical transmission in mosquitoes. In parallel, PCLV was detected from multiple organs indicating its broad tissue distribution in the infected mosquitoes. To the best of our knowledge, this is the first report of PCLV in China. Our results expanded the global biogeographic distribution of PCLV. Further investigations of PCLV on the arboviral transmission and control strategies are warranted.

1. Introduction

With advanced sequencing technologies and increasing interests into mosquito microbiome, a growing number of insect-specific viruses (ISVs) have been discovered in the recent years. ISVs naturally infect and replicate in insects or insect cells while do not replicate in vertebrates or vertebrate cells (Vasilakis and Tesh, 2015; Bolling et al., 2015; Blitvich and Firth, 2015). These ISVs are classified within the order of *Bunyavirales* and families such as *Flaviviridae, Togaviridae, Rhabdoviridae, Peribunyaviridae, Reoviridae, Mesoniviridae, Togaviridae* and the newly recognized taxon of *Negevirus* (Vasilakis and Tesh, 2015). Some ISVs were reported to modulate the replication of dual-host flaviviruses in co-infected mosquitoes or C6/36 cells, suggesting that these ISVs might indirectly impact the human health (Bolling et al., 2012; Hobson-Peters et al., 2013; Kent et al., 2010). In addition, the recombinant ISVs could be utilized as platforms for vaccine or diagnostic development (Nasar et al., 2012, 2015). Importantly, ISVs may have the potential to evolve into human pathogens, which is of substantial concern to the public health of the human community (Li et al., 2015; Cook et al., 2013; Walker et al., 2015; Marklewitz et al., 2015; Dudas and Obbard, 2015).

Most ISVs studies to date have been relatively focused on insect-specific flaviviruses (Stollar and Thomas, 1975; Cammisa-Parks et al., 1992; Zhang et al., 2017; Lutomiah et al., 2007; Calzolari et al., 2012; Tree et al., 2016; Kyaw Kyaw et al., 2018; Bittar et al., 2016; Liang et al., 2015; Blitvich and Firth, 2015). Current efforts on the discovery and characterization of insect-specific bunyaviruses are limited. The majority of them were discovered from *Culex* or *Anopheles,* including the Gouléako virus (GOLV) (Junglen et al., 2009), Cumuto virus (CUMV) (Auguste et al., 2014), Herbert virus (HEBV), Taï virus (TAIV) and Kibale virus (KIBV) (Marklewitz et al., 2013), Jonchet virus (JONV) and Ferak virus (FERV) (Marklewitz et al., 2015) as well as Badu virus (BADUV) (Hobson-Peters et al., 2016). Only two were identified from *Aedes aegypti* or *Aedes aegypti* cell line Aag-2, which included the Phasi

* Corresponding author.
E-mail address: renlizhangszcdc@aliyun.com (R. Zhang).

https://doi.org/10.1016/j.virol.2018.07.021
Received 9 May 2018; Received in revised form 18 July 2018; Accepted 18 July 2018
0042-6822/ © 2018 Published by Elsevier Inc.

Charoen virus (PhaV) (Yamao et al., 2009) and the Phasi Charoen-like virus (PCLV) (Maringer et al., 2017; Chandler et al., 2014; Aguiar et al., 2015; Zakrzewski et al., 2018). However, the previous studies on PhaV and PCLV only focused on the methods of the viral discovery and the isolation procedure in different mosquito cells. Further characterization has not been explored on important aspects including viral ecology, distribution and transmission.

In China, novel mosquito-associated viruses have been discovered mainly from *Culex, Aedes vexans,* or *Aedes dorsalis* (Xia et al., 2018). *Aedes aegypti* and *Aedes albopictus* are the major vectors for emerging arboviruses such as dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV) and yellow fever virus (YFV). *Aedes albopictus* is widely prevalent in most provinces of China, while *Aedes aegypti* is only distributed in the southern area (< 22° N), including southern Taiwan, Hainan Island, and certain regions of Guangdong and Guangxi provinces (Shi et al., 2017). Therefore, to investigate viral emergence and discover novel viruses in these two major mosquito vectors, we collected *Aedes aegypti* samples from Zhanjiang of Guangdong and *Aedes albopictus* samples from North (Beijing), Central East (Shanghai) and South (Shenzhen) regions of China in addition to samples from Zhanjiang of Guangdong (Fig. 1a). Importantly, combining high throughput sequencing (HTS) and Sanger sequencing, we discovered a novel isolate of PCLV from *Aedes aegypti* mosquitoes in Zhanjiang. Then, we further investigated the potential vectors of PCLV with different mosquito species. In addition, individual prevalence and tissue distribution of PCLV in *Aedes aegypti* were also explored.

2. Materials and methods

2.1. Mosquito collection and rearing

Mosquito eggs and larvae of wild Aedes albopictus were collected from Beijing (39°53′41.52″N, 116°42′11.93″E) and Shanghai (31°09′1.57″N, 121°06′33.56″E) (Fig. 1a). Larvae of *Aedes albopictus* and *Culex quinquefasciatus* were collected from Shenzhen (22°36′15.30″N, 114°28′24.74″E) (Fig. 1a). Larvae of *Aedes aegypti* and *Aedes albopictus* were caught in small water puddles in abandoned tires or water tanks for three times in Wushi Town (20° 55 '51"N, 109° 84 '72 "E) of Zhanjiang, Guangdong province (Fig. 1a). Mosquito larvae were fed with yeast tablets in the laboratory until they became pupae. Species were identified by morphological criteria according to the appearance of adult mosquitoes as described previously (Gaffigan et al., 2013). Adult mosquitoes were maintained in 27 ± 1 °C, 75–80% relative humidity with 12/12 h (light/dark) photoperiod and fed with cotton ball infiltrated with 10% sucrose solution (w/v) in Shenzhen Center for Disease Control and Prevention. Twenty to thirty mosquitoes in each batch were handled as one pool for further test.

2.2. Preparation for RNA and viral genome sequencing

Individual or pools of mosquitoes were homogenized in 600 μL RLT lysis buffer with one 5.0 mm pre-chilled stainless steel beads using a TissueLyzer II (Qiagen). Total RNA was extracted by using a Qiagen RNeasy mini kit (Qiagen) according to the manufacturer's instructions and sent to Beijing genome institute (BGI) for deep sequencing. Briefly, the RNA was first fragmented by using chemical method. Then cDNA library was constructed using random hexamer primer with the HiScript II (200 U/μL, Vazyme) following the manufacturer's instructions. Single-end read of 100 bp were produced on the BGISEQ-500 platform. The raw reads were processed by removing low-quality reads (SOAPnuke, with the options: -l 10; -q 0.1; -n 0.01) (https://github.com/BGI-flexlab/SOAPnuke/blob/master/src/DGECommon.h) and host contaminated reads (SOAP) (Li et al., 2009) by using the corresponding host reference genome. The filtered reads were then used to do BLASTn and reference-based assembly using MAQ (-m 3; -e 5; -b 170) (Li et al., 2008). Subsequently, Sanger sequencing was used to further confirm these assembled sequences. Sequencing primers (Table S1) were designed based on the assembled nucleotide sequences resulted from next generation sequencing.

2.3. Genome analysis and phylogenetic analysis

In silico analysis of putative PCLV Zhanjiang01 amino acid sequences was performed using the following programs as described previously (Hobson-Peters et al., 2016): Identification of the cleavage sites of the signal peptides was performed using SignalP (http://www.cbs.dtu.dk/services/SignalP/); identification of furin cleavage motif was determined using ProP 1.0 (http://www.cbs.dtu.dk/services/ProP/); estimation of protein mass was performed using the software DNAMAN 5.2.2; prediction of transmembrane domains was done by TMHMM (http://www.cbs.dtu.dk/services/TMHMM/); determination of potential N-linked glycosylation motifs was performed using NetNGlyc (http://www.cbs.dtu.dk/services/NetNGlyc/). Pairwise amino acid identity determination was performed using EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) using default settings (Blosum62, Gap Open 10, Gap extend 0.5). Amino acid sequences of RdRp of bunyaviruses were aligned with the software ClustalW (Larkin et al., 2007). Phylogenetic analysis was performed by the neighbor-joining algorithm and the Kimura two-parameter distance model using MEGA version 6.0 (Tamura et al., 2013). The reliability of the analysis was evaluated by a bootstrap test with 1000 replications.

2.4. Detection of PCLV in mosquitoes

To investigate whether there was any other potential vector besides *Aedes aegypti*, another two common mosquito species *Aedes albopictus* and *Culex quinquefasciatus* prevalent in South China were also detected for PCLV infection by RT-PCR. To explore the frequency of PCLV, three batches of *Aedes aegypti* mosquitoes were caught from Wushi town of Leizhou, Zhanjiang at different time points. For both the primary and progeny mosquitoes, part of these *Aedes aegypti* were blood-fed to produce eggs and another part of them were detected for PCLV infection in pools or individually. In addition, to determine its potential role in viral transmission or pathogenesis, we collected the heads, thoraxes, abdomens, salivary glands, midguts, ovaries and legs to identify the tissue distribution of PCLV in *Aedes aegypti*. The L, M and S segments of PCLV were amplified to detect viral carrier rate in individual mosquito and the M segment was amplified for the tissue distribution by RT-PCR. PCR products were detected by 1% agarose gel electrophoresis. Primers were designed to amplify 900–1000 bp long sequences (forward primer for L segment: 5′-AGACAGCACAAGCAAATAAAGCAAG-3′; reverse primer for L segment: 5′-AAACATGCATTGTAAGGTTTTGTCG-3′; forward primer for M segment: 5′-AAAAGTAGGAATTGATGCTGTTGC-3′, reverse primer for M segment: 5′-CTTTGAGCACTTTTGTCTAATGGC-3′; forward primer for S segment: 5′- AATGCTAACTCGAACCAATGA AAC-3′, reverse primer for S segment: 5′- TGGAAAATAAAAACAATAA AGCAATAC-3′.)

3. Results and discussion

The HTS analysis of four pools of mosquitoes generated a total of 67,459,175 to 99,653,968 single-end reads (Table S2). Clean reads of virus were gained after removing low-quality and host-contaminating reads. After doing BLASTn with these filtered reads, we found that abundant reads from the RNA sample of *Aedes aegypti* were classified into bunyavirus (Fig. 1b) and PCLV was identified to be the reference sequence (KM001085.1- KM001087.1). Through reference-based assembly, the 15,112 (0.07% of filtered reads) target virus-matched reads were assembled into the nearly complete genome of a novel isolate of PCLV, which was tentatively designated as PCLV Zhanjiang01. In addition, a small number of reads of these four mosquito RNA samples were found to be potentially related with several other viral families

Download English Version:

https://daneshyari.com/en/article/8751334

Download Persian Version:

https://daneshyari.com/article/8751334

Daneshyari.com