Evidence of West Nile virus and Saint Louis encephalitis virus in pools of mosquitoes testing negative for both viruses using real-time PCR

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Abstract

Vector-borne diseases have been on the rise during the past fifteen years in the United States. Regular surveillance of such diseases is critical for maintaining the public health of a region. Screening of mosquito pools for arboviruses using polymerase chain reaction assay (PCR) is a commonly used practice. As part of an ongoing surveillance program in northwest Texas, flavivirus-positive but West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) PCR-negative mosquito pools increased drastically in 2018 (2009-2017 average = 16.3%; 2018 = 69.0%). In an effort to determine the genetic structure of these flavivirus-positive, WNV/SLEV-negative mosquito pools (n = 20), cDNA PCR product was sent to the Los Alamos National Laboratory for Next Generation Sequencing. Analysis of amplicon products matched 95% (19/20) of our samples to WNV and/or SLEV in various databases. Though further analysis is required, our findings demonstrate the need for continued evaluation of currently accepted surveillance tools. The primers and probes employed in this study have been utilized by various studies over the years. The potential of these primers to no longer capture all WNV or SLEV positive samples is alarming and may have a negative effect on vector surveillance operations that currently utilize these primer sets.

Introduction

Vector-borne diseases have been on the rise during the past 15 years. West Nile virus is the most common locally acquired mosquito-borne disease in the United States. As such, mosquito control districts often screen local mosquito populations for such pathogens.

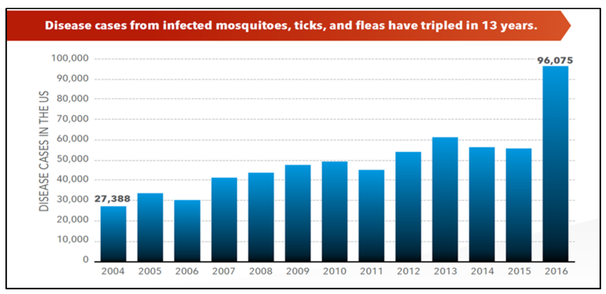
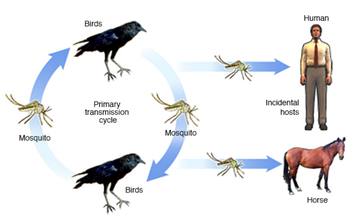
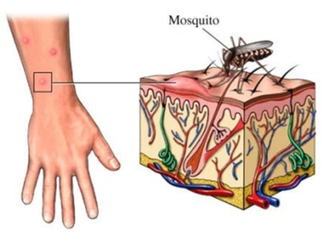


Figure 1. Number of vector-borne disease cases in the U.S. from 2004-2016

WNV and SLEV are maintained in nature in bird populations





Transmitted to humans mainly through the bite of infective mosquitoes

Figure 2. Mode of transmission of flaviviruses

Project 1: Annual Mosquito Screening

As part of an ongoing surveillance project, the Vector-borne Zoonoses Laboratory conducts a two-stage screening of mosquitoes from around Lubbock County, Texas:

1) Screen using flavivirus consensus primers via conventional RT-PCR
2) Screen WNV, SLEV, WEEV via real-time reverse transcriptase-PCR using a triplex assay

Aedes vexans

Culex quinquefasciatus

Culex tarsalis

Figure 3. Most common species screened in Lubbock, Texas

Results

During 2009-2017, 16.3% (11/68) of our mosquito pools tested positive for flavivirus but were negative for WNV or SLEV.

High: 2009 = 33.3% (2/6)
Low: 2013 and 2014 = 0% (0/8 and 0/5, respectively)
9.1% (1/11) of the flavivirus-positive WNV/SLEV-negative pools were *Aedes vexans*

In 2018, 69.0% (20/29) of our mosquito pools tested positive for flavivirus but were negative for WNV or SLEV.

65.0% (13/20) of which were *Aedes vexans*

Discussion

There was a 4.2-fold increase in the number of flavivirus-positive WNV/SLEV-negative mosquitoes pools in 2018 as well as a 7.1-fold increase in these pools that were *Aedes vexans* from previous years.

These results are concerning – potentially indicating a shift in the local viral structure.

In an effort to rule out mosquito specific flaviviruses, we investigated these samples even further in collaboration with the Los Alamos National Laboratory.

Project 2: Next Generation Sequencing

cDNA PCR product from 22 samples from 2018 were sent for Next Generation Sequencing (NGS) at the Los Alamos National Laboratory in New Mexico.

20 = flavivirus-positive and WNV/SLEV-negative pools
2 = flavivirus-positive and WNV/SLEV-positive pools

NGS results were mapped to three databases for potential identification of amplicons.

Results

95% (19/20) of the flavivirus-positive WNV/SLEV-negative pools positively mapped to either WNV or SLEV (Figure 4).

Only one sample (number 21) did not map to a flavivirus in any of the databases.

The two WNV/SLEV PCR positive samples (numbers 7 and 16) both mapped to WNV and SLEV as expected.

	Gottcha-speDB-V		pangia		bwa	
Sample	1st	2nd	1st	2nd	1st	2nd
1	WNV	N/A	WNV	N/A	WNV	*Arthrobacter* spp. QXT-31
2	N/A	N/A	SLEV	N/A	SLEV	*Ochrobactrum* spp. A44
3	WNV	N/A	WNV	N/A	WNV	*Martelella* spp. AD-3
4	WNV	N/A	WNV	SLEV	WNV	SLEV
5	WNV	N/A	WNV	N/A	WNV	*Pseudomonas putida*
6	WNV	N/A	WNV	N/A	WNV	S. albulus
7	WNV	N/A	WNV	SLEV	N/A	N/A
8	WNV	N/A	SLEV	WNV	N/A	N/A
9	WNV	N/A	WNV	N/A	WNV	*Marinobacter* spp. LQ44
10	N/A	N/A	WNV	N/A	SLEV	WNV
11	N/A	N/A	WNV	N/A	WNV	SLEV
12	N/A	N/A	WNV	N/A	WNV	N/A
13	WNV	N/A	WNV	SLEV	WNV	SLEV
14	WNV	N/A	SLEV	WNV	SLEV	WNV
15	WNV	N/A	SLEV	WNV	SLEV	WNV
16	WNV	N/A	WNV	SLEV	WNV	SLEV
17	WNV	N/A	WNV	N/A	N/A	N/A
18	WNV	N/A	WNV	N/A	WNV	*Ochrobactrum* spp. A44
19	WNV	N/A	WNV	N/A	WNV	*Aequorivita sublithincola*
20	WNV	N/A	SLEV	WNV	SLEV	WNV
21	N/A	N/A	N/A	N/A	*S. albulus*	*Martelella* spp. AD-3
22	WNV	N/A	SLEV	WNV	SLEV	WNV

Figure 4. Next Generation Sequencing results for flavivirus positive, WNV/SLEV negative samples

Discussion

These results demonstrate the need for continued evaluation of currently accepted and utilized surveillance tools as viruses have the potential to evolve via antigenic shifts and drifts.

The lack of concordance between the consensus flavivirus primer and NGS results show that there could potentially be a shift in the local viral structure.

If the genetic structure of WNV and/or SLEV is shifting and the currently utilized primer sets are not accurately capturing the true arboviral prevalence, it can result in a potential public health crisis.

Further Research

Maintain regular surveillance of vector-borne diseases, in particular arboviruses, is critical for maintaining the public health of a region.

Given that this study evaluated the amplicon product from the PCR process, only a portion of the potential viral structure was sequenced and analyzed.

Future studies will focus on shotgun sequencing of the entire RNA extract from the mosquito pools in an effort to capture the entire viral structure.