Development of rapid detection method for West Nile virus surveillance in Florida

PI: Liming Zhao
Co-PI: Barry W. Alto
Florida Medical Entomology Laboratory
Entomology and Nematology Department
College of Agricultural and Life Sciences
University of Florida
200 9th Street SE
Vero Beach, FL 32962
lmzhao@ufl.edu
bwalto@ufl.edu
ABSTRACT

West Nile virus (WNV) has become one of the most commonly transmitted mosquito-borne viruses to humans in the United States. WNV emerged for the first time in North America in New York in 1999 and spread to Florida by 2001. The most serious manifestation of WNV infection is fatal encephalitis (inflammation of the brain) in humans and horses, as well as mortality in birds, and so poses as a significant public health and veterinary threat. The natural cycle of WNV involves several species of Culex mosquitoes and wild birds. Since the arrival of WNV in Florida there have been 340 human cases with 24 cases on average per year (CDC 2014). During 2014, there were 16 cumulative human cases of WNV in Florida. Despite substantial variation in the number of human cases of WNV from year to year, there have consistently been cases every year in Florida since its arrival, thus poses as a major public health risk. Florida is in need of the development of more rapid and improved surveillance technology that can be used for early detection of West Nile Virus.

To facilitate an early warning signal to assist mosquito controls battle against emerging pathogens like WNV, we are developing rapid detection methods for surveillance of WNV in Florida using the Bio-Layer Interferometry (BLI) technology and a portable BLI-equipped BLITZ device as well as Octet System. A rapid and accurate method to identify the presence of emerging pathogens in the environment will allow for directed mosquito control to high risk areas benefiting public health of Floridians and tourists. We tested for the detection of WNV using BLI technology and a portable BLI-equipped BLITZ device using specific anti- WNV virus antibodies. The results of this project has the potential to provide a rapid, portable, and cost-effective surveillance tool for Florida Mosquito Control to detect the risk of disease transmission.

INTRODUCTION

West Nile Virus, belonging to the genus Flavivirus in the family Flaviviridae, is a mosquito-borne zoonotic arbovirus found throughout temperate and tropical regions of the world. WNV was first identified in the West Nile subregion in the East African nation of Uganda in 1937. Prior to the mid-1990s, WNV disease occurred only sporadically and was considered a minor risk for humans, until an outbreak in Algeria in 1994, with cases of WNV-caused encephalitis, and the first large outbreak in Romania in 1996, with a high number of cases with neuroinvasive disease (CDC 2011). WNV has now spread globally, with the first case in the Western Hemisphere being identified in New York City in 1999; over the next five years, the virus spread across the continental United States, north into Canada, and southward into the Caribbean islands and Latin America (CDC 2015). WNV is now considered to be an endemic pathogen in Africa, Asia, Australia, the Middle East, Europe and in the United States, which in 2012 has experienced one of its worst epidemics. In 2012, WNV killed 286 people in the United States, with the state of Texas being hard hit by this virus, making the year the deadliest on record for the United States (CDC 2014). Currently, no human vaccine for WNV is available. To improve surveillance and allow for more rapid deployment of control for this emerging pathogen, we used Bio-Layer Interferometry (BLI) technology and BLITZ/Octet System to develop rapid detection method for surveillance of WNV in Florida.
The Bio-Layer Interferometry (BLI) technology and a portable BLI-equipped BLITZ device enable real-time analysis of biomolecular interactions (e.g., amount of proteins and their interactions). BLI assays are based on interactions between an analyte and its ligand which is reported as a light wavelength shift that is a direct measure of the change in optical thickness (nm) of the biological layer. BLI has been used in several recent developmental applications including small molecule analysis and kinetic studies, such as mycotoxin deoxynivalenol, domoic acid and Shiga (Maragos 2012, McGrath et al. 2013). The portable BLI system from Pall ForteBio Co. is the BLITZ device that measures at 17.4 cm H x 15.3 cm W x 22.2 cm D, weighs 7.2 lb (3.3 kg), and uses 100-240 V AC. BLITZ has two volume settings for samples, 4 µl drop in a “dimple” board and 250 µl volume in a 500 µl microfuge tube, thus allowing flexibility in sample format being tested. The attributes of the BLITZ device described here are conducive to deployment to field labs and facilitate real-time processing of samples for surveillance. The BLI system offers a variety of sensors, including Protein L and Protein A sensors for coupling antibodies, NTA: nitrilotriacetic acid sensor for HIS-tagged proteins, Streptavidin (SA) sensor and Super streptavidin sensor for biotinylated nucleic acids and proteins. All sensors are pre-formulated and stored at room temperature with dried sucrose coating, allowing them to be used immediately after hydrating in buffer or water.

Adaptation of the BLITZ device compared to the PCR detection will show the potential for deployment of the BLITZ system for rapid, accurate and sensitive determination WNV as well as other pathogens including dengue and chikungunya viruses. We anticipate that upon successful development that the BLI/BLITZ system may be extended to other arboviruses with importance to Florida such as surveillance for Eastern equine encephalitis viruses and St. Louis encephalitis viruses. This project will benefit arbovirus surveillance and mosquito control in Florida.

MATERIAL AND METHODS

Modification of antibodies.
Biotinylation was tested to label the WNV-specific antibodies from different companies, such as Abcam, Gene Tex, MyBioSource and Santa Cruz Biotechnology. We also tested more than 5 antibodies from BEI resource. The modified biotin-MoAbs were coupled onto SA (streptavidin) sensors for a more durable probe to detect West Nile Virus in more than one sample. An “amplifying” antibody step was also added to enhance the detection signal.

Virus isolate and propagation. We used an isolate of West Nile virus (GenBank accession no. DQ983578) from Indian River County, Florida in 2003. Viral stocks were propagated in culture using African green monkey (Vero) cells using standard procedures (Alto et al. 2014a). Briefly, propagation of arboviruses to establish viral stocks and for blood meals was accomplished by inoculating confluent monolayers of Vero cells in tissue culture flasks (175 cm²) (containing 25 ml media: 199 media, 10% fetal bovine serum, 0.2% amphotericin B (Fungizone®), 2% penicillin-streptomycin) with 0.2 ml virus diluted from stock at a multiplicity of infection of 0.01 (number of viruses to cells). Tissue culture flasks with virus and media were then placed in an incubator at 35°C with a 5% CO₂ atmosphere. Freshly harvested media and virus from tissue
Zhao & Alto, 2016

culture flasks containing Vero cells was combined with blood on the day mosquitoes will be allowed to blood feed. Inoculation of tissue culture flasks with virus and media was be completed two days prior to use in blood feeding trials for WNV (Alto et al. 2014a).

**Mosquito infection.** Egg rafts of Florida *Culex quinquefasciatus* were placed in plastic rearing pans with two liters of water with equal amounts of brewer’s yeast and lactalbumin as larval food at 28°C and a 12:12 L:D photoregime. Adult mosquitoes were held in cages (0.3m³) and provided with sucrose and water from cotton wicks. Adult females were transferred to 16 oz. cylindrical cages the day before the feeding trials. Adult females aged 7-11 days old were provided with WNV infected chicken blood using an artificial membrane feeding system (Hemotek, Lancashire, United Kingdom). Alsever’s solution was added to the chicken blood which was obtained from a supplier (Hemostat Laboratories, Dixon, CA). Separate groups of mosquitoes were provided a high and low dose of WNV, representative of viremia profiles in infected birds (Guerrero-Sánchez et al. 2011, Komar et al. 2003). Aliquots of blood were stored at -80°C for later determination of virus titer by plaque assay (Alto et al. 2014a). After the feeding trials, fully engorged females were held in cages (16 oz. by volume) along with an oviposition substrate and maintained at a 12:12 hour light:dark photoperiod and 28°C for 12 days, after which they were killed and stored in an ultralow freezer at -80°C. These mosquitoes are available to be tested by BLI/BLITZ/antibody-based detection.

**RESULTS AND DISCUSSION**

We used cell culture inoculated WNV as the initial study. Firstly, we used two types of biosensors to run diagnostic tests in the BLItz system and to screen the commercial monoclonal antibodies for sensitivity and specificity for four serotypes of dengue virus. We tested Protein L (ProL) and Streptavidin (SA) Dip and Read™ Biosensors with commercial monoclonal WNV antibodies. Secondly, we used the EZ-Link® Micro-PEO4 Biotinylation Kit to modify the monoclonal antibodies anti-WNV. We develop our protocol of total six steps for the BLItz detection. 1) 1xPBS buffer was used for initial baseline. 2) The biotinylated WNV-antibody was bound to the SA Dip and Read™ Biosensors. 3) Buffer baseline to wash unbound biotinylated WNV-antibody. 4) WNV viruses or controls (*Aedes aegypti* mosquito protein or 1XPBS) were bound to the biotinylated antibody attached the biosensor. 5) Buffer baseline to wash unbound WNV or controls. 6) Use WNV antibody to detect WNV or controls.

We also tested Super Streptavidin (SSA) Dip and Read™ Biosensors with commercial monoclonal antibodies and modified antibodies including West Nile Virus fragment proteins and cell cultures.

We screened more than 15 monoclonal antibodies for West Nile Virus (including fragment proteins and cell cultures) for this project in order to identify the virus accurately and rapidly. Our biotinylated antibody worked very well for the binding to the biosensor and regenerated for the biosensor. These data are useful for the development of the new methods.

The cell cultures of the West Nile Virus were tested. We are in the last stage of these tests. This new technology, the Bio-Layer Interferometry (BLI), has never been used for the arbovirus detection before. This research has the potential to provide a rapid, portable, and cost-effective surveillance tool for Florida mosquito control.

We have improved our cell culture protocols to propagate West Nile Virus to high titers which were desirable for these assays and to successful expose and infect
mosquitoes with WNV. These findings show that Florida mosquito vectors are highly susceptible to infection and transmission of WNV after being fed high titer infected blood. Experiments with WNV are ongoing as well as testing mosquitoes using BLI/BLITZ/antibody-based detection.

If the final test is successful, we will further develop the protocol and may file a patent application and also develop the unique biosensor for West Nile Virus tests. Our final goal is to develop a rapid detection method for improved surveillance for Florida of West Nile Virus. According to our experience (Di et al., 2016), the IgM monoclonal antibody for the critical step is not commercial available. This is why delaying our development of the rapid detection method for West Nile virus surveillance. If we can generated our IgM monoclonal antibody, it requires at least 8 month to one year to obtain the purified products. This is important for our future to try, if the grant and time are available.

ACKNOWLEDGEMENTS

We thank the Centers for Disease Control and Prevention for supplying the West Nile virus isolates. We thank BEI Resources [National Institute of Allergy and Infectious Diseases (NIAID) to provide reagents, tools and information for studying WNV. M. Williams, B. Eastmond, N. Nishimura, S. Ortiz, D. Velez, K. Wiggins, and T. Stenn assisted in experimental studies. These studies were supported by Florida Department of Agricultural and Consumer Services contract 00097943 (University of Florida Project No. 0023717).

LITERATURE CITED


Zhao & Alto, 2016