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SEROSURVEY FOR EVIDENCE OF DENGUE AND WEST NILE VIRUS HUMAN INFECTIONS IN EL PASO, TEXAS

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Dedication

This thesis is dedicated to my mentors, parents, and peers for their endless support and encouragement.

SEROSURVEY FOR EVIDENCE OF DENGUE AND WEST NILE VIRUS HUMAN INFECTIONS IN EL PASO, TEXAS

by

VERONICA SUAREZ

THESIS

Presented to the Faculty of the Graduate School of

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for the Degree of

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THE UNIVERSITY OF TEXAS AT EL PASO

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Abstract

Dengue viruses (DENV), including the 4 serotypes are the cause of dengue fever and dengue hemorrhagic fever. The viruses are transmitted by *Aedes aegypti* mosquitoes in urban communities in tropical, sub-tropical, and temperate regions and are the cause of the highest rate of infection and death of all known *Flaviviruses*. DENV are endemic in United States (US) - Mexico (MX) border communities in the Rio Grande Valley and suspected of being endemic in Ciudad Juarez, a sister city to El Paso, TX. To determine if DENV were endemic in the El Paso community, cord-blood samples from mothers were tested for antibody by an enzyme-linked immunosorbent assay (ELISA), plaque reduction neutralization test (PRNT), and microsphere-immunoassay (MIA). DENV antibody positive samples were further tested for West Nile Virus (WNV) antibody for the possibility of cross-reactivity, considering that WNV is enzootic in El Paso, TX. Of the 1,472 mothers who participated in the study, 0.74% (11/1,472) were positive for DENV antibodies as evidence of a past DENV infection, 3.30% (48/1,472) were positive for WNV antibody, and 0.20% (3/1,472) of the mothers were positive for antibodies to both viruses. The results for 0.20% (3/1472) of the mothers were positive for antibody to only WNV envelope indicating undetermined flavivirus exposure. Another 0.8% (13/1472) of the plasma samples were reactive by ELISA but were negative by the PRNT and MIA. An additional 28 ELISA non-reactive samples were also non-reactive by the MIA and PRNT, thus confirming the accuracy of the ELISA results. Overall, the results for the UTEP-PRNT and the NY-MIA were in concordance for 91% (91/100) of the samples tested for DENV and WNV antibodies. Although 6 of the 11 DENV antibody positive mothers did not have a history of travel to a DENV endemic country, the findings of this survey provided further evidence of local transmission of WNV but did not support a stable state of autochthonous transmission of DENV in the El Paso community.

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I. Background

I.1 The Dengue Virus

Dengue fever and dengue hemorrhagic fever are vector-borne arboviral illnesses caused by four different dengue viral serotypes that belong to the genus *Flavivirus*, family *Flaviviridae* (Westaway et al., 1985). As the primary cause of febrile illness, DENV are the cause of classical dengue fever (DENF) and dengue hemorrhagic fever (DHF); with the incidence being the highest of any other arbovirus as a cause of human disease (Bhatt et al., 2013). The four serotypes of DENV are distributed throughout the tropical and subtropical regions and some temperate regions primarily in urban communities inhabited by the mosquito vectors, *Aedes aegypti and Aedes albopictus* and susceptible humans (Gubler, 2002). The classical dengue fever illness normally resolves in about two weeks by human host primary immune response. The disease is very similar to the symptoms of the influenza virus, causing it to be unrecognized within a community. However, secondary infection with a heterologous serotype can cause an enhanced infection that can results in more severe disease, including DHF and dengue shock syndrome (DSS) which, if left untreated can result in a 20% fatality rate (Gubler, 1998; Halstead, 1989). Outbreaks of DENF are often associated with economic burdens within endemic countries, often causing high infection and fatality rates in third world countries (Gubler and Meltzer et al., 1999; Suaya et al., 2009; Shepard et al., 2011). The DENV has a very large distribution around the world, even reaching into temperate climates, due to its vector, *A. aegypti*, a mosquito with a very long evolutionary relationship with humans (Gubler, 2002). Pandemic cases of dengue were first reported in Southeast Asia in the 1950's following rapid urbanization and globalization after World War II (Gubler and Meltzer, 1999). Epidemic activity dramatically increased during the 1970's and 1980's with a similar pattern of disease growth occurring in the Americas; this rapid global

expansion of the dengue virus is most likely due to globalization, human population growth, international transportation and global trade (Gubler, 1998). The global spread of DENV resulted in four recent outbreaks in the United States, including 2001, 2005, 2013, and 2011 outbreaks in Hawaii, Texas, and Florida respectively, but this is most likely due to the importation of the viruses by tourists traveling and returning from DENV endemic areas, (Rice, 1922; Adalja, et al., 2012). Although one of several vaccine candidates have been approved for use in selected DENV endemic countries, the vaccine is not being widely used because the vaccine has been documented to cause a secondary infection and more sever disease (Hadinegoro SR, et al., 2015). Therefore, vector control is the only way to prevent dengue, but is not effective because of it is not sustainable in most countries for various reasons, including complacency, affordability and ineffective due insecticide resistance.

I.1.1 Pathology of Dengue Fever and Causes for DHF and DSS

When humans are bitten by a DENV infected mosquito, the virus is released into the bloodstream where it is engulfed by macrophages and dendritic cells that are resident within the tissue and phagocytosed via receptor-mediated endocytosis; in the endosome, the cell will begin to process the virus for antigen presentation, to initiate the primary immune response (Uno and Ross, 2018). In addition, the pH changes caused by the cell in efforts to degrade the pathogen for antigen presentation activates proteins within the viral capsid that facilitates the release of the nucleocapsid outside the endosome where pathogenesis can occur This delivery system is due to the external structure of the virus, composed of E and M proteins (internally there is a RNA-capsid complex surrounded by a lipid bilayer), the E protein in the acidic environment associates with the endosomal plasma membrane that catalyzes viral genome release, while cleaving M proteins and NS proteins catalyzes viral replication and viral-complex formation (Modis, 2003). Protein

expression of viral proteins takes places outside the endoplasmic reticulum, where structural proteins will associate with the membrane, and NS proteins will facilitate inclusion of viral genome (Byk and Gamarnik, 2016). The infected DC will begin to excrete viruses as it makes its way into secondary lymphoid tissue where viremia is increased by infection of other phagocytes, until the primary immune system eradicates the virus from the host.

At this point, the primary immune response against DENV is working to eradicate the virus from the host, and host B-cells begin producing IgM and IgG antibodies against virus-specific antigens, such as NS1 (Guzman, 2010). Due to the nature and pathology of B-cell maturation, IgM antibody appear early in infection and peak around 2 weeks post onset of fever, while IgG antibody have low titers near the end of the acute phase of the illness, around 1 week, and rise in titer over time (Innis, 1989; Guzman, 2010). As levels of IgM and IgG antibodies reach peak titers, viremia subsides, and the patient begins to recover. The immune response includes neutralizing antibodies specifically against the infecting DENV serotype, and cross-reactive neutralizing antibodies that can enhance DENV infections (Kolitha, 2013). At this point, IgM antibody levels decrease since most B-cells have matured into plasma cells and have undergone isotype switching, most of the time producing IgG antibody instead of IgM antibody and if the host were to be infected again, the secondary immune response will elicit high IgG antibody titers during the acute phase, and low-levels of IgM (Yam-Puc, 2016).

However, though higher IgG antibody levels are present during the acute phase of the illness, this in facts worsens the impact of the virus, especially if the secondary infection is with a heterologous serotype due to uptake of the virus by Fc-receptors complexed with non-neutralizing cross-reactive antibodies that were produced in the primary immune response to another serotype, that is antigenically related (Vaughn, 2000). This causes an increase in the amount of virus

phagocytosed and accelerates the replication cycle of the virus, thus increasing the viremia and facilitating an immunopathogenic cascade that induces an exaggerated cytokine response including IFN-G, IL2, and TNFα causing micro-vascular permeability and plasma leakage to cause hemorrhaging or DHF and/or shock syndrome (Kolitha, 2013). Since NS3 attacks $CD8^+$ and $CD4^+$ T cells, cross-reactive memory T cells will also increase the severity of the infection as well as facilitate overproduction of cytokines (Kurane, 1994). Because of the nature and pathophysiology of the secondary infection, primary infection in infants is often fatal due to the enhancement of infection by passively transferred cross-reactive non-neutralizing antibodies obtained from the mother (Kliks, 1988).

I.2 Transmission of DENV by *Ae. aegypti* to Humans

Transmission of DENV to humans is thought to have originated from sylvatic cycles involving nonhuman primates and forest dwelling mosquitoes during pre-civilized times, the spillover of DENV to humans resulted in the adaptation of *Ae. aegypti* to be fully domesticated with man, allowing DENV to be maintained an endemic-epidemic cycle in urban environments (Gubler, 2014).

The known urban cycle of DENV includes only *Ae. aegypti* and to a lesser extent *Ae. albopictus* and humans; female mosquitoes are infected when feeding upon infected humans during the viremic phase of the illness (Siler, 1926). Once contracted, the virus infects the midgut and disseminates to salivary tissue, where it can then be transmitted to other hosts while the mosquito is taking a blood meal (Gubler, 2014). Due to this cycle, DENV are most concentrated in urban communities with densely populated humans, the highest incidence of dengue cases

occurs in Asia and the Americas where it is believed that half of the world's population are at-risk (Bhatt et al., 2013).

I.2.1 Endemic Transmission and Cases Across the TX-MX Border

Sporadic outbreaks of DENF and DHF have been reported since 1980 in the Rio Grande Valley of the United States (US) - Mexico (MX) border region, primarily in the urban communities of Matamoros in the Mexican state of Tamaulipas and in Brownsville, TX and surrounding communities (Halfkin et al.,1980; Reiter et al. 2003; Thomas et al., 2016). Most of the infections reported on the U.S. side of the border can be attributed to people traveling to endemic areas during the outbreak in Reynosa, Tamaulipas, Mexico (Rawlings, 1998). Although dengue cases have been reported in other communities on the U.S. side of the border such as the 93 cases during 2014 in Yuma, AZ (bordering with Sonora, MX), in which all of the cases were apparently acquired on the Mexico side of the border (Jones et al., 2016).

Most recently in 2015, a study conducted on a cohort of 77 adults in Ciudad Juarez, MX, revealed that 10.2% of this cohort acquired a recent infection by DENV serotypes 1 and 2, and 14.1% already experienced a past infection (Palermo et al., 2019). However, unlike the Rio Grande Valley region, endemic transmission involving dengue cases has not been reported in any other U.S. border community, despite the high possibility of autochthonous transmission due to a wide distribution of *Ae. Aegypti* and *Ae. Albopictus* across the border (Hahn et al., 2016; Vitek et al., 2014). The failure to report dengue cases in the El Paso area may be due to passive surveillance programs not being able to detect a prior infection in subjects or are misdiagnosed for other diseases such as influenza (Hollidge et al., 2010). In Brownsville, TX no cases of dengue were detected until the use of active surveillance in 1980 that was able to detect at least 63 cases (Hafkin

et al., 1982). This is cause for concern because it could explain the failure to detect dengue cases in the El Paso community, especially since recent evidence showed the presence of dengue in Ciudad Juarez, MX, a sister city that sees heavy traffic across the border (Palermo et al. 2019; Watts DM, 2019, unpublished data). This shows a high possibility of autochthonous transmission, presenting a critical need for an active surveillance study for early detection of these viruses. There is also no treatment or vaccine for dengue, signifying a need to control transmission before an outbreak to occur, making the need for active surveillance even more significant (Pang, 2017).

The primary aim of this study was to conduct an active serosurvey for DENV antibody in cord-blood samples obtained from mothers at the time of delivery of babies at selected hospitals to determine if these viruses were endemic in the El Paso community. The same cord-blood samples were also tested for West Nile Virus (WNV) since antibody positive samples for DENV could reflect cross-reactions due to WNV infection, the only other known *Flavivirus* endemic in the El Paso community.

II. Methods and Materials

II.1 DENV and WNV

Working stocks of DENV and WNV used in this study were prepared for use in the plaque reduction neutralization tests (PRNT) in C6/36 cells to test the cord-blood plasma samples for WNV and DENV specific antibodies. Also, lysate antigen was prepared in African Green Monkey (*Chlorocebus species*) kidney cells (Vero cells) for use to perform the enzyme-linked immunosorbent assays (ELISA) to test plasma samples for DENV and WNV IgG antibodies. DENV 1, 2 and 4 were obtained from the Dept. of Virology, U.S. Naval Medical Research Unit No. 6, Lima, Peru. DENV-1 (strain 16007) and DENV-2 (strain 16681) were isolated from patients in Thailand in 1964. DENV-1 had received 10 passages in *Aedes albopictus* cells and DENV-2 had been passaged 9 times in C6/36 cells. DENV-3 was isolated from a patient in the Philippines in 1956 and had been was passaged twice in C6/36 cells. This serotype was obtained from the World Reference Center for Emerging Viruses and Arboviruses, UT Medical Branch, Galveston, TX. DENV-4 was isolated in 1967 from a patient in Indonesia and had received 10 passaged in C6/36 cells. The WNV used in this study was provided by World Reference Center for Emerging Viruses and Arboviruses, UT Medical Branch, Galveston, TX. The virus was originally isolated from a deceased Snowy Owl (*Nyctea scandiaca*) in the Bronx Zoo during the 1999 WNV epizootic in New York City (Steele et al. 2000).

II.2 Subject Enrollment and Classification of Identities

Pregnant women who attended hospitals in El Paso, TX were enrolled in this study for multiple reasons: the location of the different hospitals provided an opportunity to obtain cord-

blood samples from study subjects from different parts of the city, cord-blood samples were very convenient to obtain as they do not require invasive procedures and does not cause any strain on the mothers who had just given birth. Also, studies in South America showed that there were no male/female bias for vector feeding and dengue case incidence. (Anker et al., 2011; Gunther et al., 2009; Garcia-Rivera and Rigau-Perez, 2003; Gamble, 2000; Travassos da Rosa et al., 2000).

Subjects who volunteered for participation in this survey were enrolled under a protocol #515533-1 that was approved by the Institutional Review Board (IRB) at the University of Texas at El Paso (UTEP) and by the participating hospitals, including the Sierra Medical Center (SMC), Sierra East Medical Center (SES), and the Providence Memorial Hospital (PMH) in El Paso, TX.

Subjects eligible for participation in the survey were expecting mothers or mothers who had recently delivered in the past 24 hours and were18 years of age or older. The participants were selected and invited to participate in the survey by a clinical coordinator who worked with hospital staff for at least four hours every week from June 2015 to June 2017. The coordinator provided an explanation of the survey to the subjects and obtain written consent for 5ml of cord blood samples. A copy of the forms was given to the hospital laboratory and the original forms were permanently stored in a locked file cabinet located in the office of Dr. Douglas M. Watts in the UTEP Biosciences Building. All subjects were administered a questionnaire to obtain their age, occupation, zip code, and travel history over the past five years.

Questionnaire

Title: "SEROSURVEY FOR EVIDENCE OF DENGUE AND WEST NILE VIRUS HUMAN INFECTIONS IN EL PASO, TEXAS"

Date: ________________

Blood Sample Number: ________________

Age: _______ **Zip Code/Colonia:** ________________ **Occupation:** ______________________

Travel history over past 5 years:

____

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Figure 1. The questionnaire was administered to mothers who volunteered to participate in the survey

Subjects donated a 5 ml cord-blood plasma sample. Each sample was transferred by the hospital laboratory staff to a 5ml ethylenediaminetetraacetic acid (EDTA) coated vacutainer and given a code number to de-identify the subjects. The blood samples were placed on ice-packs within Igloo coolers and transported to the Biosafety-Level 3 laboratory at the UTEP Biosciences building, where they were centrifuged at 1200XG at $4^{o}C$ for 10 minutes. Plasma was transferred in aliquots of 0.5 ml to sterile vials and stored at $-20^{o}C$ until tested for antibodies.

II.2.1 Confidentiality of the Subjects Used in the Survey

All participant's information was kept confidential. Dr. Watts was the only participant of the survey who had full access to original document and data, which was only identified by number that was associated with the questionnaire information, participant names were not used in the study.

II.3 Indirect ELISA for IgG Antibody

To test for DENV or WNV IgG antibodies, a DENV antigen pool (DENV1-4) and WNV antigen was prepared by infecting Vero cells with the specific viruses and lysing the cells (uninfected cells were also lysed as a control). The cell lysates were then added in duplicates to the wells of 96-well microplates in order to coat the walls of the wells with the antigens. Cord-blood samples were diluted at 1:100 with blocking buffer (5% Skim Milk and 1% Tween in phosphate buffered saline (PBS) 1x pH 7.4) and then added to the coated wells (in duplicates) and left in the 96-well microplate overnight at $4^{o}C$ (Ansari et al., 1993). The next morning, the wells were then aspirated and carefully washed with 1X PBS 0.1% Tween three times and completely aspirated and a secondary horseradish peroxidase (HRP) conjugated antibody (anti-Human IgG)

was added to each well and allowed to incubate. The plates were washed again using 1X PBS 0.1% Tween to remove any excess unbound secondary antibody. A colorimetric substrate ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt was then added to each well and incubated at 37°C to allow to catalyze for 30 min. The optical density (OD) of the reaction was then determined by a microplate spectrophotometer at 410 nm absorbance to determine the OD values of the samples; the reactivity of samples that exceeded the reference cut-off OD value calculated as the mean of six antibody-negative controls plus three times the standard deviation of the antibody negative control samples were considered to be IgG antibody positive. Any samples that had reactivity more than the cut-off value were considered antibody positive. Samples that were positive for antibody at 1:100 dilution to DENV or WNV were retested by the indirect ELISA at four-fold dilutions ranging from 1:100 to 1:6400 to determine the titer of the antibody against each virus antigen. This method was used to obtain a presumptive diagnosis, as any sample with a 4-fold or greater titer to one of the viruses were considered as presumptive evidence for that virus, and samples less than a 4-fold difference reflected possible infection by both WNV and/or DENV, or possible cross-reactivity to both viruses.

Regardless of the presumed antibody titers, all reactive ELISA samples were retested as described below for viral type specific antibody by the PRNT at UTEP and a subsample was tested by PRNT at the Arbovirus Laboratory, New York State Department of Health, Slingerlands, New York. Also, all samples were further tested by a multiplex microsphere immunoassay (NY-MIA) at the Diagnostic Immunology Laboratory at the Wadsworth Center NY State Department of Health, Albany, New York.

II.4 Indirect Immunoglobulin M (IgM) ELISA

An IgM antibody capture ELISA was used to test cord-blood samples that were positive for DENV IgG antibody for DENV IgM antibody according to a method described previously (Innis et al., 1989). Ninety-six well microplates were coated with anti-human IgM antibody and allowed to incubate at 4°C overnight. Each of the plasma samples were diluted 1:100 using blocking buffer (5% Skim Milk and 1% Tween in phosphate buffered saline (PBS) 1x pH 7.4) and were then added to the wells of the 96-well microplate after they were washed three times with 1x PBS 0.1% Tween and aspirated completely. Next, dengue viral lysate antigen was added followed by the addition of DENV specific hyperimmune ascitic fluid HRP-conjugated rabbit anti-mouse IgG antibody. After washing, ABST was added and incubated at 37°C for 30 min and the mixtures in the wells were read on a microplate spectrophotometer at 410 nm absorbance. The procedures for the OD-cutoff calculations were the same as described above for indirect ELISA against DENV and WNV IgG antibodies.

II.5 The Plaque Reduction Neutralization Test (PRNT)

The PRNT was performed at UTEP (UTEP-PRNT) to determine if the ELISA reactive cord-blood samples reflected a WNV or DENV infection or infection by both viruses. For detection of WNV specific antibodies, Rhesus monkeys (*Macaca mulatto*) epithelial (LLc-MK2) cells were used to be infected with WNV and stained with naphthol blue black to stain viable cells, this method was adapted from methods previously published from a PRNT that employed Vero cells and crystal violet stain (Juarez et al., 2013). A similar plaque assay described previously that utilized Syrian baby hamster (*Mesocricetus auratus*) kidney cells 21 (BHK-21) and naphthol blue black stain was used for the DENV PRNT (Morens et al., 1985).

First, four-fold dilutions of each plasma sample that had tested positive in the indirect ELISA for DENV and/or WNV IgG antibody were prepared at 1:20-1:2560 which were then heat-treated at 56°C for 30 minutes. These heat-treated diluted samples were then mixed with 40-50 plaque forming units (PFU's) of either DENV or WNV to yield a viral dose of 20-25 PFU. These mixtures were then incubated overnight at 4°C. Equal volumes of 40-50 PFU's were also mixed with Eagle's Minimal Essential Medium (EMEM) as a control to verify the amount of virus used in the PRNT. The next day, the plasma-virus mixtures and EMEM-virus mixture were inoculated onto LLc-MK2 cells or BHK-21 cells to test for neutralizing antibody against DENV and/or WNV. The inoculated cells were incubated for 3-7 days at 37°C 5% CO_2 , and then stained with naphthol blue black stain (SIGMA, St. Louis, MO) for viable cells. The plaques were then counted, and the dilution of the cord-blood samples that reduced the number of plaques that developed in the EMEM-virus inoculation (the viral dose) by 80% was recorded as the antibody titer for either DENV or WNV, or both viruses; antibody titer above 1:20 were considered positive for one or both viruses.

A random sub-sample (n=36) of the cord-blood plasma samples tested by UTEP-$PRNT_{80}$ and NY-MIA were retested for neutralizing antibody by the NY-$PRNT_{90}$, using the dilutions of the samples that reduced the viral dose by 90% to determine the antibody titer as described previously (Lindsey et al., 1976). The retesting of these 36 samples in the NY-$PRNT_{90}$ was performed to evaluate the results of the UTEP-$PRNT_{80}$ because the use of the $PRNT_{90}$ provides more robust conclusions; antibody titers $\geq$ 1:20 were considered positive for virus-neutralizing antibody to either DENV, WNV, or both viruses.

II.6 Multiplex Microsphere Immunoassay (MIA)

All samples tested positive for WNV and DENV antibodies by ELISA and PRNT were shipped to the Diagnostic Immunology Laboratory at the Wadsworth Center New York State Department of Health, as well as selected antibody negative samples, to be used in a multiplex microsphere immunoassay (NY-MIA) to further confirm antibody virus type specificity.

The NS1 of DENV 1-4 and ZIKV, as well as the E protein of the ZIKV were purchased as recombinant proteins from Meridian Bioscience INC. These recombinant proteins were produced in insect cells and purified by affinity chromatography; purification samples were analyzed via sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stored in 1X PBS pH 7.4 until used.

The WNV E protein antigen and the WNV NS5 were provided by Michel Ledizet of L Squared Diagnostics (L2DX) of New Haven, CT and Pei Yong Shi of UTMB, Galveston, TX respectively, while the WNV NS1 was prepared by Randall Renshaw of Cornell University Animal Health Diagnostic Laboratory, Ithaca, NY as previously described (Wong et al., 2004, Wong et al., 2003).

Altogether the NY-MIA assay utilized 9 different recombinant antigens (ZIKV E, ZIKV NS1, DEN1 NS1, DEN2 NS1, DEN3 NS1, DEN4 NS1, WNV E, WNV NS1, WNV NS5) to determine antibody virus type specificity (Wong et al., 2003, Wong et al., 2004, Wong et al., 2017). The NY-MIA was a 3-phase suspension assay; first the 9 different flavivirus recombinant antigen-conjugated bead sets were prepared by covalently coupling the proteins to 6.25 million Luminex MicroPlex carboxylate polystyrene microspheres (Luminex Corporation, Austin, TX) as described previously (Wong et al., 2007). These beads were then suspended with subject plasma at a 1:100 dilution in PBS with azide (PBN) and incubated at 37°C for 30 minutes. These sets were then

washed 3x with 1X PBS Tween-20 0.05% pH 7.4 (PBST) (Sigma Aldrich, St. Louis, MO) to which a biotinylated anti-human immunoglobulin (against IgG, M, and A) was added as a secondary and incubated at 37°C for 30 min. The beads were then washed 3x with PBST and incubated with streptavidin RPE fluorochrome (SA-PE) for 30 min at 37°C in order to analyze captured plasma antibody. The beads were then washed again 3x with PBST and completely aspirated, and resuspended in PBN, detection of captured antibody was achieved by using a flow analysis in a dual-laser Luminex 100 flow analyzer (Luminex Corporation, Austin, TX) to measure the median fluorescence intensity (MFI) to quantify antibody-antigen relationship within 100 beads of each recombinant protein/subject plasma mix. The cutoff value for MFI quantities was calculated using the mean of the MFI values of 90 selected negative samples summed with the standard deviation 3x; any MFI value under this cutoff was considered a negative sample and samples with MFI values above the cutoff were considered positive
Samples (Wong, 2004).

Along with the NY-MIA, a two-tail type 3 T-test was performed using the ages of the women within the various hospitals to determine if there was significance difference between age differences ($p=<0.05$).

III. Results

III.1 Seroprevalence of Subject Plasma Samples

A total of 1,472 mothers were enrolled in this study, including 562 from the Providence Memorial hospital (PMH), 158 from the Sierra Medical Center Main hospital, and 752 from the Sierra Medical East hospital (Table 1). Overall, 90.8 % (n=1337) of the mothers were from El Paso, Texas 3.1% (n=45) from New Mexico, 3.7% (n= 55) from Mexico, and 2.3 % (n=35) of the mothers failed to provide an address. The distribution of the mothers by age (years) revealed that 33.4 % (n=492) of the mother ranged in age from 18 – 23 years, 38.5 % (n=567) from 24 – 29, 18.1 % (n=267) from 30 – 34, 7.0 % (n=103) from 35 – 40, 1.2 % (n=17) from 41 – 45, 0.1 % (n=2) was over 46 years old, and 1.6 % (n=24) did not state their age. Although the age distribution varied among the 3 hospitals, the difference was not significant between SMC and PMH (p=0.11), between SMC and SES, (p=0.16), and between PMH and SES, (p= 0.97) (Table 1).

A total of 4.9% (72/1,472) of the plasma samples from mothers at the time of the delivery of their babies were reactive for DENV IgG antibody by ELISA, suggesting that the mothers had been infected with one or more of the 4 DENV serotypes. Since DEN viruses are antigenically closely related to WNV, reactivity for DENV IgG antibody could represent cross reactivity due to a previous infection by WNV, the samples were further tested for WNV ELISA IgG antibody. All of the 72 samples but 2 were also reactive for WNV IgG antibody, thus reflecting infection by either one or both viruses for 70 samples. Two samples were reactive only for DENV antibody. All ELISA reactive samples were then tested at dilutions ranging from 1:100 to 1:6400 and all samples were reactive at titers ranging from 1:100 to 1:6400 for DENV and/or WNV or for both

viruses. The results of testing the 72 ELISA reactive samples by the UTEP- PRNT and NY- MIA for both DENV and WNV antibodies revealed that 59 were positive to either DENV or WNV or to both viruses, or for antibody to flavivirus envelope. The other 13 ELISA reactive samples or 18% (13/72) were negative for DENV and/or WNV antibodies or to both viruses by the UTEP-PRNT and NY- MIA. An additional 28 samples that were negative for DENV and WNV ELISA IgG antibody were included for testing to evaluate the validity of the ELISA results and found to be negative for antibody by the UTEP - PRNT and the NY- MIA. Also, among the 59 antibody positive samples by the ELISA and the UTEP-PRNT and NY- MIA, 36 were selected for further testing by the NY - PRNT90 to further evaluate the validity of the results obtained by the ELISA, UTEP – PRNT80 and the NY - MIA. The results showed that all but 2 samples were in concordance for the 3 different assays. A more detailed description of these observations are described below.

Of the 59 samples that were positive for flavivirus antibody, 3.0% (45/1,472) were positive for only WNV antibody as evidence of a previous infection by this virus at the 3 hospitals (Table 1). The distribution of the WNV antibody positive samples included 1.8% (10/562) of mothers enrolled at the PMH hospital, 4.4% (7/158) at the SMC hospital and 3.7% (28/752) at the SES hospital. Antibody to only DENV was detected in 0.5% (8/1472) of the mothers as evidence of a previous infection by this virus. The distribution of DENV antibody included 0.00% (0/562) among mothers enrolled at the PMH, 1.3% (2/158) at the SMC hospital, 0.80% (6/752) at the SES hospital. If the antibody prevalence rates for both WNV/DENV are considered as reflecting infection by both viruses, then the number antibody positive mothers increased by 3 for an overall WNV antibody prevalence rate of 3.3% (48/1,472), and the overall rate increases to 0.74% (11/1,472) for DENV antibody. The results for another 5 mothers included in the summary had

antibody to flavivirus envelope of which 4 were positive by the UTEP-PRNT for WNV antibody, and one for WNV/DENV antibody. Also, 3 mothers, 2 at the SMC and one at the SES hospitals had antibody to flavivirus envelope, but negative for antibodies to DENV and/or WNV. The overall seroprevalence rates for flaviviruses for mothers at the different hospitals was 4.0% (59/1,472), including 2.1% (12/562) at the PMH, 7.0% (11/158), and 4.8% (36/752) at the SES. All 59 samples tested by the NY - MIA were negative for ZIKV antibody.

Among the 8 mothers who had DENV antibody, 6 had not traveled outside of El Paso, Texas, and one had traveled to Jamaica and one to Puerto Rico. The history of travel for the 3 mothers who were infected with both DENV and WNV indicated both had traveled to Mexico. Of the 45 mothers who had WNV antibody, 22 had not traveled outside of El Paso, and 18 had traveled to Mexico, one to Puerto Rico, one to Europe, one to Costa Rico, one to Europe, Asia and Caribbean and one did not provide an answer.

III.2 Analysis of Samples Reactive for WNV and DENV Antibodies

The results for screening of the plasma samples obtained from mothers at the PMH hospital by the ELISA for DENV and WNV IgG antibodies and for UTEP-PRNT and NY – MIA are presented in Tables 2. Among the 10 UTEP- PRNT and NY – MIA confirmed WNV and 2 WNV/DENV antibody positive plasma samples, the ELISA IgG antibody titers for 11 samples were 4-fold greater for WNV antibody. The other sample was one of the 2 WNV/DENV antibody positives that had the same ELISA titer for WNV and DENV.

The results for screening of plasma samples for mothers enrolled at the SES hospitals by ELISA for DENV and WNV IgG antibodies and for UTEP-PRNT and NY – MIA are presented in (Table 3). Of the 36 reactive samples, 28 were confirmed positive for WNV antibody by the

UTEP-PRNT and/or NY- MIA, including 19 with titers 4-fold greater to WNV, 8 with antibody titers equal to both DENV and WNV viruses, and one with a titer two-fold higher for WNV. Of the 28 WNV positive samples, 3 were positive for flavivirus envelope antibody by the MIA, but were positive by the PRNT for WNV antibody, and one flavivirus envelope antibody positive was negative to both DENV and WNV by the UTEP-PRNT. The IgG antibody titers for the 6 DENV confirmed antibody positive mothers enrolled at the SES hospital ranged from 1:1600 to 1:6400, with titers for 4 samples 4-fold higher to DENV than for WNV, and 2 samples had antibody titers equal to both DENV and WNV.

The results for screening of plasma samples for mothers enrolled at the SMC hospital by ELISA for DENV and WNV IgG antibodies and for UTEP-PRNT and NY-MIA are presented in (Table 4). Of the 11 ELISA reactive samples, 7 were confirmed positive for WNV antibody by the UTEP-PRNT and/or NY-MIA and all had titers 4-fold greater than to DENV. Of the 2 DENV antibody positive samples, one had a titer equal to both viruses and one had a titer 4-fold higher for DENV than WNV. Two sample were positive only for flavivirus envelope antibody, one with an IgG antibody titer equal for DENV and WNV, and one with a 1:200 titer to DENV and negative for WNV antibody.

Overall, the results of the ELISA predicted 82% (37/45) of the PRNT and/or MIA confirmed WNV antibody positive samples based on a 4-fold or greater titer than for DENV. Among the DENV antibody positives, 63% (5/8) had an ELISA titer of 4-fold or greater than for WNV.

As shown in Table 2, the diagnostic results for 12 ELISA IgG antibody positive samples obtained from mothers at the PMH hospital showed that 10 WNV and/or WNV/DENV antibody positive samples were in agreement between the UTEP-PRNT and NY- MIA. The results for the

other 2 samples, including sample PMH0037 as positive for antibody to flavivirus envelope by the NY- MIA, but positive for WNV antibody by the UTEP- PRNT. The other sample, PMH0167 was positive for WNV/DENV antibody by the NY-MIA and positive only for WNV antibody by the UTEP – PRNT.

Among the 36 ELISA antibody positive samples for mothers enrolled at the SES hospital, the results for 31 samples were in concordance between the NY- MIA and the UTEP - PRNT (Table 3). Of the other 5 samples, the results for 4, including samples, SES0450, SES0827, SES0654 and SES0632 were positive for flavivirus envelope antibody by the NY - MIA, but 3 were positive for antibody to WNV and one for antibody to WNV/DENV by the UTEP - PRNT. The other sample SES0146 was positive for antibody to flavivirus envelope and negative for antibody by the UTEP-PRNT.

Among the 11 ELISA antibody positive samples for mothers enrolled at the SMC hospital, the results for 9 samples were in concordance between the NY-MIA and the UTEP- PRNT (Table 4). The other 2 samples, including SMC0055 and SMC0119 were positive for antibody to flavivirus envelope by the NY - MIA, but were negative for antibody by the UTEP- PRNT. Overall, a concordance of 96% (50/52) was demonstrated between the results obtained by the UTEP-PRNT and NY- MIA. This concordance estimate does not include the 7 flavivirus antibody positive samples and is based entirely on PRNT and NS antibody results.

III.3 Summary of Disoncordant and Flavivirus Antibody Positive Results

A summary of 9 samples that yielded disconcordant results and/or were flavivirus antibody positive is presented in Table 5. Of these 9 samples, 7 were positive for antibody to the flavivirus envelope by the NY - MIA and one was positive for WNV/DENV antibody and one was positive

for WNV antibody. In contrast, the UTEP-PRNT showed that of the 7 samples positive for flavivirus envelope antibody, 4 were positive for WNV antibody, and 3 were negative. The one NY - MIA WNV/DENV antibody positive sample was positive by the UTEP –PRNT for WNV antibody and the one NY - MIA positive for only WNV antibody, was positive for antibody to WNV and DENV by the UTEP – PRNT.

III.4 Summary of ELISA Reactive but PRNT Antibody Negative Samples and ELISA Negative and PRNT Antibody Negative Samples

As mentioned above, 13 samples were reactive for ELISA IgG antibody to either or both WNV and DENV, but negative by NY- MIA and UTEP- PRNT (Table 6). Of these 13 samples, 3 were was collected from mothers at the PMH, 9 at SES and one at the SMC hospital. All were positive for DENV antibody by the ELISA with 11 having a titer of 1:400 and one had a titer of 1:200 and one had a 1:100 titer. Only 5 of the 13 DENV reactive samples were reactive to WNV, including one with a titer of 1:400 and 4 with titers of 1:100. Overall, 13 samples were reactive to DENV, and 5 of the 13 were also reactive to WNV.

An additional 28 non-reactive samples by the ELISA were selected, including 3 collected from mothers at the PMH hospital, 18 at the SES and 7 at the SMC for testing by the NY- MIA and UTEP- PRNT to evaluate the accuracy of the ELISA (Data not shown). The 28 samples were negative for DENV and WNV antibody by the NY- MIA and UTEP- PRNT, thus confirming that the ELISA antibody negative results were in concordance with the NY - MIA and UTEP - PRNT negative results.

III.5 Testing of a Subsample of Non-reactive and Antibody Positive Plasma Samples by an External Laboratory

A subsample of 36 of the 72 plasma samples, including 15 antibody negative samples and 14 WNV antibody and 6 DENV antibody and one WNV/DENV antibody positive samples were randomly selected for further testing by a NY- PRNT using a 90% reduction of the virus dose (Table 7). The rationale for testing samples by the NY-PRNT was to have an external laboratory test selected samples to further evaluate the NY- MIA and UTEP - PRNT results as an enhanced robust testing scheme. All 36 samples were concordant except for 2 by the 3 assays. The 2 disconcordant samples included sample SES0475 that was positive for WNV/DENV by the NY-PRNT, and positive only for WNV by the NY- MIA and UTEP - PRNT. The other sample, SES0654 was positive for WNV antibody only by the NY-PRNT, but positive for both WNV/DENV antibody by the UTEP- PRNT.

Overall, the results for the UTEP-PRNT and the NY-MIA were in concordance for 50 of 52 flavivirus neutralizing antibody positive samples, for 13 of 13 ELISA reactive samples that were negative by the UTEP – PRNT and the NY-MIA, and for 28 ELISA negative samples that were negative by both the NY-MIA and UTEP- PRNT. The total concordance was 98% (91/93) for the samples tested by the NY-MIA and the UTEP-PRNT. Among the 36 samples, including 13 WNV and 7 DENV antibody positive samples and 16 negative samples by the UTEP-PRNT and the NY-MIA, the results of testing these samples by the NY – PRNT were in concordance for 94% (34/36) of the samples.

IV. Discussion

DENV are emerging arboviruses that cause more human disease than any other mosquito-borne viruses, yet DENV are either not recognized or under-recognized in many tropical and sub-tropical regions due to lack off or unreliable surveillance programs. Surveillance for human DENV infection along the U.S. - MX border has been conducted primarily during or after sporadic outbreaks of dengue involving endemic transmission in selected southern most urban communities in the Rio Grande valley of TX. Seroprevalence rates as evidence of past DENV infection ranged from 23% in Laredo, TX (Reiter et al., 2003) to 38% in Brownsville, TX (Ramos et al., 2008). More recently, observations indicated that during 2015, 14.1% of a selected cohort of 77 adults had serological evidence of a past DENV infection and 10.2% (5/61) seroconverted as evidence of a recent DENV 1 and 2 infection the El Paso adjoining sister community of Ciudad Juarez, MX (Palermo et al., 2019). Other than the Rio Grande valley region of TX, autochthonous transmission of DENV has not been reported from any other U.S. border communities (Hahn et al., 2016). A possible explanation for the perceived absence of DENV in other urban border communities is that cases may not have been detected by passive surveillance, and therefore, this survey was conducted to determine if these viruses were endemic in the El Paso, TX community. The findings revealed that 0.74% (11/1,472) of cord blood plasma samples obtained from mothers at the PMH, SES and SMC hospitals in El Paso were positive for DENV 1 and/or 2 IgG antibody and 3.3% (48/1,472) were positive for WNV antibody. The identity of these virus-type specific antibody was confirmed by a robust testing scheme that involved the screening of samples by an ELISA and by analyzing the ELISA reactive samples by the more specific assays, including the NY - MIA and the UTEP - PRNT80 and the NY - PRNT90. The use of these different diagnostic tests was employed because the serological diagnosis of virus type specific flavivirus antibody is often confounded by the

antigenic similarities among the viruses of the Flavivirus genus (Calisher et al., 1989; Qureshi &Trent, 1973).

Among the 11 DENV antibody positive cord-blood plasma samples, 2 were obtained from mothers enrolled at the PMH and one from a mother enrolled at the SES hospital; all 3 were positive for antibody to both DENV and WNV. The other 8 DENV antibody positive mothers included 7 enrolled at the SES and 2 at the SMC hospital. Of the 11 antibody positive mothers, 8 resided in East El Paso, 2 in Central and one in West, El Paso. The observation that most of the DENV antibody positive mothers were from East El Paso was in the area of the city where the population density of *Aedes aegypti* females was the highest from 2015 – 2017 with estimates of 205 females per month for at a total of 3,768 female mosquitoes over the summer months of the 3 years. (Watts DM, 2019 unpublished data). Also, as a potential vector of DENV, data accumulated from 2014 - 2017 indicated that this species was distributed throughout the El Paso community and was second to *Culex quinefasctiatus* as the most abundant species. Further suggestive evidence of the possibility that the mothers may have acquired a local DENV infection was supported by the travel history. Of the 11 mothers, 6 had not traveled outside of El Paso, Texas, and 3 had traveled Mexico, one to Puerto Rico, and one had traveled, but no to a DENV endemic country. These observations and the more recent evidence that supported endemic transmission among a human cohort DENV infection in Ciudad Juarez, MX, the urban adjoining sister community with El Paso suggested that the mothers could have acquired DENV infections in the El Paso community (Palermo et al. 2019). Although DENV infection in the El Paso community cannot be excluded, the location and date of infection are inconclusive. Blood samples were not obtained from the mothers; therefore, it was not possible to test for IgM antibody as possible evidence of a recent DENV infection. Also, our results that showed the 11 DENV IgG antibody positive cord-

blood samples were negative for IgM antibody was consistent with general knowledge that IgM antibody rarely crosses the placental barrier (Brambell et al. 1960). However, DENV IgG antibody has been well documented to be transferred across the placental barriers in several DENV endemic countries. In support of these observations, the results of several studies documented that DENV IgM antibody was rarely or not maternally transferred at all, but that IgG antibody were transferred at a frequency of 90% or higher to neonates in Thailand (Perret et al. 2005; Pengsaa et al. 2006), Brazil (Ribeiro CF et al. 2017; Argolo et al. 2013, and Malaysia (Mohamed et al. 2014). Therefore, these results suggested that antibody rates in cord-blood samples may provide a reliable estimate of the prevalence of DENV infection in the female population for child bearing age cohort but may or may not reflect the rate among the same male cohort. Estimates of the distribution of cases by gender in 6 countries in Southeast Asia found a consistent and significant excess of dengue cases among males > 15 years of age (Anker et al. 2011). However, studies in South and Central America found that dengue cases were equally distributed among male and female, or a greater proportion of female cases. (Gunther et al. 2009; Trravassos da Rosa et al. 2000, Garcia-Rivera and Rigau-Perez, 2003). Also, in the US-Mexico border community of Reynoso, MX, 58% of dengue cases during an outbreak between 2014 - 2016 were among females (Laredo - Tiscareño et al. 2018). These findings represented observations in DENV endemic countries, and therefore, the relevance, if any to the prevalence rate and the gender distribution of DENV antibody in the El Paso community will depend on whether not the El Paso results reflected endemic DENV transmission.

The frequency of DENV IgG antibody transfer across the placental barriers may or may not be representative of the prevalence of DENV infection in the mothers, or the El Paso community at large as possible evidence of endemic transmission. However, the exceptionally low DENV antibody prevalence rate of 0.74% in El Paso in comparison to much higher rates in DENV

endemic areas argue against endemic transmission. For example, existing observations for the US - MX border region revealed that the DENV seroprevalence rate was 14.1% in the El Paso adjoining urban community of Ciudad Juarez, MX with preliminary evidence of autochthonous DENV 1 and 2 transmission. In other border communities with confirmed autochthonous DENV transmission, the seroprevalence rate was 38% in Brownsville, TX and 77% Matamoros, MX (Ramos et al. 2008). The rate for past DENV infection was 23% for Laredo, TX, and 48% in Nuevo Laredo, MX, and 62.7% in Reynoso, MX (Reiter et al. 2003; Laredo-Tiscareno et al. 2018). The DENV seroprevalence based on a regional analysis among all age groups and adults (>15 years old) involving a sample size that varied from 46 to 5,669 was 64.4% in the Americas, 46.2% in Asia and 18.1% in Africa (Fritzell et al. 2018). The rates in other selected DENV endemic urban communities varied, for example, the seroprevalence rate for DENV ranged from 61% to 97% in Zhejiang Province, China (Luo et al. 2018), Pune, India (Mishra et al. 2018), Central and Southern Thailand (Vongpunsawad et al. 2017), Medellin, Colombia, (Carabali et al. 2017), Bangkok, Thailand (Burke et al, 1988), Managua, Nicaragua (Balmaseda et al. 2006), and Iquitos, Peru (Morrison et al. 2014). Overall, these higher DENV seroprevalence rates in well documented DENV endemic regions suggested that the exceptionally low prevalence rate of DENV antibody in the El Paso community did not reflect stable autochthonous transmission of DENV.

Active surveillance for human cases of dengue has not been conducted, and cases have not been reported based on passive surveillance in the El Paso community. Also, as further evidence that argues against endemicity of DENV, these viruses were not isolated during 2015 – 2017 from a total of 3,768 female *Aedes aegypti* collected in East El Paso where DENV antibody was detected in 7 of the 11 DENV antibody positive mothers enrolled in this survey (Watts DM, 2019, unpublished data). Although the reported DENV infection rates in endemic countries varied for

Aedes aegypti, estimates indicated that the population density and number tested in El Paso were likely to be enough to detect DENV, if these viruses were endemic in this community. Estimates of minimum field DENV infection rates (MFIR) in *Aedes aegypti* based on the testing of 1000 mosquitoes in a DENV hyper-endemic urban community of Bangkok Thailand varied from 0.4 to 8.2 isolates per 1000 female mosquitoes around houses with positive dengue cases and 0.4 per 1000 near houses with no dengue cases (Yoon et al., 2012). In other DENV endemic countries, the MFIR/1000 mosquitoes ranged from 0.15 to 26.0 in Puerto (Barrera et al. 1971), 0.5 to 32 in Singapore (Chan et al, 1971; Chung and Pany, 2002), 5.8 in New Delhi, India (Vikram et al. 2015), 16.2 in Brazil (Medeiros et al. 2018), 15.9 in Venezuela (Urdneta et al. 2005), 18.0 in MX (Garcia - Rejon et al. 2008) and the rate in Colombia was 11.7 mosquitoes during an epidemic and decreased to 3.56 after the epidemic (Mendez et al. 2006).

Our initial observations indicated that a total of 72 sample of 1,472 were reactive at a 1:100 dilution in the ELISA for IgG antibody to DENV. Estimate of titers of these samples using the ELISA revealed that 70 of the 72 samples reacted to both WNV and DENV with titers ranging from 1:100 to 1:6400 and one samples reactive at 1:200 and one sample reactive at 1:400 titer to only DENV. Among the 72 samples, 59 were confirmed by the NY-MIA and the UTEP-PRNT as being positive for antibodies to either WNV and/or DENV, thus supporting a very high frequency of cross reactivity among the ELISA IgG antibody elicited by viruses of the Flavivirus genus (Besselaar et al. 1989). However, of the 59 samples confirmed for WNV and/or DENV antibody, the ELISA based on a 4 – fold difference in titer predicted 35 samples to be positive for only WNV antibody and 5 to be positive for only DENV antibody. Also, 2 samples that were confirmed positive for both WNV/DENV antibody had ELISA titers 4-fold higher to WNV for an overall 71% (42/59) ELISA predictive rate of virus type specific antibody. The other 17 samples that were

confirmed for either WNV or DENV antibody but did not have a 4 – fold difference in titer by the ELISA included 9 WNV confirmed antibody positive samples that had equal ELISA titers to WNV and DENV. The other 8 samples included one WNV/DENV confirmed positive sample that had an equal ELISA titer to WNV/DENV; one WNV confirmed positive sample that had an ELISA titer 2-fold higher for WNV; 3 flavivirus envelope confirmed antibody positive, one with an equal WNV/DENV ELISA antibody titers; one with a 1:400 ELISA antibody titer only to DENV, and one had a 1:200 titer only to DENV. Of the other 3 confirmed samples for only DENV the ELISA IgG antibody titers were equal for both DENV and WNV.

The results for the NY –MIA and the UTEP – PRNT were evaluated by selecting and testing 36 samples by the NY – PRNT90. The results for all but 2 samples by the NY- PRNT were in concordance with the results of the NY – MIA and the UTEP-PRNT. One of the samples was positive for WNV/DENV by the NY- PRNT, and positive only for WNV by the NY- MIA and UTEP - PRNT. The other sample was positive for WNV antibody only by the NY-PRNT, but positive for both WNV/DENV antibody by the UTEP- PRNT and positive for only WNV antibody by the NY- MIA. Overall, the diagnostic results, obtained by the 3 different tests together provided conclusive evidence of the estimates of the frequency of maternal transfer of DENV and WNV specific antibodies among child-bearing mothers in the El Paso community.

In conclusion, our serosurvey was the first to be conducted in the El Paso community for serological evidence of DENV infection. Although the survey showed that 72 of 1,472 human plasma samples were reactive for DENV, the more specific serological tests demonstrated that only 11 samples were positive for DENV antibody and 48 were positive for WNV antibody. While the findings were based on the detection of antibody in cord-blood samples, the seroprevalence

rate for WNV was consistent with the rates reported from other WNV enzootic areas and regions. However, the very low seroprevalence rate for DENV was inconsistent with much higher rates reported for DENV in endemic countries, and therefore suggested that this virus was not likely to be endemic in the El Paso community. However, the reported endemic transmission of DENV in the adjoining sister urban community of Ciudad Juarez, MX (12) and the possibility that the antibody rate using cord-blood samples was not representative of the population warrant further studies to obtain more conclusive understanding of whether DENV is endemic in the El Paso community.

V. Tables

Table 1. Seroprevalence rate for DENV and WNV antibody in 59 cord blood samples from mothers at the time of delivery of newborns in selected El Paso, Texas hospitals.

Antibodies	Providence Memorial Hospital	Sierra Medical Center Main Hospital	Sierra Medical East Hospital	Seroprevalence Rate
West Nile	**10 (1.8 %)**	**7 (4.4 %)**	**28 (3.7 %)**	**45 (3.0%)***
Dengue	**0**	**2 (1.3%)**	**6 (0.8 %)**	**8 (0.5 %)****
Dengue/West Nile	**2 (0.4 %)**	**0**	**1 (0.1 %)**	**3 (0.2 %)**
Flavivirus Envelope only	**0**	**2 (1.3%)*****	**1 (0 .1%)*****	**3 (0.2%)**
Total	**2.1% (12/562)**	**7.0% (11/158)**	**4.8% (36/752)**	**4.0% (59/1,472)**

*- three additional samples positive for antibody to both DENV and WNV were added to the 45 WNV antibody positive mothers to equal 48 or 3.3% of the total samples tested

**- three additional samples positive for antibody to both DENV and WNV were added to the 8 DENV antibody positive mothers to equal 11 or 0.75% of the total samples tested.

***-seven samples were positive for antibody to flavivirus by the MIA, 4 of which were positive for WNV antibody and 3 were negative by the PRNT for both WNV and DENV antibody.

Table 2. Summary of West Nile and dengue virus antibody detected by enzyme-linked immunoassay (ELISA), New York multiplex microsphere assay (NY - MIA) and the University of Texas at El Paso plaque reduction neutralization test (UTEP - PRNT) in 12 plasma samples obtained from mothers at the time of delivery of newborns in the Providence Hospital, El Paso, Texas.

			Median Fluorescence Intensity Values of samples reactive with West Nile virus envelope and/or West Nile and dengue virus nonstructural antigens								UTEP PRNT$_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	NY - MIA	Antibody Titers		PRNT$_{80}$
Sample Code	**DENV**	**WNV**	**232***	**212**	**6680**	**808**	**746**	**615**	**405**	**Diagnosis antibody**	**DENV**	**WNV**	**Diagnosis antibody**
PMH0004	400	3200	1399	596	2193	251	171	107.5	367	WNV	$\leq$20	80	WNV
PMH0012	1600	6400	6484	5240	1984	119	197.5	936	881	WNV	$\leq$20	160	WNV
PMH0037	400	1600	1004	171	459	14	10	41.5	17	Flavivirus Envelope	$\leq$20	320	WNV
PMH0118	6400	6400	7965	8392	2429	1529	1116	4684	10051	WNV/DENV	DENV 2 (320)	320	WNV/DENV-2
PMH0149	1600	6400	5942	2020	2963	38	83	1243	217	WNV / DENV	DENV 2 (640)	640	WNV/DENV-2
PMH0167	1600	6400	5523	1048	1300	2498	2630	8207	2944	WNV/DENV	$\leq$20	1280	WNV
PMH0171	1600	6400	2999	913	1124	23	28	86	170	WNV	$\leq$20	1280	WNV
PMH0249	1600	6400	3085	1037	1113	208	334	253	200	WNV	DENV2 (160)	1280	WNV
PMH0278	1600	6400	3122	462	1082	26	20	136	71	WNV	$\leq$20	1280	WNV
PMH0279	400	6400	2733	1484	2825	32	52	169	140	WNV	$\leq$20	1280	WNV
PMH0288	1600	6400	2549	863	608	19	23	349	165	WNV	DENV 1 (80)	320	WNV
PMH0411	400	1600	1026	367	1643	205	110	69	61.5	WNV	$\leq$20	320	WNV

*- Median fluorescence intensity cut-off values; sample intensity values above cutoff value were recorded as antibody positive and below were antibody negative; values positive for WNV envelope (E) protein only were recorded as antibody to flavivirus envelope, values positive for WNV-E and to one or more WNV non-structural proteins were recorded as positive for WNV antibody, and values positive for WNV-E and to one or more DENV non-structural proteins were recorded as positive for DENV antibody, and samples that were reactive for WNV envelope and for one or more WNV non-structural proteins and one or more DENV non-structural proteins were considered positive for antibody to both WNV/DENV.

ELISA cut-off values for DENV IgG antibody = 0.24-0.29, WNV IgG antibody cut off = 0.11 - 0.16, samples positive for both DENV and WNV antibodies with the same antibody titers or less than 4-fold difference = antibody positive to both viruses, virus with 4-fold or greater antibody titer considered antibody positive for the virus with the highest antibody titer.

Table 3. Summary of West Nile and dengue virus antibody detected by enzyme-linked immunoassay (ELISA), New York multiplex microsphere assay (NY - MIA) and the University of Texas at El Paso plaque reduction neutralization test (UTEP - PRNT) in 36 plasma samples obtained from mothers at the time of delivery of newborns in the Sierra East Hospital, El Paso, Texas.

			Median Fluorescence Intensity Values of samples reactive with West Nile virus envelope and/or West Nile and dengue virus nonstructural antigens								UTEP $PRNT_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	NY - MIA	Antibody Titers		UTEP $PRNT_{80}$
Sample Code	DEN	WN	232	212	6680	808	746	615	405	Diagnosis antibody	DENV	WNV	Diagnosis antibody
SES0002	400	1600	866	338.5	923	28	42	77	29	WNV	≤20	40	WNV
SES0031	800	6400	2409	985	1659	20	20	208	144	WNV	≤20	320	WNV
SES0032	400	3200	1082	1176	1278	12	12	70	195	WNV	≤20	40	WNV
SES0045	800	3200	2487	841	1438	28	20	96	225	WNV	≤20	160	WNV
SES0059	200	6400	1401	563	640	81	269	71	58	WNV	≤20	640	WNV
SES0068	6400	1600	3346	109	305	7553	3894	11714	20151	DENV	DENV1 (160), DENV2 (640), DENV3 & 4 (40)	160	DENV 2
SES0072	800	6400	3355	1303	1448	27	37	174	93	WNV	≤20	NT	WNV
SES0073A	3200	6400	6501	2570	706	43	178	303	391	WNV	≤20	40	WNV
SES0111	6400	6400	3483	168	1139	4563	1447	5767	2489	DENV	DENV1 (1280), DENV2 & 3 (40)	160	DENV 1
SES0146	400	≤100	737	262	2862	488	382	860	345	Flavivirus Envelope	≤20	≤20	NEG
SES0206	6400	6400	6289	1856	2219	67	60	412	514	WNV	≤20	80	WNV
SES0242	6400	1600	2420	81	461	8446	1331	6304	6354	DENV	DEN1 (640), DEN2 & 3 (160)	80	DENV1
SES0248	6400	800	2675	82	1355	2332	1775	5808	1501	DENV	DEN1 (160), DEN2 (640), DEN3 (160),)	40	DENV2
SES0251	400	3200	3325	421	581	29	37	179	38	WNV	≤20	160	WNV
SES0297	400	1600	NT	NT	NT	NT	NT	NT	NT	WNV	≤20	640	WNV
SES0412	1600	6400	3687	995	563	12	22	112	36	WNV	≤20	160	WNV
SES0426	6400	6400	3194	1390	2808	41	42	185	148	WNV	DEN2 (160)	1280	WNV
SES0447	6400	6400	4051	62	781	6981	488	1829	10645	DENV	DEN1 (160), DEN2 (320	≤20	DENV1 & 2
SES0450	400	6400	364	81	629	19	29	20	19	Flavivirus Envelope	≤20	1280	WNV

*- Median fluorescence intensity cut-off values for test samples equal to or higher representing antigen – antibody reactivity, ELISA cut-off values DENV IgG antibody cut off = 0.24-0.29, WNV IgG antibody cut off= 0.11-0.16, samples positive for both DENV and WNV antibodies with the same antibody titers or less than 4-fold difference = antibody positive to both viruses, virus with 4-fold or greater antibody titer considered antibody positive for the virus with the highest antibody titer.

Table 3. (continued) Summary of West Nile and dengue virus antibody detected by enzyme-linked immunoassay (ELISA), New York multiplex microsphere assay (NY - MIA) and the University of Texas at El Paso plaque reduction neutralization test (UTEP - PRNT) in 36 plasma samples obtained from mothers at the time of delivery of newborns in the Sierra East Hospital, El Paso, Texas.

			Median Fluorescence Intensity Values of samples reactive with West Nile virus envelope and/or with West Nile and dengue virus (DEN) nonstructural antigens								UTEP $PRNT_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	NY - MIA	Antibody Titers		UTEP $PRNT_{80}$
Sample Code	DEN	WN	232	212	6680	808	746	615	405	Diagnosis antibody	DENV	WNV	Diagnosis antibody
SES0475	6400	6400	3081	756	576	22	139	73	51	WNV	DENV2 (160)	1280	WNV
SES0477	1600	6400	1791	1691	889	12	12	50	260	WNV	≤20	160	WNV
SES0496	6400	6400	4778	2192	1190	29	31	391	179	WNV	≤20	640	WNV
SES0500	6400	400	522	54	636	406	839	2809	124	DENV	DENV2 (320)	≤20	DENV2
SES0625	6400	6400	1703	313	412	31	56	115	31	WNV	DENV4 (40)	320	WNV
SES0629	6400	6400	2616	1284	1257	59	54	175	296	WNV	DENV4 (160)	1280	WNV
SES0632	400	6400	620	179	504	19	15	62	31	Flavivirus Envelope	≤20	1280	WNV
SES0635	1600	6400	2256	859	2996	15	122	48	97	WNV	≤20	320	WNV
SES0647	1600	6400	3249	1532	3716	29	33	123	188	WNV	DENV4 (80}	320	WNV
SES0654	1600	6400	2343	249	1053	46	53	55	46	WNV	DENV3 (160), DENV 4 (640)	320	WNV/DENV
SES0658	1600	6400	1996	386	1424	30	24	75	43	WNV	DENV3 (160)	1280	WNV
SES0664	6400	6400	2794	1072	583	36	31	106	76	WNV	≤20	1280	WNV
SES0669	400	6400	2343	709	1027	22	25	200	76	WNV	DENV4 (40)	160	WNV
SES0670	400	6400	1314	490	2042	14	15	88	85	WNV	DENV4 (40)	640	WNV
SES0675	400	6400	4496	953	1544	27	28	176	85	WNV	≤20	160	WNV
SES0827	1600	1600	273	53	658	273	113	461	78	Flavivirus Envelope	≤20	320	WNV
SES0836	6400	6400	3649	2515	1510	125	85	177	680	WNV	≤20	320	WNV

*- Median fluorescence intensity cut-off values for test samples equal to or higher representing antigen – antibody reactivity, ELISA cut-off values DENV IgG cut off = 0.24-0.29, WNV IgG cut off=0.11-0.16, samples positive for both DENV and WNV antibodies with the same antibody titers or less than 4-fold difference = antibody positive to both viruses, virus with 4-fold or greater antibody titer considered antibody positive for the virus with the highest antibody titer.

Table 4. Summary of West Nile and dengue virus antibody detected by enzyme-linked immunoassay (ELISA) , New York multiplex microsphere assay (NY - MIA) and the University of Texas at El Paso plaque reduction neutralization test (UTEP - PRNT) in 11 plasma samples obtained from mothers at the time of delivery of newborns in the Sierra Main Hospital, El Paso, Texas.

			Median Fluorescence Intensity Values of samples reactive with West Nile virus envelope and/or with West Nile and dengue virus nonstructural antigens								UTEP $PRNT_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	NY - MIA	Antibody Titers		UTEP $PRNT_{80}$
Sample Code	DEN	WN	232	212	6680	808	746	615	405	Diagnosis antibody	DENV	WNV	Diagnosis antibody
SMC0006	400	6400	NT	NT	NT	NT	NT	NT	NT	WNV	$\leq$20	160	WNV
SMC0008	1600	6400	4019	1105	1194	17	215	74	93	WNV	$\leq$20	320	WNV
SMC0013	800	6400	964	335	235	19	20	44	26	WNV	$\leq$20	320	WNV
SMC0040	1600	6400	2914	1378	2264	39	44	254	333	WNV	$\leq$20)	80	WNV
SMC0053	1600	6400	1104	589	944	30	33	62	39	WNV	$\leq$20	1280	WNV
SMC0055	1600	1600	407	50	439	12	10	107	15	Flavivirus Envelope	$\leq$20	$\leq$20	Negative (NEG)
SMC0067	6400	1600	2517	70	773	910	1149	4124	2195	DENV	DENV2 (2560)	1280	DENV2
SMC0068	400	1600	2894	754	2055	203	177	147	91	WNV	$\leq$20	1280	WNV
SMC0070	400	1600	2343	593	885	46	238	229	377	WNV	$\leq$20	1280	WNV
SMC0114	6400	6400	5049	76	1221	11128	2276	8388	4198	DENV	DENV1 (2560), DENV2 (320)	320	DENV1
SMC0119	200	$\leq$100	851	33	331	569	149	310	71	Flavivirus Envelope	$\leq$20	$\leq$20	Neg

*- Median fluorescence intensity cut-off values for test samples equal to or higher representing antigen – antibody reactivity, ELISA cut-off values DENV IgG cut off = 0.24-0.29, WNV IgG cut off=0.11-0.16, samples positive for both DENV and WNV antibodies with the same antibody titers or less than 4-fold difference = antibody positive to both viruses, virus with 4-fold or greater antibody titer considered antibody positive for the virus with the highest antibody titer

Table 5. Summary of West Nile and dengue virus neutralizing antibody results between the New York multiplex microsphere assay (NY - MIA) and the University of Texas at El Paso plaque reduction neutralization test (UTEP - PRNT) for 9 of 59 cord-blood plasma samples obtained from mothers at the Providence, Sierra East, Sierra Main hospitals, El Paso, Texas

			Median Fluorescence Intensity Values of samples reactive with West Nile virus envelope and/or West Nile and dengue virus (DEN) nonstructural antigens								UTEP $PRNT_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	NY - MIA	Antibody Titers		$PRNT_{80}$
Sample Code	DENV	WNV	232*	212	6680	808	746	615	405	Diagnosis antibody	DENV	WNV	Diagnosis antibody
PMH0037	400	1600	1004	171	459	14	10	41.5	17	Flavivirus Envelope	≤20	320	WNV
PMH0167	1600	6400	5523	1048	1300	2498	2630	8207	2944	WNV/DENV	≤20	40	WNV
SES0146	400	100	737	262	2862	488	382	860	345	Flavivirus Envelope	≤20	≤20	Neg
SES0450	400	6400	364	81	629	19	29	20	19	Flavivirus Envelope	≤20	1280	WNV
SES0632	400	6400	620	179	504	19	15	62	31	Flavivirus Envelope	≤20	1280	WNV
SES0654	1600	6400	2343	249	1053	46	53	55	46	WNV	DENV3 (160), DENV4 (640)	WNV (320)	WNV/DENV
SES0827	1600	1600	273	53	658	273	113	461	78	Flavivirus Envelope	≤20	320	WNV
SMC0055	1600	1600	407	30	430	12	10	107	15	Flavivirus Envelope	≤20	≤20	Neg
SMC0119	200	≤100	851	33	331	564	149	310	71	Flavivirus Envelope	≤20	≤20	Neg

*- Median fluorescence intensity cut-off values for test samples equal to or higher representing antigen – antibody reactivity, ELISA cut-off values DENV IgG cut off = 0.24-0.29, WNV IgG cut off=0.11-0.16, samples positive for both DENV and WNV antibodies with the same antibody titers or less than 4-fold difference = antibody positive to both viruses, virus with 4-fold or greater antibody titer considered antibody positive for the virus with the highest antibody titer

Table 6. Summary of 13 plasma samples obtained from mothers at the Providence, Sierra East, Sierra Main hospitals, El Paso, Texas that were positive for West Nile and dengue virus antibody by the ELISA, but negative by the multiple microsphere assay (MIA) and the UTEP plaque reduction neutralization test (PRNT).

			Median Fluorescence Intensity Values of samples reactive with West Nile virus envelope and to West Nile and dengue virus nonstructural antigens								UTEP $PRNT_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	MIA	Antibody Titers		UTEP $PRNT_{80}$
Sample Code	**DEN**	**WN**	**232**	**212**	**6680**	**808**	**746**	**615**	**405**	**Diagnosis antibody**	**DENV**	**WNV**	**Diagnosis antibody**
PMH0034	400	≤100	194	199	790	115	93	461	162	Neg	≤20	≤20	Neg
PMH0105	400	≤100	18	51	424.5	11	12	61	17	Neg	≤20	≤20	Neg
PMH0124	400	400	300	56	1293	90	150	439	26	Neg	≤20	≤20	Neg
SES0061	100	100	4	34	249	8	6	22	13	**Neg**	≤20	≤20	**Neg**
SES0204	400	≤100	11	55	559	25	181	35	15	**Neg**	≤20	≤20	**Neg**
SES0214	400	≤100	11	69	1158	11	18	38	19	**Neg**	≤20	≤20	**Neg**
SES0247	200	≤100	16	49	399	28	16	47	16	Neg	≤20	<20	Neg
SES0279	400	100	76	71	561	39	24	180	51	Neg	≤20	≤20	Neg
SES0282	400	≤100	17	56	1148	21	14	49	26	Neg	≤20	≤20	Neg
SES0284	400	≤100	8	32	292	6	6	48	12	Neg	≤20	≤20	Neg
SES0451	400	100	295	91	921	52	36	227	68	Neg	≤20	≤20	Neg
SES0804	400	≤100	141	84	939	251	66	228	219	Neg	≤20	≤20	Neg
SMC0017	400	100	10	46	1526	33	27	29	17	Neg	≤20	≤20	Neg

*- Median fluorescence intensity cut-off values for test samples equal to or higher representing antigen – antibody reactivity, ELISA cut-off values DENV IgG cut off = 0.24-0.29, WNV IgG cut off=0.11-0.16, samples positive for both DENV and WNV antibodies with the same antibody titers or less than 4-fold difference = antibody positive to both viruses, virus with 4-fold or greater antibody titer considered antibody positive for the virus with the highest antibody titer**.**

Table 7. Summary of DENV and WNV antibody results for 36 cord blood samples obtained from mothers at the time of delivery of newborns in the Providence Memorial, Sierra East, and Sierra Main hospitals, El Paso, Texas. Samples were assayed by the New York plaque reduction neutralization test (NY – PRNT) and had previously been tested by the New York multiplex microsphere assay (NY-MIA) and the University of Texas at El Paso plaque reduction neutralization test UTEP – PRNT, including 13 WNV and 7 DENV antibody positive and 16 antibody negative samples.

	NY- PRNT$_{90}$					UTEP PRNT$_{80}$	NY- MIA
Sample Code	**DENV-2 antibody screen**	**DENV-2 antibody titer**	**WNV antibody screen**	**WNV antibody titer**	**Diagnosis antibody**	**Diagnosis antibody**	**Diagnosis antibody**
PMH0014	Neg	<20	NEG	<20	Neg	Neg	Neg
PMH0034	Neg	<20	NEG	<20	Neg	Neg	Neg
PMH0105	Neg	<20	NEG	<20	Neg	Neg	Neg
PMH0124	Neg	<20	NEG	<20	Neg	Neg	Neg
PMH0149	Positive (Pos)	20	POS	1280	WNV/DENV	WNV/DENV 2	WNV/DENV
PMH0247	NEG	<20	NEG	<20	Neg	Neg	Neg
PMH0279	Neg	<20	POS	320	WNV	WNV	WNV
SES0031	Neg	<20	POS	80	WNV	WNV	WNV
SES0045	Neg	<20	POS	320	WNV	WNV	WNV
SES0059	Neg	<20	POS	80	WNV	WNV	WNV
SES0068	Pos	160	Neg	<20	DENV	DENV-2	DENV
SES0073A	Neg	<20	POS	640	WNV	WNV	WNV
SES0214	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0228	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0248	Pos	160	Neg	<20	DENV	DENV 2	DENV
SES0252	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0264	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0286	Neg	<20	Neg	<20	Neg	Neg	Neg

Table 7. (Continued) Summary of DENV and WNV antibody results for 36 cord blood samples obtained from mothers at the time of delivery of newborns in the Providence Memorial, Sierra East, and Sierra Main hospitals, El Paso, Texas. Samples were assayed by the New York plaque reduction neutralization test (NY – PRNT) and had previously been tested by the New York multiplex microsphere assay (NY-MIA) and the University of Texas at El Paso plaque reduction neutralization test UTEP – PRNT, including 13 WNV and 7 DENV antibody positive and 16 antibody negative samples.

SES0288	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0402	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0405	Neg	<20	Neg	<20	Neg	Neg	Neg
SES0447	Pos	80	Neg	<20	DENV	DENV1 & 2	DENV
SES0475	Pos	20	Pos	320	WNV/DENV	WNV	WNV
SES0500	Pos	80	Neg	<20	DENV	DENV-2	DENV
SES0654	Neg	<20	Pos	320	WNV	WNV/DENV	WNV
SES0664	Neg	<20	Pos	320	WNV	WNV	WNV
SES0669	Neg	<20	Pos	80	WNV	WNV	WNV
SES0670	Neg	<20	Pos	160	WNV	WNV	WNV
SMC0008	Neg	<20	Pos	320	WNV	WNV	WNV
SMC0040	Neg	<20	Pos	640	WNV	WNV	WNV
SMC0041	Neg	<20	Neg	<20	Neg	Neg	Neg
SMC0067	Pos	80	Neg	<20	DENV	DENV-2	DENV
SMC0068	Neg	<20	Pos	640	WNV	WNV	WNV
SMC0070	Neg	<20	Pos	80	WNV	WNV	WNV
SMC0114	Pos	320	Neg	<20	DENV	DENV-1	DENV
SMC0451	Neg	<20	Neg	<20	Neg	Neg	Neg

Table 8. Summary of DENV antibody positive samples detected by enzyme-linked immunoassay, New York multiplex microsphere assay and the University of Texas at El Paso plaque reduction neutralization test in plasma samples in mothers enrolled at the Providence Memorial, Sierra East Medical and Sierra Main Medical Center hospitals in El Paso, Texas.

			Median Fluorescence Intensity Values of samples reactive with West Nile virus (WNV) and dengue virus (DENV) nonstructural antigens								UTEP $PRNT_{80}$ Titers		
	ELISA IgG Antibodies		WNV-E	WNV-NS1	WNV-NS5	Den 1 NS1	Den 2 NS1	Den 3 NS1	Den 4 NS1	NY – MIA	Antibody Titers		UTEP $PRNT_{80}$
Sample Code	DEN	WN	232	212	6680	808	746	615	405	**Diagnosis antibody**	**DENV**	**WNV**	**Diagnosis antibody**
SES0068	6400	1600	3346	109	305	7553	3894	11714	20151	DENV	DENV1 (160), DENV2 (640), DENV3 & 4 (40)	160	DENV 2
SES0111	6400	6400	3483	168	1139	4563	1447	5767	2489	DENV	DENV1(1280) DENV2 & 3 (40)	160	DENV1
SES0242	6400	1600	2420	81	461	8446	1331	6304	6354	DENV	DEN1 (640), DEN2 & 3 (160)	80	DENV1
SES0248	6400	800	2675	82	1355	2332	1775	5808	1501	DENV	DEN1 (160), DEN2 (640), DEN3 (160),)	40	DENV2
SES0447	6400	6400	4051	62	781	6981	488	1829	10645	DENV	DEN1 (160), DEN2 (320	$\leq$20	DENV1 & 2
SES0500	6400	400	522	54	636	406	839	2809	124	DENV	DENV2 (320)	$\leq$20	DENV2
SMC0067	6400	1600	2517	70	773	910	1149	4124	2195	DENV	DENV2 (2560)	1280	DENV2
SMC0114	6400	6400	5049	76	1221	11128	2276	8388	4198	DENV	DENV1 (2560), DENV2 (320)	320	DENV1
PMH0118	6400	6400	7965	8392	2429	1529	1116	4684	10051	WNV/DEN V	DENV 2 (320)	320	WNV/DEN V2
PMH0149	1600	6400	5942	2020	2963	38	83	1243	217	WNV / DENV	DENV 2 (640)	640	WNV/DEN V2
SES0654	1600	6400	2343	249	1053	46	53	55	46	WNV	DENV3 (160), DENV 4 (640)	320	WNV/DEN V

*- Median fluorescence intensity cut-off values for test samples equal to or higher representing antigen – antibody reactivity, ELISA cut-off values DENV IgG cut off = 0.24-0.29, WNV IgG cut off=0.11-0.16, samples positive for both DENV and WNV antibodies with different titers, virus with 4-fold or greater antibody titer considered diagnostic virus antibody,

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VI. Vita

Veronica Suarez was born in El Paso, TX and reside in Ciudad Juarez, MX; she attended high school at Father Yermo Catholic School where she would attend UTEP for her Undergraduate Degree in Biology.

Veronica would be placed on the College of Science Dean's List Plus for all six semesters of her degree, and join Women United for Juarez Association, Eucharistic Minister at Tepeyac Institute, National Honor Society (NHS), Medical Professional Organization (MPO,) Catholic Daughters of the Americas (CDA), The National Society of Collegiate Scholars, Honors House Program, Society of Women Engineers (SWE), Candle Lighters, and PHPP/A-PRIME TIME Organization.

While studying towards her degree, Veronica worked with Charlotte Vines, Ph.D. studying the g-protein coupled receptor CCR7 and its effects in breast cancer, and Douglas Watts, Ph.D. studying the ecological parameters of endemic mosquito populations in the El Paso community and understanding transmission of arboviral diseases from several vectors such as *Aedes aegypti* and *Culex* mosquitoes. After graduating with a B.S. in Biology, Veronica joined the UTEP Graduate School to achieve a M.S. in Biology under Douglas Watts, Ph.D. to further study Dengue Fever, a vector-borne arboviral illness, and it is possible presence in the El Paso community.

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