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Identification of Arbovirus Surveillance Sites At Maritime And Air Ports to Reduce the Risk of Export of Eastern Equine Encephalitis and Import of Zika Viruses into Coastal Georgia.

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Abstract: *Eastern Equine Encephalitis virus (EEEV) is an arbovirus which, although rare, causes significant mortality and morbidity to humans and horses. Zika virus (ZIKAV) is an emerging infectious disease that is spreading rapidly across the globe. Southeast Georgia, USA has temperature and habitat suitable for survival of several invasive mosquito species. Maritime and air ports are the most important sources of invasion for vector species. Aedes albopictus is the most successful invasive mosquito species and may serve as a bridge vector of EEEV and vector of ZIKAV. The Bioagent Transport and Environmental Modeling System was used to identify areas at high risk to EEEV. BioTEMS was validated against EEEV records in Chatham County, GA and was 100% accurate to within 100 m, 74% to within 30 m and 50% within 5 m. During the writing of this manuscript, three horses in three other counties tested positive for EEEV infection. The BioTEMS recommended surveillance sites were located within 5, 9 and 63 m from the locality of the euthanized horses. This study identifies optimal placement of surveillance and control sites in the two maritime ports located in Georgia and also airports in Chatham and Glynn counties in order to mitigate the import and export of mosquitoes, and if utilized may reduce the risk of export or import of EEEV and ZIKAV, respectively.*

I. Introduction

Eastern equine encephalitis virus (EEEV) poses a serious risk to humans, domestic and wild mammals, and some avian species originating from non-endemic areas. The mortality rate in humans ranges from 33 to 75% (Petersen and Gubler, 2003), and in horses the mortality is even higher (Mohler, 1940). Although it was not identified as a distinct disease until 1933, EEEV has most likely caused disease in North America for over 150 years (Beadle, 1952). Eastern equine encephalitis virus is primarily found in the western hemisphere from eastern Canada, along the Atlantic coast of North America, the Caribbean, and Central and South America (Zacks and Paessler, 2010); it has also been found inland in the U.S. (Mukherjee et al., 2012). However, a documented case of imported EEE into Europe has been reported (Harvala et al. 2009). In addition to the natural occurrence of EEEV, there is concern of its use as a biological weapon (BW) due to its high infectivity, ease of production, stability, potential for aerosolization, and consistent induction of debilitating disease (Steele and Twenhafel, 2010). Avian species are the primary reservoir hosts, however there is evidence reptiles may serve as overwintering hosts (White et al. 2011). The mosquito, *Culiseta melanura*, is the principal vector of EEEV but several other mosquitoes are capable of serving as bridge vectors (Armstrong and Andreadis, 2010; Lord, 2010). Other arthropods might possibly serve as vectors, e.g. mites and biting midges (Durden et al., 1993; Capinera, 2011).

The principle factor responsible for the invasion of disease vectors is through air and ship transport (Tatem et al. 2006). In the laboratory, *Aedes albopictus* was an efficient vector of EEEV (Turrel et al. 1994, Serdelis 2002); this species is also a vector of ZIKAV. The transmission efficiency of ZIKAV varies among populations of *Ae. aegypti* and *Ae. albopictus*, ranging from10-60 percent (Luca et al. 2016, Chouin-Carneiro et al. 2016). *Aedes albopictus* is found throughout Georgia and could play an important role of exporting EEEV from or importing ZIKAV through port areas. Chatham and Glynn Counties, Georgia, USA have both maritime and air ports. The maritime port of Savannah, located in Chatham County, is the largest single container port in North America with the largest number of import distribution centers on the east coast (Georgia Ports Authority, 2016). The maritime port of Brunswick, located in Glynn County, is the busiest seaport for cars in the United States (Georgia Ports Authority, 2016). Savannah-Hilton Head International Airport, Hunter Army Airfield, both international airports, and a private/regional airfield are located in Chatham County. There are three private/regional airports in Glynn County. These facilities are all located within the humid sub-tropical climate zone (Peel et al. 2007) which offers suitable climate and habitat for invasive species (Kollars et al. 2016). Because of the volume of international trade in the maritime ports and volume of air traffic, these ports pose significant risk to both the import and export of invasive mosquito species and the pathogens they transmit. Because of this risk, optimal surveillance sites were identified using the Bioagent Transport and Environmental Modeling System (BioTEMS).

II. Methods and Results

BioTEMS analyzes abiotic and biotic factors to produce risk and vulnerability assessments for biological agents and infectious diseases. BioTEMS has been used to assist in the design of infectious disease and vector surveillance systems for consequence management and surveillance in the U.S. and internationally for military/government facilities, presidential/national conventions and for public health in several countries, including Libya, Italy, Honduras, Sierra Leone and the United Arab Emirates (Kollars, 2015; Kollars et al. 2016). BioTEMS has also be used with the Hazard Prediction and Assessment Capability (HPAC) and Biological Integrated Detections System (BIDS). In this study the BioTEMS model was used to optimize surveillance placement for EEEV and ZIKAV at maritime and air ports in Chatham and Glynn Counties, Georgia, USA. Output was validated by using historical data from positive EEEV mosquito pools and sentinel chickens, where more than one year tested positive. BioTEMS accurately predicted all positive sites to within 100 m, 65% falling within 30 m, and 50% of sites within 5 m, with a mean of 23. 4 m. The areas identified at highest risk for EEEV represent 0.01% of the total area in Chatham and Glynn counties. ArcGIS and Google Earth Pro were used to analyze and visualize data. Several sites in both counties were identified for surveillance and vector control to reduce the risk export of EEEV and possible import of ZIKAV (Figures 1, 2).

III. Discussion

The primary habitat for the maintenance cycle of EEEV is freshwater hardwood swamps, where *Cs. melanura* feeds upon and infects reservoir avian hosts (Dalrymple et al. 1972). *Culiseta melanura* occasionally feeds upon mammals and possibly humans (Molaei et al. 2015). Transmission of EEEV to humans and other mammals likely requires a bridge vector such as *Aedes*, *Coquillettidia* or *Culex* species. *Aedes sollicitans* were able to transmit EEE to chicks. Crans et al. 1986, Cupp et al. 2003). Other mosquito species such as *Ae. vexans, Cq. perturbans*, and *Oc. canadensis* are implicated as epidemic/epizootic bridge vectors from viremic birds to horses and humans. These Culiseta. melanura and several suspected bridge vectors are found in the coastal area of Georgia. In Chatham County, active surveillance of mosquitoes and sentinel chickens is conducted (Lewandowski and Moulis, 2008), however no other counties in Georgia conduct sentinel surveillance. Inadequate and improperly placed arbovirus surveillance sites may put human and domestic animal populations at higher risk and may increase the risk of infected mosquitoes being imported or exported without detection.

As can be seen in Figures 1 and 2, maritime and air ports in Chatham County have a higher number of sites at risk of imported and exported infected mosquitoes than those identified in Glynn County. Several maritime and all airports in Chatham County have areas where EEEV surveillance is recommended. One site at the maritime port in Glynn County was identified for EEEV surveillance. The two airports located on the two islands in Glynn County do not appear to be at risk from EEEV, however the inland airport has several areas where EEEV surveillance is recommended. This is probably good news for the Professional Golfers Association (PGA) and other tourists that frequently visit these islands. However, there may be a risk of ZIKAV on the barrier islands, should local *Ae. aegypti* or *Ae. albopictus* be present and become infected. While mosquitoes are being tested for EEEV at the recommended surveillance sites, simultaneous testing for ZIKAV to detect import of infected mosquitoes can be conducted.

In Georgia, EEEV and reactive antibodies have been found in several species of wild and domestic animals. In 2001, EEE was diagnosed in an adult male white-tailed deer (*Odocoileus virginianus*) from Houston County, GA (Tate et al 2005). In 2015, 6 cases of EEEV were reported in horses in Georgia (APHIS, 2015). However, in 2003 there were 83 cases reported from horses in Georgia and two cases in humans, one fatal from Glynn County. (GA AGR, 2008; Promed, 2003). No human cases of EEEV have been reported in Chatham County, however, horses, *Cs. melanura* and *Cx. erraticus* mosquitoes, and sentinel chickens have tested positive through culture, polymerase chain reaction, or serology (Georgia Department Health, 2013; Moulis, 2014). Laboratory experiments have established *Ae. albopictus* as an efficient vector of EEEV and wild caught specimens have been positive. In addition to EEEV, *Ae. albopictus* is an efficient vector of ZIKAV, which to date, has only been found in humans returning to Georgia.

Aedes albopictus is probably the most successful invasive mosquito species, it is also quite catholic in its feeding and may pose a significant risk of arbovirus transmission whereever it colonizes. Chatham County, was one of the first areas in North America to be invaded by *Ae. albopictus*. Chatham County Mosquito Control has the largest mosquito control and mosquito-borne disease surveillance program in the state of Georgia. To the best of my knowledge, there is no active sentinel chicken or mosquito collection and testing being conducted locally or systematically by the government of Georgia. Unfortunately, mosquito control across the rest of the state is often under-staffed and poorly funded. During the writing of this manuscript, three horses in three other counties tested positive for EEEV infection; all three horses were euthanized. The BioTEMS EEEV model is a useful tool in identifying high risk areas as well as optimizing surveillance for arboviruses. For example, the sites recommended for EEEV surveillance were validated with independent data and were located within 5, 9 and 63 m from the locality of the euthanized horses. The extensive road/interstate system, rail and air distribution of products and travelers entering and exiting the ports in Georgia, pose a risk of export of EEEV and import and ZIKV, particularly in Savannah.

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Figure 1. Recommended surveillance and control sites around maritime and air ports in Chatham County, Georgia, USA (based on BioTEMS).

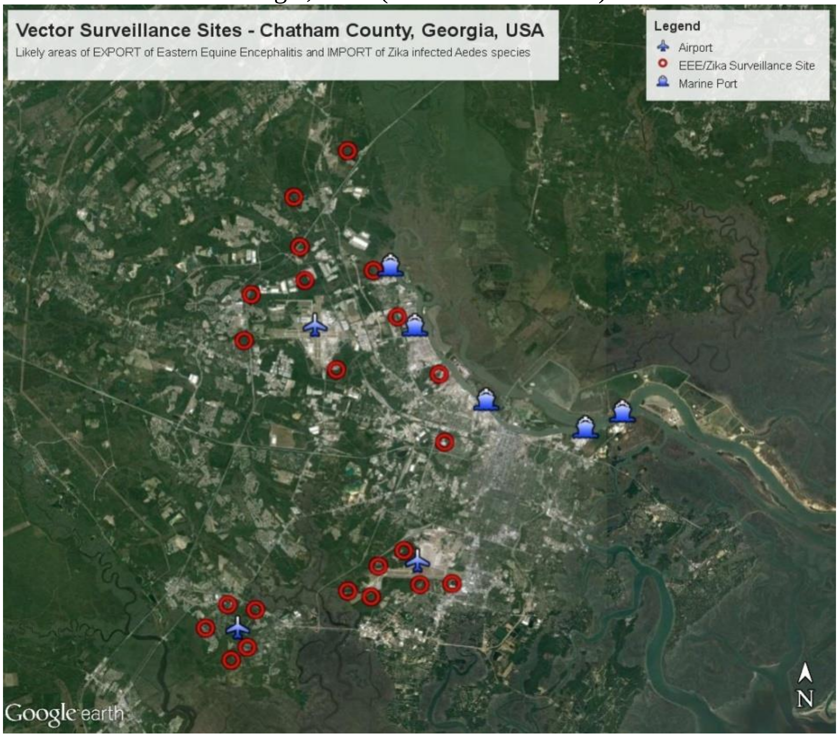

Figure 2. Recommended surveillance and control sites around maritime and air ports in Glynn County, Georgia, USA (based on BioTEMS).