WEST NILE VIRUS CONTROL IN ALLEGHENY COUNTY: DIFFICULTIES IN UTILIZATION OF THE VECTOR INDEX FOR PREDICTION

by

Kathleen Long

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This thesis was presented

by

Kathleen Long

It was defended on

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and approved by

Thesis Director: Lawrence A. Kingsley, DrPH, Professor, Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh

Mackey R. Friedman, PhD, Assistant Professor, Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh

Kristen J. Mertz, MD, PhD, Medical Epidemiologist, Epidemiology Graduate School of Public Health, University of Pittsburgh

Lawrence A. Kingsley, DrPH

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ABSTRACT

West Nile Virus is a mosquito-borne pathogen first discovered in Uganda in 1937. The virus was detected in the new world in 1999 in New York City, and has since spread throughout the continental United States as well as parts of Canada, Mexico, and South America. Because of the high percentage of asymptomatic cases, human surveillance alone is inadequate for West Nile Virus. Therefore, programs in the United States, including one in Allegheny County, Pennsylvania perform mosquito surveillance activities in order to find high-risk areas for human transmission. In this study, the mosquito surveillance program findings are presented for 2015. In addition, the vector index measure is used as a possible quantitative method for comparing West Nile Virus risk between the eastern neighborhoods of Pittsburgh. By aiming to increase understanding of the West Nile Virus risk in Allegheny County in 2015 and testing a quantitative method of risk comparison, this study provides public health relevance. Between May and September 2015, 562 gravid mosquito traps were set in Allegheny County. West Nile Virus was detected in samples from 172 of these traps; the highest percentage of positive traps seen in Allegheny County since the surveillance program began. The vector index measure was calculated each week for a subset of eleven different zones in eastern Pittsburgh, with values ranging from 0-5.8. While this method provided values representing risk, they were not useful for comparison because of the low sample size. If the sample size were large enough to allow the calculation of a vector index value for each zone each week, the method would be more useful.

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PREFACE

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1.0 INTRODUCTION

West Nile Virus is a single-stranded RNA virus of the flavivirus family and is most closely related to Japanese encephalitis virus and St. Louis encephalitis virus [1, 2]. Its mode of transmission is by mosquito, most commonly by species of the *Culex* genus [3]. The reservoir and amplifying hosts are birds, while humans are incidental, dead-end hosts [1]. In temperate regions, transmission most commonly occurs in August and September but in tropical areas can be sustained all year [2]. West Nile Virus was not identified until the 20th century, but since its emergence it has spread virtually around the world and is currently the most widespread arbovirus in the world.

1.1 HISTORY

West Nile Virus was first discovered in the West Nile region of Uganda in 1937 during the study of a yellow fever outbreak [1, 4]. The patient from which the virus sample was taken displayed only fever, however preliminary laboratory experiments on the new virus showed that central nervous system involvement was possible [4]. It was not until outbreaks in areas near the Mediterranean during the 1950s that scientists were first able to study West Nile Virus infection in-depth in humans [5]. During this time there were several outbreaks in Egypt, as well as one in a small town in Israel. In the outbreak in Israel, a high percentage of residents were infected with the virus, with a disproportionately high infection rate in children. Although a large proportion of inhabitants were infected in these outbreaks, there were no reported deaths, and the symptoms of the illness were relatively mild, including fever, headache, aches, anorexia, abdominal pain, and vomiting [4]. In the mid-1950s a study was done in the upper Nile Delta region of Egypt that indicated that approximately 60% of the population had been infected, and that West Nile Virus was a common self-limiting childhood disease that did not involve neurologic symptoms as the scientists had projected in the preliminary 1937 studies. Therefore, West Nile Virus was not thought of as a serious disease, and little further research or preventative measures were put in place [1]. Importantly, however, during this time a few studies confirmed the mode of transmission to be mosquitoes, primarily of the *Culex* genus. In addition, in this time period it was found that West Nile Virus was not just a disease of humans but could also be detected in other non-human mammals and birds, with an especially high fatality rate in equines [6].

Although the disease continued to be thought of as a mild childhood illness, during subsequent small West Nile Virus outbreaks in the 1960s and 1970s a few patients reportedly displayed neurological symptoms, including meningitis and encephalitis, which had previously only been seen in laboratory animals and patients who had undergone experimental infection. These symptoms were reported in elderly citizens in a town in Israel, as well as in rare cases during small outbreaks in France, South Africa, Russia, Spain, and India. Throughout the 1970s and 1980s, the pattern of small outbreaks causing mild febrile illness and rare cases of neurological involvement continued [2].

In the 1990s, specifically between 1994 and 1996, there was a shift in the epidemiology of West Nile Virus, in which experts believe a new, more virulent sub-type of the virus first emerged [1, 4]. The virus suddenly became more dangerous, causing larger outbreaks with a higher percentage of patients experiencing severe neurological complications [1]. Specifically, there were outbreaks in Bucharest, Romania, as well as Israel, Russia, Morocco, Tunisia, and Italy that involved a higher percentage of patients experiencing neurological symptoms than had been seen in the past. These outbreaks were also different because they were happening more frequently than past outbreaks, and they were taking place in areas that were more urban than previous outbreak locations had been. In addition, it was found that there was an overall lower rate of mild febrile illness than past outbreaks, even though the rate of neurological manifestations was higher than in outbreaks of the past [4].

During this time period, in 1999, West Nile Virus was first discovered in the new world, specifically in the borough of Queens in New York City. In the summer of 1999, a cluster of eight encephalitis cases occurred, with a simultaneous increase in the number of dead birds being found in the city, although early in the outbreak it was not suspected that these patterns were connected [4]. Within one month of the first report of the outbreak, West Nile Virus had been identified as the cause, and by the end of the summer a total of 62 human cases were confirmed [4]. In a post-outbreak study, it was estimated that there were also 110 asymptomatic and 30 mild febrile cases for each of the 62 severe confirmed cases [3]. Unfortunately, this outbreak appeared to involve the virus sub-type that was responsible for the more severe outbreaks from the earlier 1990s, involving a high rate of neurological symptoms especially in older patients [4].

West Nile Virus continued to spread in North America, reaching the west coast of the United States in 2002 [1]. At the time, the 2002 outbreak was the largest West Nile Virus outbreak ever recorded by far, with 4,156 confirmed cases and 284 deaths [4]. By 2003, the virus had been detected in almost every state in the continental United States [5] and by 2005, there was sustained transmission in virtually the entire range between central Canada and Argentina, earning West Nile Virus the title of most widely distributed arbovirus in the world [7]. Once the virus finished its rapid expansion over this range, the yearly outbreaks began to fluctuate, which is a pattern that is still seen today [5]. From 2008 to 2011 the seasonal outbreaks were mild, leading some to believe the danger of West Nile Virus was coming to an end. However, the 2012 season was very severe, with infection rates and deaths back up to where they were during the 2003 outbreak [5, 8]. As of 2013, 96% of all counties in the United States have reported evidence of West Nile Virus transmission [7].

1.2 IMPORTANT SPECIES FOR TRANSMISSION

As of 2012, the virus has been detected in over 65 species of mosquitoes in the United States, however it is thought that only three species are responsible for the bulk of the transmission, including *Culex pipiens* in the north, *Culex quinquefasciatus* in the west, and *Culex tarsalis* in the south [7]. While not as important to sustained transmission as the three species listed above, other significant species that are able to transmit West Nile Virus are *Culex salinarious*, *Culex restuans*, *Culex nigripalpus*, and *Culex stigmatasoma*.

While humans and other mammals can be infected with the virus as incidental, dead-end hosts, birds are the most important animals in the transmission cycle of West Nile Virus [9]. It is thought that birds are the reason the virus was able to spread so quickly throughout North and South America. The American robin has been specifically named in several studies because it is thought to be an important amplifying host, even in areas where the animal is present in very low densities [7]. In general, it is thought that birds do not often die as a result of infection with West Nile Virus, and they often develop immunity to the virus [10]. Another method used to detect the presence of West Nile Virus in a geographical area is to detect the virus in dead birds, but that method is not a focus of this project.

1.3 TREATMENT

Supportive care is the only treatment available for West Nile Virus [11]. Because approximately 80% of cases are asymptomatic and 19% of cases display only influenza-like symptoms, little treatment is needed for these patients. Pain relievers can be used to reduce aching and fever when symptoms are mild, and in rare cases intravenous fluids are used if a case includes severe vomiting. Treatment is generally only necessary in cases with neurological involvement, which only occurs in less than 1% of cases. Deaths due to West Nile Virus occur in about 10% of cases where neurological symptoms are present, or approximately .1% of all cases. Neuroinvasive symptoms can occur in patients of any age, but they are more likely to occur in patients who are elderly or otherwise immunocompromised [10]. Severe symptoms can include meningitis, encephalitis, and paralysis, and the treatment used is no different from that given to patients who display these symptoms from other causes. Treatment for meningitis includes pain relief for severe headaches and rehydration after vomiting. Encephalitis treatment involves hospitalization for monitoring of pressure within the cranium and possible seizures. Paralysis often involves long hospital stays and rehabilitation time, as well as close monitoring and possible ventilator support [11]. 80-90% of patients with confirmed neuroinvasive West Nile Virus disease are hospitalized [10].

The development of new antiviral drugs as well as the use of existing antiviral drugs to treat West Nile Virus cases have been unsuccessful so far [12]. However, published studies have largely been case series studies, which have a very small sample size by definition. Physicians and researchers are continuing to test old and new treatment methods for the purpose of finding an effective treatment method for the neurologic symptoms associated with West Nile Virus infection [12].

Similarly, there is no vaccine approved to prevent West Nile Virus in humans. However, a vaccine does exist that is approved to prevent West Nile Virus in horses [13]. The equine vaccine was made available in 2001, but was not widely used until the next year. There was a sharp drop in the number of equine West Nile Virus cases, from 15,000 in 2002 to 5,100 in 2003, which researchers believe was as a result of vaccine use [13]. The success of the equine vaccine provides hope that someday soon a human vaccine will also be available. In addition, safe and effective vaccines are available for other flaviviruses, including Japenese encephalitis virus, so researchers believe a human West Nile Virus vaccine is possible [14]. Currently in 2015, several clinical trials are underway for a few different types of West Nile Virus vaccines, including inactivated, live attenuated, live chimeric, and DNA vaccines [6, 14, 15]. Although a vaccine seems to be close at hand, the question of practicality has been raised regarding widespread vaccination. West Nile Virus can be a very serious disease, but the relatively low number of reported cases and deaths (41,762 cases and 1,765 deaths have been reported in the United States between 1999 and 2014 [16]) may not warrant a widespread vaccination campaign if the vaccine has significant side effects or if the cost is too high [14]. Until these issues are resolved and a vaccine is approved for humans, West Nile Virus will continue to be managed through mosquito surveillance and mosquito control activities.

1.4 NATIONAL VECTOR CONTROL RECOMMENDATIONS

The US Centers for Disease Control and Prevention have published recommendations for the purpose of providing guidance to local agencies which are responsible for vector control in their community. The guidelines, most recently updated in 2013, state that environmental surveillance in mosquitoes and birds should be the cornerstone of a control program for West Nile Virus because these measures are able to detect an increase in West Nile Virus risk before an increase in human cases is seen. For this reason, the guidelines state the importance of a program utilizing Integrated Vector Management (IVM), especially in large metropolitan areas with a history of West Nile Virus, such as Allegheny County. IVM includes regular (weekly) mosquito monitoring, which includes gathering information that can be used to find mosquito infection rates and abundance, as well as responsible, efficient use of pesticides if they are necessary to mitigate West Nile Virus risk. As the data is collected, the CDC guidelines recommend that local vector control professionals use a quantitative measure, either the WNV infection rate or the vector index measure, to quantify the West Nile Virus level in mosquito populations [7].

1.5 LOCAL VECTOR CONTROL PROGRAM

Beginning in 2000, the Pennsylvania Department of Environmental Protection (DEP) coordinates and consults on vector control activities across the state, of which West Nile Virus is the disease with the most focus. The DEP works to maintain consistency statewide by providing the West Nile Virus database, which is used by all the vector control professionals in

Pennsylvania. In order to do the actual on-the-ground work, the DEP awards grants to some counties in the state to conduct their own vector control activities, and performs these control activities for counties that do not receive a grant. Each grantee county is expected to perform mosquito surveillance and control activities as prescribed by the DEP, with the understanding that each is different and therefore has different needs, such as urban versus rural counties. Allegheny County has received a grant each year beginning in 2001, and the West Nile Virus Control Program, which is run through the Allegheny County Health Department, uses this grant to fund its activities. The grant is able to fund one full-time staff member and one seasonal intern position, as well as supplies such as pesticides and mosquito traps.

1.5.1 Allegheny County West Nile Virus Control Program

In Allegheny County, the summer activities of the West Nile Virus Control Program begin in April with the treatment of wetlands to prevent large populations of *Aedes Canadensis* and *Aedes vexans*, which are nuisance mosquitoes. Wetland areas are treated with larvicide, which efficiently controls mosquito populations by killing mosquito larvae before they become adults. This is efficient because a smaller amount of pesticide is needed to kill larvae than to kill adult mosquitoes. In addition, only adult mosquitoes can breed, so by killing the larvae also prevents future generations of mosquitoes. Wetland areas are chosen for treatment based on the previous experience of the Allegheny County Health Department vector control program, which has been in existence since 1975. Wetlands have been found using maps of the county, and also through citizens' mosquito nuisance complaints. By targeting wetlands that have been large mosquito breeding areas in the past and treating them early in the season, the breeding cycle in these wetlands is interrupted, which significantly reduces the number of adult mosquitoes in the area for the whole summer season.

Next, during one week early in June, catch basins within the City of Pittsburgh are treated with pesticide. Because the purpose of catch basins is to catch rain runoff, they are located everywhere in the city, they hold a lot of standing water, and they are well protected, which makes them a perfect habitat for mosquitoes to breed. In addition, because of the combined sewer system in Pittsburgh the catch basins can hold sewage as well, making these environments organic and nutrient-rich, which attracts the *Culex* mosquitoes. Therefore, each year the city catch basins are treated with pesticide in order to reduce the mosquito population. In order to treat as many catch basins as possible, approximately twenty employees from the Allegheny County Health Department assist with this task. Catch basin treatment takes place over approximately five weekday evenings. All the participating ACHD employees are split into groups of two or three and are assigned a city ward in which to treat as many catch basins as possible. Treatment is done by dropping a block of pesticide into each catch basin and then marking the outside of the catch basin with spray paint, in order to ensure that each basin is not treated more than once and to communicate to the public that the catch basin has been treated.

Beginning in mid-May, the focus of the West Nile Virus Control Program shifts to the *Culex* species of mosquito with the start of mosquito surveillance activities. While surveillance for many diseases is done though recording the number of human cases, this approach is impractical for West Nile Virus because of its low manifestation in humans [7]. Because most cases of human West Nile Virus are asymptomatic, the number of reported cases is significantly less than the number of actual cases, making human surveillance an ineffective method for representing the true level of West Nile Virus in a population. In order to get a better idea of the level and location of the virus within Allegheny County, mosquito surveillance is used. A few different mosquito collection methods are used, including gravid traps, light traps, Biogents' (BG) sentinel traps, and dipping for larvae.

1.5.1.1 The Gravid Trap

The trapping method used most regularly by the West Nile Virus Control Program is the gravid trap. The gravid trap is used because it is meant to specifically attract *Culex* mosquitoes. This type of trap uses stagnant bait water to attract female mosquitoes searching for a place to lay their eggs (gravid female mosquitoes). The bait solution used in the gravid traps consisted of water, hay, yeast, and lactalbumin. A small motor runs a fan that creates negative pressure inside the trap, so as the mosquitoes fly close to the bait water to lay eggs, they are sucked up through a tube into the trap. Gravid traps are set in the afternoon and picked up in the morning, when the sample of live mosquitoes is removed from the trap. During the summer, two staff members each set twenty gravid traps per week at predetermined sites within Allegheny County.

After the samples are picked up, they are processed and entered into the Pennsylvania West Nile Virus database, and then sent overnight to the Pennsylvania DEP Vector Management Laboratory for testing in Harrisburg. Results are returned in approximately one week via the West Nile Virus database, as well as reported to the public through the Pennsylvania West Nile Virus website.

If the laboratory PCR test of a sample comes back positive for West Nile Virus, more mosquito surveillance is done in the area surrounding the original trap location, called response trapping. At least four gravid traps are set in the area immediately surrounding the location of the positive trap, in order to better determine the extent of the virus in the area.

With the use of the results from the response traps, a decision is then made about whether adult mosquito control is necessary in the area where the positive samples were caught. If it is decided that adult mosquito control is warranted to prevent human cases of West Nile Virus, then control spraying is done. The adult mosquito control process does not reduce the percentage of mosquitoes infected with the virus; its purpose is to simply reduce the overall number of adult mosquitoes in the area, therefore decreasing the likelihood that a person would be bitten by any mosquito, infected or not.

1.6 THE VECTOR INDEX MEASURE

The vector index is one of five mosquito-based surveillance indicators the CDC recommends using to quantify West Nile Virus risk. The other four indicators are vector abundance, number of positive pools, percent of pools positive, and infection rate. The vector index measure is considered to be a better predictor of West Nile Virus risk than the other measures because it takes into account both the vector abundance and the infection rate. A vector index value is generally calculated for each geographical area for each week of the peak West Nile Virus season. The value is calculated by multiplying the vector abundance (the average number of mosquitoes caught per trap per night) by the minimum infection rate (assuming that only one mosquito in each positive pool was infected). This value is calculated separately for each mosquito species and summed to find the final vector index measure [7] (Figure 1). The vector index is usually a positive, single-digit number.

$$\mathbf{VI} = \sum_{i=\text{species}} \bar{N}_i \, \hat{P}_i$$

N=Average density
(Avg. # mosquitoes per trap)
P=Estimated Infection Rate
(Assuming smallest number of positive mosquitoes possible)

Figure 1. Vector Index Formula

Previously, once per year a vector index value was calculated for the areas of Allegheny County and the sub region of the City of Pittsburgh, for each week from mid-May through September. These values were used to compare West Nile Virus risk between counties and years, and to determine during which week risk was the highest within Allegheny County. However, because of time constraints, these calculations were always done after the mosquito collection season had ended, meaning that they could not be used to influence the vector control efforts while they were happening. In addition, the areas of Allegheny County and the City of Pittsburgh are very large, and West Nile Virus risk varies widely within them. Therefore, this project focuses on calculating the vector index measure for smaller areas, in order to examine the measure's practicality in assisting with decision-making regarding vector control during West Nile Virus season. In order for the vector index measure to be a practical tool for the Allegheny County West Nile Virus Control Program to use, it must be simple and quick to calculate and robust enough to handle a small sample size.

1.7 OBJECTIVES

The first part of this project involved performing various descriptive statistics in order to give the reader an understanding of the large amount of data collected by the West Nile Virus Control Program in the summer of 2015.

The second part of the project aimed to assist the Allegheny County West Nile Virus Control Program staff in the future by attempting to determine the usefulness of the vector index measure in the vector control decision-making process. Currently, the decision about when and where to spray to efficiently control the adult mosquito population is done very thoughtfully and logically, however there is no exact, quantifiable method used. In the future, it is hoped that a measure of West Nile Virus risk can be used to determine a threshold value above which adult mosquito control will be performed.

2.0 METHODS

Within Allegheny County, two afternoons per week, ten gravid traps were set at specified site locations by two staff members. In general, one staff member set traps only within the city, while the other set traps county-wide. Within the city, traps were set along one of eight predetermined routes. The routes and site locations were originally established in 2002 and 2003, and were based on the residences or other probable infection locations of people who were diagnosed with West Nile Virus during those years. Since their establishment, the routes and site locations have been adapted in order to find other places where West Nile Virus is being spread, and to better conceal the traps to prevent tampering by the public. Outside the city limits, a similar routine is followed, however there are more routes because there is more area to cover.

Each morning, each staff member picked up the ten gravid traps they had set the previous afternoon and brought them back to the Allegheny County Health Department for processing. The mosquito samples were processed by first being placed in insulated coolers with dry ice. The samples were left in the coolers for at least 30 minutes to "knock down" the mosquitoes (this will kill the mosquitoes eventually, but within 30 minutes they are still alive), both for ease of counting and for preservation. Next, any non-mosquito organisms (e.g. flies, slugs, midges, spiders) were removed from the sample and discarded. The total number of mosquitoes caught in each trap were then estimated without being separated by species or sex, in order for the laboratory technicians to ensure they are testing the correct sample. Next, the data was entered into the statewide West Nile Virus database. The information that was entered included the type of trap set, the name of the collector, the code on the label placed on each processed sample, the date the trap was set, the number of hours the trap was collecting mosquitoes, the time the trap was picked up, the approximate number of mosquitoes caught in the trap, the location of the trap, the habitat where the trap was set (e.g. urban, wooded, recreational area, landfill, wetland, etc.), the number of tires nearby if applicable, and any additional comments. Finally, the processed samples were placed in a small cooler with dry ice, and sent by courier to the DEP laboratory in Harrisburg, where they were separated and counted by species, then tested for West Nile Virus by PCR. The mosquitoes from each trap were separated into pools of no more than 100 mosquitoes for testing. Each pool was homogenous by species, with the exception of 44 pools which contained both *Culex pipiens* and *Culex restuans* mosquitoes due to small sample sizes in those traps. Once the laboratory processing and testing was finished, the results were posted in the online database. Information entered by the laboratory personnel included the date and time the sample was received, the date and time the sample was processed at the laboratory, the exact number of mosquitoes of each species in the sample, the results of the PCR test of each mosquito pool, and any additional comments.

Any time a pool tested positive, at least four response traps were set around the area where the positive sample was trapped. The same protocol was followed for processing and testing the response traps as the traps set on the normal routes. (However, if a response trap is found to be positive, four more response traps were not set around that location.) Based on the results from testing the response traps, a decision was made about whether it was necessary to spray to control adult mosquito populations in the area.

Spraying to control adult mosquito populations was done with a truck-bed-mounted Ultra Low Volume (ULV) pesticide sprayer. Spraying was only done between 8:00pm and 10:00pm, because that is when the highest number of adult mosquitoes are flying. Because there was only one sprayer available in the county for adult mosquito control, as well as a short two-hour timeframe for spraying, the amount of area that can be covered in one evening was limited.

2.1 VECTOR INDEX CALCULATION

For the vector index portion of this project, a subsection of the sampling area was chosen in order to make the scope of the project more manageable. The eastern neighborhoods of the City of Pittsburgh were chosen, as well as Wilkinsburg and Edgewood, which border the city to the east. This area was chosen because it was the largest contiguous area where traps were set consistently. In the chosen area, borders were drawn so that the area was split into eleven zones. In general, the area was broken up by neighborhood, but in some cases smaller neighborhoods were combined in order to create zones of relatively comparable size. The final eleven zones were Squirrel Hill, Point Breeze, Wilkinsburg/Edgewood, Homewood, Shadyside/East Liberty/Larimer, Lincoln-Lemington, Highland Park, Morningside, Bloomfield/Garfield, Stanton Heights/Allegheny Cemetery, and Lawrenceville. The intent was that each zone would be large enough to contain several trapping sites, but small enough that the whole zone could be treated to control adult mosquitoes within only one evening of spraying.

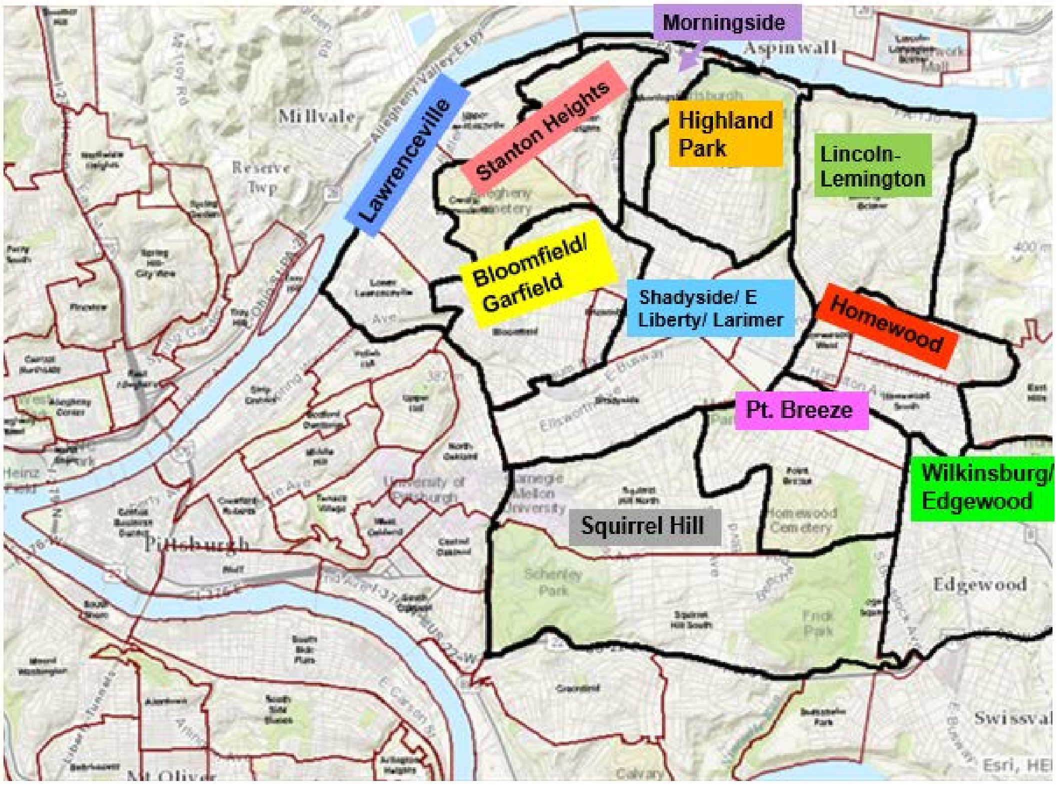


Figure 2. Map of eleven zones for which vector index values were calculated.

Once the area was divided, a vector index value was calculated for each zone for each week mosquito samples were collected from that zone. The formula from Figure 1 was used, and Microsoft Excel was used for the calculations. Generally, when a vector index is calculated, a value is calculated for each CDC Morbidity and Mortality Weekly Report (MMWR) week, so that was done for this project as well.

3.0 RESULTS

Between May and September 2015, 562 gravid traps were set in Allegheny County. Samples from 124 traps were not tested for a few reasons. Eight traps were excluded because of trap failure (five traps were not on at pick-up, two traps were vandalized, and one trap was stolen). Samples from 116 traps were not tested because of a small sample size, so the extremely low risk of missing a positive sample was outweighed by the benefits of saving time and money. The laboratory staff members made the decisions about which samples were worth testing and which were not. Samples that were not tested were generally caught early in the season and contained a small number of mosquitoes. After the above exclusions, samples from 438 gravid traps were tested for West Nile Virus, of which 172 contained at least one positive mosquito pool. Of the 172 traps that tested positive, 128 traps contained only one positive mosquito pool, 32 contained only two positive pools, ten contained only three positive pools, one contained only four positive pools, and one contained six positive pools.

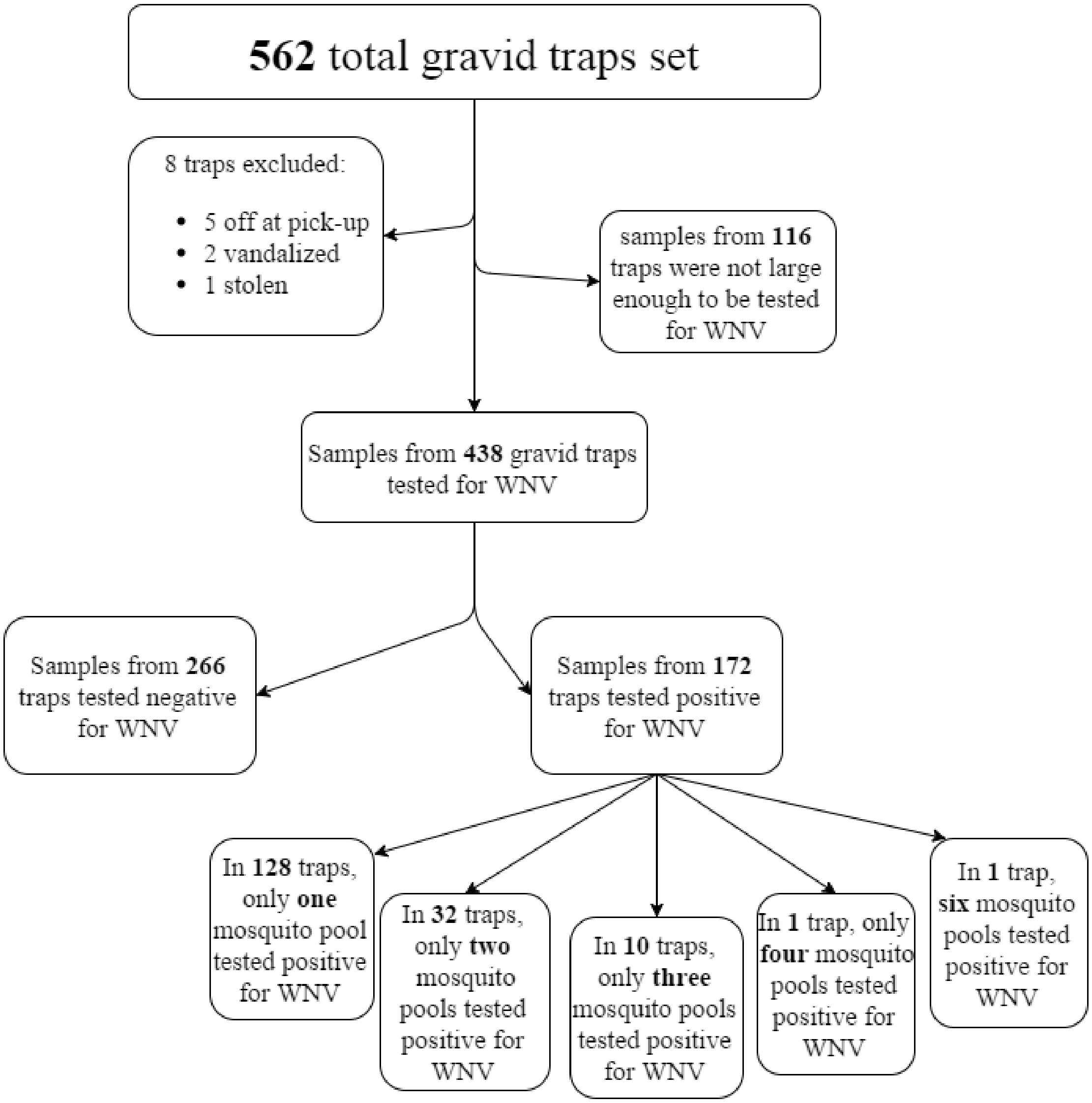


Figure 3. Summary of all gravid traps set within Allegheny County in 2015.

In the 2015 season, 30.6% of the total traps set were found to contain at least one pool of mosquitoes that tested positive for West Nile Virus. When compared with data from the last ten years of the Allegheny County West Nile Virus Control Program, 2015 was the year with the highest percentage of positive traps. The next highest percentage was 28.3%, which was found in the 2012 season. The remaining eight seasons had values under 15%. Although the program has existed in some form since 2001, the sample size was significantly smaller during the first few years. Therefore, only data from 2005 onward was included for this portion.

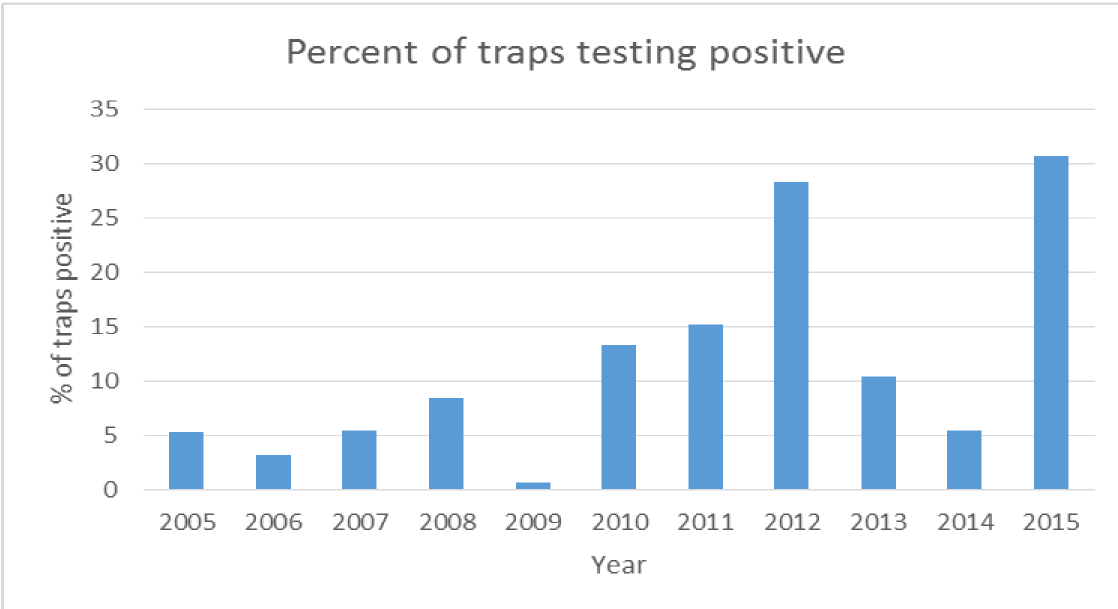


Figure 4. Percent of total traps set that tested positive for West Nile Virus from 2005-2015.

Of the mosquito pools that were tested for West Nile Virus using PCR, 49.7% contained only *Culex pipiens* mosquitoes, 39.4% contained only *Culex restuans* mosquitoes, 5.1% contained a combination of both *Culex pipiens* and *Culex restuans* mosquitoes, and 5.8% contained only *Aedes japonicus* mosquitoes.

Of the pools tested, 39.3% of the homogenous *Culex pipiens* pools tested positive, 13.9% of the homogenous *Culex restuans* pools tested positive, 34.1% of the combined *Culex pipiens-Culex restuans* pools tested positive, and 8.0% of the *Aedes japonicus* pools tested positive.

Table 1. Summary of mosquito pool data by species.

Species	# pools tested (%)	# positive pools	% positive pools
Culex pipiens	427 (49.7%)	168	39.34%
Culex restuans	338 (39.4%)	47	13.91%
Culex pipiens-restuans (pools contained both species)	44 (5.1%)	15	34.09%
Aedes japonicus	50 (5.8%)	4	8.00%
All species	859 (100%)	234	27.24%

There was a clear peak in the number of positive traps in mid to late August, with the highest number of positive traps being set during week of August 23rd, when samples from 28 traps tested positive for West Nile Virus. During the month of May, there were no positive traps tested, and during the month of June, only two traps were found to be positive. In July, the number of positives began to increase, with 31 positive traps total, seventeen of which were set during the last week of the month. The number of positive traps continued to increase in August, with thirteen positive traps set during the week of August 2nd, fifteen positive traps set during the week of August 9th, 23 positive traps set during the week of August 16th, and 28 positive traps set during the week of August 23rd. In September, the number of positive traps decreased until trapping was ended on September 25th. Sixteen positive traps were set during the week of August 30th, fifteen positive traps were set during the week of September 6th, sixteen positive traps were set during the week of September 13th, and thirteen positive traps were set during the final week of trapping, which was the week of September 20th. The week of August 23rd had the highest percentage of positive traps, at just over 75%.

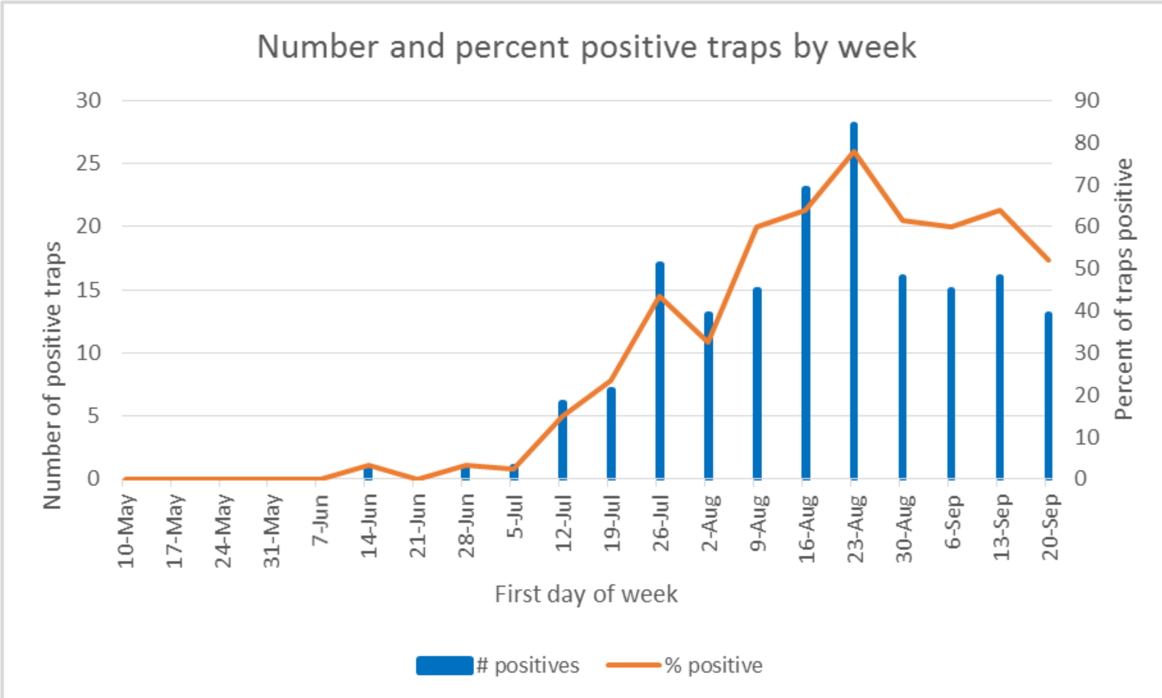


Figure 5. Number of traps testing positive each week from May 10-September 20.

Of the 562 total gravid traps set within Allegheny County, 373 (66.4%) of them were set within the City of Pittsburgh and 189 (33.6%) of them were set outside the city limits. 127 (34.1%) of the 373 traps set within the City of Pittsburgh tested positive for West Nile Virus. 45 (23.8%) of the 189 traps set outside the City of Pittsburgh tested positive for West Nile Virus.

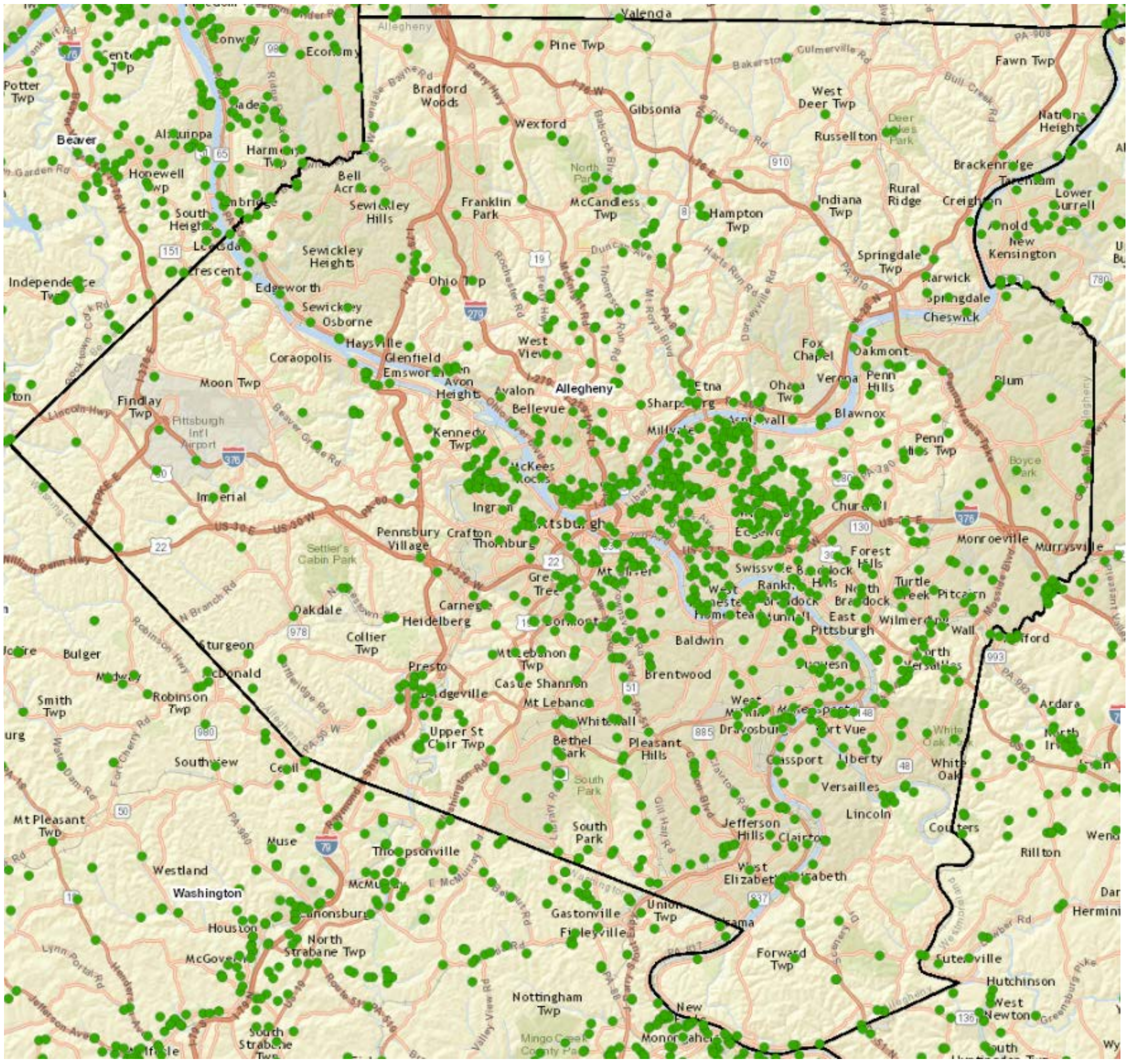
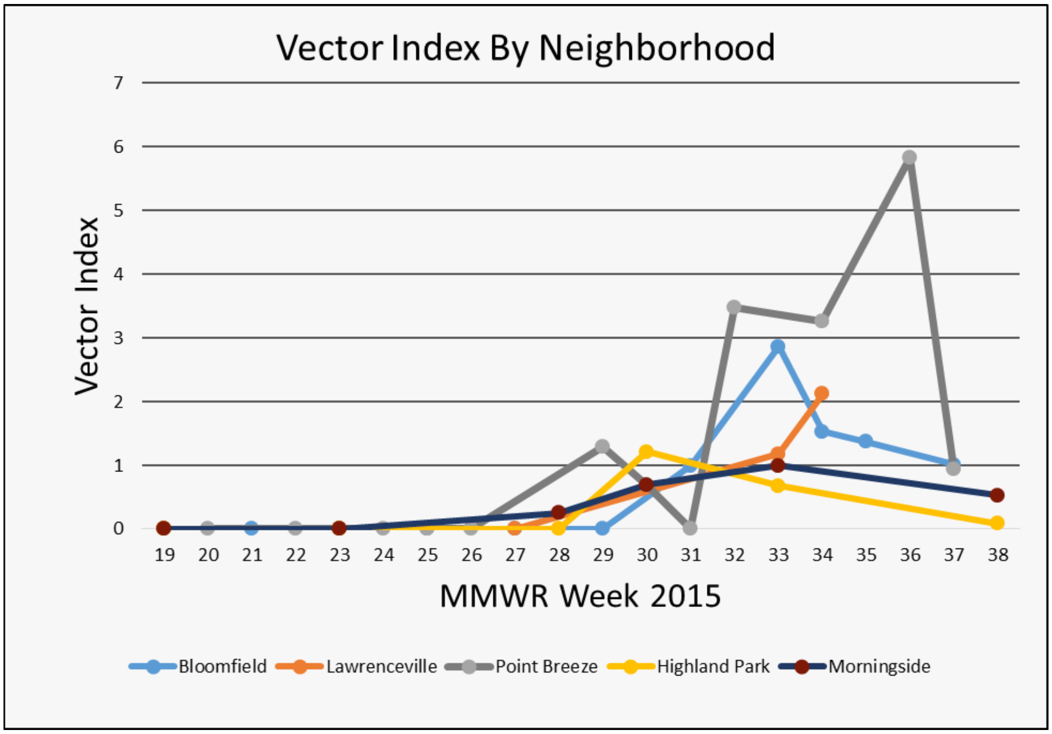


Figure 6. Map of Allegheny County with green points representing trap locations set since 2001.

The City of Pittsburgh is directly in the center of the county.

3.1 VECTOR INDEX

The next part of this project involved finding the vector index measure for eleven zones on the eastern side of the City of Pittsburgh. The vector index is usually reported by MMWR week, as it is in this project. Because there were eleven zones and only two people setting and collecting gravid traps, there was no available data for some zones during some weeks. For each MMWR week between May 10th (MMWR week 19) and September 20th (MMWR week 38), a vector index was able to be calculated for one to four zones. The lowest vector index was zero and the highest vector index calculated was 5.8, in Point Breeze during MMWR week 36 (September 5-11). The general trend was that vector index values increased as the summer went on, with a sharp decrease in early September as the summer season came to an end.



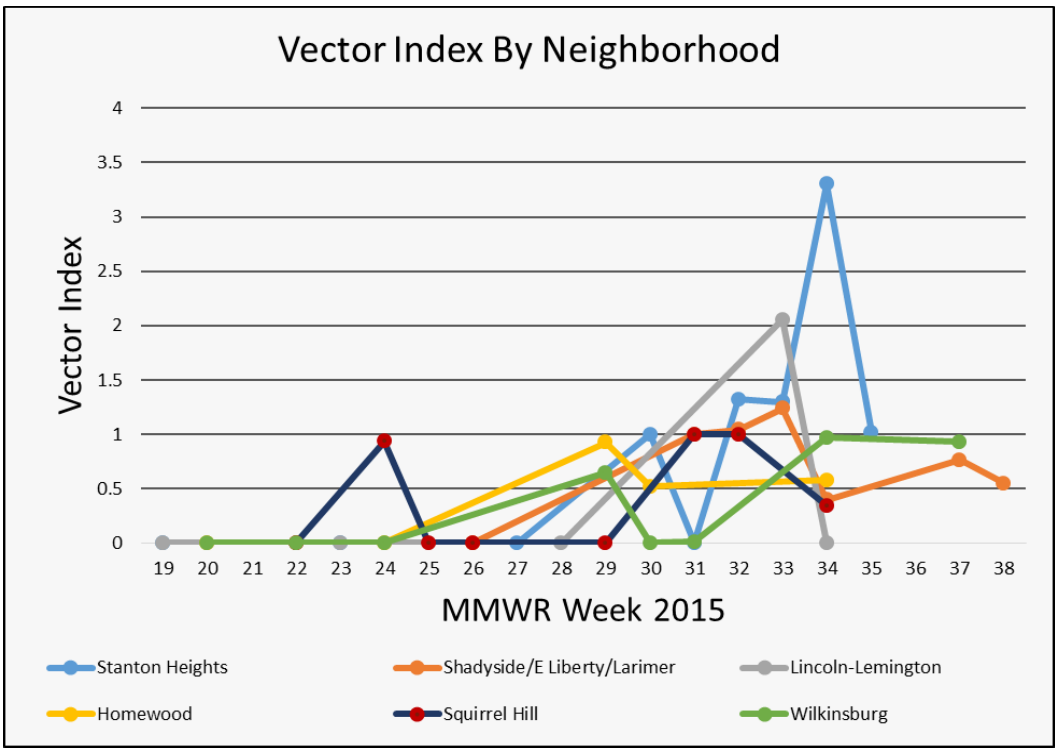


Figure 7. Vector Index values within the eleven zones, MMWR weeks 19-38.

4.0 DISCUSSION

Compared to previous years, 2015 was a high-risk year for West Nile Virus in Pennsylvania and Allegheny County. The percentage of gravid traps that tested positive for West Nile Virus within Allegheny County at 30.6% was the highest it has been for the past ten years in Allegheny County. This season closely resembled the summer of 2012, during which 28.3% of all gravid traps set in Allegheny County tested positive for West Nile Virus. All the other years within the past decade have had a maximum of 15% positive traps, which is half of the value from 2015. Factors that affect the West Nile Virus risk each season are not yet well understood and there is no clear pattern of risk between years. Researchers believe different combinations of factors including temperature, rainfall, mosquito species' feeding behavior, and vector competence affect the West Nile Virus risk each year [1, 17]. Based on the data from this study alone it is unclear why the risk was so high in 2015 compared to other years. This is an area for further investigation, including looking closer into weather patterns and bird and mosquito behavior.

In the 2015 season, there were three laboratory-confirmed human cases of West Nile Virus in Allegheny County, which is the highest since 2005, when there were six human cases. This seems like a very low number without taking into consideration the large percentage of asymptomatic or subclinical West Nile Virus cases in humans. Based on data from 1999-2014, it is estimated that 41-61% of the reported human West Nile Virus cases were considered neuroinvasive [10, 18]. Because it is estimated that less than 1% of human cases result in neurological disease, it can be gathered that for every reported case of West Nile Virus, there are approximately 50 human cases that go unreported.

From past data collection, it is known that *Culex pipiens* and *Culex restuans* mosquitoes are the most common West Nile Virus vectors in Allegheny County, and in Pennsylvania as well. The data from this project continued to support this. Both species prefer to bite birds and do not often bite humans until late in the summer, when birds begin their migration southward [7]. Because birds are the reservoir for West Nile Virus, it makes sense that mosquito species that most often bite birds are at the most risk for carrying and transmitting the pathogen. As the birds begin to migrate late in the summer, the *Culex* species of mosquito look to humans for blood meals. This is why it is common to see a peak in human West Nile Virus cases at the end of the season, in late August and September. In 2015, the human cases were all reported within this expected time frame.

In 2015, there were four pools of *Aedes japonicus* mosquitoes that were found to be positive for West Nile Virus. While this species is a competent West Nile Virus vector, it is relatively unusual to find positive samples in this area. In general, *Aedes japonicus* mosquitoes tend to bite humans rather than birds, so therefore they are less likely to carry the virus because of their limited contact with the reservoir host. However, because this species tends to bite humans often, it is very important to note the positive pools of *Aedes japonicus* mosquitoes. Because they often bite humans, infected *Aedes japonicus* mosquitoes are more likely to transmit the virus to humans compared to *Culex* species mosquitoes. Therefore, it is important to continue collecting mosquitoes using gravid traps and to continue testing *Aedes japonicus* mosquito pools for West Nile Virus.

More gravid mosquito traps were set within the city limits of Pittsburgh (66.37%) than outside the city limits (33.63%). Because the area within the city is smaller than the area outside of the city, this means that the traps were set much more densely within the city limits. In part, this was due to the set-up of the program, with one staff member setting traps only inside the city and the other setting traps county-wide. In addition, the literature indicates that West Nile Virus risk is higher in urban areas than in suburban or rural areas [7], so the West Nile Virus Control Program chooses to allocate more of their limited time and resources within the city for this reason. Also, because the staff of the control program have been working within Allegheny County for over a decade, they understand from experience that areas within the city of Pittsburgh are often higher risk for West Nile Virus than areas outside of the city.

A limitation of this study was the method used to test the mosquito samples. A PCR test was used to detect any West Nile Virus DNA in a sample of no more than 100 mosquitoes. However, the test is not able to detect the number of mosquitoes in the pool that are carrying the virus. Valuable information is still taken from the PCR testing, but it is important to note this limitation.

4.1 VECTOR INDEX

The vector index values calculated showed a slow increase in West Nile Virus risk as the summer progressed, with a sharp decrease during the last two weeks of the season. Very early in the season, low numbers of mosquitoes were caught, and the first sample to test positive for West Nile Virus was caught during the third week of June, five to six weeks into the trapping season. Mosquito population numbers and the number of positive traps continued to steadily increase as the season went on. Mosquitoes continued to breed creating larger numbers, and more of the mosquito population became infected as they continued to bite infected birds. During these last few weeks of summer, although positive samples were still being found, the number of mosquitoes caught in each trap was rapidly decreasing. At this time in the season the nighttime temperatures were beginning to drop, which was believed to cause the sharp decrease in the average number of mosquitoes caught per trap. The data collection was stopped during the last week in September as per statewide instructions by the Pennsylvania DEP. At least two to three more weeks of sample collection could have been beneficial, as positive samples were still being trapped during the last week of sampling. It would have been interesting to see data from the very end of West Nile Virus season, to find out how long it took until the mosquito populations disappeared for the winter. This is an area of possible future study improvement.

A vector index value was able to be calculated for each of the eleven zones for at least four of the twenty weeks of the surveillance program. The values that were calculated for each week were based on data from between two and eight different traps. The vector index values calculated were within the expected range of values. However, the goal of using the vector index to compare West Nile Virus between neighborhoods was largely unsuccessful due to the limited sample size. Vector index values could be calculated for each of the eleven zones, however because of limited time and resources every zone could not be reached every week. Therefore, although it was possible to see patterns over the whole season, it was not particularly useful for comparing one neighborhood to another during the same week.

In order for this method to be of use for direct and timely comparison of West Nile Virus risk between neighborhoods, a larger sample size is needed. In order to have reliable numbers for each of the eleven zones for each week of the season, approximately 50 or more traps would need to be set every week. In 2015 with the current available time and resources, the two staff members who sampled using the gravid traps were only able to set a maximum of 40 traps per week. Therefore, to make the vector index method possible, at least one more staff member would be needed in order to gather enough data. In addition, these eleven zones only represented approximately one third of the geographical area of the City of Pittsburgh. Therefore, in order to gather enough data to use the vector index for zone comparison, at least 150 traps would have to be set per week throughout the city. Further, the area within the limits of the City of Pittsburgh only represents approximately 10% of the area of Allegheny County, so it is clear to see how expensive and improbable a project like this could be if it is applied to an area as large as a county.

An alternative to increasing the number of traps set each week would be to increase the area of each zone, so that fewer, larger areas could be compared, requiring fewer traps. On a very large scale, this is already being done in Allegheny County, as a vector index value is calculated for the entire county by week each year. However, the problem with this is that the values calculated are not as useful for decision-making. The objective in creating these small, neighborhood-sized zones was that data could be used to determine where adult mosquito control spraying operations are most needed. If a zone is larger than the area that can be sprayed during one evening, the method will not work as intended. Also, although the pesticide used is very safe, it is not in anyone's best interest to spray over a larger area than necessary.

If a quantitative method for vector control decision-making is to be used in the future, further investigation is necessary to find an efficient and reliable method. In order for the vector index to be of use, either a much larger sample size or much larger areas will need to be used. Each of these options have significant disadvantages, as detailed above. It is important to have a quantitative measure to prioritize areas to receive more mosquito control activities as well as to help determine a risk threshold below which pesticide treatment is not beneficial. Although finding an efficient method will be difficult, it is worthy of further investigation.

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