Examining the Effects of Precipitation and Temperature on Mosquito Population Density and West Nile Virus Positive Samples in Allegheny County from 2016-2022

by

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Abstract

Vector-borne diseases continue to rise as a serious public health threat throughout the world. West Nile Virus (WNV) is a mosquito-borne virus that is endemic throughout the United States. While most cases of WNV are asymptomatic, certain individuals face severe neurological symptoms and in rare cases, fatality. Outside of humans, several avian and horse populations are affected by the virus as well. Many states' including Pennsylvania created vector control programs to monitor WNV and several other vector-borne diseases. Allegheny County Health Department's (ACHD) Housing and Community Environment Office (HCE) are responsible for conducting the program in Allegheny County. Surveillance of WNV is conducted by the setting of gravid traps specifically designed to trap *Culex pipiens* and *Culex restuans* which are the primary mosquito species that transmit WNV. Starting in 2016, 25 fixed gravid sites were established mainly within the City of Pittsburgh. These traps were set weekly from May-September of each year. The data collected from these traps was used to determine when control efforts such as pesticide distribution were necessary. While the data is currently only used for surveillance purposes, it holds value for research as well. Many variables affect mosquito population density and WNV incidence, with precipitation and temperature being two of the most influential factors. These variables have the potential to increase the programs' understanding of what is affecting WNV in Allegheny County. This study aimed to determine if there were any correlations between precipitation and/or temperature and mosquito population density and WNV positive samples from 2016-2022. While

no significant correlations were found, it is important that research continues to examine these variables, as it may aid public health programs in their efforts against WNV. If vector control programs had the power of a localized predictive model for WNV severity based on variables like weather patterns, they would have a greater ability to proactively protect the public from the threat of WNV and other diseases.

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Preface

To start off, I woud like to take the time to thank my committee for dedicating their time and energy in making this essay possible. I would like to send my sincerest gratitude to ACHD's housing department. I will forever be gratful for the time spent working alongside you. Your hardwork and dedication to the residents of Allegheny County should be recognized and appreciated. All of this would not have been possible without the support of my family and friends. To my parents and my sister Stephanie, thank you for always being there to keep me sane during this incredible process. Nana, Pappy, Grandma, and Grumpy, thank you for being the most supportive grandparents anyone could ask for. I hope I have made you all proud. To my best friend Jess, there are not enough words to express my gratitiude for all you have done for me. I am so thankful to have you in my corner through every stage of my life. Lastly, to my friends here at Pitt, what a wild journey we have been on. Thank you from the bottom of my heart for supporting me these past two years. My time here at Pitt would not have been nearly as enjoyable without you.

1.0 Introduction

In the past few decades, West Nile Virus (WNV) has become a significant public health issue on a global scale. WNV was first isolated in Northern Uganda in 1937 (Smithburn et al., 1940). WNV is indigenous to Africa, Asia, Europe, and Australia (Campbell et al., 2003). Israel saw the first recognized epidemic of the virus in 1951 (Bernkopf et al., 1953). In 1996, an outbreak occurred in Bucharest Romania, marking the first urban-centered outbreak (Tsai et al., 1998). In 1999, North America detected the virus for the first time in Queens, New York City (Nash et al., 2001). Several severe encephalitis cases resulted in an epidemiologic investigation which led to the discovery (Nash et al., 2001). Since then, the virus has been detected several avian species and horses throughout the continental United States (Johnson et al., 2001).

WVN is classified as a mosquito-borne flavivirus. It is also known as a human, avian, and equine neuropathogen (Campbell et al., 2003). The *Culex* genus of mosquitos is the primary vector for WNV transmission. The virus goes through an enzootic cycle between mosquitoes and avian reservoir hosts (Nguyen et al., 2019). Symptoms range from no symptoms to severe neuroinvasive symptoms. As of 2021, there have been more than 51,000 clinical cases and more than 2,300 deaths due to WNV in the US (Ronca et al., 2021).

Mosquitos require water to lay their eggs. Precipitation and temperature patterns influence mosquito populations throughout their breeding season. Global warming has led to warmer summers and shorter winters which correlate with increased blood feeding and breeding activity in mosquitoes (Yoo el al., 2016). These trends have been identified on a global scale, however there is a gap of literature in Pennsylvania and Allegheny County specifically. WNV is a serious public health threat. Changing weather patterns combined with the virus's genetic and epidemiologic variation, there is potential for an increase in mosquito populations and WNV (Mencattelli et al., 2022).

There are a variety of stakeholders that could benefit from this project including but not limited to, Allegheny County residents, Allegheny County Health Department, Pennsylvania State Government, Allegheny County Government, health care workers, and researchers in the field. The health department could benefit from the information as it has the potential to provide a greater insight on the local impact of weather patterns on mosquito populations and WNV transmission. This benefits residents as the health department may be able to improve the efficiency of preventative measures to protect the community from WNV. Pennsylvania's state government could benefit from increased efficiency of surveillance and preventive measures, given that many vector control programs are state funded. Local government could use this information to assess if infrastructural faults related to weather conditions exacerbate mosquito populations and take action to negate these problems. Health care workers could benefit from this information if it helps reduce the number of human WNV cases. Researchers could benefit from this information as it provides a localized view on these issues in comparison to global trends.

1.1 Specific Aims

This project will compare global mosquito, weather, and WNV data to Allegheny County data. It will help fill a local gap in literature regarding how weather patterns in Allegheny County may have affected mosquito populations and WNV prevalence since the county began collecting data on WNV. This information could help establish local predictive models on mosquito populations and the transmission and distribution of WNV. Data will include mosquito population density and distribution, number of positive WNV mosquito samples, temperature, and precipitation. Data will be collected from the Department of Environmental Protection of Pennsylvania (DEP) and the National Oceanic and Atmospheric Administration (NOAA). The primary focus will be data collected from 25 fixed gravid mosquito traps throughout Allegheny County from 2016-2022. The specific aims for this project are:

1. Investigate the effects of temperature and precipitation on mosquito population density and WNV prevalence in mosquito populations.
2. Discuss other key drivers involved in mosquito population density and WNV prevalence such as genetics, urbanicity, climate change, and land structure.
3. Discuss the importance of WNV surveillance in public health.

2.0 Literature Review/Background

2.1 History of West Nile Virus

2.1.1 West Nile Virus Structure and Genetics

WNV is classified as a mosquito-borne flavivirus, specifically a Japanese Encephalitis virus (Pesko & Ebel, 2012). Other members of the Flaviviridae family include Zika, dengue fever, and yellow fever viruses. WNV virion consists of a single strand of positive sense RNA, a spherical shape, and an envelope (Pesko & Ebel, 2012).

There are ten genes in the approximately 11,000 nucleotide-long genome that are surrounded by non-coding regions (NCR). The stem-loop structures that form within the NCR of WNV's genome are important for replication (Chancey et al., 2015). Only three structural proteins are present in WNV: envelope, precursor of membrane/membrane, and capsid proteins (Heinz & Stiasny, 2012). The other seven proteins play various roles within replication as well as regulating cell signaling and host immune response. In WNV specifically, NS1 protein disrupts the immune response by blocking signal transducer and activator of transcription (STAT) activation and TLR3 signal transduction (Liu et al., 2005; Wilson et al., 2008).

Host cell lipids are commonly used in the replication of flaviviruses as they provide energy and fatty acids (Liebscher et al., 2018). The parts of the replication complex include a specialized membranous organelle required for viral replication, viral structural components, and metabolic precursors. Flavivirus replication is heavily dependent on a cell's endoplasmic reticulum (ER). Formation of paracrystalline arrays and convoluted membranes is aided by the ER (Westaway et al., 1997). These components are where viral protein translation and proteolytic processing occurs (Westaway et al., 1997). The ER also forms small vesicles through invagination of the ER membrane. These vesicles, also known as replication complexes, contain viral mechanisms necessary for replication as well as double-stranded RNA (Liebscher et al., 2018). Flavivirus virion assembly is initiated in the lumen of the rough endoplasmic reticulum (Mackenzie & Westaway, 2001).

WNV has several genetic lineages that vary geographically in prevalence. The exact number of lineages is unknown. As many as nine biologically distinct strains have been proposed. Lineage one is the only strain known to be globally distributed and has the most associations with neurological disease among outbreaks in Europe, Africa, and the Americas (Fall et al., 2017).

2.1.2 West Nile Virus Transmission and Life Cycle

Whereas birds are the reservoir, mosquitos are the vector. Mosquitos that take a blood-meal from both birds and humans are considered bridge vectors because they transmit WNV from birds to humans. Mosquitoes in the Culicidae family are the primary vector in the transmission cycle. Culex pipiens, C. quinquefasiatus, C. nigripalpus, and C. tarsalis are the main WNV drivers in the United States (Campbell et al., 2002). Mosquitoes become infected by consuming a blood-meal from an infected bird. For WNV to be spread from mosquitoes to a host, it must be able to replicate within the vector. The virus first replicates in the midgut epithelia. From there it makes its way to the salivary glands through the mosquito’s hemolymph. Once WNV viremia reaches a certain level within the salivary glands, it can then be spread through the saliva to mammals (Girard et al., 2005; McGee et al., 2010; Richards et al., 2012). The virus is maintained in both mosquito and bird populations throughout the summer. In geographic areas that experience cold winters, the virus has been found in overwintering Culex species (Nasci et al., 2001). This means that come spring, mosquitoes aid in reestablishing the enzootic transmission cycle (Nasci et al., 2001).

The last step of the transmission cycle is between mosquitoes and mammalian hosts such as humans and horses. Mammals are considered incidental hosts as well as dead-end hosts. An infected mosquito spreads the virus by biting a host. Saliva is transferred to the host as the proboscis enters a blood vessel (Styer et al., 2007). WNV antigen has been found in the skin throughout an infection (Schneider et al., 2006). In a mouse model it has been shown that the virus was present in the skin as well as the central-nervous system (CNS) at fourteen days post inoculation (Appler et al., 2010). Once the virus has entered a host it must bind to specific sites on the host's cell to initiate replication. WNV enters mammalian host cells through receptor-mediated endocytosis of clathrin-coated pits (Chu & Ng, 2004). The receptor for WNV is a protease-sensitive, N-linked glycoprotein (Chu & Ng, 2003). As infected cells produce viral proteins, the virus's ability to inhibit the antiviral defense response increases (Brinton, 2013). To this day, not all mechanisms for WNV replication in humans are known.

The mode of transmission of WNV for humans is from birds to mosquitos to a new susceptible host. An enzootic cycle between birds and mosquitoes maintains the viral load within the avian reservoir. WNV replicates well within birds, resulting in a high enough viremia for mosquitoes to become infected (Chancey et al., 2015). Depending on the species of bird, the transmission efficiency of WNV can vary. One study used WNV-infected American robins and common grackles to determine the transmission efficiency to naïve mosquitoes. When looking at equivalent viremia levels, they found that robins infected far more mosquitoes than grackles. Furthermore, they concluded that viremia is not the only determining factor of infectiousness. Other factors include vector factors such as vector competence, temperature of environment, and feeding behaviors (Vaughan et al., 2022). This study highlights that different avian populations may have a significant impact on WNV prevalence in mosquitos.

2.1.3 The Origin of West Nile Virus and its Geographic Scope

WNV was first discovered in 1937 in the West Nile district of Northern Uganda (Smithburn et al., 1940). The patient exhibited symptoms indicative of yellow fever. However, when researchers inoculated mice with serum from the patient, a new virus was found (Smithburn et al., 1940). Other studies soon followed and found that WNV's pathology involved the CNS (Sejvar, 2003). As time went on, several outbreaks of WNV occurred all over the world. Israel saw the first officially recognized epidemic of WNV in 1951 (Bernkopf et al., 1953). As outbreaks occurred across Africa and the Mediterranean Basin, researchers began to define clinical features, the epidemiology, and the ecology of WNV.

WNV first reached North America in 1999. An epidemic of meningoencephalitis in New York City led to the discovery (Nash et al., 2001). In the USA, 142 neuroinvasive WNV cases were reported from 1999-2001 (Campbell et al., 2002). The virus was quickly becoming a serious public health threat. Today, WNV is found in every continental state in the US (*CDC*, 2022). According to the World Health Organization (WHO), it is also common in Africa, Europe, the Middle East, North America, and West Asia (WHO, 2017).

2.2 Environmental Factors Effect on West Nile Virus

2.2.1 Mosquito Breeding Habitat and Life Cycle

The typical mosquito breeding season starts in early spring and ends in late fall. A mosquito’s breeding habitat is dependent on its species. A commonality among all species is that water is necessary for a female mosquito to lay her eggs. Two breeding habitat-based categories are permanent water mosquitoes and floodwater mosquitoes. Permanent water mosquitoes prefer to lay their eggs in standing water such as lakes, ponds, swamps, vernal pools, and artificial containers (CDC, 2022). Floodwater mosquitoes will lay their eggs in flood prone areas. The eggs dry out until a flood event occurs. These areas can include temporary pools, floodplains, irrigated fields, artificial containers, and tree holes (CDC, 2022).

C. pipiens prefer a breeding habitat such as catch basins, sinkholes, artificial containers, and stagnant water in low-lying areas (Shaman et al., 2010). With such a broad range of breeding habitats, these mosquitoes thrive in urban environments which have an abundance of habitats such as storm drains.

The lifecycle of a mosquito has four main stages, egg, larva, pupa, and adult (US EPA, 2013). The first three stages are all aquatic. A female adult mosquito must consume a bloodmeal to produce eggs. These eggs are laid in the preferred breeding habitat where they either quickly hatch or desiccate until a precipitation event occurs. Larvae emerge from the eggs and undergo four instar stages. Throughout the first three instar stages, larvae feed on organic material such as bacteria, algae, and protozoa (Souza et al., 2019). After the fourth instar stage the larva develops into a pupa, at which point feeding stops. Adult mosquitoes emerge from the pupal case after anywhere from two days to one week in the pupal stage. The total cycle takes a few days to an entire month to complete depending on the species (US EPA, 2013). For *C. pipiens,* their adult lifespan is between 12-132 days (Andreadis et al., 2014). Differences in temperature result in the variability of lifespan, as shorter lifespans are associated with higher temperatures and longer lifespans are associated with lower temperatures (Andreadis et al., 2014).

2.2.2 Weather Conditions

Weather conditions play a vital role in mosquito breeding and WNV transmission. Temperature and precipitation in conjunction with land structure have been studied in various geographic locations. A study done in the United States from 2004-2012 found that higher annual temperatures led to an increased probability of WNV incidence in most of the country (Hahn et al., 2015). A similar study conducted in Europe found that above average July temperatures led to higher WNV incidence as well (Tran et al., 2014). A Chicago-based study observed that there was an increased risk of a positive WNV case when higher than average temperatures are reported in the previous weeks (Karki et al., 2020).

In terms of precipitation, it was found that lower than average precipitation led to higher disease incidence in the eastern US but found the opposite for the western US (Hahn et al., 2015). Another study found that in the United States, higher incidence may be the result of above average precipitation in the previous year and below average precipitation in the current year (Landesman et al., 2007). Researchers from this study hypothesized that ecologic differences between mosquito vectors might contribute to the different effects precipitation had on WNV outbreaks in the United States (Landesman et al., 2007).

Many studies have suggested that weather patterns could potentially be used to create predictive models. A study conducted in Toronto found a strong correlation between weather variables which could predict *C. pipiens* population density in the study region (Yoo et al., 2016). A limitation to that study is the results do not account for the dynamic changes between the environment and *C. pipiens* (Yoo et al., 2016). These changes include variability in mosquito interactions with weather, land-use, vegetation coverage, blood meal availability, and control efforts (Yoo et al., 2016).

2.3 Impacts of West Nile Virus

2.3.1 West Nile Virus Disease Symptoms

Currently, around 80% of people who contract WNV will be asymptomatic (Chancey et al., 2015). 20%-40% of those infected develop a mild infection known as West Nile fever. Symptoms for this infection include fever, headache, body aches, vomiting, diarrhea, fatigue, and skin rash (Donadieu et al., 2013). These symptoms typically last for a few days. Less than 1% of WNV cases develop neuroinvasive symptoms that affect the CNS such as encephalitis and/or meningitis (Koch et al., 2021). Cases with WNV that affect the CNS have a mortality rate of about 10% (*CDC*, 2021). Neuroinvasive symptoms have been found to still affect those infected with WNV for up to eight years after infection (Murray et al., 2014). There is no vaccine available for WNV. Treatment centers around symptom management such as pain control for headaches. Patients with severe neuroinvasive symptoms are often hospitalized for better monitoring (*CDC*, 2021).

According to the CDC, 52,532 WNV cases were reported between 1999-2020, with 2,456 deaths reported. A total of 25,849 of these cases were neuroinvasive (*CDC*, 2022). This is concerning, as close to 50% of all cases are resulting in life-altering symptoms. Since a majority of WNV cases are asymptomatic, these numbers most likely do not reflect the actual number of infections due to underreporting (Clark & Schaefer, 2022).

2.3.2 Risk Factors of WNV Disease

2.3.2.1 Geographic Location

One of the most important risk factors associated with WNV is geographic location. An area's climate, ambient temperature, precipitation, relative humidity, landscape and land-use, and avian population, can affect the prevalence of WNV (Paz & Semenza, 2013). One specific study conducted in Houston Texas found that living near slow moving and stagnant water sources is associated with an increased risk of contracting WNV (Nolan et al., 2012). They also found that living near faster moving bodies of water had a decreased risk of contracting WNV (Nolan et al., 2012). Another study conducted in Houston, which included homeless individuals, found that increased outdoor exposure is also associated with a higher risk (Meyer et al., 2007). A seroprevalence of 6.8% was found in their total sample and a 16.4% seroprevalence was reported in those who had been homeless for longer than a year (Meyer et al., 2007). This is significantly higher than a seroprevalence of 4.7% found in students, faculty, and staff at the University of Texas (Meyer et al., 2007).

2.3.2.2 Individual Host Risk Factors

There are both population and individual host risk factors that affect the chances of infection and severity of infection. A retrospective study found that having a history of hypertension and cardiovascular disease leads to an increased risk of developing encephalitis

(Murray et al., 2006). Also, chronic renal disease, hepatitis C virus, and immunosuppression increase the risk of death from WNV (Murray et al., 2006).

Multiple studies have also investigated genetic risk factors. One study used a novel *ex vivo* model and found that OAS1 SNP rs10774671 is a risk factor for WNV infection (Lim et al., 2009). The OAS1 gene is an antiviral gene, and the presence of WNV reduces its ability to function (Lim et al., 2009). SNPs in RFC1, SCN1a, ANPEP have also been connected to severe neurological disease related to WNV (Loeb et al., 2011). The CCR5 gene has produced mixed results in relation to WNV infection risk and severity. The most current research points to CCR5Δ32 being associated with more severe clinical outcomes but not as a risk factor for initial WNV infection (Lim et al., 2010). Interestingly, this deletion is known to be protective against HIV (Lopalco, 2010). These studies were candidate gene association studies and there are currently no Genome-Wide Association Studies (GWAS) published on WNV infection or disease risk.

Researchers have also investigated age as a risk factor. A serum sample study conducted in Ohio found that children had a higher chance of infection but a much lower risk of neuroinvasive disease (Mandalakas et al., 2005). Older populations have an increased risk of morbidity and mortality when infected with WNV (Qian et al., 2011). One reason for this is that aging lowers the production of type I IFN in dendritic cells which results in a weakening of the blood brain barrier (Kong et al., 2008).

2.4 Public Health Response to West Nile Virus

2.4.1 Public Health Programs

There is a wide variety of WNV public health initiatives occurring globally. Within the United States, programs differ from state to state. WNV was first detected in Pennsylvania in the year 2000 (Revesz & Wu, 2006). The Pennsylvania Departments of Health, Environmental Protection, and Agriculture created a plan that focused on surveillance, mosquito population management, and education. This program is conducted in thirty-eight counties, including Allegheny County, with the highest risk for mosquito-borne diseases.

Education is a valuable tool in WNV prevention. A study conducted in Kansas evaluated various methods used to distribute WNV information to the public in the beginning of the disease's emergence in the US (Averett et al., 2005). They found that the most influential factors that affected understanding of WNV campaigns were, message content, type of media used, and delivery (Averett et al., 2005). Mass media coverage and person-person conversations were the most effective delivery method while healthcare providers, magazines, the internet, and brochures were not as successful (Averett et al., 2005). One recommendation for the best public health practices regarding WNV, was to lessen barriers that were causing undesired behaviors that increase one's risk for contracting WNV (Averett et al., 2005). The most important barrier to remove was the public's feelings towards the safety of DEET, an ingredient found in many insect repellents (Averett et al., 2005).

California's public health initiatives were evaluated in a study that looked at the effectiveness of the surveillance and response plans from 2009-2018 (Danforth et al., 2022). Data related to environmental surveillance was used to create a model that assessed the overall risk level of WNV and how that is associated with human cases (Danforth et al., 2022). Overall, it was found that California's surveillance system and response plan improved their ability to predict incidence of human WNV cases (Danforth et al., 2022). Many states solely use the vector index (VI), an estimate of the average number of WNV infected mosquitoes in each area, to predict incidence of WNV (Danforth et al., 2022). While the study's model showed a positive relationship between incidence and VI, California's risk level system had an even stronger correlation with predicting WNV cases (Danforth et al., 2022). This was largely due to the inclusion of temperature, dead birds, and sentinel chickens into their predictive models (Danforth et al., 2022). While this model was more successful in its predictiveness than other models, more information would need to be collected to specify it as an "accurate" model (Danforth et al., 2022).

Recently, a framework was created that analyzed the quality of statistical models used to predict human cases in the United States. Thirteen models including California's Risk Assessment model, were compared to achieve the studies goal which was to eliminate gaps in model development (Keyel et al., 2021). Their framework identified the value of each model in WNV-based decision-making and the correct spatial and temporal scope for each model (Keyel et al., 2021). They found that models with a county level resolution are far more common and that there is a need for short-term planning models (Keyel et al., 2021). Another finding suggests that the localization factor on many of the models severely limits the generalizability of these models (Keyel et al., 2021). This strengthens the idea that multiple models should be used to aid vector control and public health practices, to ensure the most effective methods are in use (Keyel et al., 2021).

In Canada, specifically Ontario, all municipalities are mandated to conduct surveillance and risk assessments for human infection (Bamotra et al., 2020). This surveillance data is used to develop vector-borne disease management strategies (Bamotra et al., 2020). A study analyzed the effectiveness of a specific risk-assessment tool used by the Region of Peel. They found that virus isolation rate in the vector, and local WNV activity were strongly associated with human WNV cases. Along with these variables, temperature and the final risk-assessment score were associated with a mosquito trap being positive for WNV (Bamotra et al., 2020). A limitation to this and similar models, is that low WNV positive human cases make the predictiveness hard to evaluate (Bamotra et al., 2020).

WNV is also endemic in many areas of Europe, including northern Italy (Marchino et al., 2021). Italy has a national plan for WNV surveillance that creates the framework for implementation of regional level programs (Marchino et al., 2021). In Italy, surveillance follows a multi-disciplinary “One Health” approach that includes experts from animal, human, and environmental health (Rizzo et al., 2016). A study evaluated the effectiveness of this approach and found that inter-institutional and interdisciplinary practices aid in the formation of effective surveillance (Marchino et al., 2021). The use of transdisciplinary research approaches in evaluating surveillance systems has the potential to increase the effectiveness of these symptoms (Marchino et al., 2021).

3.0 Materials and Methods

3.1 Mosquito Collection

Allegheny County's vector control program conducts its mosquito collection season from early May until the end of September. This collection period coincides with the peak mosquito breeding season in this area. Throughout the season, twenty-five fixed gravid trap sites were placed each week. These sites were broken up into four regions, North, East, South, and West. Traps were set on Monday's and Wednesday's and collected on Tuesdays and Thursdays. Each trap was set for approximately 24 hours.

Gravid traps were used for the collection of mosquitoes. As the name implies, this type of trap specifically targets female *Culex* mosquitoes searching for an egg-laying habitat. The parts of a gravid trap include a motor, fan, battery, collection chamber, PVC pipe, and a toolbox like shell. The trap is set on a tray that contains a liquid called gravid water or "stink juice". This liquid consists of water, hay, lactalbumin, and yeast. It is designed to mimic an egg-laying habitat. Once the trap is set, female mosquitoes are attracted to the gravid water. When they approach the liquid to lay their eggs, they are sucked up through the PVC pipe by the fan and are trapped in the collection chamber.

After 24 hours, each trap is collected. The traps are examined to see if they are still running or if they have been tampered with. Once this is done, the collection chamber containing the mosquitoes is removed and labeled with the time and location of collection. After all traps are collected, the mosquitoes are brought to ACHD's Housing and Community Environment (HCE) office for initial processing.

3.2 Mosquito Processing

The initial processing of the mosquito samples started with placing the specimens on dry ice. This immobilizes the mosquitoes for ease of sorting. Each sample spent approximately 1 hour on ice. A unique sample identifier (USI) was created for each sample. Information on these USI's included site location, county, date, name of collector, habitat type, and mosquito count. Next, the samples were sorted based on their categorization as a mosquito or non-mosquito. Mosquitoes were counted and placed into a small vial. These vials were then placed into a larger bottle containing the USI. The bottles were stored on dry ice until Thursday of each week when they were shipped to the DEP lab in Harrisburg for WNV testing.

USI's were tracked via Greenport, a database used by the DEP. Collectors entered each USI into the database. Once mosquito samples had been shipped after approximately 1 week, WNV results were available. The state lab used polymerase chain reaction (PCR) testing to screen for WNV. They also identified the species of each mosquito in the sample, as well as the exact count. Once a sample was processed by the state, the USI's in Greenport are updated. WNV+ samples were reported to ACHD via email.

3.3 Weather Data

Weather data for Pittsburgh Pa from 2016-2022, was gathered from NOAA using their NOWData past weather tool. Average monthly temperature and average monthly precipitation were obtained from each year. Only the months from May-September were used as they coincide with the vector control season.

4.0 Statistical Analysis

First, a mosquito positivity rate for WNV was calculated using mosquito count and WNV+ data from each year, $Rate = \frac{\#of\ WNV+}{Mosquito\ Count} \times 1000$.

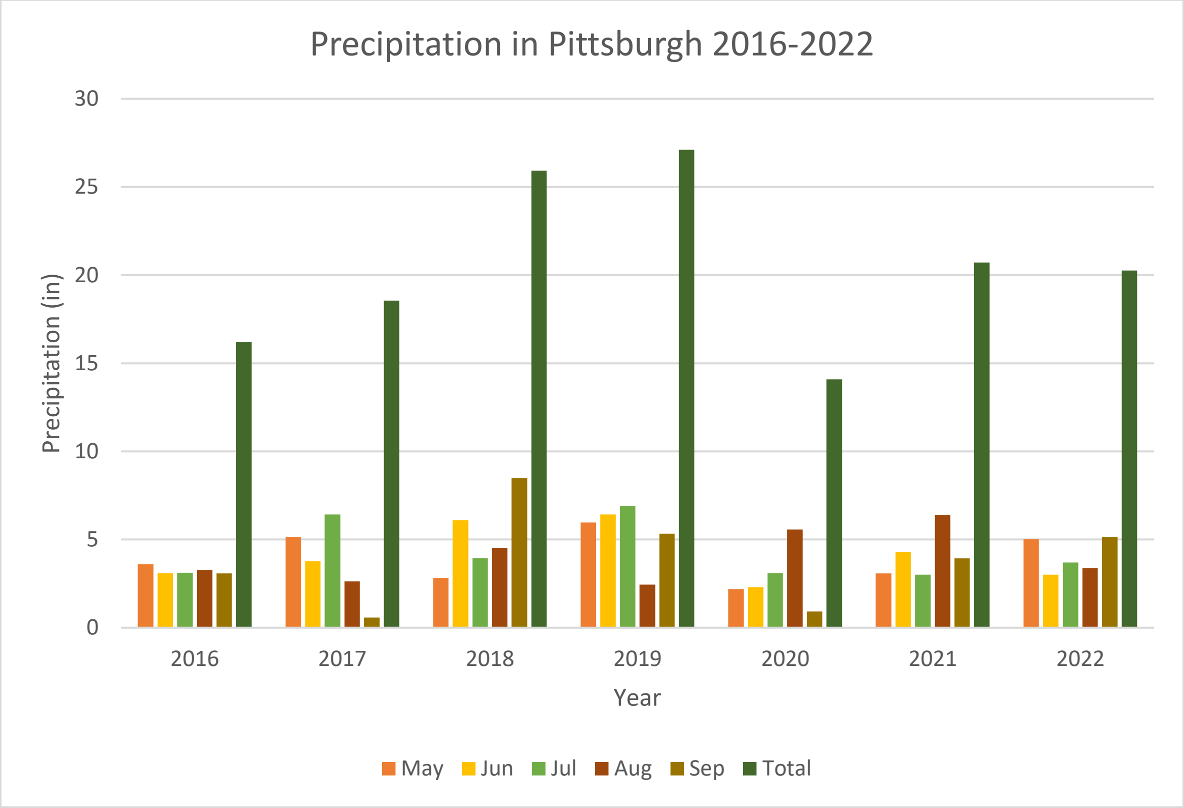
For each year, multiple relationships between variables were tested. The number of traps set, temperature, and precipitation represented the independent variables in this study. Mosquito count and WNV+ samples represented the experimental variables in this study. Multiple regressions were conducted which analyzed the relationship between the number of traps set and mosquito count, as well as WNV+ samples. Relationships between mosquito count and precipitation/temperature were examined. WNV+ samples were also analyzed with temperature and precipitation. Multiple linear regressions were run using Microsoft Excel. Regressions with p-values ≤ 0.05 were considered significant. Data from NOAA and the DEP were used for these calculations.

5.0 Results

Table 1 Average Precipitation (in) in Pittsburgh

Year	May	Jun	Jul	Aug	Sep	Total
2016	3.61	3.1	3.12	3.29	3.08	16.2
2017	5.15	3.78	6.42	2.63	0.58	18.56
2018	2.83	6.1	3.96	4.53	8.5	25.92
2019	5.97	6.42	6.92	2.45	5.34	27.1
2020	2.19	2.3	3.1	5.57	0.92	14.08
2021	3.09	4.29	3	6.41	3.93	20.72
2022	5.03	3	3.69	3.39	5.15	20.26
Average	3.98	4.14	4.32	4.04	3.93	20.41

Figure 1 Precipitation in Pittsburgh 2016-2022

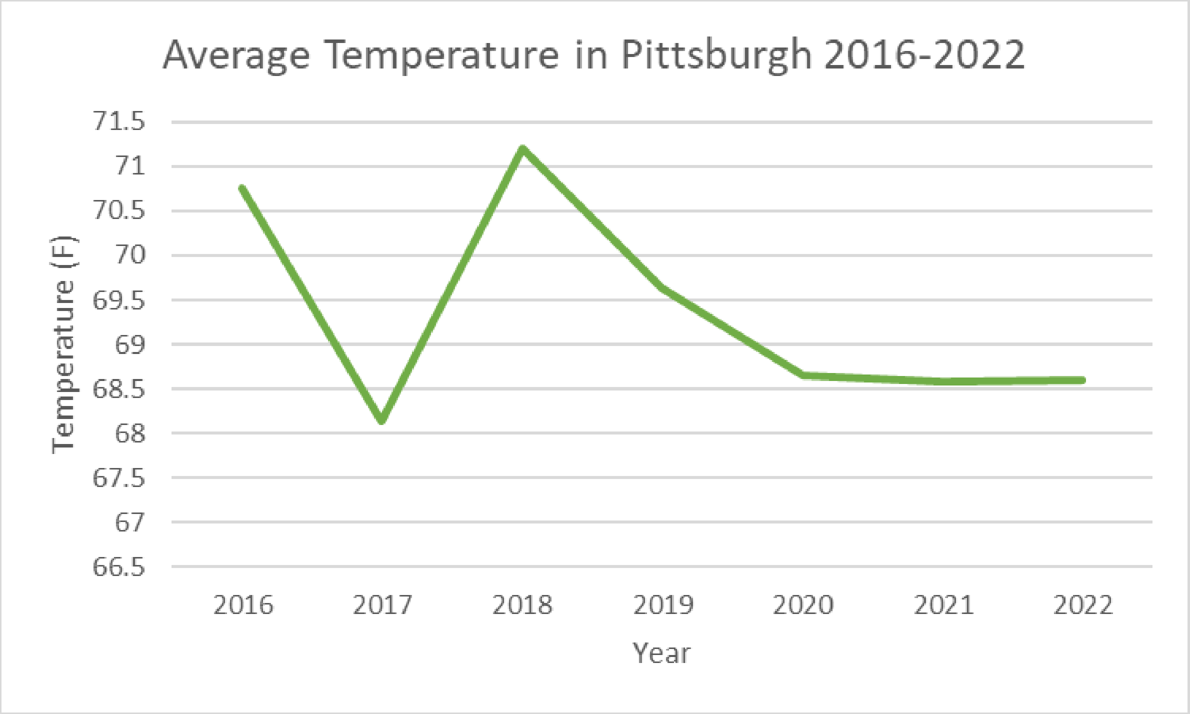


Precipitation totals during the vector control season ranged from 14.08 inches in 2020 and 27.1 inches in 2019. The average rainfall from 2016-2022 was 20.41 inches. June and July saw the highest monthly average per year at 4.14 inches and 4.32 inches respectively (Table 1, Figure 1).

Table 2 Average Temperature (F) in Pittsburgh

Year	May	Jun	Jul	Aug	Sep	Average
2016	61.2	71.6	75.5	76	69.5	70.76
2017	60.6	69.7	73.7	70.3	66.4	68.14
2018	69	70.8	73.5	73	69.7	71.2
2019	64	68.3	75	71.9	69	69.64
2020	58.2	70	77.3	73.2	64.6	68.66
2021	58.5	71.2	72.5	74.4	66.3	68.58
2022	62.8	69.6	74.2	72.1	64.3	68.6
Average	62.04	70.17	74.53	72.99	67.1	69.37

Figure 2 Average Temperature in Pittsburgh 2016-2022



Average temperature per year during the vector control season ranged from 68.14°F in 2017 to 71.2°F in 2018. The average temperature from 2016-2022 was 69.37°F. July and August saw the highest average temperature per year at 74.53°F and 72.99°F respectively (Table 2, Figure 2).

Table 3 Traps Set per Year

Year	# Traps Set
2016	404
2017	392
2018	396
2019	399
2020	579
2021	501
2022	499

From 2016-2022 there was a total of 3,170 fixed gravid traps. 2017 saw the fewest traps set with 392 and 2020 saw the most traps set with 579 (Table 3).

Table 4 Mosquito Count and WNV+ Samples per Year in Pittsburgh

Year	Mosquito Count	WNV+ Samples	Positivity Rate per 1000 Mosquitoes
2016	25170	142	5.64
2017	21613	72	3.33
2018	41407	214	5.17
2019	27113	26	0.959
2020	30756	101	3.28
2021	32205	138	4.29
2022	25676	133	5.18

Mosquito counts between 2016-2022 ranged from 21,613-41,407. 2017 had the lowest number of mosquitos reported and 2018 had the highest number of mosquitos reported. WNV+ samples ranged from 26-214, with 2019 seeing the fewest and 2018 seeing the most (Table 4).

Table 5 # of Traps Set Vs. Mosquito Count

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.089271973
R Square	0.007969485
Adjusted R Squar	-0.190436618
Standard Error	80.40297078
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	259.6685952	259.668595	0.04016754	0.849050368
Residual	5	32323.18855	6464.63771		
Total	6	32582.85714			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	423.2245878	150.9442422	2.80384718	0.03782158	35.21006055	811.23912	35.21006055	811.239115
Mosquito Count	0.001017103	0.005074896	0.20041841	0.84905037	-0.012028332	0.0140625	-0.012028332	0.014062537

Table 6 # Traps Set Vs. WNV+ Samples

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.02407397
R Square	0.00057956
Adjusted R Square	-0.1993045
Standard Error	80.7018879
Observations	7

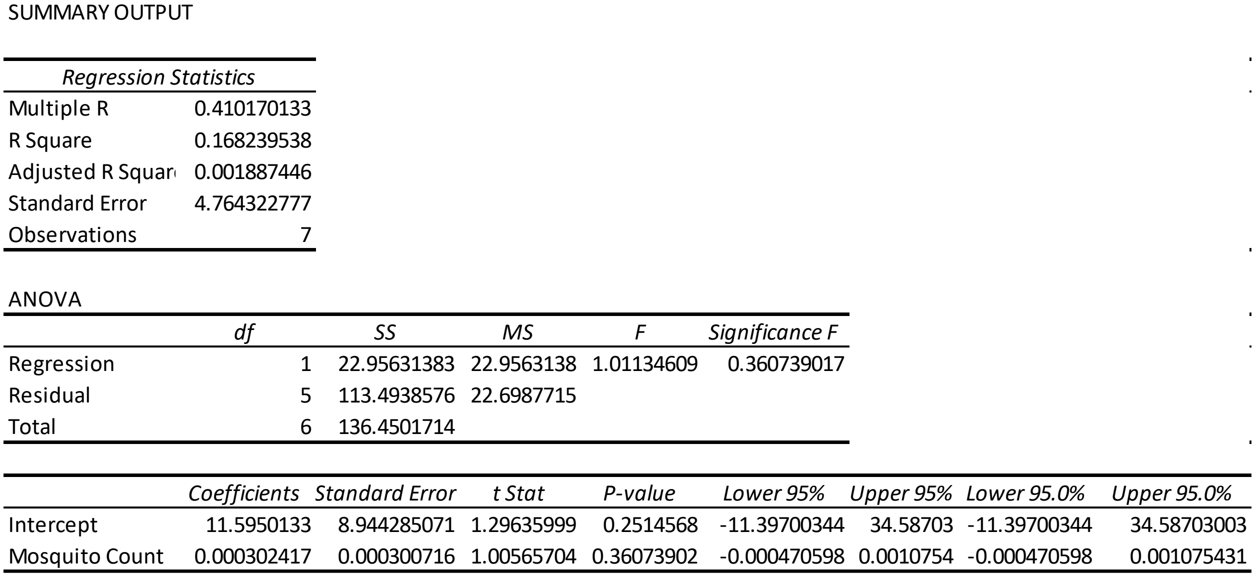
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18.88358546	18.88359	0.0029	0.959142608
Residual	5	32563.97356	6512.795		
Total	6	32582.85714			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	449.342532	72.04631442	6.236857	0.00155	264.1415851	634.54348	264.1415851	634.5434795
WNV+	0.02978484	0.553142038	0.053847	0.95914	-1.392112041	1.4516817	-1.39211204	1.451681711

A regression was calculated from the number of traps set per year vs. their respective mosquito counts. The number of traps set had little to no influence on mosquito count, $R^2 = 0.008$, $P = 0.849$ (Table 5). Another regression was calculated from the number of traps set per year vs. their respective WNV+ samples. The number of traps set had little to no influence on WNV+ samples $R^2 = 0.0006$, $P = 0.959$ (Table 5, Table 6).

Table 7 Total Precipitation (in) Vs. Mosquito Count



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.410170133
R Square	0.168239538
Adjusted R Squar	0.001887446
Standard Error	4.764322777
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	22.95631383	22.9563138	1.01134609	0.360739017
Residual	5	113.4938576	22.6987715		
Total	6	136.4501714			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	11.5950133	8.944285071	1.29635999	0.2514568	-11.39700344	34.58703	-11.39700344	34.58703003
Mosquito Count	0.000302417	0.000300716	1.00565704	0.36073902	-0.000470598	0.0010754	-0.000470598	0.001075431

A regression analysis was calculated using the total precipitation per year vs. their respective mosquito count. Total precipitation had little to no influence on mosquito count, $R^2 = 0.168$, $P = 0.361$ (Table 7).

Table 8 Average Temperature (F) Vs. Mosquito Count

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.53285257
R Square	0.28393186
Adjusted R Square	0.14071823
Standard Error	1.10900959
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.438374371	2.438374	1.98258	0.218151833
Residual	5	6.149511344	1.229902		
Total	6	8.587885714			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	66.4970664	2.081995359	31.9391	5.7E-07	61.14512696	71.849006	61.14512696	71.84900587
Mosquito Count	9.8561E-05	6.99988E-05	1.40804	0.21815	-8.13765E-05	0.0002785	-8.1377E-05	0.000278499

A regression analysis was calculated using the average temperature per year vs. the respective mosquito count. Average temperature little to no influence on mosquito count, R^2 = 0.284, P = 0.218 (Table 8).

Table 9 Total Precipitation (in) Vs. WNV+ Samples

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.005339583
R Square	2.85111E-05
Adjusted R Squar	-0.199965787
Standard Error	5.223911965
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00389035	0.00389035	0.00014256	0.990935372
Residual	5	136.4462811	27.2892562		
Total	6	136.4501714			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	20.35526798	4.663628246	4.36468494	0.00725822	8.367029926	32.343506	8.367029926	32.34350604
WNV+	0.000427511	0.035805424	0.01193984	0.99093537	-0.091613261	0.0924683	-0.091613261	0.092468284

A regression was calculated using the total precipitation per year vs. their respective WNV+ samples. Total precipitation had little to no influence on WNV+ samples, $R^2 = 2.851$, P = 0.991 (Table 9).

Table 10 Average Temperature (F) Vs. WNV+ Samples

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.52741975
R Square	0.27817159
Adjusted R Square	0.13380591
Standard Error	1.11346126
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.388905854	2.388906	1.92685	0.223774287
Residual	5	6.198979861	1.239796		
Total	6	8.587885714			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	68.118501	0.994038454	68.52703	1.3E-08	65.56324377	70.673758	65.56324377	70.67375815
WNV+	0.01059382	0.007631819	1.388112	0.22377	-0.009024399	0.030212	-0.0090244	0.030212034

A regression was calculated using the average temperature per year vs. their respective WNV+ samples. Average temperature had little to no influence on WNV+ samples, R^2=0.278, P=0.224 (Table 10).

6.0 Discussion

6.1 Mosquito Population Density

6.1.1 Traps Set

Each year there was a varying amount of fixed gravid traps set. These differences could be the result of a variety reasons including but not limited to severe weather conditions, trap tampering, staff availability, and DEP adjustments to the trap number requirements. No significant differences ($p = 0.849$) were observed with the number of traps set per year in relation to mosquito count. While no significant influence was found, the number of traps set per year could theoretically begin to influence results in the number set drastically changes from year to year.

6.1.2 Precipitation

As stated previously, precipitation is known to affect mosquito population density. Pittsburgh saw an average of 20.42 inches of precipitation per year from 2016-2022 (Table 1). This analysis suggests that from 2016-2022 precipitation alone did not have a significant influence on mosquito population density ($p = 0.360$; Tab 7). This was an unexpected result, as precipitation is commonly known to influence mosquito population density. This may be due to the data being limited to seven years as fixed trapping only began in 2016. Other weather conditions not examined here such as storm intensity and frequency, droughts, and humidity, may have also impacted these results. Additional studies including these variables should be performed.

6.1.3 Temperature

Temperature is considered a determining factor of mosquito population density. The average temperature per year in Pittsburgh from 2016-2022 remained steady (Fig 2). No month, or year stood out as a potential outlier and the analysis suggested that temperature alone did not have a significant influence on mosquito population density (p = 0.218; Tab 8). This was an unexpected result, as temperature is commonly known to influence mosquito population density.As previously stated, this may be due to limitations in the time span of available data. This analysis did not examine weather conditions such as storms, droughts, and humidity, all of which may have an influence on results.

6.2 WNV+ Samples

6.2.1 Traps Set

As done with mosquito population density, a regression was computed to determine if the number of traps set influenced the number of WNV+ samples reported (p = 0.959; Tab 5). This value is not significant and therefore suggests that the number of traps set did not influence the number of WNV+ samples. This could change if the number of traps set dramatically changed from year to year. If the numbers set do dramatically change, further analysis should be conducted to account for this difference.

6.2.2 Precipitation

A regression was computed to determine if precipitation totals per year influenced the number of WNV+ samples per year (p = 0.991; Tab 6). This value is not significant and suggests that precipitation totals alone did not have an influence on the number of WNV+ samples reported. This was an unexpected result, as precipitation is known to influence WNV prevalence in mosquito populations. A second regression was computed without the values from 2019 to determine if the low value of 26 WNV+ samples was influencing the results. The p-value remained statistically unsignificant.

6.2.3 Temperature

A regression was computed to determine if average temperatures per year influenced the number of WNV+ samples per year (p = 0.224; Tab 7). This value is not statistically significant and suggests that temperature alone did not have an influence on the number of WNV+ samples reported. This was an unexpected result, as temperature is known to influence WNV prevalence in mosquito populations. Another regression without 2019's data was computed, and the p-value remained statistically unsignificant.

6.2.4 Limitations and Next Steps

As mentioned previously, an important limitation to this study is only having seven years of data available. The DEP database does have data available starting in 2002, however fixed site trapping only began in 2016. Prior to 2016, trapping locations were considerably more inconsistent than the use of fixed locations. However, it is possible for this data to be used to supplement this study and represents a possible next step. As the vector control program continues collecting data in future years, this study could be replicated to determine if precipitation and or temperature are significantly influencing mosquito population density and the number WNV+ mosquito samples over time.

Another potential limitation is the lack of site-specific weather data. NOAA's weather data, while quite accurate, is limited to regional averages. The vector control program's focus is surveillance, so it is not necessary for site-specific weather data to be collected. However, future studies should consider monitoring this data to create an accurate picture of what is occurring site-to-site. Other variables not included in this study that could be examined include land-use, elevation, urbanicity score, and avian data.

Lastly, this study did not account for the frequency of control measures such as pesticide use and habitat elimination, which may affect mosquito number. Commonly used pesticides often target mosquitoes at larval stages, therefore driving down the number of adult mosquitoes. Habitat elimination such as waste cleanup can also reduce the number of adults mosquitoes. Lower mosquito counts and WNV prevalence could be the result of controls measures instead of changes in temperature and precipitation.

6.3 Conclusions

Vector control surveillance programs and research are vital to public health. Vector-borne diseases pose a serious risk to individuals all over the world. A multitude of variables including reservoir availability, climate, weather, urbanicity, and prevention measures, affect how these diseases spread. For WNV, avian migrations, weather patterns, urbanicity, land-use, and human intervention influence the impact of the virus on human populations. Examining these variables at a local level helps with public health prevention efforts, understanding the extent of the virus, and possibility predicting the severity of the virus. WNV may not pose an immediate risk to Allegheny County at this time given its low incidence rate, however, mutations, climate change, and habitat changes may alter this risk. It is important that the local government, health department, and residents are prepared for this should the situation arise. Preparedness is made possible by the continual monitoring of WNV by ACHD as well as continuing research on the factors influencing the virus and mosquito populations.

While the results in this study did not show temperature or precipitation as having a significant impact on mosquito population density and WNV+ samples in Allegheny County, it is imperative that these variables be monitored. Pittsburgh’s urbanicity, proximity to water sources, weather, climate, and land-use can all be examined as influential factors of WNV’s presence within the city and county. Studying these variables in conjunction with one another rather than individually may yield more significant results.

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