Master of Public Health

Integrative Learning Experience Report

WASTEWATER MONITORING FOR WEST NILE VIRUS TRENDS IN KANSAS

by

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MASTER OF PUBLIC HEALTH

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Summary/Abstract

Purpose and Scope: As part of my Applied Practice Experience at the Kansas Department of Health and Environment, this project explored the application of wastewater monitoring for West Nile Virus (WNV), a mosquito-borne virus with a high rate of asymptomatic infection. The goal was to assess whether wastewater data could enhance traditional surveillance methods and provide additional insight for public health professionals and evaluate the global implementation of similar approaches.

Activities and Methods: My activities included statistical and spatial analyses using reported human WNV case data, wastewater monitoring data, serological data, and online mosquito search trends to explore wastewater monitoring for WNV from Kansas Department of Health and Environment. Autoregressive Integrated Moving Average models were used to model human WNV cases and assess utility of wastewater monitoring data, and Geographic Information System (GIS) tools were applied to visualize global incidence rate patterns and clustering.

Findings: Wastewater WNV detection was moderately associated with reported human WNV cases at a five-month lag. The best model, which included wastewater, Google Trends, and immunoglobulin G testing data, outperformed models without the wastewater input. Spatial analysis showed regional clustering in North America and Europe, with mean centers near the Midwest and Mediterranean. A literature review identified over 25 countries exploring wastewater monitoring globally.

Significance: These findings suggest wastewater monitoring supports WNV surveillance efforts in Kansas. Public health agencies should consider expanding wastewater-based monitoring, particularly in areas with underreported human cases. Continued research is needed to validate these findings over multiple seasons and inform policy decisions around integrated mosquito-borne disease monitoring.

Subject Keywords: Wastewater, West Nile Virus, Mosquito-borne Disease, Wastewater Monitoring, Wastewater-based Epidemiology, Arbovirus

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Chapter 1 - Literature Review

As part of my Applied Practice Experience (APE), I worked with the Kansas Department of Health and Environment (KDHE) in Topeka, Kansas, which represents the Kansas population. The agency's mission is to "protect and improve the health and environment of all Kansans." (KDHE, 2025). I worked with the Informatics and Molecular Epidemiology Program and Infectious Disease Epidemiology & Response, under the guidance of Dr. John Anderson, PhD, Dr. Erin Petro, DVM, MPH, DACVPM, and Chris Carter, MPH. I explored the potential application of wastewater monitoring to enhance the surveillance of West Nile Virus (WNV) in Kansas. This project aimed to evaluate whether wastewater surveillance could complement existing tools—such as clinical case reporting, syndromic surveillance, and mosquito monitoring—in detecting trends in WNV incidence.

The motivation for this project stems from the growing need to strengthen surveillance systems for mosquito-borne diseases, which are often underreported due to high rates of asymptomatic infections and limited clinical testing. Given WNV's endemic presence in Kansas and limited ability of traditional systems to capture its true incidence, KDHE initiated efforts to assess wastewater monitoring as an additional surveillance system. This project also included a global review of WNV trends and wastewater implementation, as well as communication strategies to convey technical findings to public health professionals and stakeholders.

Wastewater Monitoring

Wastewater monitoring, also known as wastewater surveillance, is a tool that public health professionals utilize to monitor different pathogens (e.g. influenza A & B virus, Respiratory Syncytial Virus (RSV), and measles virus) in a community. This process includes collecting samples from community or institutional sewer systems and detecting harmful pathogens from the samples. In the 1930's, it was discovered that the polio virus could be detected in sewage; allowing epidemiologists to understand the virus and identify areas of transmission and trace local outbreaks (Diamond et al., 2022). Wastewater monitoring has been successfully used during the COVID-19 (SARS-CoV-2) pandemic to monitor the presence of the SARS-CoV-2 virus in communities (Diamond et al., 2022; Singer et al., 2023). Analyzing the genetic code of SARS-CoV-2 from wastewater provided public health professionals with early insights into different lineages of SARS-CoV-2 circulating within the community—sometimes before the virus was detected in patients (Diamond et al., 2022).

Wastewater monitoring captures data regardless of whether individuals seek healthcare or show symptoms. Public health professionals can use wastewater monitoring to complement traditional case reporting and clinical data to better detect and monitor the spread of disease. Wastewater monitoring is a cost-effective method for identifying potential outbreaks, as well as the emergence, or re-emergence of diseases within communities (Diamond et al., 2022). A pilot implementation and evaluation study at 17 Air Force bases in the United States (US), found that the cost of monitoring wastewater for pathogens was between US $10.5 - $18.5 million less expensive annually compared to clinical diagnostic surveillance (Sanjak et al., 2024). In Kansas, wastewater samples are collected at several wastewater treatment plants and represent SARS-CoV-2, Influenza A & B, RSV, Measles, and West Nile Virus (WNV) present in the entire sewershed (i.e. area where all the wastewater flows in the sewer system) of the treatment plant. Globally, the end of the COVID-19 public health emergency has allowed jurisdictions to apply wastewater-based epidemiology to other diseases, such as arboviruses, in their communities (Lee et al., 2022).

There are challenges and opportunities using wastewater to monitor arboviruses. Arboviruses are pathogens transmitted by arthropods, such as mosquitoes, ticks, fleas, and others. More than 500 arboviruses have been identified and more than 100 can cause disease in humans (Lee et al., 2022). A few examples of arboviruses that can cause disease in humans include Dengue virus, WNV, Yellow Fever virus, and Eastern Equine Encephalitis virus (Lee et al., 2022). In the US, the spread of these viruses is monitored through case reporting, laboratory syndromic surveillance, vector surveillance, and animal surveillance. However, these methods of surveillance underestimate the true incidence of arboviruses in humans because they require contact with a healthcare provider, correct diagnosis, and reporting of the results. Mosquito-borne pathogens are increasing in humans and are spreading around the world, except Antarctica, leading to considerable strain on societies and economies (Lee et al., 2022). Therefore, the application of wastewater-based monitoring for arboviruses could be an effective tool for public health professionals to identify and monitor arboviral spread across the world (Lee et al., 2022).

West Nile Virus

West Nile Virus was first introduced to the US in 1999 in New York City and has become a significant threat to humans, horses, and birds (Vazquez-Prokopec et al., 2010). The Centers for Disease Control and Prevention (CDC) estimates that 80% of individuals infected with WNV are asymptomatic, while 1% or less of infected individuals have severe neurological symptoms (CDC, 2021; Kuhn et al., 2025). The most common type of mosquito that spreads WNV in the United States is the *Culex* mosquito. These mosquitoes are often found in urban areas and are spreading to new areas, making WNV an important emerging arbovirus (Gorris et al., 2021; Kuhn et al., 2025). According to Kuhn et al., WNV is the top leading mosquito-borne disease and has a higher risk of potential local outbreaks compared to other arboviruses.

There are control measures that eliminate mosquitoes, that the public and agricultural individuals can use or apply to reduce their exposure to WNV ("Public Education", n.d.). These include:

- Using United States Environmental Protection Agency (EPA) approved mosquito repellent, pesticides, and insecticides along with following the label directions for product application.
- Repair door, window screens, faucets, and Air conditioning units.

- Schedule irrigation and allow the water to soak completely into the soil before applying more water (“Public Education”, n.d.).
- Proper disposal of old tires, cans, buckets, bottles, or any other containers that hold water to reduce breeding sites in the community.

Local and state governments can also help communities by using these control measures. It is important to keep individuals informed on appropriate vector control measures to help reduce mosquito breeding sites and WNV risk in the community (“Public Education”, n.d.).

In the US, it is important to know the different species of *Culex* mosquitoes and their host feeding patterns to help deter them from feeding off humans and animals. There are several different *Culex* (*Cx.*) species that are seen in the US. These species are *Cx. pipiens*, *Cx. tarsalis*, *Cx. quinquefasciatus*, *Cx. restuans*, and *Cx. salinarius*. According to the Northeast Regional Center for Excellence in Vector-Borne Diseases, *Cx pipiens* can be found in suburban areas and live in the northeast, northwest, and north midwestern regions of the US (Gorris et al., 2021). However, these mosquitoes are not likely to feed on humans (Rochlin et al., 2019). *Culex pipiens* usually take blood meals from birds and other mammals (Molaei et al., 2006). *Culex quinquefasciatus* live in urban areas in the southern part of the US, closer to Mexico (Gorris et al., 2021). *Culex tarsalis* tend to thrive in hot and dry and rural locations on the western side of the US, and do not normally feed humans. They are more likely to take a blood meal from birds or other mammals (Rochlin et al., 2019). *Culex salinarius* are more opportunistic in their blood meals than the other species and are more likely to feed on humans (Molaei et al., 2006). *Culex salinarius* are most often found in urban Midwest and Eastern portions of the US (Gorris et al., 2021). The differences with vector competency—the ability of Culex mosquitoes to transmit WNV—and their host feeding preferences shape human transmission patterns in different geographic areas.

Wastewater and West Nile Virus

Public health professionals, especially epidemiologists, can use wastewater as a method to monitor WNV spread in the communities they work with. An observational study conducted in Atlanta, Georgia, found that WNV was spatially clustered in areas with combined sewer overflows (i.e. a wastewater term for sewer systems that are overloaded with rainwater and sewage and causing it to be released in the environment) and had a higher number of *Culex* mosquitoes (Vazquez-Prokopec et al., 2010). These areas seemed to have a higher risk of WNV infections and *Culex* mosquitoes, along with human and bird cases (Vazquez-Prokopec et al., 2010).

Another observational study increased knowledge of different emerging pathogens that could be identified in wastewater. Between February 2021 - April 2023, researchers collected two samples from two wastewater treatment plants in the San Francisco Bay Area, California. These two wastewater treatment facilities are 45.6 miles from each other (Google Maps, n.d.). The researchers tested for human rotavirus, adenovirus group F, norovirus GI and GII, hepatitis A virus, enterovirus, enterovirus D68, *Candida auris*, and WNV (Boehm et al., 2023). All but one of these pathogens in the study was detected either at both facilities, one facility, or for a certain time of the year; however, WNV was the only virus not detected at all (Boehm et al., 2023).

An observational study in Miami, Florida, applied wastewater monitoring to identify Dengue virus. Dengue is an arbovirus spread by mosquitoes, similar to WNV. The authors took samples from three wastewater treatment plants in the Miami-Dade County area, which covers approximately 95% of the county's population (Wolfe et al., 2023). Then, to compare Dengue cases with the wastewater samples, the authors acquired a weekly arbovirus report from the Florida Department of Health (Wolfe et al., 2023). The authors were able to detect one of the Dengue serotypes in all three wastewater treatment plants. These results aligned with the human reported cases from the Florida Department of Health. There was no detection of mosquitoes carrying the specific serotype, showing that wastewater was able to detect Dengue before mosquito pool surveillance. The types of mosquito pool surveillance used to compare wastewater detection were not specified in the study (Wolfe et al., 2023).

Another observational study demonstrated the detection of WNV in wastewater samples across Oklahoma (Kuhn et al., 2025). The authors aimed to develop and establish a long-term wastewater monitoring tool for WNV in the state of Oklahoma. Wastewater samples were collected from sixteen counties from July-September in 2023 and then analyzed using a digital droplet PCR at the University of Oklahoma. The wastewater monitoring results were compared with simultaneously reported WNV cases in humans, horses, and mosquito pools (Kuhn et al., 2025). West Nile Virus was detected in three counties, which represents approximately 1.6 million people in Oklahoma. The wastewater-based WNV detection overlapped with human and mosquito pool surveillance data, with half of the counties aligning with wastewater and mosquito monitoring. In contrast, there was no WNV detection from five rural wastewater samples where no human WNV cases were reported. Additionally, there was no WNV detection in rural counties, possibly due to lack of centralized sewage infrastructure and due to private septic systems. Finally, there were no findings of horse fecal material detected in wastewater, suggesting that horse infections did not contribute to wastewater-based WNV detections (Kuhn et al., 2025).

There are several studies that show wastewater monitoring being applied as a surveillance tool for many different pathogens in a community. However, there are limited studies detecting WNV in wastewater. Given the emerging use of wastewater-based monitoring for WNV, it may take time to develop laboratory methods that are optimized for the detection of WNV in wastewater.

An estimated 30 countries are applying wastewater-based epidemiology to monitor pathogens in their communities (Singer et al., 2022). Most countries have not applied wastewater monitoring for WNV to reduce WNV exposure; however, wastewater monitoring is still being explored. Integration of WNV wastewater-based monitoring with current prevention strategies could help public health professionals have a more accurate method to reduce WNV spread within their communities. The virus is endemic (i.e. disease is consistently present is a specific area/population) to the United States, but only 1% or less of infected individuals have severe symptoms (CDC, 2021). The CDC states that the use of passive surveillance systems (i.e. reported diseases voluntarily or routinely by healthcare providers, laboratories, or institutions—without outreach) for WNV is likely underreporting the true incidence of WNV. Other studies estimate that 30-70 non-neuroinvasive WNV cases occur for every reported WNV neuroinvasive case (CDC, 2025b). However, detecting a virus like WNV is proving to be a challenge; only one study has detected WNV in wastewater. Overall, there is a need to further study wastewater monitoring as a tool for detecting WNV in our communities so that we can limit the amount of severe WNV disease that our communities experience.

Chapter 2 - Learning Objectives and Project Description

The following learning objectives guided the activities of my APE at KDHE, developed both my technical and communication skills in the context of multiple surveillance tools for WNV. My first objective was to gain hands-on experience using KDHE's electronic disease surveillance system to access and utilize relevant epidemiological data for my project. I also aimed to strengthen my data management and analysis skills by cleaning and working with complex, real-world datasets that included wastewater, clinical, serological, and mosquito surveillance data. A key objective was to apply statistical methods—including time-series and spatial analysis—to explore how interpreting multiple data sources could improve the understanding of WNV incidence in Kansas. In addition to data analysis, I sought to improve my ability to communicate technical concepts related to wastewater monitoring and WNV to public health professionals through written materials and oral presentations. Another important objective was to identify and summarize foundational resources related to wastewater-based epidemiology and vector-borne diseases, which aimed to support the development of practical tools and educational materials. Finally, I sought to apply a systems thinking approach by creating diagrams that visually represented the WNV and wastewater surveillance processes to aid public health officials' understanding of different monitoring tools for WNV.

Data Collection

There were multiple different data sets for Kansas used during research. The wastewater-based WNV detection was collected from the Kansas Health and Environment Laboratories from April 2024-July 2025. Human and animal WNV cases from 2002-July 2025 were collected from public data; however, only human WNV cases were used in our study (KDHE, 2025). The syndromic surveillance data includes Immunoglobulin G (IgG) and Immunoglobulin M (IgM) testing data from the Kansas Syndromic Surveillance Program. The Google Trends data were downloaded from Google using the "Mosquito" topic, a collection of search terms relating to mosquitoes, filtered to searches in the “Home & Garden” category. The bird observation data was downloaded from the Cornell Lab on crow, blue jay, and robin bird sightings.

Statistical Analysis

Using R and RStudio, we combined wastewater, syndromic surveillance, Google Trends, and bird observation data into one table. From this table, we were able to train an Autoregressive Integrated Moving Average (ARIMA) model for our statistical analysis. The ARIMA model allows us to produce forecasts and understand the impact of predictor variables on model fit and forecast accuracy. The Autoregressive (AR) means that the model identifies whether the data is correlated with its past values in the time series itself (Hyndman & Athanasopoulos, 2021). The Integrated (I) part of the ARIMA model uses differencing to make the time-series of the data stationary. Differencing is used to transform non-stationary data into stationary data. An ARIMA model requires the data to be stationary, otherwise the model could identify patterns when there are none (Hyndman & Athanasopoulos, 2021). The Moving Average (MA) calculates past prediction errors and then uses those errors to create better forecasts (Hyndman & Athanasopoulos, 2021).

The first step was to run the Kwiatkowski-Phillips-Schmidt-Shin (KPSS) unitroot test to confirm that the data was stationary to continue with the ARIMA model. The KPSS test measures if the data is stationary, meaning that we had a constant mean and variance over time and no long-term trends were in our data. The KPSS test is the Integrated section of the ARIMA model. After the KPSS test, we then ran a time-series cross-correlation to find strong and appropriate lags between the outcome and predictor variables. These lags show an increase in the predictor variable (e.g. wastewater WNV concentration, Google Trends) leads to an increase in our outcome variable (human WNV cases). The cross-correlation function (CCF) measures how strongly two time series are correlated at various lags, which is used for building our ARIMA model for the best possible predictive model. Once we confirmed that our data was stationary and we had appropriate lags from our time series, we could start building the ARIMA model.

We started with a baseline ARIMA model—or a control model—using the human WNV case data to see if the past human cases could predict future ones. After our baseline model, we started to add the predictor variables—such as the wastewater WNV concentration, Google Trends, syndromic surveillance, and the bird observations—to discover if the model could improve its ability to predict future human WNV cases. To understand which of the predictors were the better fit, we started with all the predictor variables and then performed a backwards selection (i.e., removed one predictor variable at a time). After we were down to one predictor variable, we tested each predictor individually against the human WNV cases. This allowed us to identify which predictors or combination of predictors were the best fit for forecasting, along with knowing if the wastewater data was helpful.

After training the ARIMA models, we needed to assess which model was the best fit and most accurate. To assess the best model, we used the Akaike Information Criteria (AIC) and the Root Mean Square Error (RMSE). The AIC is a model selection tool that helps to choose the best fit with minimal complexity (Hyndman & Athanasopoulos, 2021). The RMSE measures prediction accuracy; how far off are the model's predictions from the actual observed values. Discovering the best fit and most accurate model includes finding the smaller AIC and RMSE value (Hyndman & Athanasopoulos, 2021). The final step in our statistical analysis involved a Ljung-Box portmanteau test on our ARIMA model residuals (errors) to check for remaining autocorrelation. The Ljung-box test helps confirm whether the model has captured all meaningful time-based patterns. A non-significant result indicates that the residuals are random (Hyndman & Athanasopoulos, 2021).

Geographic Information Systems Analysis

Using ArcGIS Pro version 3.4.0, we were able to analyze and get an overview of the impact of West Nile Virus in other countries, and if those countries have wastewater monitoring or have studied the use of wastewater monitoring. Data was acquired from multiple dashboards, articles, and past seasonal epidemiological reports. Africa and Asia did not have publicly available reported human WNV case data, but multiple articles discovered which countries are impacted by WNV. If the country had or has human WNV cases, they were marked with 'Yes' and all the other countries were marked 'No'. I created point feature classes in a geodatabase using the Create Feature Class tool. I chose the geometry type as point, and named one feature class, "Wastewater Surveillance Present" and the other point feature class, "Environmental Surveillance Present". These point feature classes were created for the Africa and Asia maps. The Europe map also had a point feature class, but it was for the "Wastewater Surveillance Present".

Publicly available data on reported human WNV cases from previous years were available from the US, Canada, and Europe to apply to the spatial analysis. We analyzed data from previous years because 2025 did not provide a representative sample of human WNV cases, as most cases typically occur in August and September. We collected 2024 reported human WNV case data from the US from the CDC. The European CDC contained publicly available 2024 human WNV case data in every country; however, Canada inhibited publicly available reported human WNV case data up to 2023 in each province. Then we calculated a incidence rate of WNV human cases for every US state, Canada province, and European country from the years 2023 and 2024.

Once the reported human WNV incidence rates were mapped, I utilized the average nearest neighbor tool to measure the distribution pattern of the incidence rates in North America—excluding Mexico—and Europe. The average nearest neighbor tool compares the observed mean distance between each point to the expected random distribution utilizing Euclidean distance. The average nearest neighbor tool can also be a precursor to using the spatial autocorrelation tool. After using the average nearest neighbor tool, I was then able to run the spatial autocorrelation tool. The spatial autocorrelation, Global Moran's I tool, measures whether similar values occur near each other in space, and determines if reported human WNV incidence is significantly clustered or dispersed. For the Global Moran's I analysis, the spatial matrix was identified using an inverse distance band conceptualization with the distance band set to the observed mean distance calculated from the average nearest neighbor tool. I also performed a mean center and standard distance spatial analysis to spatially visualize reported human WNV incidence rate. The mean center measures the average location of the reported human WNV incidence rates. The standard distance shows how spread out the reported human WNV incidence rates are around the mean center.

In addition to the international and national analyses, a focused spatial analysis was performed for Kansas. Human WNV incidence rates were calculated by region using reported 2024 case data and regional population estimates. I then applied the average nearest neighbor and spatial autocorrelation tools on Kansas' regional WNV incidence data to evaluate spatial patterns and significant clustering or dispersion. The mean center and standard distance tools were utilized to visualize the central tendency and dispersion of incidence across Kansas. All spatial statistics for Kansas were based on regional boundaries and mapped accordingly.

For the wastewater surveillance facilities in the US and Canada, we collected the location of the wastewater facilities from the US Wastewater Sewer Coronavirus Alert Network (SCAN) Dashboard and Canada's Wastewater monitoring dashboard. Wastewater SCAN is a national leader in wastewater-based epidemiology, transforming the tool into a globally recognized disease surveillance method since the COVID-19 pandemic. Once the facilities names were found, we used Google maps for longitude and latitude to visualize the facilities on the map. Then we determined which wastewater facilities in Canda and the US monitor WNV. We also created a feature class for the state of Oklahoma because their wastewater facilities monitored for WNV but were not part of the SCAN dashboard.

Chapter 3 - Results

The wastewater WNV concentration, reported human WNV cases, bird observations, and syndromic surveillance data displays temporal trends between January 2024 – July 2025 in Figure 3.1. Human and animal WNV cases were first reported in June and peaked in August and September, consistent with late-summer seasonality of mosquito-borne transmission. The proportion of positive IgG tests peaked sharply in February, while the proportion of positive IgM tests for WNV remained low throughout the year. There were small increases in the IgM positive tests in June, September, and November. The Google Trends search activity related to mosquitoes increased substantially from April through July, peaking in May and June with a search index of over 60 (out of 100). Bird counts—including crows, jays, and robins—were highest from February through April, then declined during the summer months. The wastewater WNV concentrations were highest in April with small detections in July, September, and October. The April wastewater WNV concentration preceded the increase in reported human and animal WNV cases.

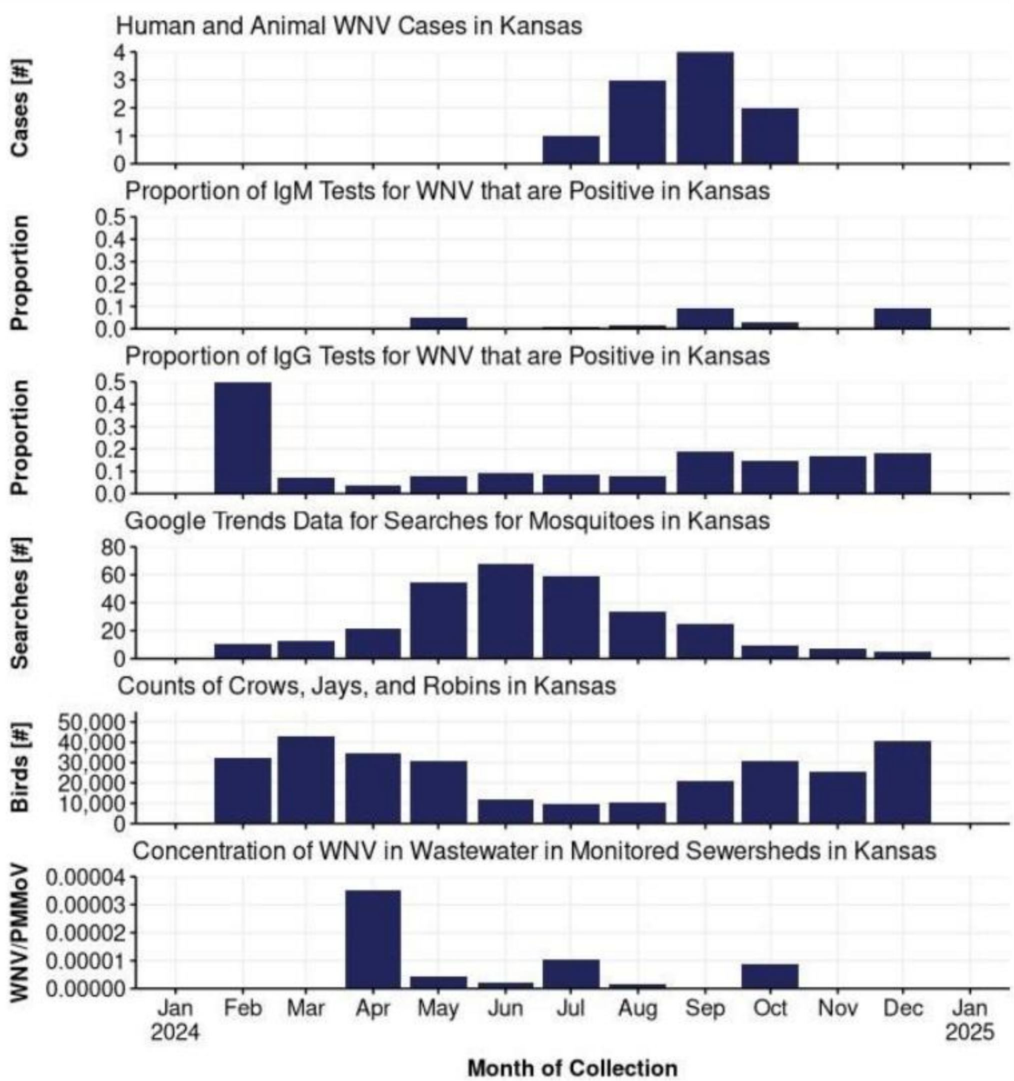

Figure 3.1: An overview of the human and animal WNV cases, proportion of IgM and IgG positive tests for WNV, Google Trends on mosquito and WNV searching, the observation counts of crows, robins, blue jays, and the concentration of WNV in the wastewater in Kansas, 2024.

The KPSS test results indicated that all variables were stationary ($p < 0.05$), meaning each time series had a stable mean and variance over time and did not exhibit a significant trend. As a result, no differencing was required during ARIMA modeling, and the differencing order (d) was set to 0 for all series. The time-series cross-correlation analysis revealed that wastewater WNV concentrations were most strongly correlated with human WNV cases at a lag of five-month, meaning the increases in WNV levels in wastewater tended to precede increases in human cases by approximately five-month (seen in Fig. 3.2). The Google Trends time-series cross-correlations were most moderately correlated with human WNV cases at a lag of two-month, and the IgG positivity rate had a moderate correlation with human WNV cases at a lag of seven-month. The IgM testing and bird observations did not have strong cross-correlations with human WNV cases.

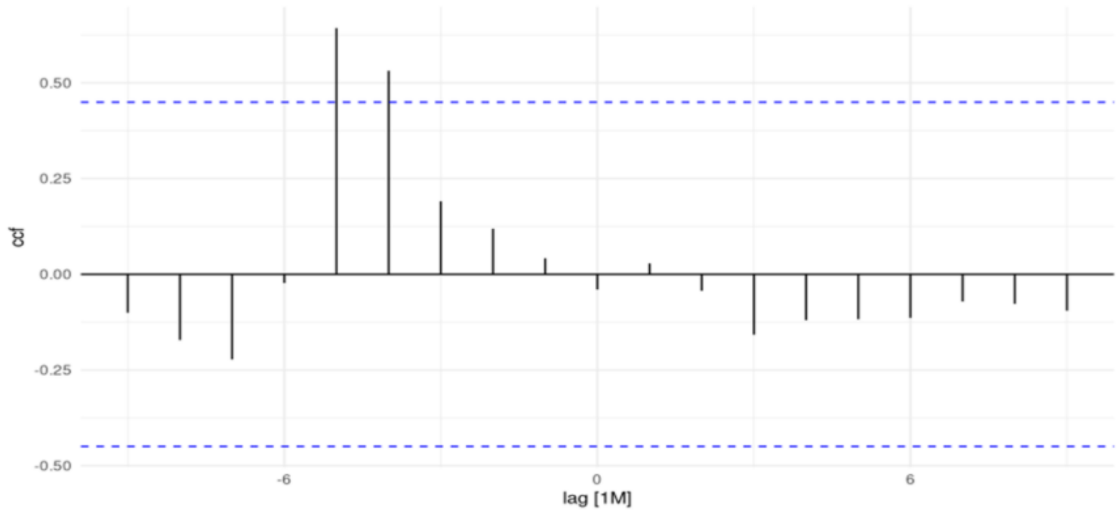

Figure 3.2: Time-series cross-correlation plot for Human WNV cases and Wastewater detection

The baseline ARIMA model (1,0,0) using only reported human WNV cases showed relatively limited predictive ability (AIC = 49.0, RMSE = 0.727), indicating that past human cases alone may not be sufficient for accurate forecasting. Among the models tested, the best-performing ARIMA model (1,0,0) included three predictors: wastewater WNV concentration, Google Trends, and IgG testing results. This model had the lowest AIC (14.9) and a relatively low RMSE (0.273), suggesting a good balance between fit and accuracy (Fig. 3.3). The second-best model included all predictors—wastewater WNV concentration, Google Trends, IgG/IgM testing, and bird observations—and while it achieved a slightly lower RMSE (0.257), its higher AIC (17.6) indicated a more complex model with less optimal fit when accounting for complexity (fig. 3.3).

To assess the specific contribution of the wastewater monitoring data, we also performed an ARIMA model with Google Trends, and IgG testing variables but did not include the wastewater data. This model had a higher AIC (26.8) and worse RMSE (0.515) compared to the model that included wastewater (Fig. 3.3). Finally, the Ljung-Box test applied to the residuals of the four models passed ($p < 0.05$), suggesting that the models fully accounted for the autocorrelation and did not leave significant temporal patterns unexplained.

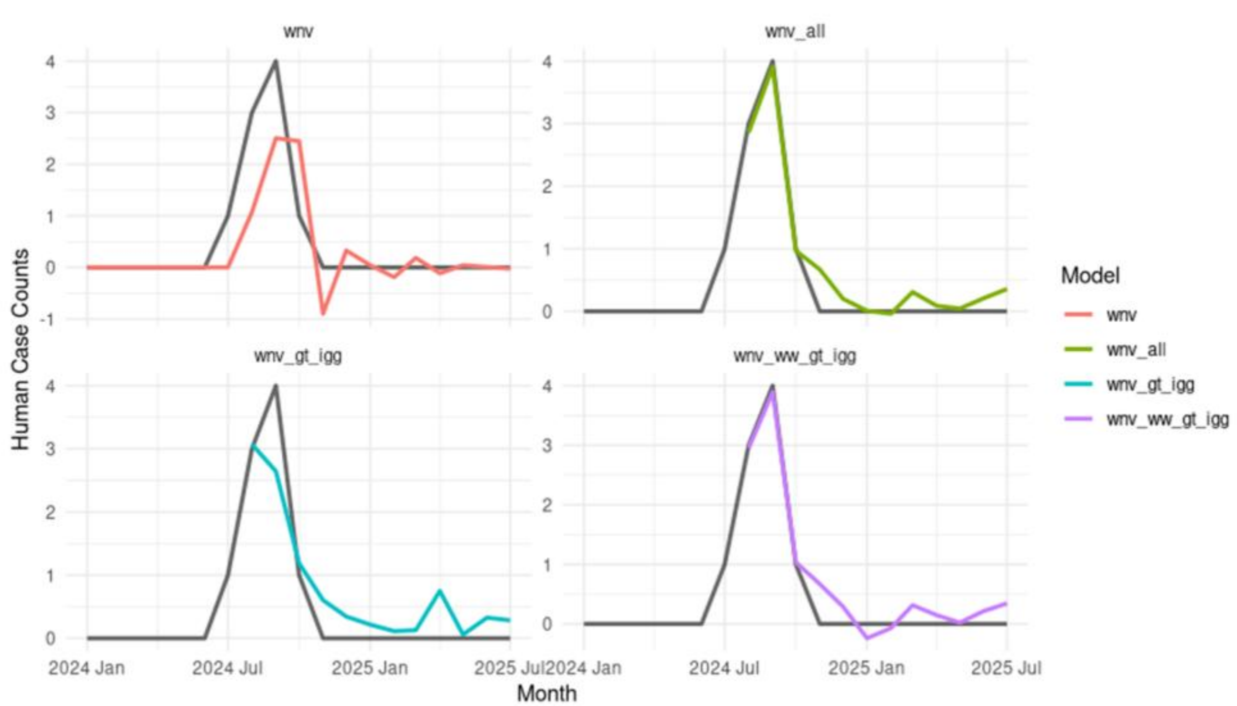

Figure 3.3: The “wnv” ARIMA model is the baseline of reported human WNV cases. The “wnv_all” predictive graph shows the second-best fitting model, which includes all the predictor variables. The "wnv_gt_igg” graph shows the predictive model without the wastewater detection data. The "wnv_ww_gt_igg” model is the best fitting model, including wastewater detection, Google Trends, and IgG testing.

An overview of impacted countries with human WNV cases presented in Figures 3.4 and 3.5. Through a literature review, Africa and Asia countries were categorized as human WNV present (“Yes”) or not present (“No”). Africa and Asia do not have publicly available case data, but available resources reported which countries are known to have human WNV circulation in those areas (Abbas, et al., 2025; Chancey et al., 2015; Eybpoosh, et al., 2019; Harty, 2024; Holland et al., 2024; Mencattelli, et al., 2022).

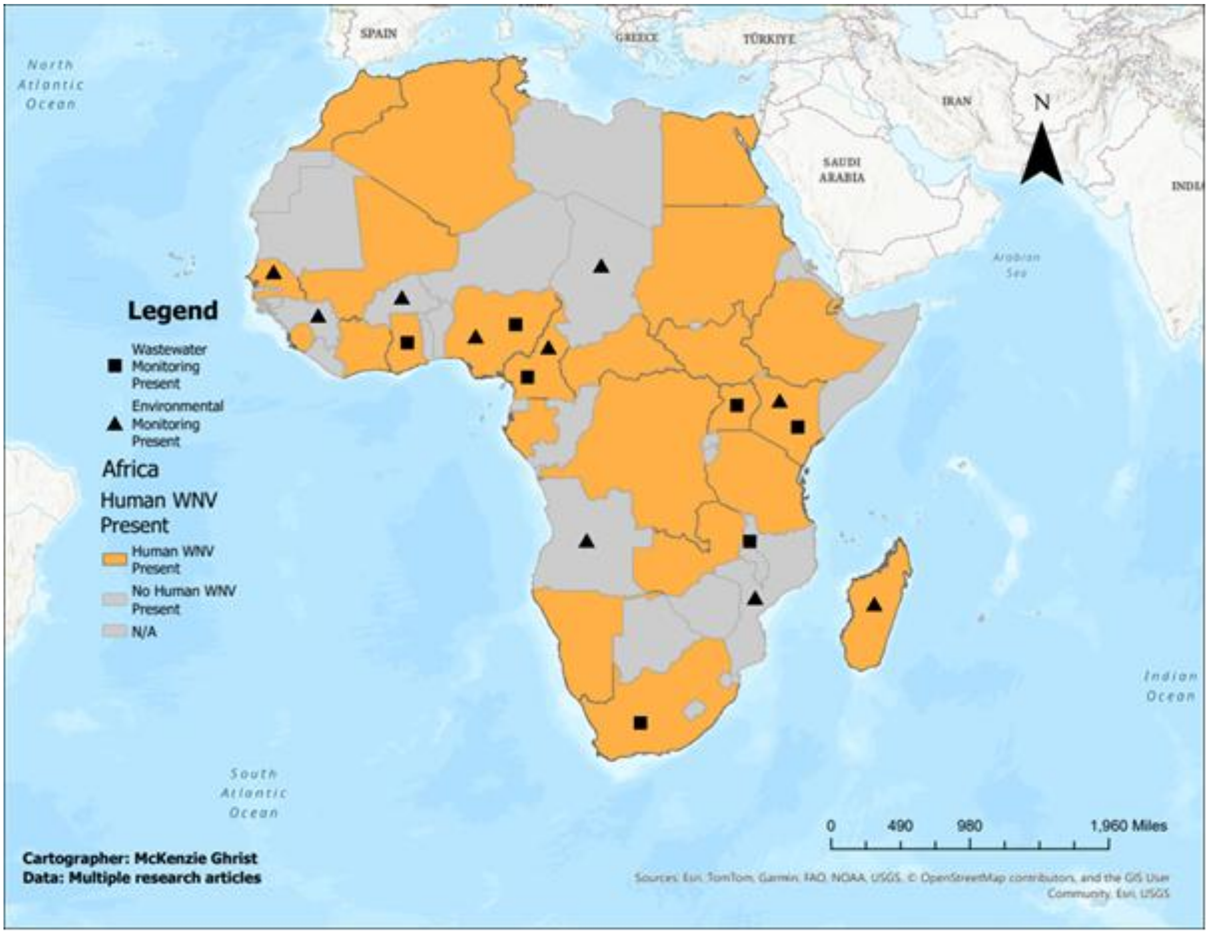

Figure 3.4: A map of present human WNV cases vs non-present human WNV cases, along with wastewater monitoring and environmental monitoring presence in Africa.

Figure 3.5: A map of present human WNV cases vs non-present human WNV cases, along with wastewater monitoring and environmental monitoring presence in Asia.

Spatial statistics were performed for areas with publicly available 2024 human WNV incidence rate data for Europe and the US, while publicly available 2023 human WNV incidence rate data was collected for Canada. Each region's mean center and standard distance were calculated to evaluate spatial dispersion. The standard distance results presented below represent standard distance, typically encompassing an estimated 63% of observations around the mean center, assuming a roughly normal spatial distribution (Esri, 2024). The mean center, in Figure 3.6, was identified on the borders of Nebraska and Iowa. The standard distance presented human WNV incidence dispersion across the central region of the US and Canada. The mean center, in Figure 3.7, was identified on the western border of Albania. The standard distance presented human WNV incidence dispersion across the southeastern region of Europe. In Figure 3.10, the mean center was located between the Northwest and Southwest regions of Kansas, with the standard distance representing spatial dispersion around the western side of Kansas. Wastewater monitoring sites and reported human WNV incidence rates were visualized by region.

The average nearest neighbor statistic was calculated for Kansas, North America—not including Mexico—and Europe to evaluate the spatial distribution of countries impacted by WNV. Regions are reported using a confidence level of $\alpha = 0.05$, meaning any p-value ≤ 0.05 is considered statistically significant. For North America, the analysis was conducted using the NAD 1983 UTM Zone 15 projection. The North American nearest neighbor ration (R) was 1.361915, with a z-score of 5.582049 and a p-value < 0.000001 indicating a statistically significant dispersed pattern. Kansas applied the same projection system as North America. The Kansas R value was 2.234171. The z-score was 5.783384 with a p-value < 0.000001 indicating a statistically significant dispersed pattern. Europe spatial analysis was utilizing the ETRS89 projection. The R value was 0.781057, the z-score was -4.530601, and the p-value was < 0.000006, indicating statistically significant spatial clustering.

Global Moran's I was used to assess the distribution of human WNV incidence rates across North America, Europe, and Kansas through spatial autocorrelation (i.e., clustering or dispersion). Results were evaluated at a confidence level or $\alpha = 0.05$, where p-values ≤ 0.05 are considered statistically significant. The Moran's I test returns values between –1 and +1, where values close to +1 indicate clustering, values near –1 suggest dispersion, and values near 0 suggest randomness. North America, the Moran's I was 0.824372, with a z-score of 6.315949 and a p-value < 0.000001, indicating a statistically significant clustering. Kansas' Moran's I was 0.485904, with a z-score of 0.976361 and a p-value of 0.328885, indicating random distribution. Europe had a Moran's I value of 1.286096, with a z-score of 8.859773 and a p-value $<$ 0.000001, indicating statistically significant clustering.

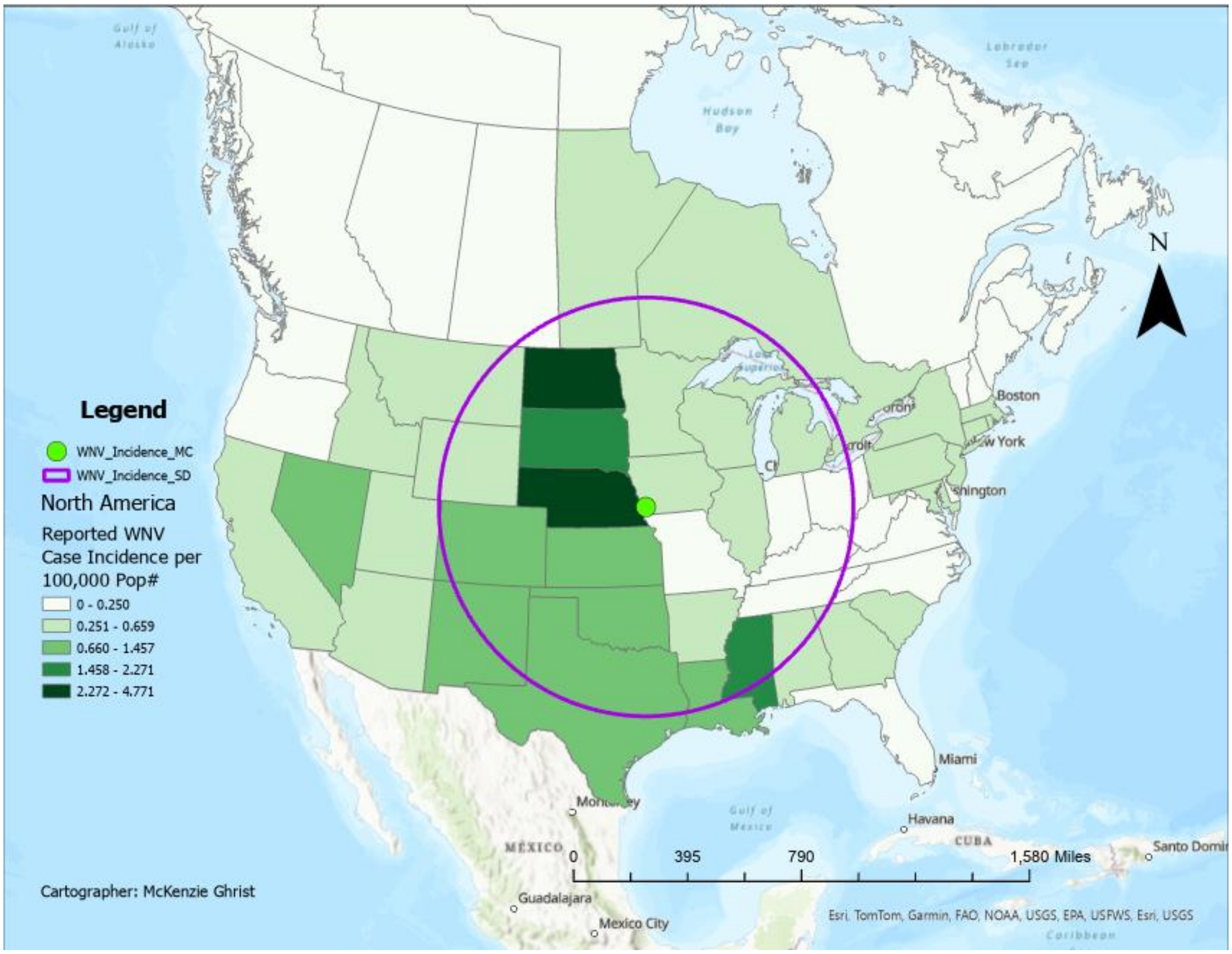

Figure 3.6: A map of the mean center and standard distance of reported human WNV incidence rates in the US (2024) and Canada (2023).

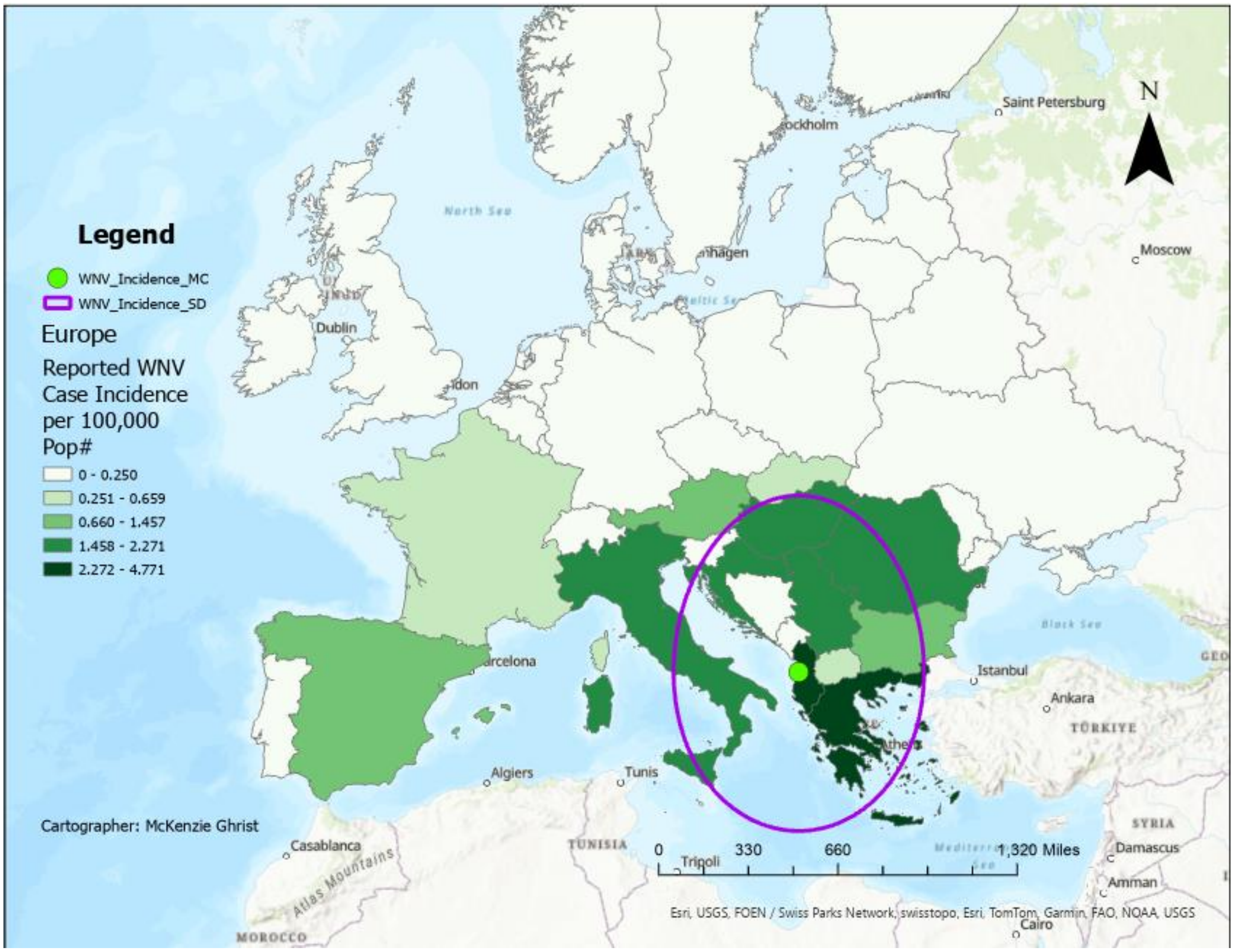

Figure 3.7: A map of the mean center and standard distance of reported human WNV Incidence rates in Europe (2024).

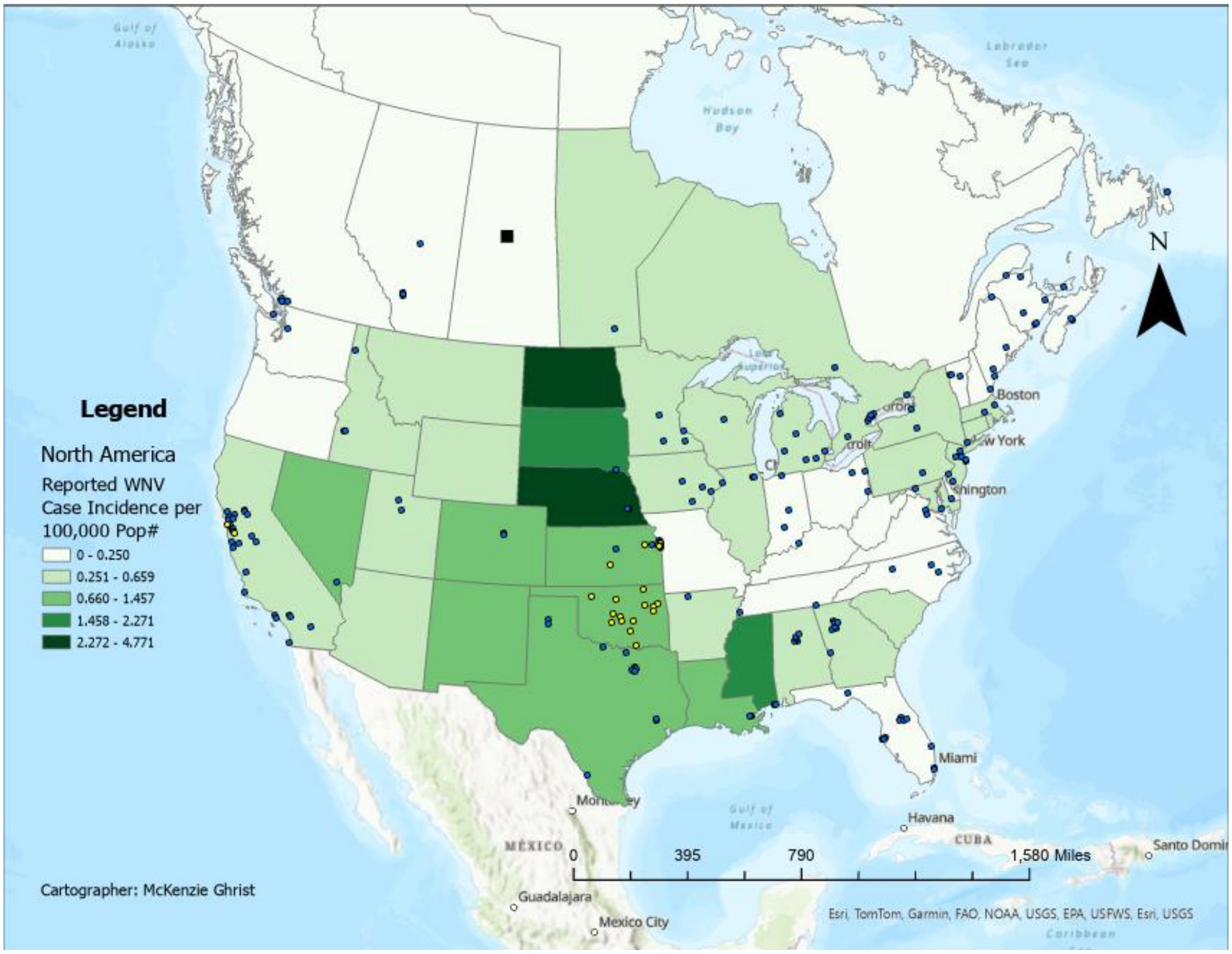

Figure 3.8: A map of reported human WNV incidence rates, along with wastewater treatment facility locations (2025) in the US (2024) and Canada (2023).

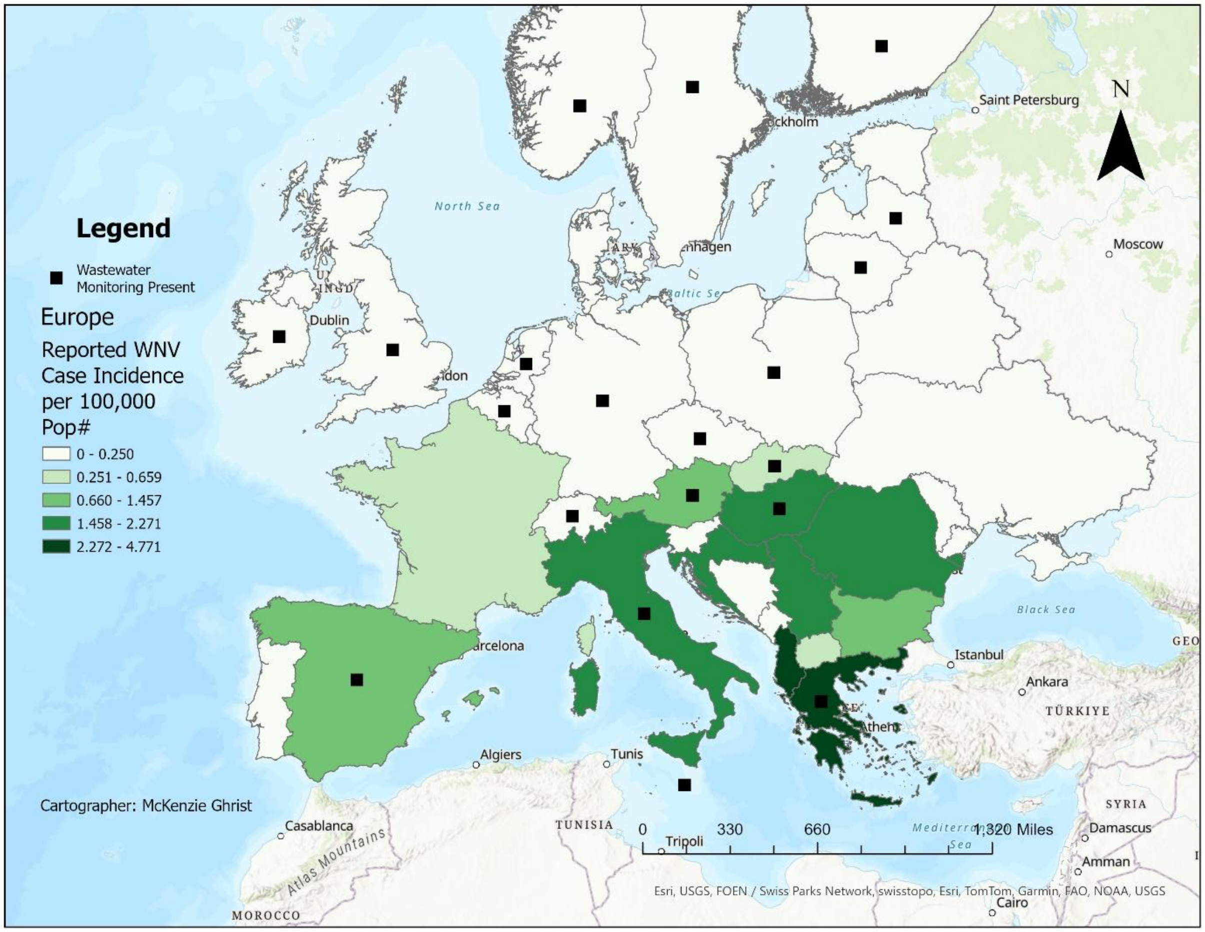

Figure 3.9: A map of reported human WNV incidence rates, along with wastewater monitoring presence (2025) in Europe (2024).

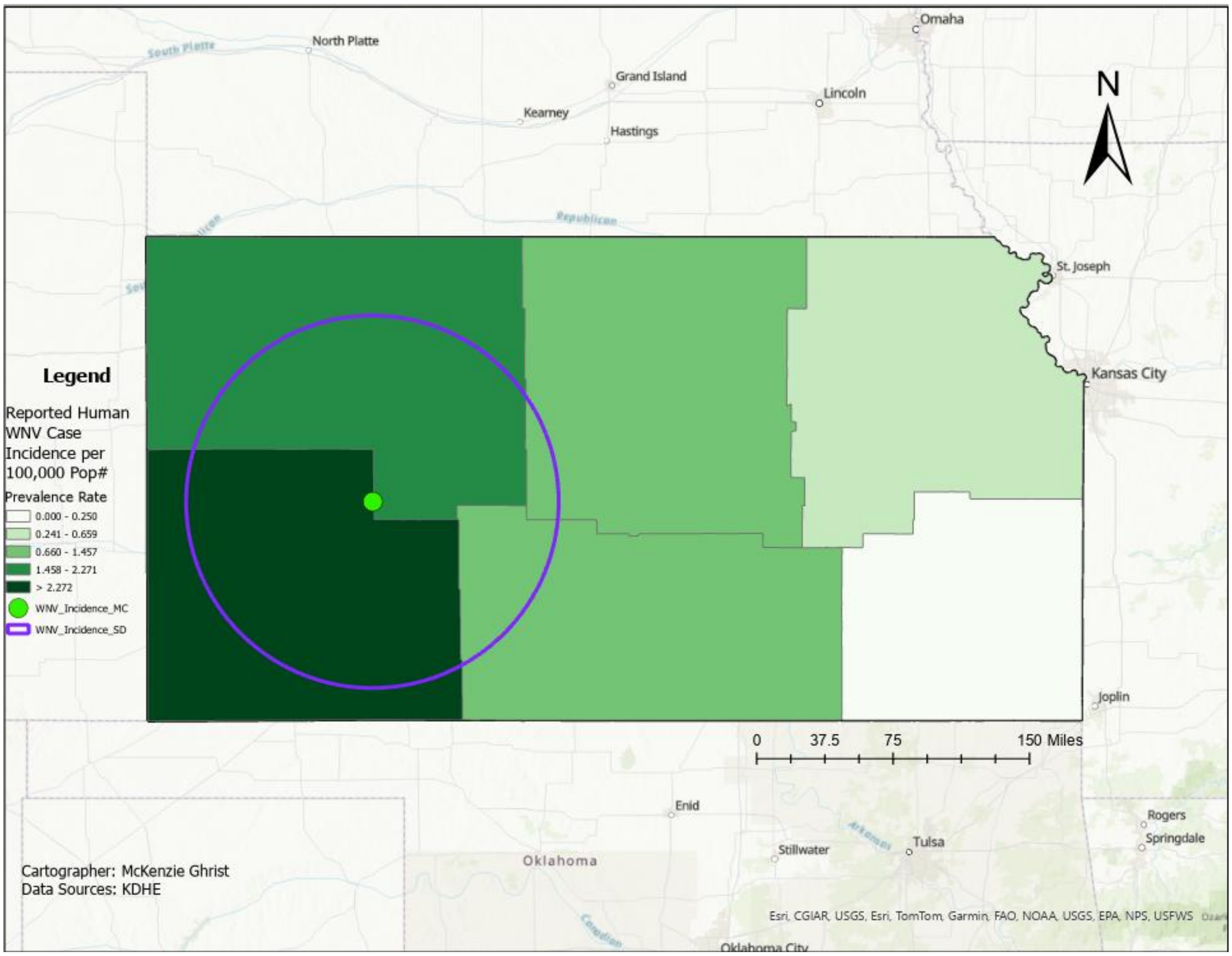

Figure 3.10: A map of the mean center and standard distance of reported human WNV incidence rates in Kansas (2024).

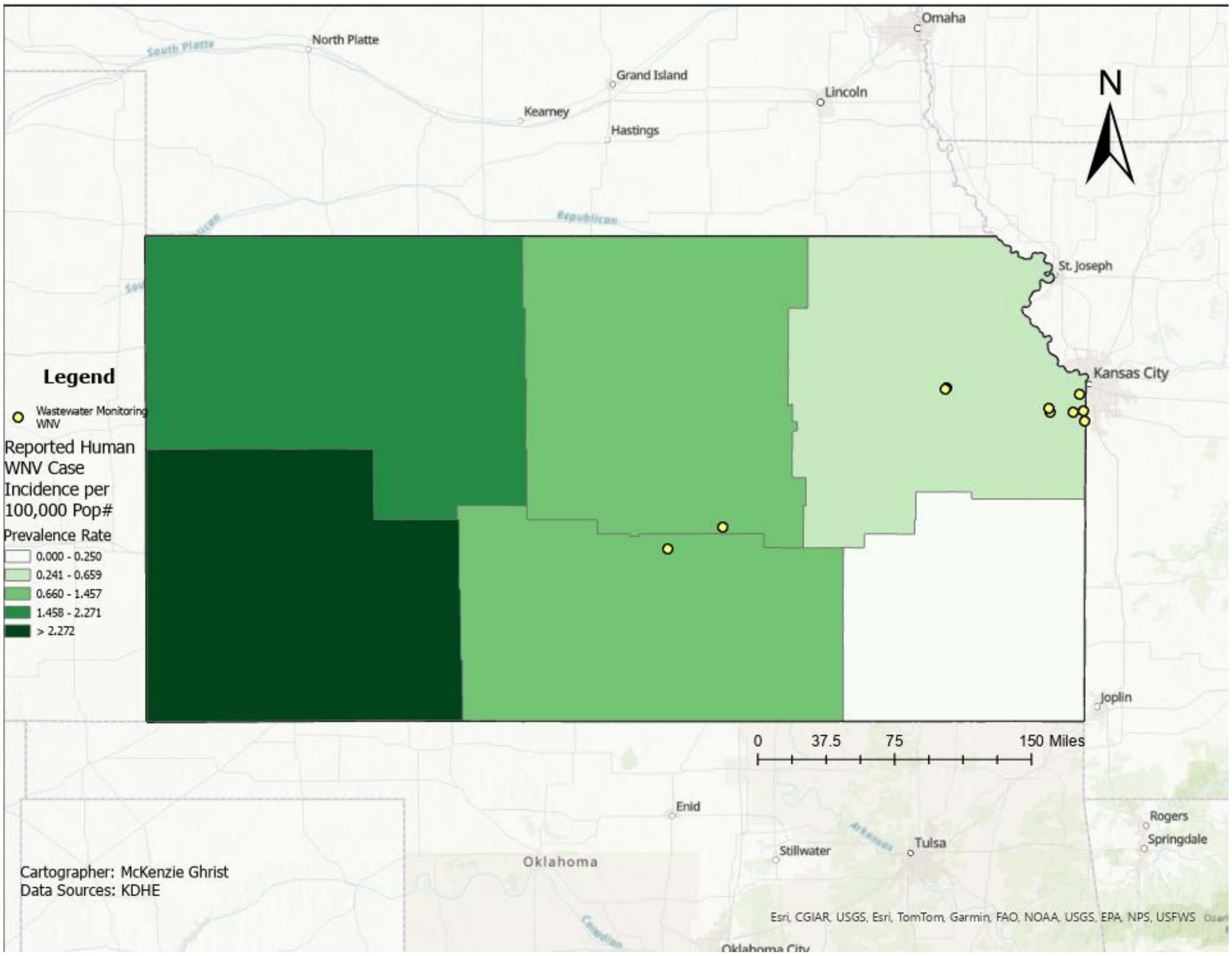

Figure 3.11: A map of reported human WNV incidence rates, along with wastewater monitoring (2025) of WNV sites in Kansas (2024).

Chapter 4 - Discussion

This study contributes to the growing field of wastewater-based epidemiology by evaluating the potential application of wastewater monitoring for detecting WNV in Kansas. Using statistical and spatial analyses, our findings provide important initial insight into how wastewater monitoring could complement traditional surveillance systems, such as clinical case reporting, syndromic surveillance, and vector monitoring. Through this APE, the objectives of applying statistical and spatial analysis, interpreting surveillance trends, and communicating findings to different audiences were addressed.

The time-series cross-correlation analysis revealed that the wastewater WNV concentration was moderately associated with human WNV cases at a lag of five months. This suggests that increases in wastewater WNV detection occurred several months before reported human cases increased. This temporal lead highlights the potential of wastewater monitoring as an early detection tool for public health agencies. In addition to the wastewater WNV detection data, the Google Trends data displayed an increase two months before an increase in reported human WNV cases. Immunoglobulin G testing had a moderate association with the reported human WNV cases at a lag of seven months. However, the ARIMA model that included Google Trends and IgG testing performed poorly, with the higher AIC and RMSE values compared to models that included wastewater detection data. The best-performing ARIMA model combined wastewater WNV detection, Google Trends, and IgG testing data. This suggests that the Google Trends and IgG testing data alone are insufficient for accurately providing more insight on human WNV trends, and that wastewater monitoring contributes to understanding human WNV cases for public health agencies.

In addition to understanding the potential use of wastewater monitoring for WNV, we utilized GIS software for a global overview of the impact of WNV and which countries have researched or implemented wastewater monitoring. Although Africa and Asia lacked publicly available case data, the literature review revealed the countries that confirm human WNV circulation, reinforcing the global distribution of WNV. There are also twenty-seven countries between Africa and Asia that have implemented wastewater monitoring systems or researched the use of wastewater monitoring. These countries are utilizing wastewater monitoring for SARS-CoV-2 within their communities. Along with wastewater monitoring, fifteen countries researched environmental monitoring. This process includes testing other environmental factors instead of testing sewage and water impacted by humans alone. An observational study in Mozambique, Africa researched the application of environmental monitoring for vector-borne diseases at the Infulene River Basin (Monteiro et al., 2025).

In North America, the average nearest neighbor results indicated a statistically significant dispersed pattern, meaning the states with reported human WNV incidence rates were spaced farther apart than expected under random distribution. However, the Global Moran's I showed statistically significant positive autocorrelation, suggesting that regions with similar WNV incidence rates were spatially grouped. In other words, while the physical locations of reporting states were dispersed, the reported human WNV incidence rates themselves were clustered, indicating the presence of regional patterns of high and low incidence rates.

In Kansas, the average nearest neighbor also indicated strong spatial dispersion. However, the Global Moran's I tool did not show statistically significant spatial autocorrelation; suggesting that although reporting locations were spread out geographically, human WNV incidence rates were randomly distributed with no consistent pattern of clustering or dispersion across the state. In contrast, Europe exhibited consistent spatial results, indicating spatial clustering. The average nearest neighbor revealed that reporting countries were closer together than expected, and the Global Moran's I confirmed strong positive spatial autocorrelation (i.e., clustering). These findings suggest that countries with higher reported human WNV incidence rates were near other high-incidence countries, and those with low incidence were similarly grouped. Overall, these results indicate that Europe exhibited the strongest clustering of reported human WNV incidence rates, North America displayed a mixed pattern, and Kansas showed random spatial structure.

The high clustering of reported human WNV incidence rates in Europe, can be observed towards the Mediterranean Sea area reflects the mean center in Albania. The mean center for North America falls along the border of Nebraska and Iowa, with the Midwest having higher reported human WNV incidence rates. At the state level, Kansas' mean center was located between the Northwest and Southwest region border, and the standard distance illustrated human WNV cases spread across the western region of Kansas. These findings highlight the potential value of expanding wastewater monitoring in Kansas to fill gaps in regional monitoring and improve Kansas public health officials' understanding of human WNV trends.

In relation to existing literature, this project aligns with broader findings on the utility of wastewater surveillance complementing other surveillance tools for detection of pathogens like COVID-19 and, more recently, Dengue in the community (Boehm et al., 2023; Wolfe et al., 2023). While few studies have confirmed wastewater detection of WNV, observational studies in Oklahoma and Georgia have demonstrated potential for identifying WNV presence in wastewater systems (Kuhn et al., 2025; Vazquez-Prokopec et al., 2010). Our study extends this work by providing spatial and temporal analyses on wastewater WNV detection and complementing other surveillance methods.

Limitations

There are several limitations in this study to consider. While our study offers insight into the use of wastewater monitoring for WNV, it lacks multiple years of data. Having a year of wastewater data limits the evaluation of long-term trends or consistency of wastewater WNV detection within Kansas. The limited time series also affects model stability, and the ARIMA approach can be sensitive to non-stationary data, particularly when seasonal trends are not well represented. The south-central regional area of Kansas reports the most WNV cases; however, Harvey County (i.e. a county in the south-central region) has wastewater monitoring, but does not send the samples regularly (KDHE, personal communication, Sep. 22, 2025). There also appears to be a higher human WNV incidence rate on the western side of Kansas and no wastewater monitoring sites that send samples to KDHE. In addition, approximately 20% of Kansas households use septic systems rather than centralized wastewater facilities, which limits the representativeness of wastewater surveillance in rural areas (Moser, 2023).

Additionally, WNV is likely underreported due to the estimated 80% of individuals that are asymptomatic and 1% or fewer cases develop severe neurological symptoms (CDC, 2021). Surveillance that relies on clinical case reporting may not accurately reflect the true incidence of infection within the population, limiting direct comparisons with wastewater detection trends. The spatial analysis was also limited by data availability. Africa and Asia do not have publicly accessible data on reported human WNV cases, which restricted the inclusion of these regions in our spatial analysis. This result may impact the global distribution of reported human WNV incidence rates, particularly in areas with limited reporting or surveillance.

Next Steps

While this study offers initial insight into wastewater monitoring for WNV, additional research is needed to fully evaluate wastewater utility in Kansas. To assess long-term trends and seasonal variability, KDHE would benefit from collecting and analyzing multi-year wastewater data. This expanded dataset could support more robust comparisons and inform decisions on integrating WNV monitoring into routine public health practices. A key next step is increasing surveillance coverage in western Kansas, where spatial analysis indicates higher concentrations of reported human WNV incidence rates. Establishing additional monitoring sites in the Northwest and Southwest regions would provide more geographically representative data. The Kansas Department of Health and Environment may also consider working with Harvey County to encourage regular submission of wastewater samples, improving consistency in statewide monitoring reliability. Additionally, based on 2024-2025 wastewater data, an unexpected rise in wastewater WNV detection was observed in April. As a result, the agency is considering initiating sample collection as early as March in future seasons to capture better wastewater monitoring for WNV. Together, these actions could enhance the agency's understanding of wastewater-based WNV monitoring and support its potential adoption as a long-term tool for public health.

Conclusion

Overall, this study provides initial insights into the use of wastewater monitoring for WNV in Kansas but should be interpreted cautiously. As the first effort to apply this approach at the Kansas Department of Health and Environment (KDHE), our results demonstrate the potential of wastewater data to complement existing WNV monitoring strategies. In addition to our work, other recent studies have explored wastewater-based monitoring for arboviruses, expanding the information available to public health agencies. Our spatial analysis highlights the global burden of WNV and identifies with higher reported incidence, as well as those investigating wastewater-based surveillance. While these findings are promising, it is important to consider the study's limitations when interpreting the results. Future efforts at KDHE should incorporate multi-year data to better assess temporal trends and the reliability of wastewater signals in tracking WNV activity.

Chapter 5 - Competencies

Student Synthesis and Integration of MPH Foundational Competencies

Competency 1 – Geographic Information Systems software is a valuable tool in epidemiological practice for analyzing the spatial distribution of disease. I incorporated epidemiological methods using ArcGIS Pro to assess the global impact of human WNV and the extent of wastewater monitoring implementation. By calculating incidence rates based on reported human WNV cases and population data, I visualized and analyzed disease patterns across US states (specifically Kansas), Canada provinces, and European countries. I compiled spatial statistical tools—including mean center and standard distance—to evaluate the geographic distribution and central tendency of reported human WNV incidence rates in North America, Europe, and Kansas.

To further assess spatial patterns, I integrated average nearest neighbor and spatial autocorrelation (Global Moran's I) analyses to determine whether human WNV incidence rates were statistically clustered, dispersed, or randomly distributed. These spatial analyses provided insight into potential epidemiological hotspots and helped identify potential areas for closer surveillance. Due to data limitations, I supplemented quantitative analyses with a literature review of human WNV circulation in Africa and Asia countries, classifying them as "Yes" or "No" based on available peer-reviewed sources.

To explore the global use of environmental surveillance, I combined geospatial data from wastewater monitoring dashboards in the US, Canada, and Europe; and conducted literature reviews for Africa, and Asia. I created point feature classes to map countries that implemented or researched wastewater or environmental monitoring, effectively integrating spatial statistic methods and qualitative epidemiological data to inform a broader understanding of reported human WNV incidence and public health monitoring efforts worldwide.

In several of my classes within the Master of Public Health (MPH) program, I selected epidemiological methods to analyze diverse public health data in a variety of practical settings. In Geographical Information Systems I (GEOG 508), I used ArcGIS Pro to conduct spatial analyses that integrated quantitative data, a skill I later incorporated during my APE at KDHE. This course strengthened my ability to assess disease patterns and environmental monitoring strategies using geospatial methods. In Introduction to Biostatistics (MPH 701) and Regression/Analysis of Variance (STAT 705), I utilized RStudio to conduct multiple regression analyses evaluating the relationship between environmental factors and human WNV cases. Drawing on publicly available national datasets from 2021 to 2023, I modeled the impact of average monthly temperature and precipitation on WNV incidence, developing statistical techniques to support epidemiologic inference. In my STAT 705 class, I expanded these skills by using advanced statistical methods to further explore relationships within public health data.

Through my APE and MPH courses, I synthesized the ability to apply core epidemiological methods—including spatial analysis, statistical modeling, and quantitative research—to investigate complex public health issues. My work on WNV incidence and wastewater monitoring strengthened how I integrated multiple data sources and analytic tools to inform both local and global public health surveillance and response.

Competency 19 – At the end of my internship with KDHE, I delivered a professional presentation to public health staff with expertise in WNV and wastewater monitoring. I presented the results of our statistical analysis, illustrating that wastewater could be a valuable addition to understanding human WNV trends. I explained the ARIMA model outputs, supported my analysis with predictive graphs, and discussed the next steps for KDHE's wastewater monitoring systems for WNV. In addition to the oral presentation, I developed a literature review to communicate the background and significance of wastewater monitoring for diseases, specifically WNV. I structured the review to inform both the KDHE team and public health professionals unfamiliar with wastewater monitoring and WNV, emphasizing how wastewater monitoring complements disease trends. Through both my oral presentation and literature review, I improved my ability to convey complex public health data in a clear, engaging, and actionable way, tailored to a professional audience.

Apart from my APE with KDHE, I incorporated my ability to communicate public health content effectively through both written and interpersonal formats. In the Multidisciplinary Thought and Presentation (DMP 815) course, I developed a news release on wastewater monitoring for WNV, using plain language to ensure clarity for readers with a high school education level. This required translating technical information into clear, accessible content while maintaining scientific accuracy. As a teaching assistant for the Introduction to Epidemiology (MPH 754) course, I worked directly with students to explain epidemiological concepts and equations. I adapted my communication in real time based on their learning needs and knowledge, helping them grasp material through clear, student-specific explanations. Both experiences highlight my ability to deliver audience-appropriate public health messages and concepts through writing and oral communication.

My APE and MPH program experiences allowed me to refine my communication approach depending on the audience—whether simplifying complex public health concepts for the public, supporting students in learning technical material, or presenting statistical findings to professionals—enhancing my ability to apply this competency across diverse settings.

Table 5.1 Summary of MPH Foundational Competencies Synthesis and Integration

Number and Competency		**Description**
1	Apply epidemiological methods to the breadth of settings and situations in public health practice	I incorporated spatial epidemiological methods to analyze the global impact of reported human WNV incidence rates and the global distribution of wastewater

		monitoring, producing maps and insights to inform KDHE epidemiologists.
19	Communicate audience-appropriate public health content, both in writing and through oral presentation to a non-academic, non-peer audience with attention to factors such as literacy and health literacy	I communicated the wastewater WNV monitoring results to public health professionals through an oral presentation and conveyed findings from a literature review on the potential application of wastewater monitoring for WNV.

Table 5.2 MPH Foundational Competencies and Course Taught In

22 Public Health Foundational Competencies Course Mapping	MPH 701	MPH 720	MPH 754	MPH 802	MPH 818
Evidence-based Approaches to Public Health					
1. Apply epidemiological methods to the breadth of settings and situations in public health practice	x		x		
2. Select quantitative and qualitative data collection methods appropriate for a given public health context	x	x	x		
3. Analyze quantitative and qualitative data using biostatistics, informatics, computer-based programming and software, as appropriate	x	x	x		
4. Interpret results of data analysis for public health research, policy or practice	x		x		
Public Health and Health Care Systems					
5. Compare the organization, structure and function of health care, public health and regulatory systems across national and international settings		x			
6. Discuss the means by which structural bias, social inequities and racism undermine health and create challenges to achieving health equity at organizational, community and societal levels					x
Planning and Management to Promote Health					
7. Assess population needs, assets and capacities that affect communities' health		x		X	
8. Apply awareness of cultural values and practices to the design, implementation, or critique of public health policies or programs					x
9. Design a population-based policy, program, project or intervention			x		
10. Explain basic principles and tools of budget and resource management		x	x		
11. Select methods to evaluate public health programs	x	x	x		
Policy in Public Health					
12. Discuss the policy-making process, including the roles of ethics and evidence		x	x	X	
13. Propose strategies to identify relevant communities and individuals and build coalitions and partnerships for influencing public health outcomes		x		X	
14. Advocate for political, social or economic policies and programs that will improve health in diverse populations		x			x
15. Evaluate policies for their impact on public health and health equity		x		X	

22 Public Health Foundational Competencies Course Mapping	MPH 701	MPH 720	MPH 754	MPH 802	MPH 818
Leadership					
16. Apply leadership and/or management principles to address a relevant issue		x			x
17. Apply negotiation and mediation skills to address organizational or community challenges		x			
Communication					
18. Select communication strategies for different audiences and sectors	DMP 815				
19. Communicate audience-appropriate public health content, both in writing and through oral presentation to a non-academic, non-peer audience with attention to factors such as literacy and health literacy	DMP 815				
20. Describe the importance of cultural humility in communicating public health content		x			x
Interprofessional Practice					
21. Integrate perspectives from other sectors and/or professions to promote and advance population health		x			x
Systems Thinking					
22. Apply a systems thinking tool to visually represent a public health issue in a format other than a standard narrative			x	X	

Student Synthesis and Integration of MPH Emphasis Area Competencies

Throughout my APE and MPH courses, I integrated and synthesized the five competencies from the Infectious Disease and Zoonoses emphasis area. My APE focused on the application of wastewater monitoring for WNV, requiring application and knowledge in pathogen biology, host immune response, vector ecology, environmental factors, and disease surveillance systems. Along with my project, several classes I took throughout my MPH experience contributed to the integration of these five emphasis area competencies. The following sections describe how each of the five emphasis area competencies were addressed through my project and courses.

Competency 1: Pathogens/Pathogenic Mechanisms – In my project, it was important to understand both the transmission cycle and shedding patterns of WNV to evaluate the potential application of wastewater monitoring. Through my APE, we researched viral shedding and discovered that WNV can be shed in feces and urine, emphasizing WNV detectability in wastewater systems. We also examined the transmission cycle, which primarily involves circulation between birds and mosquitoes. Humans and horses are considered dead-end hosts, meaning they do not contribute to the primary transmission cycle, which is important for surveillance designs. In support of this competency, I synthesized key concepts in microbial survival, adaptation, and environment transmission through my Microbial Ecology (BIOL 687) course. This understanding informed my interpretation of WNV detection in wastewater monitoring and enhanced my ability to connect environmental microbiology with public health surveillance.

***Competency 2: Host Response to Pathogens/Immunology* –** I combined serological data—specifically, IgG and IgM antibody testing—to evaluate population-level immune responses in relation to wastewater WNV detection and human WNV case data. The IgM positivity typically indicates recent or active infection, while IgG positivity reflects past exposure. However, we discovered that WNV antibodies can persist in the body for 15 months. Our time-series analysis revealed that IgG positivity was strongly correlated with human WNV cases at a seven-month lag, reinforcing the importance of incorporating serological surveillance when interpreting WNV trends. To support this competency, I cultivated foundational concepts from the Domestic Animal Immunology (DMP 850) course, which deepened my understanding of both the innate and adaptive immune response to pathogens. Although the course was focused on animals, the immunological principles directly enhanced my ability to interpret serological data in a public health context. Integrating this knowledge into real-world surveillance strengthened the inclusion of wastewater monitoring as a tool for understanding WNV transmission and community-level immunity.

Competency 3: Environmental/Ecological Influences – As part of my APE ecological analysis, we examined bird sighting data—specifically crows, blue jays, and robins which are known to play a role in WNV amplification—whose observational data was used to contextualize trends in WNV surveillance. I also integrated *Culex* mosquito data to create a systems thinking diagram explaining the process of mosquito surveillance to better understand WNV trends/transmission rates in Kansas.

I synthesized this competency throughout my MPH program. In the MPH 701 (biostatistics course), I conducted a regression analysis project evaluating how temperature and precipitation influenced human WNV cases across the US. I discovered that temperature was a significant factor influencing WNV case counts, reinforcing the role of environmental conditions in arboviral disease transmission. This project enhanced my understanding and interpretation of climate and ecological variables that shape vector behavior and disease risk. Additionally, coursework in Environmental Health (MPH 802) and Introduction to One Health (DMP 850), enabled me to critically evaluate the interconnectedness among environmental, animal, and human health. These courses helped me synthesize ecological and environmental drivers into my knowledge of infectious disease dynamics, supporting a systems-level perspective of WNV transmission. Together, these experiences equipped me with the ability to contextualize disease trends within broader environmental and ecological frameworks when assessing public health surveillance strategies.

Competency 4: Disease Surveillance – My APE is directly aligned with this competency, as it requires the application of wastewater monitoring to complement other WNV surveillance tools for Kansas. I compiled traditional surveillance data (reported human WNV cases), serological surveillance data (IgG/IgM testing), environmental data (bird observations), public awareness through Google Trends, and wastewater surveillance to examine the utilization of multiple surveillance tools in adding an understanding of WNV trends. By developing time-series models, I evaluated the relative performance of each surveillance stream and demonstrated that wastewater detection provided a moderate association at a five-month lag for reported human WNV cases. Reinforcing the potential value of wastewater monitoring for human WNV trends, particularly in areas with underreported infections or limited healthcare access.

To support this work, I built upon foundational knowledge from Introduction to Epidemiology (MPH 754) and Intermediate Epidemiology (DMP 854), where I critically examined core principles of disease surveillance, including case definitions, sensitivity, specificity, and evaluation metrics. These courses equipped me with the ability to interpret the strengths and limitations of diverse data sources and integrate surveillance theory with practical experience in real-world public health contexts.

***Competency 5: Disease Vectors* –** Although I did not directly analyze mosquito surveillance data into the wastewater WNV detection analysis for Kansas. I compiled research on the biology and ecology of *Culex* mosquito species, including their geographic distribution, host feeding behavior, and ability to transmit WNV. The knowledge on these vectors enhanced my ability to conceptualize how different surveillance systems interact, which I generated into my systems thinking diagrams. These diagrams included mosquito surveillance, wastewater surveillance, human and horse surveillance, and bird surveillance; illustrating the various components contributing to WNV monitoring and control.

To support this competency, I completed BIOL 687, which explored microbe-environment interactions relevant to vectors, such as mosquitoes. In MPH 802 and DMP 710, I developed a broader understanding of environmental conditions—such as temperature, standing water, and habitat disruption—shape vector populations and influence zoonotic disease transmission. Through this combination of coursework, research, and systems thinking application; I incorporated the knowledge of disease vectors into a broader framework of WNV surveillance. While mosquito surveillance data was not directly analyzed, my ability to integrate vector ecology into the conceptual framework of WNV surveillance reflects a systems-level understanding of how vectors contribute to disease dynamics and inform public health responses.

Table 5.3 Summary of Synthesis and Integration of MPH Emphasis Area Competencies

MPH Emphasis Area:		
Number and Competency		**Description**
1	Pathogens/pathogenic mechanisms	Evaluate modes of disease causation of infectious agents.
2	Host response to pathogens/immunology	Investigate the host immune response to infection.
3	Environmental/ecological influences	Examine the influence of environmental and ecological forces on infectious diseases.
4	Disease surveillance	Analyze disease risk factors and select appropriate surveillance.
5	Disease vectors	Investigate the role of vectors, toxic plants and other toxins in infectious diseases.

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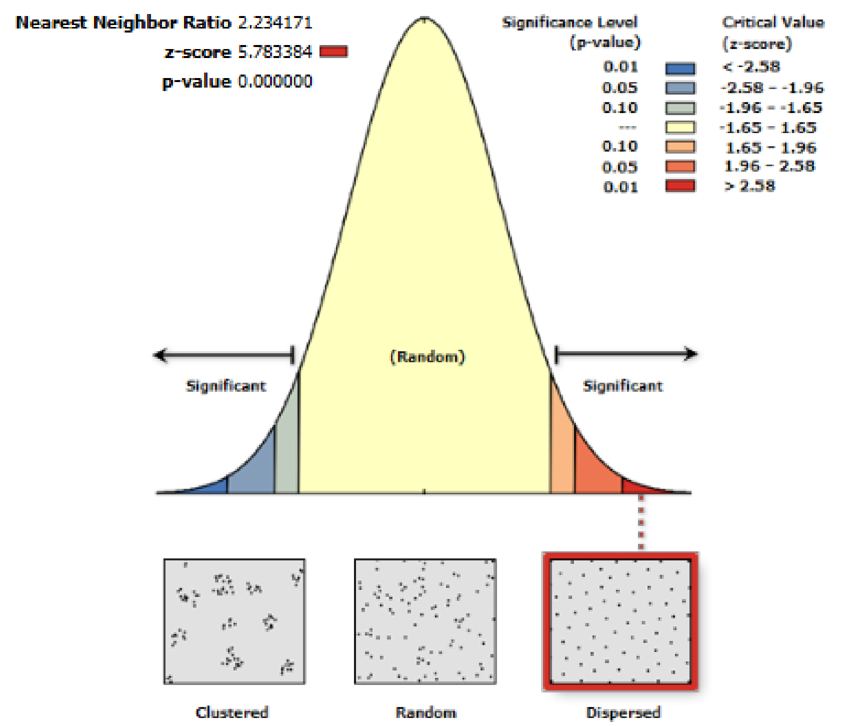
Appendix

Appendix 1 – Average Nearest Neighbor Reports

Average Nearest Neighbor Summary



Given the z-score of 5.783384, there is a less than 1% likelihood that this dispersed pattern could be the result of random chance.

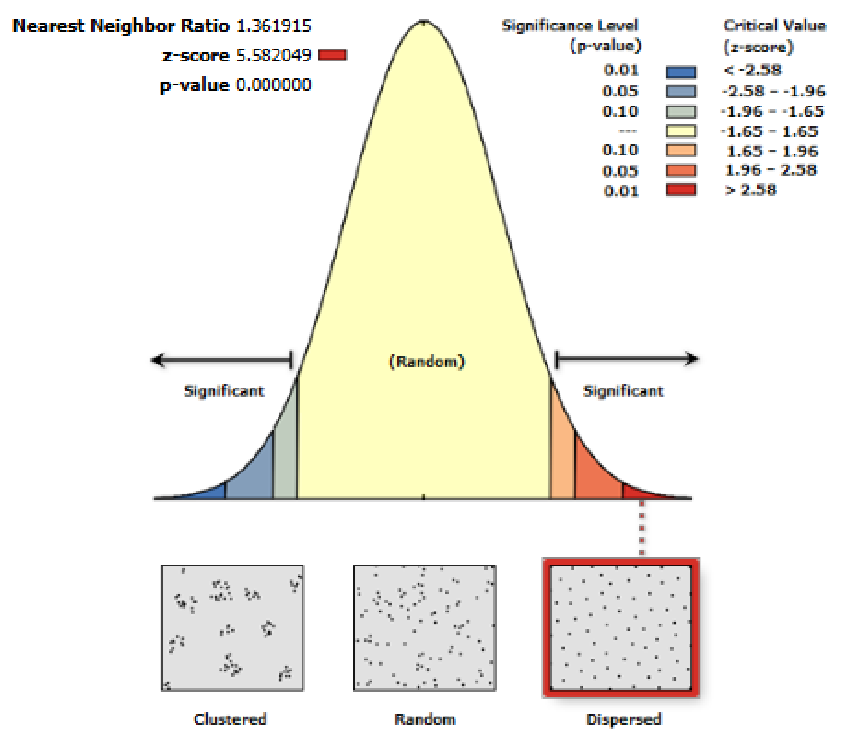
Average Nearest Neighbor Summary

Observed Mean Distance	210524.1229 meters
Expected Mean Distance	94229.1688 meters
Nearest Neighbor Ratio	2.234171
z-score	5.783384
p-value	0.000000

Dataset Information

Input Feature Class:	Reported Human WNV Case Prevalence per 50,000 Pop#
Distance Method:	EUCLIDEAN
Study Area:	213099270000.000000
Selection Set:	False

Average Nearest Neighbor Summary



Given the z-score of 5.582049, there is a less than 1% likelihood that this dispersed pattern could be the result of random chance.

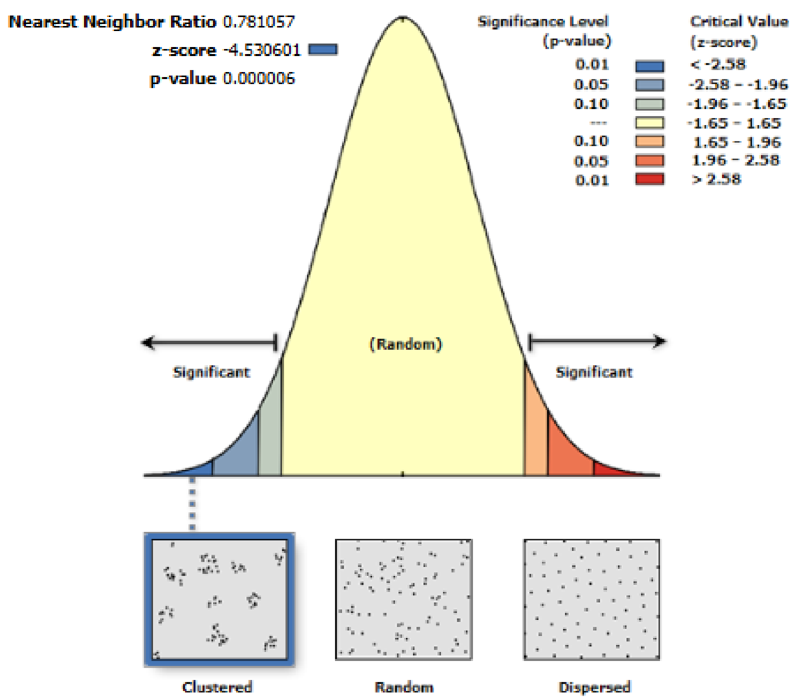
Average Nearest Neighbor Summary

Observed Mean Distance	360735.3507 meters
Expected Mean Distance	264873.6999 meters
Nearest Neighbor Ratio	1.361915
z-score	5.582049
p-value	0.000000

Dataset Information

Input Feature Class:	North America
Distance Method:	EUCLIDEAN
Study Area:	18241100000000.000000
Selection Set:	False

Average Nearest Neighbor Summary



Given the z-score of -4.530601, there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

Average Nearest Neighbor Summary

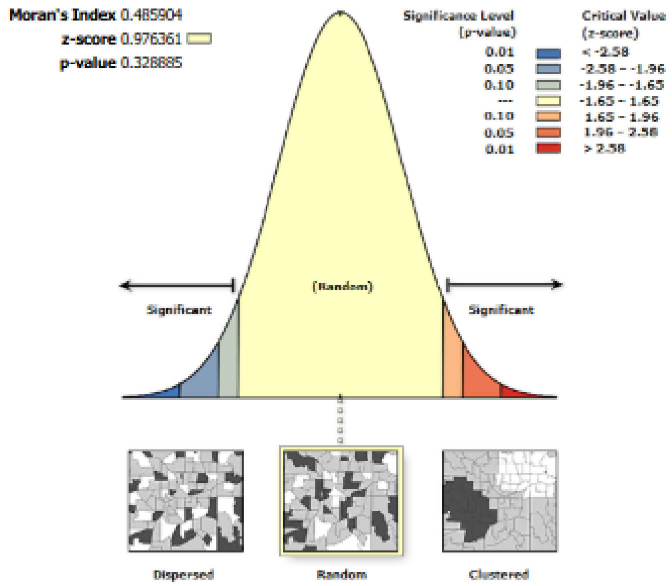
Observed Mean Distance	115228.8891 meters
Expected Mean Distance	147529.5132 meters
Nearest Neighbor Ratio	0.781057
z-score	-4.530601
p-value	0.000006

Dataset Information

Input Feature Class:	Europe
Distance Method:	EUCLIDEAN
Study Area:	10186000000000.000000
Selection Set:	False

Appendix 2 – Spatial Autocorrelation Reports

Spatial Autocorrelation Report



Given the z-score of 0.976361, the pattern does not appear to be significantly different than random.

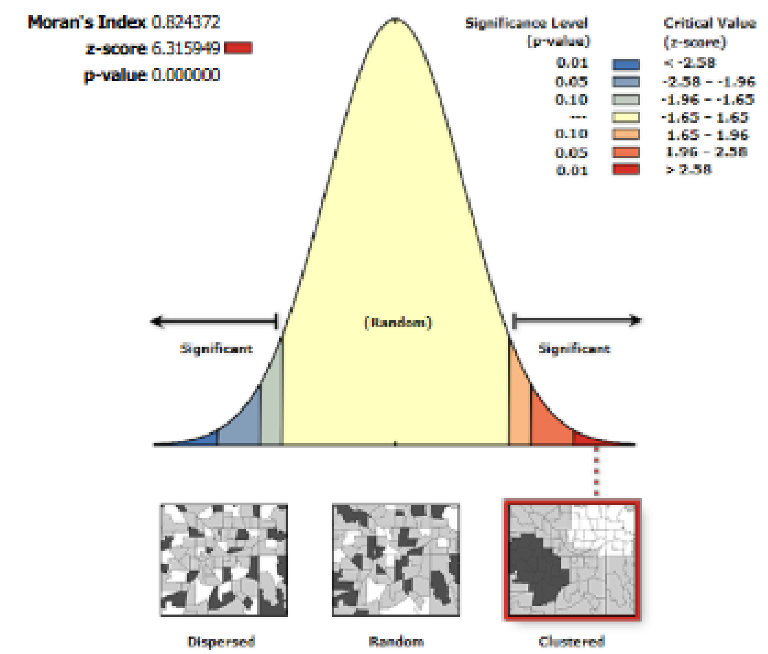
Global Moran's I Summary

Moran's Index	0.485904
Expected Index	-0.200000
Variance	0.493520
z-score	0.976361
p-value	0.328885

Dataset Information

Input Feature Class:	Reported Human WNV Case Incidence per 100,000 Pop#
Input Field:	T_WNV_PR$_.PREVALENCE_RATE
Conceptualization:	INVERSE_DISTANCE
Distance Method:	EUCLIDEAN
Row Standardization:	True
Distance Threshold:	210524.1229 meters
Weights Matrix File:	None
Selection Set:	False

Spatial Autocorrelation Report



Given the z-score of 6.315949, there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

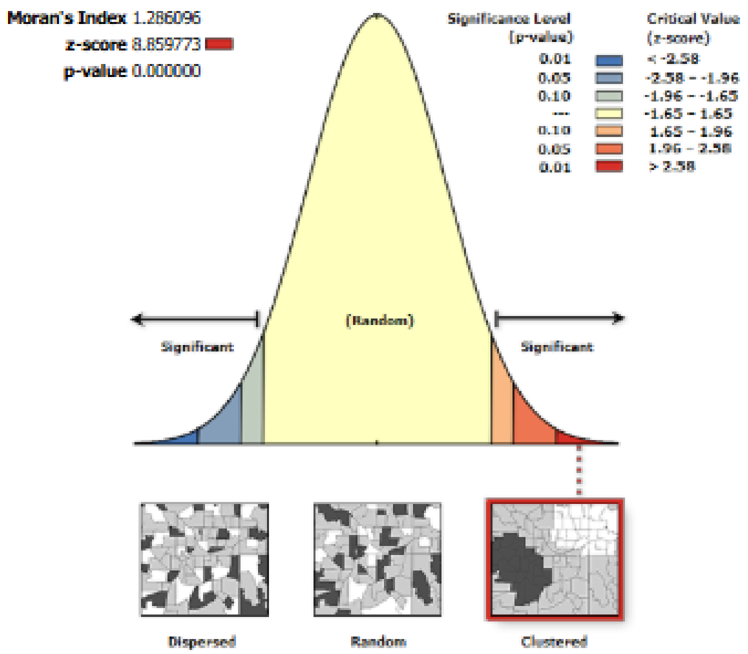
Global Moran's I Summary

Moran's Index	0.824372
Expected Index	-0.016129
Variance	0.017709
z-score	6.315949
p-value	0.000000

Dataset Information

Input Feature Class:	North America
Input Field:	NA_WNV_PREVALENCE$.PREVALENCE_PER_100_000_POP_
Conceptualization:	INVERSE_DISTANCE
Distance Method:	EUCLIDEAN
Row Standardization:	True
Distance Threshold:	360735.3507 meters
Weights Matrix File:	None
Selection Set:	False

Spatial Autocorrelation Report



Given the z-score of 8.859773, there is a less than 1% likelihood that this clustered pattern could be the result of random chance.

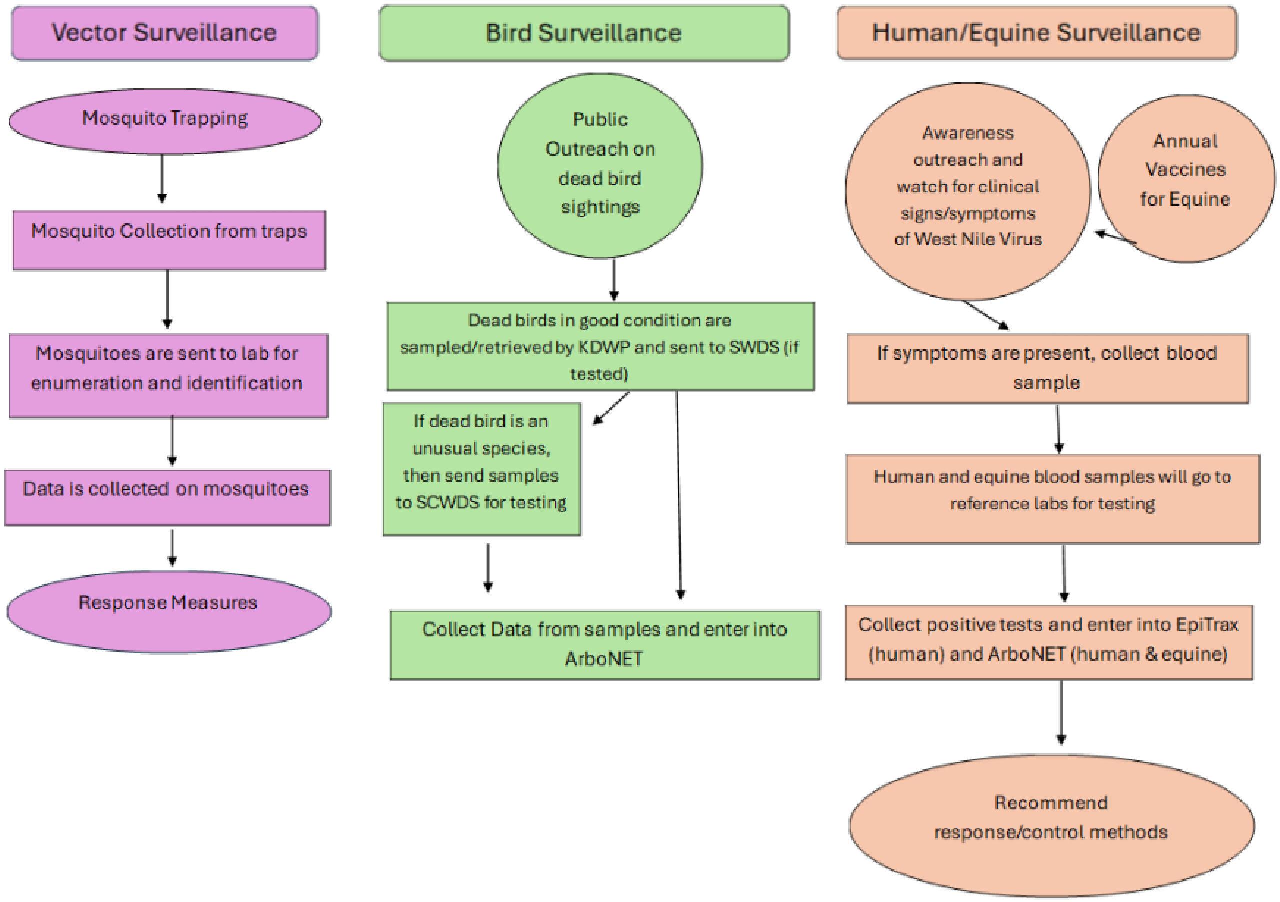
Global Moran's I Summary

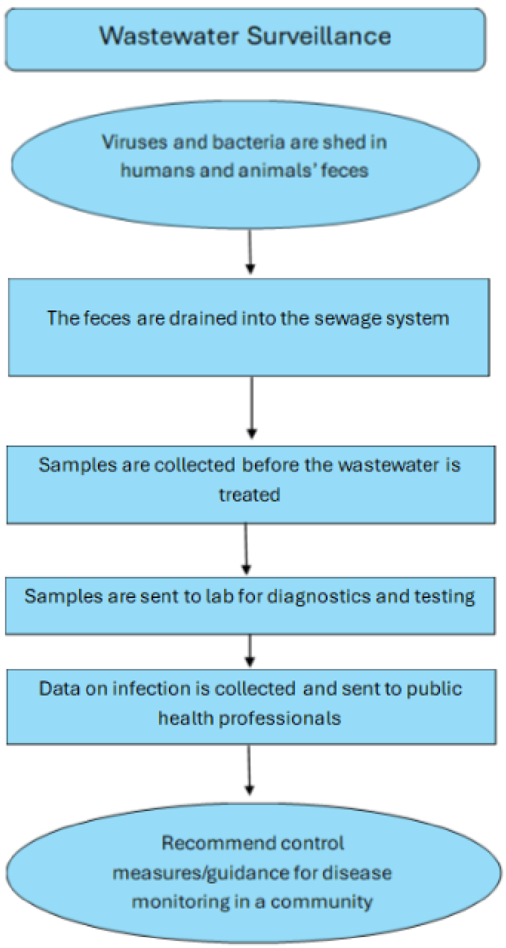
Moran's Index	1.286096
Expected Index	-0.009901
Variance	0.021397
z-score	8.859773
p-value	0.000000

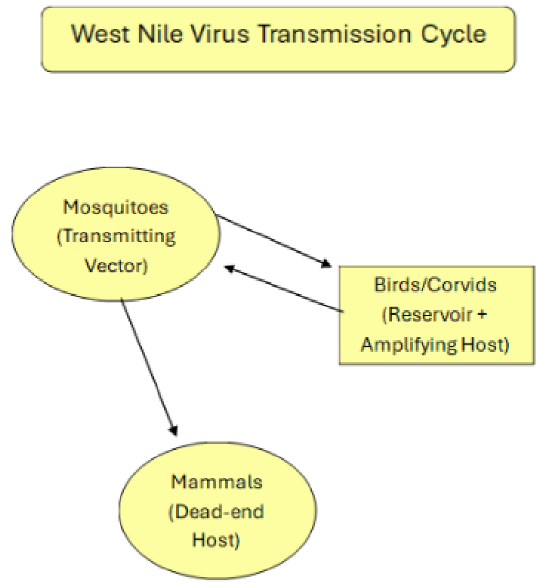
Dataset Information

Input Feature Class:	Europe
Input Field:	EU_WNV_PREVALENCE$.PREVALENCE_PER_100_000_POP_
Conceptualization:	INVERSE_DISTANCE
Distance Method:	EUCLIDEAN
Row Standardization:	True
Distance Threshold:	115228.8891 meters
Weights Matrix File:	None
Selection Set:	False

Appendix 3 – Systems Thinking Diagrams







Surveillance Sections	**Item**	**Description**	**Waiting Period**
Vector	Trapping	CDC light traps are deployed out into the field to collect mosquitoes. Light traps attract indiscriminately, and non-blood fed mosquitoes will be trapped.	
	Mosquito Collection	The following morning the light traps will be collected and stored in a cold environment by local health departments. Local health departments send the frozen mosquitoes over to KDHE.	
	Laboratory Testing	Mosquitoes are then enumerated and identification at KDHE.	

	Data Collection from samples	Data is collected and saved locally on the H drive and VectorSurv, but only on the enumerated and identification of the mosquitoes. Then that data and information is sent back to the local health departments.	
	Response measures	A "risk level" is calculated based on mosquito numbers and species quantity and then recommendations and responses are based on the "risk level".	
Bird	Public outreach of dead birds	Reach out and educate the public, wildlife conservatories, state and national parks, and fishing and game workers about reporting any dead birds to state health departments and state wildlife agencies.	
	Dead birds in good condition are sampled/received	After reporting dead birds, if the bird is in good condition, it may be retrieved and sampled by Kansas Department of Wildlife & Parks (KDWP) and sent to Southeastern Cooperative Wildlife Disease Study (SCWDS) for possible testing. Good condition means not scavenged and no obvious decomposition or maggot infestation.	
	Unusual Species	If the sampled dead bird is an unusual species to find, like a hawk or eagle, then KDWP sends those birds to SCWDS in Athens, GA.	
	Collect data from samples	Once those samples are tested, the data will be sent to KDHE and entered into ArboNET.	
Human/Equine	Annual Vaccinations for Horses	Horses should get vaccinated every year for West Nile Virus. There are 4 licensed vaccines.	
	Watch for clinical signs/symptoms of West Nile Virus	Healthcare providers and veterinarians must be aware of signs and symptoms that are present with West Nile Virus in their patients. Public Health professionals will want to do awareness outreach to the public on reducing their risk of West Nile Virus.	2-14 days

	Blood Sample Collection	If healthcare provider and veterinarian see clinical signs and symptoms for West Nile Virus, then a blood sample will need to be collected.	1 day
	Send blood sample to labs for diagnostics and testing	Once blood sample is collected, then it will need to be sent to commercial labs for humans testing of West Nile Virus. KHEL can do an IgM test if requested. Equine blood samples are sent to reference labs (commercial, veterinary, and USDA).	2-10 days
	Collect positive tests and then enter into ArboNET/any other software	If blood samples come back positive, labs are reported instantly (electronically) for human cases to public health professionals. Equine cases are reported directly to the state veterinary epidemiologist from the Kansas Department of Agriculture, again highly variable timeframe. These samples are then entered into EpiTrax (human only) and ArboNET (human and horses).	0 days
	Recommend response/cont rol methods	Public health professionals update risk levels every week from June to October. If there is an early rise in cases, high cases, or higher proportion of fatal cases then a press release would be sent out. In Kansas, local health departments have the authority to administer public health response in their county. Some of these counties may have a lower threshold for response, could be guided by mosquito surveillance, or may not have any control and response measures.	Varia ble
Wastewat er	Viruses and bacteria are shed in humans and animals' feces	Humans and other animals shed pathogens in their feces, urine, and vomit even when they are asymptomatic. It can also be shed through washing hands or body.	

	The feces are drained into the sewage system	The viruses shed from humans and animals are then drained into the sewage system or are drained into rivers/streams along sewage system.	
	Samples are collected before wastewater is treated	Samples of untreated wastewater are collected at the Wastewater Treatment Plant.	
	Samples are sent to lab for diagnostics and testing	Collected untreated wastewater samples are sent to the lab for diagnostic testing of West Nile Virus (or other viruses).	
	Data on infection is collected and sent to public health professionals	Positive wastewater samples are then reported to public health professionals. It can take 5-7 days from sample collection until public health professionals get the results.	5-7 days
	Recommend control measures/guidance for disease monitoring in a community	Public health professionals will receive the data and then implement control methods or provide guidance in those areas on monitoring and reducing the risk of disease. If samples do not find West Nile Virus, then public health professionals will monitor them and won't recommend or implement control measures.	

Appendix 4 – KDHE Presentation Slides

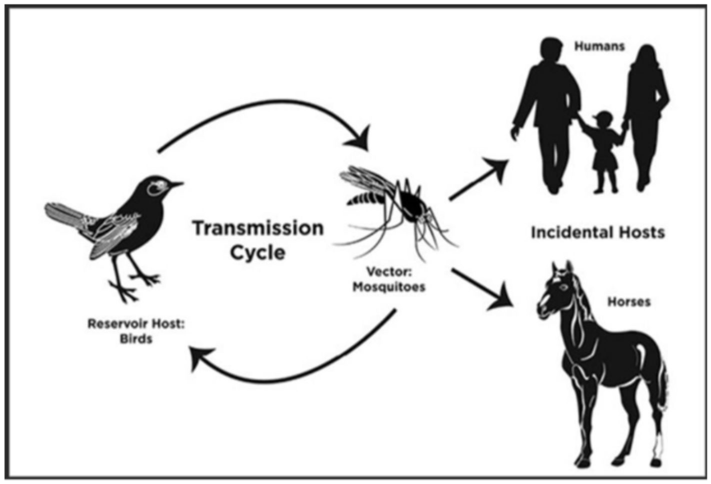
Overview

- Introduction
  - West Nile Virus
  - Significance of Wastewater Monitoring
  - Wastewater Monitoring for West Nile Virus
- Methods
- Results
  - Maps
- Conclusion
- Acknowledgements
- Questions

To protect and improve the health and environment of all Kansans

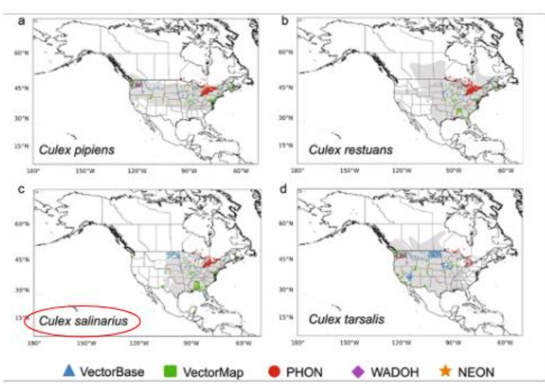
West Nile Virus (WNV)

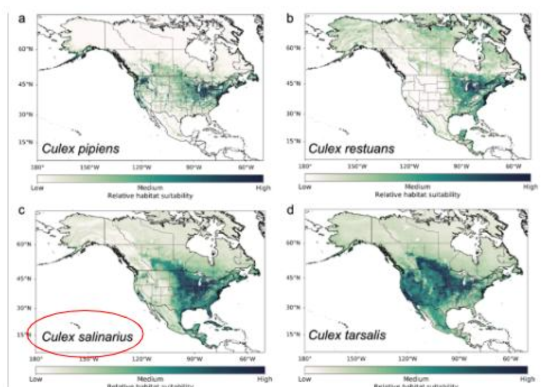
- Spread by Culex mosquitoes
- Spatially spreading
  - Increase temperatures = increase human WNV risk
- 80% of infected humans are asymptomatic
  - ~19% have mild flu-like symptoms
  - ~1% have neuroinvasive symptoms



(Vazquez-Prokopec et al., 2010 & Soucheray, 2025)

Culex Mosquitoes





(Gorris et al., 2021)

Cx. Salinarius are more opportunistic with their blood meals – more likely to feed on humans (Molaei et al., 2006).

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Wastewater Monitoring

1

1930: Polio Campaigns

2020: SARS-CoV-2 variants & outbreak

(Diamond et al., 2022)

2

Cost Effective: US saves $10.5 - $18.5 million

(Sanjak et al., 2024)

3

Possible use for Arboviruses

(Lee et al., 2022)

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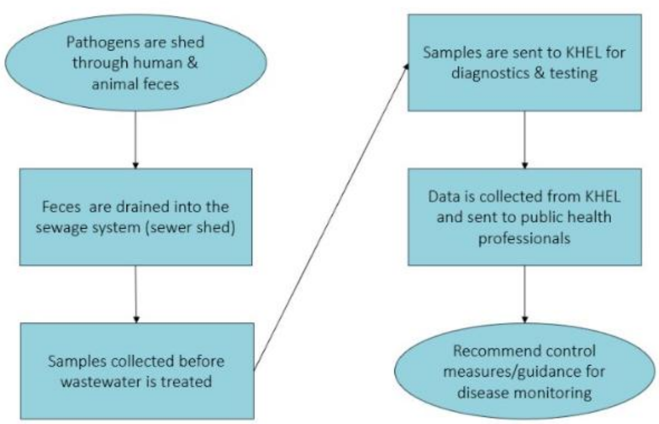
Wastewater Flow Diagram



Collected samples = pathogens present for entire sewer shed of treatment plant

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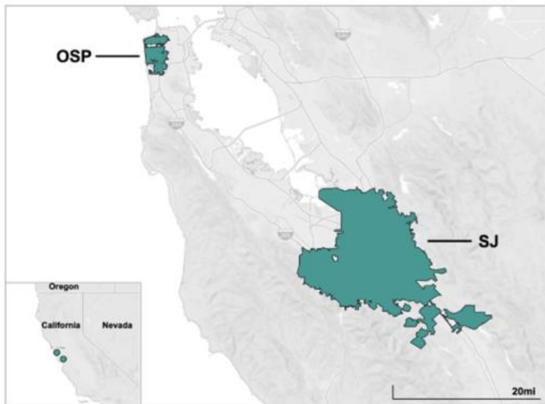
San Francisco Bay Area, CA Study



- Collected wastewater samples from Feb. 2021 – Apr. 2023
  - Tested for multiple pathogens
  - Included WNV
- No detection of WNV
- Detected other pathogens

*Wastewater Scan Dashboard

(Boehm et al., 2023)

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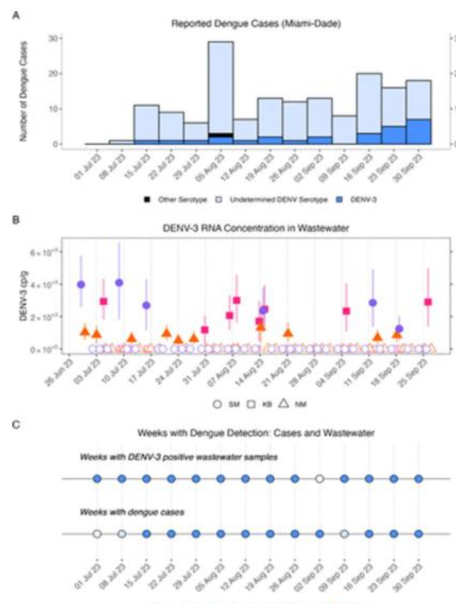
Miami, FL Study

- Collected samples from 3 WWTF
  - Represents 95% county pop.
  - Tested Dengue serotypes 1-4
- Acquired weekly human Dengue reports
  - Acquired mosquito surveillance
- Detected Dengue serotype 3
  - Serotype 3 in humans
  - No serotype 3 in mosquitoes

*Wastewater Scan Dashboard

(Wolfe et al., 2023)



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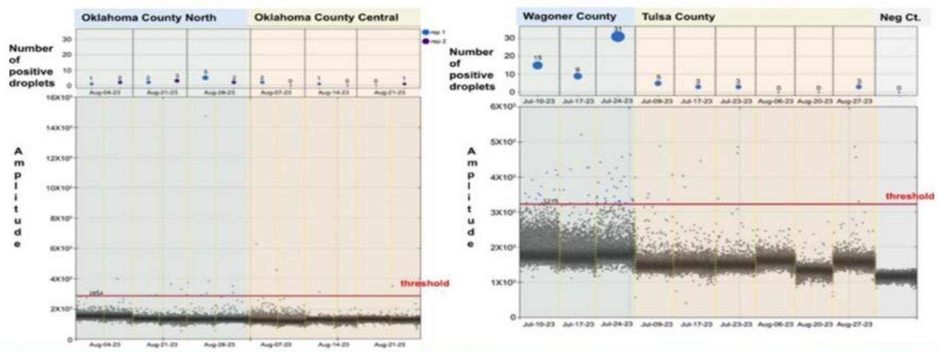
Oklahoma Study

- Goal: develop a long-term wastewater monitoring tool for WNV
- Acquired human, horse, and mosquito data
- WW detection aligned w/human cases
  - WNV detected in 3 counties = 1.6 million pop.
  - Aligned with mosquito testing



(Kuhn et al., 2025)

To protect and improve the health and environment of all Kansans

Methods

Kansas Department of Health and Environment
Division of Public Health

GIS Analysis & Mapping

Collected Boundary Shapefiles:
- North America – not including Mexico
- Europe
- Asia
- Africa

Created Feature Classes:
- Wastewater Monitoring Present
- Environmental Surveillance Present

Prevalence Rate of Reported WNV human cases
- Ranged from 0.000 – 4.771

Mean Center & Standard Distance

Spatial Autocorrelation
- Average Nearest Neighbor

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Statistical Analysis

- Collected Multiple Data sets:
  - Human & animal reported cases
    - Only used the human cases
  - Wastewater WNV detection
  - Google Trends
  - IgG & IgM rates
  - Crow, blue jay, & robin bird counts
- Time series cross correlation
- Kwiatkowski-Phillips-Schmidt-Shin (KPSS)
- ARIMA models
  - AIC & RMSE
- Ljung-Box portmanteau test

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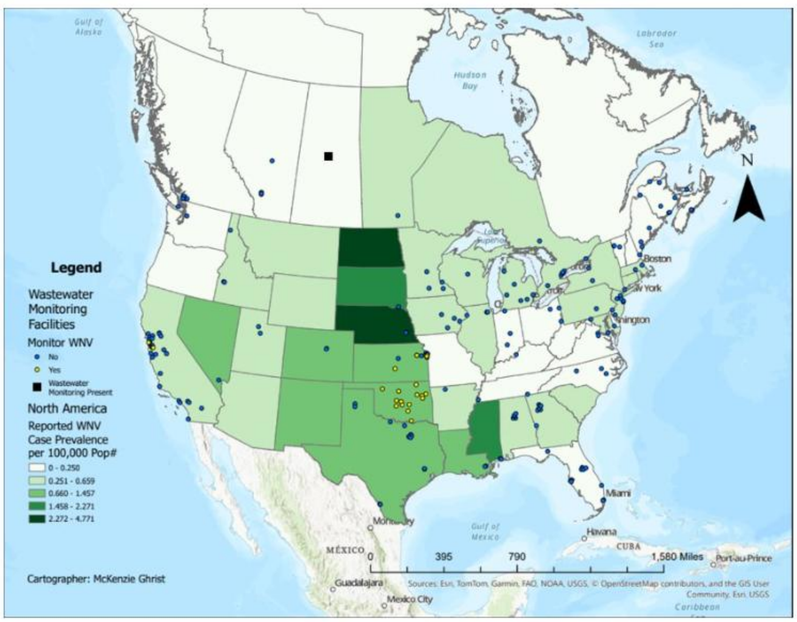
Results



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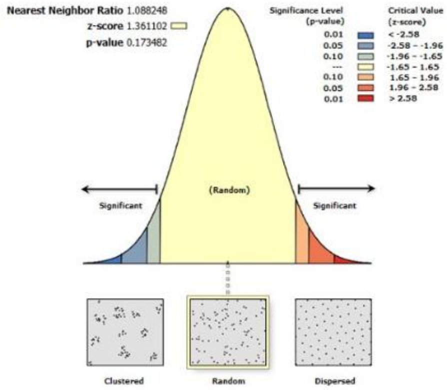
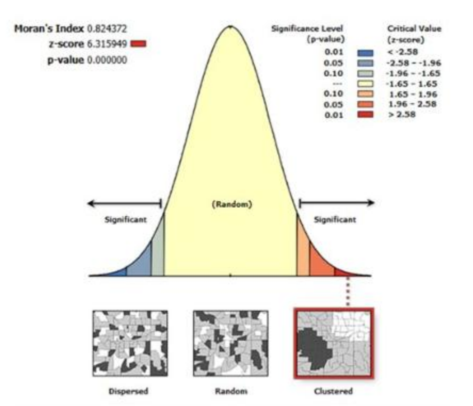
Spatial Autocorrelation – North America





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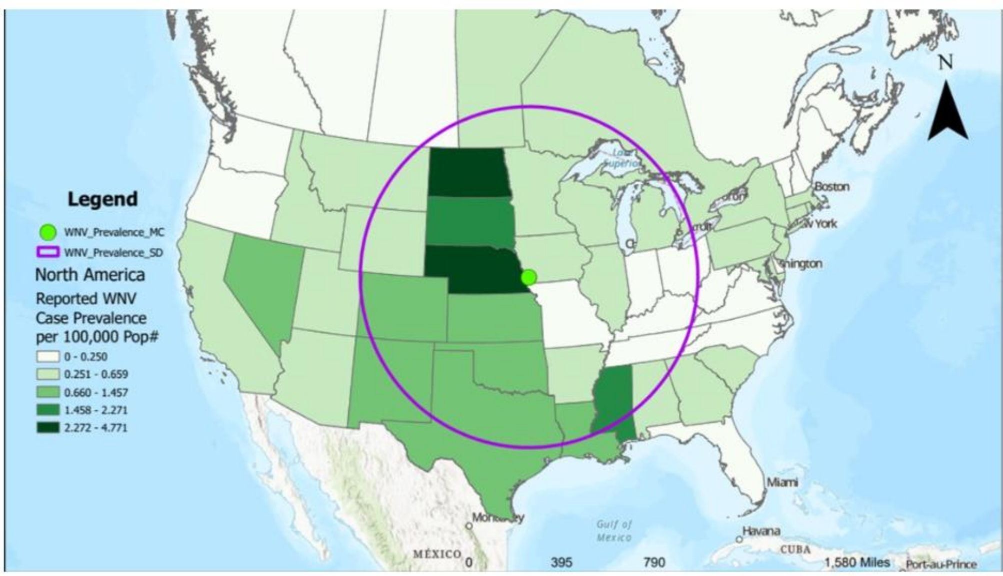

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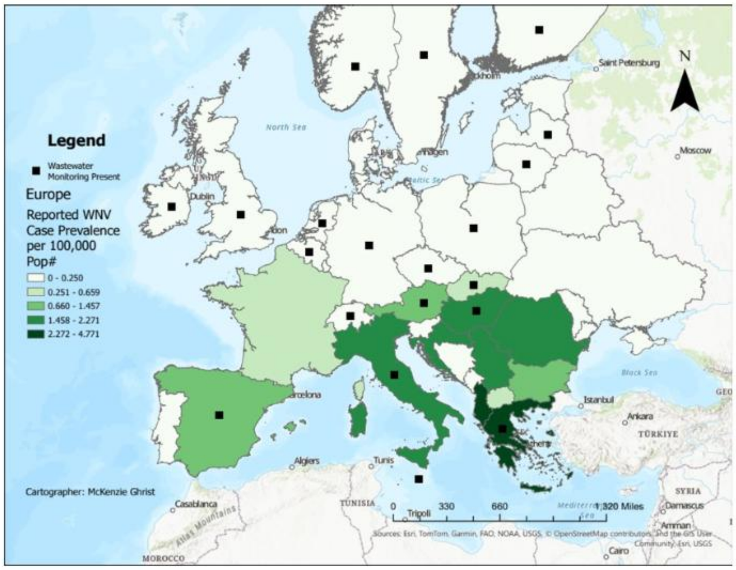

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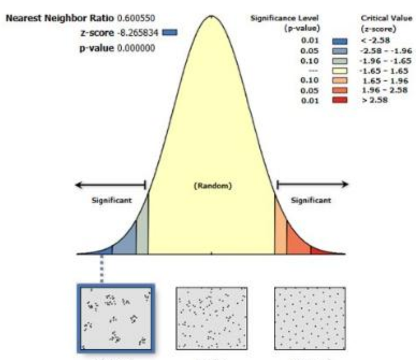
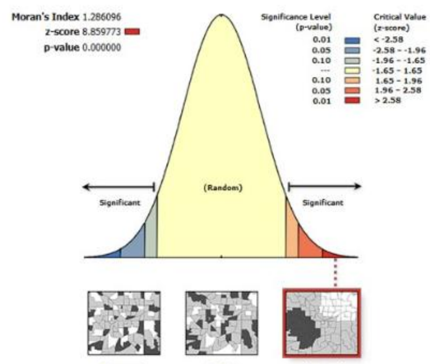
Spatial Autocorrelation - Europe





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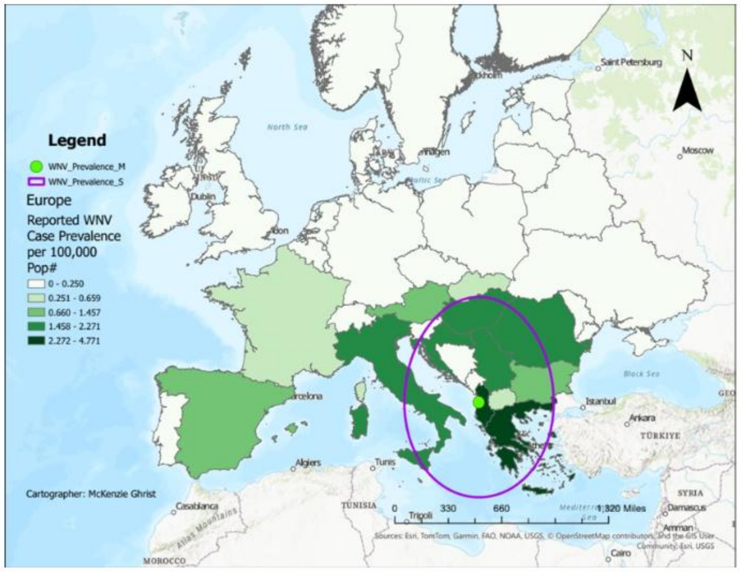

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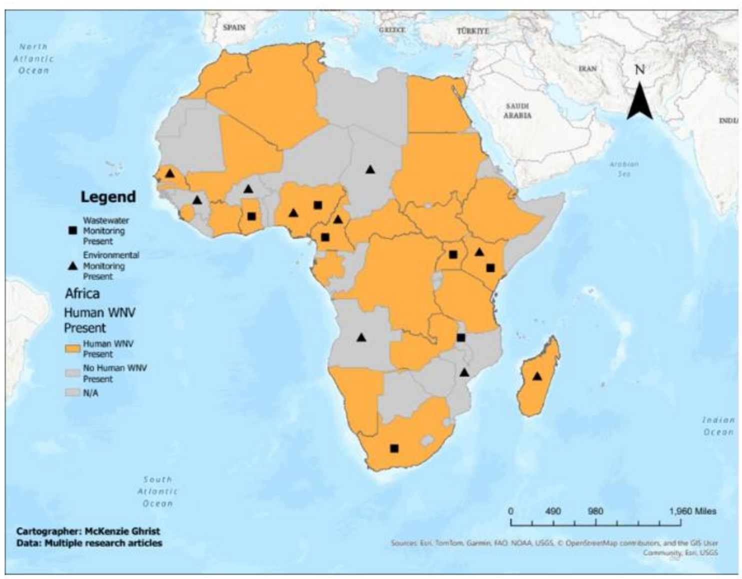

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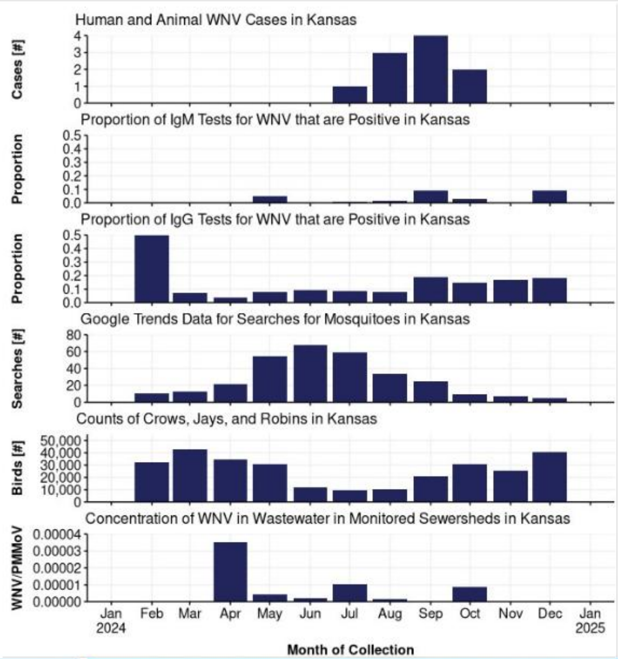
Overview of the Data we used



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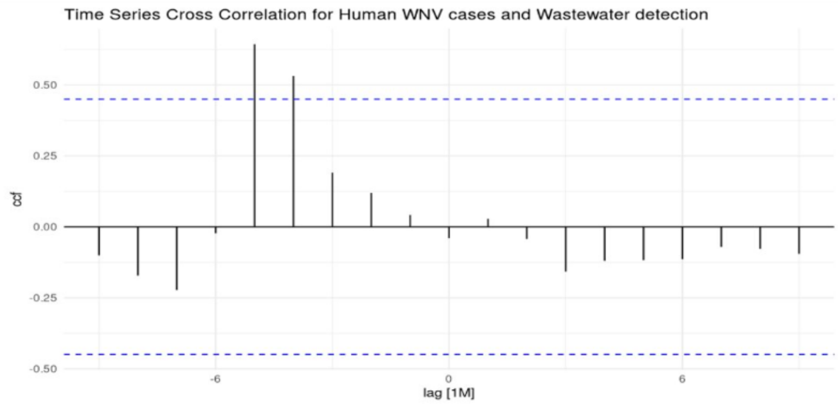
ARIMA Model: Time Series



AIC & RMSE Values

```
# A tibble: 4 × 8
  .model          sigma2 log_lik   AIC  AICc   BIC ar_roots   ma_roots
  <chr>            <dbl>   <dbl> <dbl> <dbl> <dbl> <list>     <list>
1 wnv_ww_gt_igg 0.0598     -2.47  14.9  19.5  19.7 <cpl [1]> <cpl [0]>
2 wnv_all       0.0610     -1.81  17.6  27.8  24.2 <cpl [1]> <cpl [0]>
3 wnv_gt_igg    0.199      -9.41  26.8  29.7  30.6 <cpl [1]> <cpl [0]>
4 wnv           0.591     -21.5   49.0  50.6  51.9 <cpl [0]> <cpl [2]>
```

```
# A tibble: 4 × 10
  .model        .type        ME  RMSE   MAE   MPE  MAPE  MASE RMSSE    ACF1
  <chr>         <chr>     <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>   <dbl>
1 wnv_all       Training -0.132 0.257 0.182   NaN   Inf  1.27 0.680 0.0606
2 wnv_ww_gt_igg Training -0.131 0.273 0.209   NaN   Inf  1.46 0.723 0.127
3 wnv_gt_igg    Training -0.144 0.515 0.370  -Inf   Inf  2.59 1.36  0.0396
4 wnv           Training  0.189 0.727 0.405   NaN   Inf  2.83 1.92  0.0303
```

AIC:

wnv_ww_gt_igg: fits the best

14.9 AIC

wnv_all: fits the second best

17.6 AIC

RMSE:

wnv_all: fits the best

0.257 RMSE

wnv_ww_gt_igg: fits the second best

0.273 RMSE

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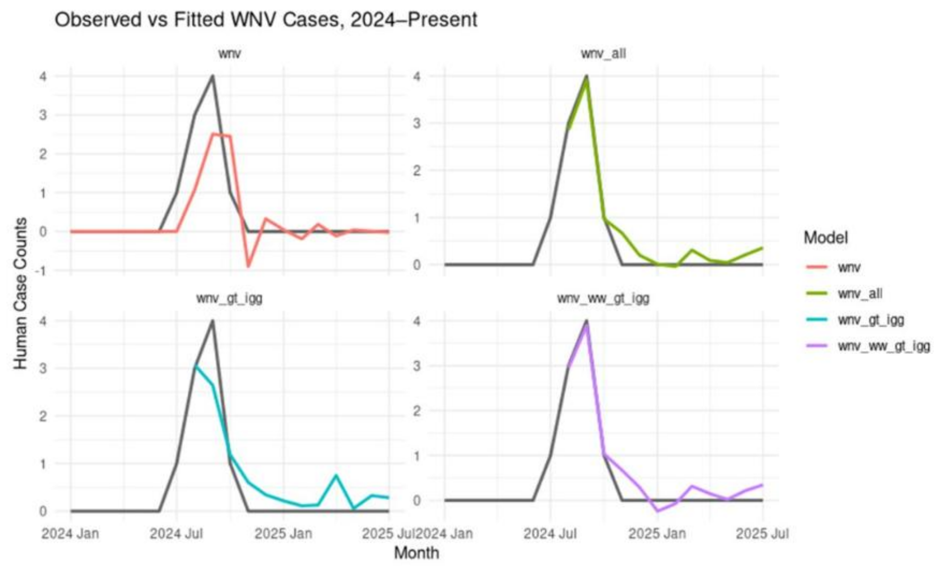

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Conclusions

- North America Map: Midwest has the most impact
- Europe Map: Countries around the Mediterranean has the most impact
- Asia and Africa are impacted by WNV
    - Some of the countries have WW monitoring
    - Didn't have case data available
- Wastewater can add information
- Further study is needed

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Thank You/Questions

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Appendix 5 – Blog Posts

Blog Post #1: Wastewater Surveillance as a Tool for West Nile Virus

By: McKenzie Ghrist, Kansas State University

West Nile Virus (WNV) is a disease transmitted by mosquitoes, more specifically *Culex* mosquitoes. Around 80% of infected individuals will not experience any symptoms, however some individuals can experience more serious symptoms. As climate change becomes an increasing issue, these mosquito-borne viruses are also increasing in incidence (Kuhn et al., 2024). Public health professionals report this virus through clinical cases and mosquito trapping. Although, WNV is possibly being underrepresented in the population due to most individuals being asymptomatic. A possible solution to better represent the population and monitor WNV cases could be through Wastewater surveillance (Lee et al., 2022).

In order for me to write a paper on using wastewater surveillance for WNV, a literature review needs to be conducted. This means finding previous literature that has tested wastewater for WNV. In my literature review most studies were not able to detect WNV in the wastewater, except for one. It is a challenge to detect WNV in wastewater due to their fragile genomes, and that individuals shed the virus at a lower rate than can be detected (Lee et al., 2022). A study in Oklahoma was able to detect WNV in several of their wastewater samples. This study is not yet published, but undergoing review as of now. They used a digital droplet PCR test that was able to detect the virus. However, it was noted that only 3 counties (which represented 1.6 million people) detected WNV in the wastewater, other counties were not able to detect the virus. This could be due to the fact that there were no human reported cases in those areas, or possibly a false negative (Kuhn et al., 2024).

After conducting a literature review, I have found that it can be detected by a certain PCR test. This is helpful to know as the KDHE starts to test for WNV. It was noted in Lee et al. article that there are other tests that could be a better fit. I would need to do more research on those tests, but as of now I know a digital droplet PCR test can work well. It is interesting that WNV is hard to detect, but that lets us know that we may not get positive tests back and that could be due to a number of things, which will be helpful in a limitations section. This literature review also prepares me to present on the topic during my defense at Kansas State University.

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MPHTC Student Field Placement Blog Post #2

Title: Mapping Wastewater Treatment Plants in Countries impacted by West Nile Virus

By: McKenzie Ghrist, Kansas State University

Geographical information systems (maps) have increased in the public health world. It can be important for individuals that are not familiar with data or public health terms to see the significance of a public health issue. Maps are a great way for lay people to visualize the data and to see whether they should be worried about an issue or not. It can also be helpful to public health professionals to see the data and know where they can implement control measures or just keep monitoring in those communities.

My responsibility is to create these maps that show what countries are impacted by West Nile Virus and to show where wastewater treatments, that monitor disease, can be found across the world. Using West Nile human cases data and having wastewater treatment plant's location across the world. However, getting data in Third World countries is proving to be harder because these countries are not reporting cases, or they don't have the data publicly available. There are studies that have found West Nile Virus cases in these Third World countries that are not reporting data. Even though the data is not present, it is still possible to highlight these countries because they are still impacted by West Nile Virus.

What I have found in my research is that Canada, Europe, Africa, Asia, and the United States are the countries that are the most impacted by West Nile Virus. This virus thrives in urban settings, like Europe, Canada, and the US. These countries also have better wastewater monitoring systems than Africa and Asia. I am still creating the maps because this takes some time. I will have to manually highlight the different countries in Asia and Africa. I will also need to find shapefiles for the different countries and wastewater treatment plants (if there are any shapefiles for wastewater treatment plants). However, once I have those maps, I will add them either to the third blog post or they can be found in my report!

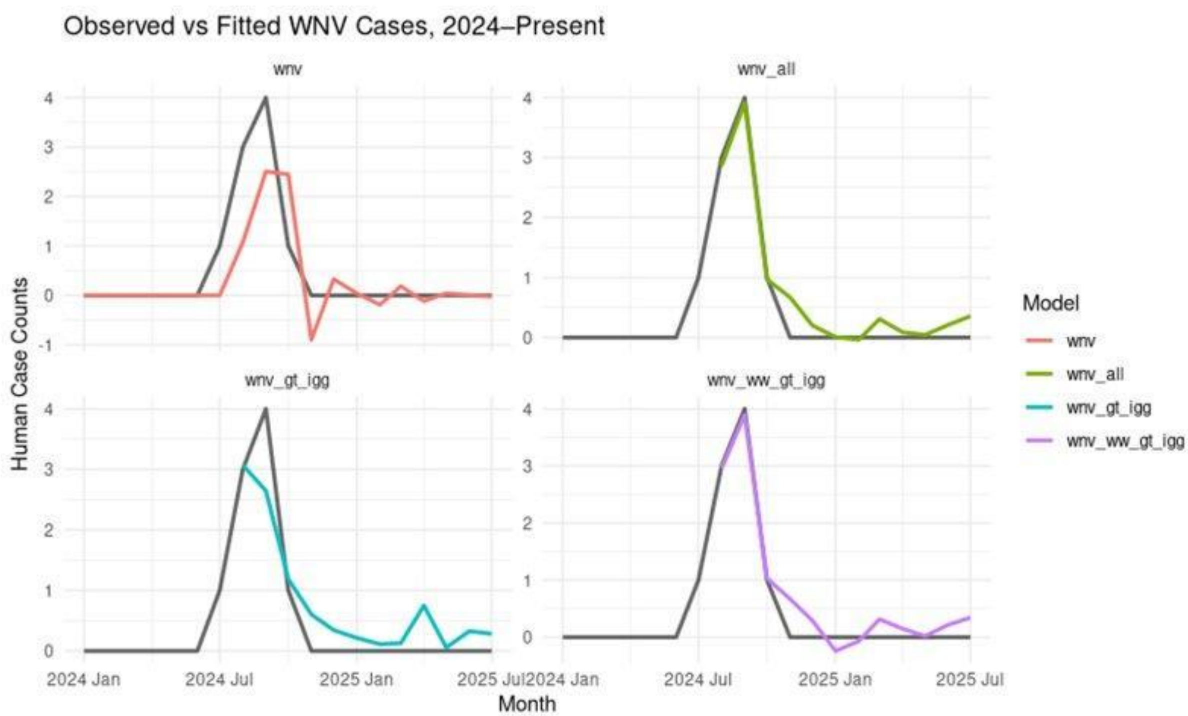
Blog Post #3: ARIMA Modeling: A Statistical Analysis

By: McKenzie Ghrist, Kansas State University

In Kansas, wastewater monitoring for West Nile Virus (WNV) started in 2024 around April-October. These months are during mosquito surveillance as well and WNV is a seasonal virus. Because we did not have a lot of data, we ran an Autoregressive Integrated Moving Average (ARIMA) model to use 2024 data and some of the 2025 data for a time series forecasting (prediction). An ARIMA model allows data analytics to see if current and past variables are correlated, handle trends and noise, along with forecasting the data (Hyndman & Athanasopoulos, 2021).

We collected data from multiple sources on WNV concentration in wastewater, google trends on mosquitoes in Kansas, serological testing (IgG and IgM), human WNV cases, and Crow, blue jay, and robin sightings in Kansas. We combined all the data into one table and from there were able to perform ARIMA models. We first started with a Kwiatkowski-Phillips-Schmidt-Shin (KPSS) root test to assess if our data was stationary, which our data was. In an ARIMA model it is important for a time series to see that the data would look the same at any time (Hyndman & Athanasopoulos, 2021). Then we ran a time series correlation to estimate appropriate/strong lags between our outcome and predictor variables for a predictive model. The time series were also helpful in our ARIMA models to determine which lags would be the best to test. After that, auto ARIMA models were built to look at human WNV cases (control) and then fit that data with the other variables in different combinations and individually. Through those models, we were able to use the Akaike Information Criterion (AIC) and the Root Mean Square Error (RMSE) to find which model was the best fit. And as a final step, we ran a Ljung-Box portmanteau test to confirm model adequacy.

In the end we found that the model with wastewater, google trends, and the IgG serological testing and the combination with all the variables were the best fit models. Then we ran a model without wastewater to show that wastewater can help in providing more information on WNV for public health professionals. Below are the predictive modeling graphs we produced.



Observed vs Fitted WNV Cases, 2024–Present
wnv
wnv_all
wnv_gt_igg
wnv_ww_gt_igg
Human Case Counts
Month
2024 Jan
2024 Jul
2025 Jan
2025 Jul
Model
wnv
wnv_all
wnv_gt_igg
wnv_ww_gt_igg