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## Viral Particle Detection In Wastewater

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# VIRAL PARTICLE DETECTION IN WASTEWATER

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by

Katelyn Clark, BS, MPH

2023

VIRAL PARTICLE DETECTION IN WASTEWATER

by

KATELYN CLARK

BS, North Carolina State University, 2020

Presented to the Faculty of The University of Texas

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# VIRAL PARTICLE DETECTION IN WASTEWATER

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School of Public Health, 2023

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Wastewater is an underutilized resource for recognizing the epidemiology of pathogen transmission and infections within a community that has the potential to mitigate disease outbreaks. Pathogens of public health concern, such as adenovirus, norovirus, and SARS-CoV-2, occur in wastewater from excretion of infected individuals. Despite the potential for wastewater pathogen data to predict future outbreaks, the relationship between viral shedding patterns in wastewater and case counts of infectious disease remains unclear. An evaluation of the peer-reviewed literature was conducted to compile characteristics of adenovirus, norovirus, and SARS-CoV-2 in the wastewater environment. These characteristics were considered in prioritizing the key pathogens for wastewater monitoring programs. SARS-CoV-2 is recommended to have the highest priority due to the recent

pandemic, in addition to high caseload, shedding volumes, and contagiousness. Adenovirus receives the next highest priority because the positive PCR detections have been increasing (indicating there may be an increase in cases), the large case burden, and pathogen hardness in wastewater. Norovirus receives the final ranking of the three but should still be a priority due to high caseload, shedding volume, and contagiousness. Wastewater-based epidemiology is gaining recognition as a substantial sector of epidemiology and public health research. This work provides a criteria platform to inform future quantitative microbial risk assessments that will build a framework for interpreting wastewater pathogen monitoring data. This framework will aid community leaders in public health decision-making based on the occurrence of specific pathogens detected in sewage.



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## INTRODUCTION

### *Wastewater-based Epidemiology*

Wastewater is an underutilized resource for recognizing the epidemiology of pathogen transmission and infections within a community that has the potential to mitigate devastating outbreaks. Pathogens of public health concern, such as adenovirus, norovirus, and SARS-CoV-2, occur in wastewater from excretion of infected individuals. Despite the potential for wastewater pathogen data to predict future outbreaks, the relationship between viral shedding patterns in wastewater and case counts of infectious disease remains unclear. Wastewater-Based Epidemiology (WBE) uses wastewater surveillance to create community health profiles (Dutta, 2021). Wastewater monitoring of microbial hazards provides a generally unbiased estimate of disease prevalence compared to conventional case diagnostics. This method of monitoring also accounts for asymptomatic and undiagnosed caseloads. In order to apply this more complete picture of disease burden, the link between microbial particles in wastewater and case trends needs to be better understood.

The relationship between viral shedding patterns in wastewater and case counts of infectious diseases, while requiring more research, has potential to predict infections in a community and prevent outbreaks. The objective of this study is to evaluate viral particle shedding patterns of select pathogens detected in sewage. The study has identified virus-specific excretion parameters to inform the design of a framework that can be used to estimate community infections from a range of pathogens. Adenovirus, norovirus, and SARS-CoV-2 occur in wastewater in high numbers due to environmental hardiness of the viruses, high shedding volumes from infected individuals, and typically high caseloads. This framework will provide guidance to community leaders as they interpret wastewater surveillance data for public health preparedness. Further, the

proposed paradigm will identify the characteristic properties that make a pathogen an ideal candidate for monitoring in community wastewater.

### *Quantitative Microbial Risk Assessment (QMRA)*

The viral characteristic property data generated will inform a quantitative microbial risk assessment (QMRA) approach that will estimate the number of specific viral infections circulating in the neighborhoods served by that particular wastewater treatment plant. QMRA is a systematic approach to estimating the probability of infection, illness, and death from exposure to a disease-causing microorganisms. The dynamic, four-component framework of hazard identification, dose-response assessment, exposure assessment, and risk characterization is an iterative process that comprehensively evaluates the pathogen-host interaction (Haas, 1999). The pathogen is the focus of a QMRA, with data generated from field and/or laboratory studies to inform its occurrence in the environment, its survivability and virulence properties, and transmission pathways. Previous human dose-response studies are available for many pathogens transmitted through environmental sources (such as air, water, food, and fomites), and best-fit mathematical models have been determined to represent infection probabilities for specific microorganisms (Haas, 2014). Such information will be used to evaluate how the number of viral particles detected in wastewater may relate to the number of community infections.

QMRAs are an effective tool to predict human health impacts associated with exposure to pathogens. Likewise, a *reverse* QMRA can be applied to estimate the number of infections in a community based on pathogen occurrence in an environmental source, such as microbial particles circulating in a wastewater system. The study results will be a critical reference for risk assessors to define QMRA parameters that will determine the state of viral transmission in the community. The QMRA will translate the viral wastewater data to meaningful information

regarding. These data driven decisions are being implemented at institutions like the Center for Advancing Microbial Risk Assessment (CAMRA) at Michigan State University.

### *Transmission & Shedding Characteristics*

Transmission methods discussed include person to person, foodborne, waterborne, droplet, and airborne. There are various ways to quantify transmission of disease. Statistical methods of transmission include transmission probability ratio, basic reproduction number, effective reproduction number, transmission rate, etc. (Mubayi et al., 2021; Delamater et al., 2019; Gaythorpe et al., 2017; Woolhouse, 2017). The transmission statistic discussed in this thesis is the basic reproduction number ( $R_0$ ).  $R_0$  is the predicted number of infections possible from one infected individual placed in an entirely susceptible population.  $R_E$ , the effective reproduction number, considers possible limitations of the pathogen when the entire population is not susceptible (Delamater et al., 2019; Gaythorpe et al., 2017).

Pathogens are most effective when they induce symptoms in their host to aid transmission. For example, rabies is transmitted through saliva (typically in bite wounds), and it causes esophageal paralysis which, in turn, results in more saliva remaining in the mouth. Respiratory pathogens are transmitted through the air or droplets, aided by sneezing and coughing. Gastrointestinal pathogens are often transmitted through the fecal-oral route while causing vomiting and/or diarrhea. Terminology such as super-shedder or super-spreader describe qualities of transmission from individuals rather than communities. Super-shedders are infected individuals that shed the disease in large volumes and are therefore highly contagious. Super-spreaders are infected individuals that have many opportunities to transmit the disease (Woolhouse, 2017). Certain pathogens of particular concern cause illness, but the importance is amplified by the sequelae experienced from recovered individuals.

## *Research Aims*

Wastewater-Base Epidemiology (WBE) is a way to better characterize infectious disease trends in a community. Current events demonstrate the great potential in studying SARS-CoV-2 in wastewater. SARS-CoV-2 can be shed in feces although there have not been any reported cases attributed to direct contact with treated or untreated wastewater (Dutta et al., 2021). Pathogens of public health concern, such as adenovirus, norovirus, and SARS-CoV-2, have all been identified in wastewater facilities; however, very little is understood about the public health implications of viral particles shed into wastewater. Despite the potential for these data to predict future outbreaks, the relationship between viral shedding patterns in wastewater and case counts of infectious diseases remains unclear. This study aimed to determine how viral shedding patterns reflect case trends and how they can be utilized in earlier prevention implementations. The current objective is to evaluate viral particle shedding patterns of this group of pathogens of public health concern in wastewater facilities by identifying its impact on quantitative microbial risk assessments. The long-term goal of this study is to decrease morbidity and mortality of infectious disease by using the connection between viral shedding patterns in wastewater and case trends to predict the spread of disease in any community equipped to study wastewater facilities. The central hypothesis is that viral shedding patterns of these pathogens found in the wastewater will predict case trends. The aims to test the hypothesis are as follows:

**Aim 1.** To identify viral shedding patterns of distinct pathogens in wastewater facilities. This study will be based on a literature review categorizing viral particle shedding patterns of the selected group of pathogens. Historical data will be accumulated on viral particle load, incubation period, modes of transmission, and documented outbreaks.



**Aim 2.** To identify drivers of health risk based on the characteristics of distinct pathogens.

Information on virulence, shedding patterns and community parameters will inform QMRAs of pathogens detected in wastewater. Wastewater monitoring works outside of testing accessibility and availability of healthcare (CDC, 2022). This type of monitoring also accounts for cases with and without symptoms to indicate more complete case trends.

The successful completion of these aims has provided information to build a framework that will potentially predict case trends earlier for a variety of pathogens of public health concern. Earlier prediction of trends will lead to improved preparedness responses for communities at risk. The ability to predict case trends via viral particle detection in the wastewater would greatly improve the ability to decrease morbidity. In the future, this impact will expand basic epidemiological knowledge and prevent outbreaks of these viruses and decrease morbidity and mortality from diseases of top public health concern.

## METHODS

### *Literature Review*

The aims of this research were executed by conducting a review of peer-reviewed literature using PubMed and Elsevier databases regarding adenovirus, norovirus, SARS-CoV-2, and wastewater-based epidemiology. The review highlights pathogen characteristics pertinent to wastewater-based epidemiology. The purpose of this review was to obtain and centralize information to characterize viral pathogens in terms of dose-response relationships and excretion patterns from a variety of sources. This information will be used to develop a guide for community wastewater monitoring interpretation and application.

### *Searching Mechanisms*

Boolean search methodologies were applied during the initial literature accumulation phase from PubMed and Elsevier. Peer-reviewed literature was broadly queried using combinations of “adenovirus”, “norovirus”, “SARS-CoV-2”, and “wastewater-based epidemiology”. Additionally, the terms “shedding pattern(s)”, “excretion”, “dose-response”, and “shedding mechanism(s)” were used to yield results regarding shedding patterns of the suite of pathogens. The search was refined further by the addition of the terms “wastewater”, “wastewater monitoring”, “sewage”, “storm water runoff”, “wastewater surveillance”, “wastewater facility”, and “water treatment”. The truncation symbol (\*) was used to broaden the search to ensure relevant literature would not go unnoticed. For example, “adeno\*” was used to find results relevant to adenovirus, adenoviral, adenoviridae, etc. After the preliminary literature recruitment, searches were simplified by the addition of the key/subject terms from the initial articles.

### *Organization Tools*

Articles were added to Zotero via a web extension from the databases. Zotero is an open source citation managing software. Here, PDFs of the articles can be annotated. This software can create folders and subfolders for easy organization of literature. Additionally, Zotero has the capability to host an RSS feed for related articles from linked databases. After culling articles that the RSS feed brought in, it ultimately accrued *eight* additional articles that were included in the final product. The various routes of literature accrual resulted in over 226 different articles ranging from the years 1978 to 2023. Articles were evaluated based on publication year, how many times it has been cited, and relevancy to the topic. In total this thesis is the culmination of 52 peer-reviewed articles and 3 web sources (WHO, CDC, Britannica).

### *Data, Measurements, and Statistical Analysis*

Data were abstracted from a CDC reporting website for each pathogen, except SARS-CoV-2 (CDC). Due to the timeline of this thesis, data abstraction for SARS-CoV-2 morbidity and mortality reports was not feasible, as the pandemic and associated data collection are ongoing. The COVID-19 pandemic, while it remains of high public health concern, is not comparable to the other viruses discussed. For the purposes of this thesis, coronavirus OC43 is used to represent a “typical” coronavirus in the discussion for comparison of seasonal trends and typical occurrence to other pathogens. Data were compiled and visualized in Microsoft Excel. Percent positive PCR detection data were input as a line graph with time as the X-axis to show how the percentage of positive tests increases or decreases over time (Figures 1, 3, 4). These data were collected from March 5<sup>th</sup>, 2022 to March 5<sup>th</sup>, 2023 to represent the most current trends available.

For norovirus, there was an additional database available with morbidity and mortality tallies reported monthly from 2013 to 2020. Data from the years 2021 and 2022 are still being entered into the NORS database. These data were sorted by pathogen in Microsoft Excel because the database included morbidity and mortality information from a variety of foodborne pathogens. All non-norovirus pathogens were removed, and the remaining data were sorted by month and year. These data were entered into a new spreadsheet and a conditional formatted heat map showed the variation in color based on a numerical threshold (Figure 2). The resulting heatmap visually aids in demonstrating the seasonal trend of norovirus in the United States.

## RESULTS

### ADENOVIRUS

#### *Characteristic Properties*

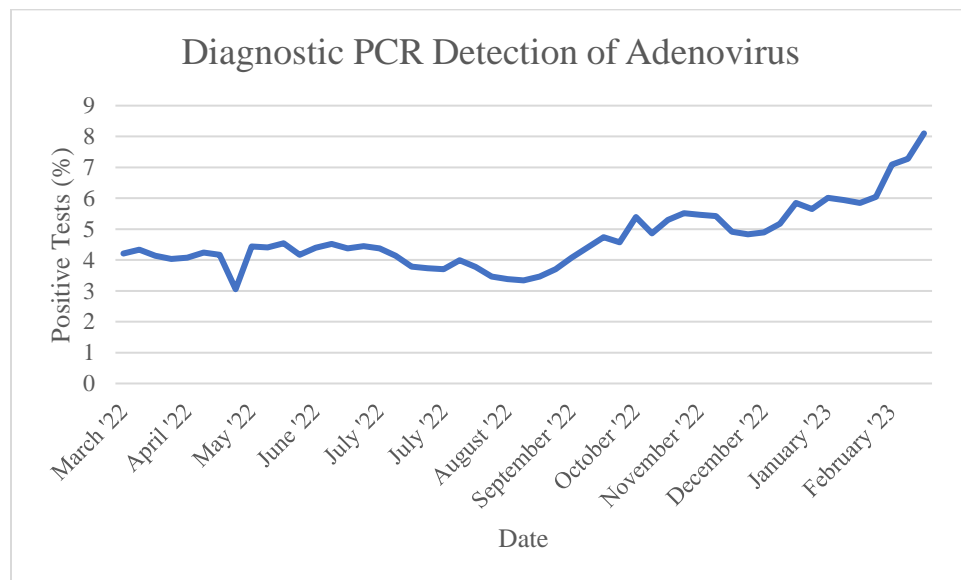
Adenovirus is aptly named from the Adenoviridae family which was originally isolated from human adenoid tissue (Doerfler, 1996). It is a non-enveloped, double-stranded linear DNA virus, which means it falls into classification I of the Baltimore scheme (Doerfler, 1996). DNA is more stable than RNA, so adenoviruses may be more resistant to disinfection processes and inhospitable environments (Dhingra et al., 2019). Adenoviridae is known to have six genera and 47 species that are categorized alphabetically and numerically. Of the human adenovirus species, there are 88 known genotypes. The largest species group, D, contains 57 of the genotypes (Britannica). These genotypes are mostly categorized by type (previously “serotypes”), but as adenoviruses continue to emerge, using genotypic definition with novel sequences is necessary. Adenoviruses of species C, though limited, are already more clinically significant than species B and D. Species C can particularly affect immune compromised individuals. More than half of adenovirus infections are caused by either C type 1 or 2, and mainly manifest in the respiratory system (Dhingra et al., 2019). Very few of the many known adenoviruses actually cause illness in humans. Adenoviruses mainly cause respiratory symptoms, but different strains can cause gastrointestinal symptoms (Gholipour et al., 2022).

#### *Occurrence & Transmission*

Adenovirus is reportedly responsible for 2 to 5% of all the world’s respiratory infections (Akello et al., 2020). This virus is mostly present in children with nearly all children having been infected by the age of 10 (Doerfler, 1996, CDC). Transmission of this virus is typically via respiratory

droplets from person to person. Adenovirus is typically diagnosed via differential diagnosis due to the quick duration in acute cases (Doerfler, 1996, CDC). Acute cases will have symptoms similar to the common cold or flu. Severe cases can have fatality up to 20% due to pneumonia and bronchitis. In 2021, an outbreak of hepatitis in children emerged and it is thought to be possibly linked to adenovirus (Reyne et al., 2023). Immune compromised individuals are at a higher risk for developing complications because of the adenovirus infection residing in tonsil, adenoid tissue, and intestines—all major components of the human immune system. Adenoviruses are extremely contagious with  $R_0$  as high as 5.1 in one study (Guo et al., 2020). Adenovirus is considered a pathogen of high public health concern as percentages of positive tests increase (Figure 1) due to the genetic diversity and high variation rate (Gholipour et al., 2022). A summary of basic adenovirus characteristics can be found in Table 2 at the end of the results section.

Figure 1: Percent Positive PCR Detection of Adenovirus



### *Dose Response & Shedding*

The various strains an individual may contract determines the type of symptoms that occur. Adenoviruses can be primarily shed through two routes: respiratory droplets and fecal-oral. According to Teunis et al., there are several variables that impact the dose-response relationship. Virus type, characteristics of the host, and mode of transmission are the most important variables that affect this relationship (Teunis et al., 2016). This claim insinuates the dose-response relationship is different if an individual is exposed via the fecal-oral route versus respiratory droplets. The study by Teunis et al. revealed that while adenoviruses are generally highly infectious, manifestation of gastrointestinal symptoms requires a higher inoculum than respiratory manifestations (Teunis et al., 2016). An infectious dose study by Yezli et al., revealed that as few as 6.6 adenovirus type 4 particles is the minimum requirement to establish an infection in 50% of those exposed to respiratory droplets (Yezli, 2011). Adenovirus type C DNA can enter latency, and asymptomatic shedding can occur in feces for years after infection (Kosulin et al., 2016). Individuals typically recover from adenovirus infections without medical intervention, though infection in an immunocompetent individual can be lethal on rare occasions. A summary of shedding properties of adenovirus can be found in Table 1 at the end of the results section.

### *Wastewater-based Epidemiology*

The systematic review by Gholipour et al. used the “PICO” strategy for organizing their data. PICO stands for population, intervention, comparison, and outcome. This review defined populations as viruses or viral particles, while the intervention was wastewater composites. The comparison and outcomes looked at the presence of viral particles that remained after exposure to the “interventions”. Adenovirus was the most frequently detected enteric virus in wastewater

samples, followed by norovirus (Gholipour et al., 2022). Adenoviruses have been found to be abundant in many different environmental samples (Teunis et al., 2016). The capsid protein is responsible for high resistance to environment in abundance (Teunis et al., 2016). Additional hardiness attributes of adenoviruses are that they can be detected in chlorinated drinking water and survive up to 132 days in wastewater (Gholipour et al., 2022).

## NOROVIRUS

### *Characteristic Properties*

Norovirus is a member of the caliciviridae family, which is a class IV virus. The class grouping, from the Baltimore Scheme, is based on size, viral genome, and other structural similarities. There are some structural similarities among all group IV viruses, but the grouping denotation does not mean the viruses are functionally the same. The only common denominator among all viruses in this classification is the positive-sense, single stranded RNA genome ((+)ssRNA) (Berman, 2012). When a virus is positive-sense, it means that the RNA strand is the infectious particle and they do not need encapsulation (capsid, envelope or enclosed protein) to infect a human cell (Berman, 2012). Genera in the caliciviridae family other than norovirus are sapovirus, lagovirus, vesivirus and nebovirus. Both sapovirus and neboviruses are known causes of gastroenteritis in other mammal species including humans, though not as commonly as noroviruses (Desselberger, 2019). Other class IV viruses include picornaviridae, togaviridae, coronaviridae, hepeviridae, flaviviridae and astroviridae. Further, there is a division of the class IV viruses among enveloped (flaviviridae, togaviridae, and coronaviridae) and non-enveloped (caliciviridae, picornaviridae, hepeviridae, and astroviridae) (Berman, 2012). A summary of basic norovirus characteristics can be found in Table 2.



### *Occurrence & Transmission*

Young children and older adults are especially vulnerable to norovirus infection complications due to the severe dehydration that can follow the progression of symptoms. On average, there are 900 deaths among the 65 and older group and 19-21 million cases of vomiting and diarrhea each year in the US. Norovirus is responsible for over half of the foodborne illness acquired in the US and an estimated \$2 billion in productivity loss and healthcare expenses (CDC). The typical season for norovirus spans from November to April (Figure 2).

There is a strong seasonal pattern of norovirus outbreaks during the winter months in the United States, earning norovirus the moniker the “winter vomiting disease” (Gaythorpe et al., 2017). Cases and fatalities trend upward during the winter season from 2013 to 2020. Data for the years 2021-2023 are still currently being collected and collated into the National Outbreak Reporting System (NORS) and CaliciNet, but historical data have revealed several notable patterns (Figure 2). The highly infectious nature of the virus and shedding patterns of those infected make close-quarters situations, such as cruise travel and hospital and nursing facilities, prime grounds for outbreaks. Norovirus has an extremely low infectious dose and the capability to produce high shedding volumes. This is a dangerous combination with cases shedding billions of viral particles into the environment and only requiring 18 particles to establish an infection (Robilotti et al., 2015).

Figure 2: Heatmap of Norovirus Seasonal Morbidity and Mortality (*National Outbreak Reporting System (NORS) Dashboard* /CDC, n.d.).

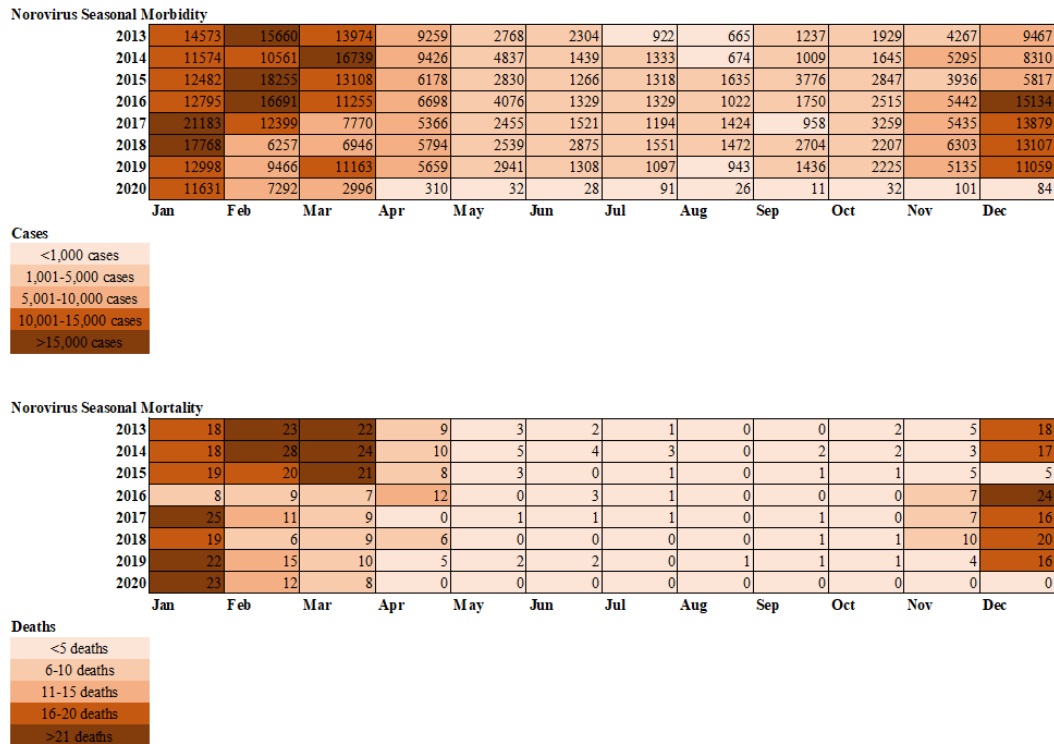
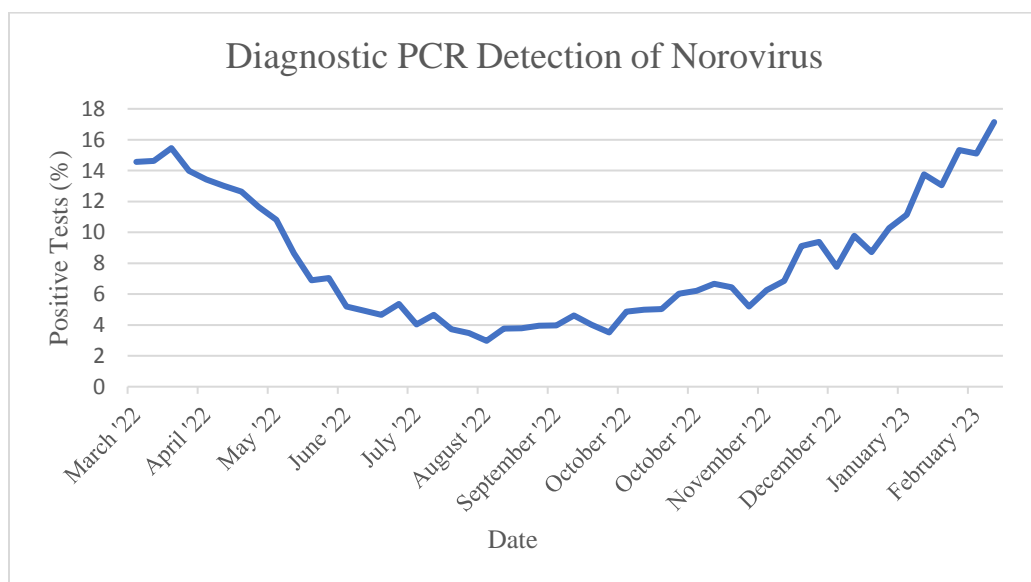


Figure 3: Percent Positive PCR Detection of Norovirus



Transmissibility is quantifiable with the basic reproduction and effective reproduction numbers known as  $R_0$  and  $R_E$ , respectively (Delamater et al., 2019). Norovirus  $R_0$  calculations are slightly more complex because norovirus typically occurs in outbreaks. In the scenario of outbreaks, each duration is different, and populations are highly specific. It makes each outbreak a unique scenario that is difficult to obtain information that is generalizable. The  $R_0$  estimate is greater than 2, which means it is a highly contagious pathogen (Gaythorpe et al., 2017).

### *Dose Response & Shedding*

Noroviruses remain a constant, worldwide public health threat for viral gastroenteritis. The primary mode of transmission is fecal-oral, which is facilitated by the profuse vomiting and diarrhea that can occur as symptoms. Norovirus can also be transmitted through contaminated food, water, and surfaces. The average incubation time of noroviruses ranges from 12 hours to 48 hours, but an infected individual can continue shedding the virus up to two weeks after the presentation of the last symptom. The severity of illness has been assessed in several dose-response relationship studies. Like many pathogens, a higher attack rate is common among those exposed to a higher number of viral particles (Thornley et al., 2011).

On average up to 30% of cases are estimated to be asymptomatic (Miura et al., 2018). The percentage of asymptomatic individuals can have a large range with each outbreak being unique. Asymptomatic individuals shed the virus in an amount that is not significantly different from symptomatic individuals. Symptomatic individuals are more efficient at shedding the virus and likely contribute more to transmission than asymptomatic individuals. However, in identical durations, asymptomatic individuals have the potential to produce and shed viral particles in similar volumes as symptomatic individuals (Teunis et al., 2015). A summary of other shedding properties of norovirus can be found in Table 1 at the end of the results section.

Since there is no known treatment or vaccine available, prevention of transmission and supportive care are important. An effective vaccine for noroviruses would have a significant public health impact, however, no effective options are yet available. Noroviruses are responsible for a large portion of the gastroenteritis burden and economic impact of morbidity and mortality. Human noroviruses are genetically very diverse, with five genogroups and 35 sub-genotypes. The dominant strain of human noroviruses responsible for the most infections is genogroup II, genotype 4 (GII.4). GII.4, a strain of high public health impact, is a priority for vaccine development. Unfortunately, noroviruses can easily evolve and produce different variants and are not compatible with growth in cell culture. In previous vaccine candidate studies, there have been inconsistent immune responses in the animal models for an undetermined reason (Tan, 2021). Developing a human norovirus vaccine remains an important endeavor in public health research even though there are many obstacles like, genetic diversity and culture capabilities. Due to the complicated nature of vaccination and treatments of noroviruses, monitoring and prevention are critical.

The most promising potential drug therapy for noroviruses are protease inhibitors (Chang et al., 2019). Proteases are enzymes that break up peptide bonds in target proteins, rendering them inoperable. A protease inhibitor targeting the capsid structure of noroviruses would be an ideal candidate for drug therapy. Though encapsulation is not required to cause infectivity in class IV viruses, the presence of a variety of encapsulation mechanisms assists the virus in the environment. This structure increases the likelihood of survival in the environment and against the host immune system. The capsid structure is dynamic and highly flexible, which inhibits studying the host immune response and potential vaccine targets (Smith & Smith, 2019). The capsid structure of norovirus is one plausible explanation for its high volume in wastewater.

## *Wastewater-based Epidemiology*

Wastewater-based epidemiology is a method to explore the incidence of human norovirus infections. The amount of ribonucleic acid (RNA) contributed to wastewater by shedding is an indication of the prevalence of norovirus infections in the served community. This prevalence estimation method has great potential to decrease morbidity and mortality of norovirus, and infectious disease in general. Back-estimation research attempts to calculate quantities of viral particles in wastewater (Guo et al., 2022). Requiring numerous cycles of isolation to get a clean sample in RT-PCR, significant loss of volume of the genetic material is a concern. The main aspect hindering back-estimation is the sensitivity to analytical detection (Guo et al., 2022). Other factors, like seasonal temperature changes, are known to influence RNA decay rates, but this is negligible for norovirus. No evidence has shown that temperature severely affects decay rates. Norovirus RNA is also so numerous in wastewater that the amount lost to RNA decay is not a concern. Biofilms have an unknown impact on norovirus RNA decay rates. The microbiome of wastewater facilities potentially impacts the likelihood of detecting norovirus. The magnitude of this impact has yet to be studied and it is unclear how it will be addressed in the future (Guo et al., 2022).

The detection of norovirus viral particles in wastewater can be observed increasing during the winter season. Preliminary studies have also shown that concentrations of viral particles in the wastewater correlate with case numbers. Norovirus is shed through the feces and vomit of cases and asymptomatic infections. This shedding pattern can cause particularly high concentrations of norovirus particles in wastewater. Huang et al. performed a meta-analysis across 46 studies on norovirus detection in wastewater. After controlling for factors like genogroup and community mortality rates in a multifactor regression, the  $R^2$  was 52.81%. The norovirus genotype and

mortality information significantly affected the analysis results. Norovirus genotype II was detected in wastewater at a higher rate. This meta-analysis also confirmed that noroviruses were detected at higher rates over the winter than in the summer seasons. (Huang et al., 2022).

In the quantitative report of viral enteric pathogens by Jahne et al., norovirus genogroup II was the most commonly detected virus in wastewater. This detection was made via ddPCR (digital droplet). This method was used over quantitative real time PCR, because ddPCR was more sensitive in comparison. Although wastewater analysis is not standardized yet, sample handling at individual wastewater facilities (that likely varies from location to location) is not thought to hinder the results (Jahne et al., 2020).

## SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

### *Characteristic Properties*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of the coronaviridae family. Corona, meaning crown in Latin, is named due to the halo-like effect coronaviruses appear to have under an electron microscope. This halo is caused by viral surface proteins embedded in the lipid membrane of the envelope. The coronavirus genome is a positive-sense, single strand RNA ((+)ssRNA) (Ludwig, 2020). Like norovirus, this is a class IV virus meaning it does not require any encapsulation to infect human cells. The surface protein (S-glycoprotein) attaches to the angiotensin-converting enzyme 2 (ACE2) on human cells. This attachment site is what allows human to human transmission of coronaviruses as it is found in the lower respiratory tract (Jia et al., 2005).

There are four genera in the coronaviridae family: alpha, beta, gamma, and delta. The genera alpha and beta primarily infect mammals. Porcine epidemic diarrhea (PED) virus is a

coronavirus as well that is extremely important to veterinary medicine (Ludwig, 2020). PED causes extreme diarrhea and can eliminate entire herds. SARS-CoV-2 belongs to the beta genera. Genera gamma and delta primarily infect birds. There are several genomic recombination errors that commonly take place in coronaviruses, adding to their vast diversity. This diversity is what allows coronaviruses to infect many different species. Diversity is also believed to be responsible for the zoonotic transmission of coronaviruses (Ludwig, 2020). The other notable outbreaks of coronaviruses traced back to bats were severe acute respiratory syndrome (SARS) in 2003 and Middle East respiratory syndrome (MERS) in 2005 (Drexler, 2014). A summary of the basic characteristics of SARS-CoV-2 can be found in Table 2.

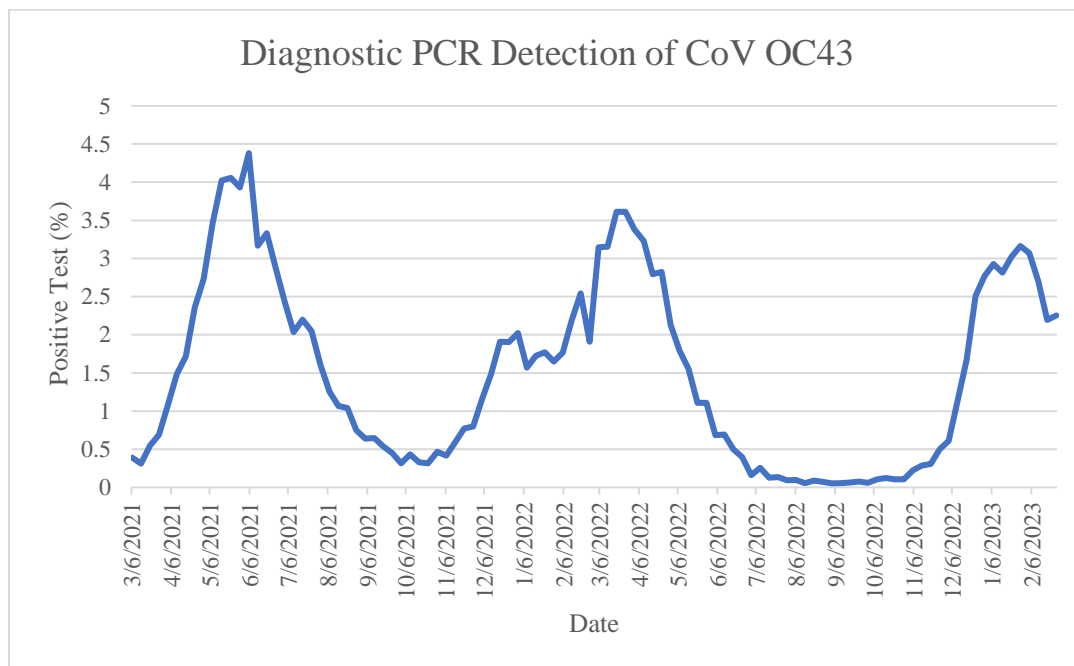
#### *Occurrence & Transmission*

SARS-CoV-2 is the causative agent of Coronavirus Disease 2019 (COVID-19) and emerged in December 2019. It soon spread globally and was declared a pandemic by the World Health Organization (WHO) in March 2020. As of March 2023, there have been over 100,000,000 cases and over one million deaths in the United States (CDC, 2023). It is assumed to have followed a similar origin pattern to SARS and MERS via bat transmission to other mammals (Ludwig, 2020). As of March 2021, the best estimate for the  $R_0$  of SARS-CoV-2 is about 2.5 (CDC, 2022) which indicates a highly contagious pathogen. Studies and simulations have exhibited an  $R_0$  range from 2 to 4 (D'Arienzo, 2020; CDC, 2022). A basic reproductive number of 2.5 indicates a highly contagious pathogen. Like norovirus, the  $R_0$  for SARS-CoV-2 is difficult to estimate in the context of waves and variants.

Upon emergence of SARS-CoV-2, a three-wave pattern was noticed worldwide. The first wave was the initial emergence of the pathogen, the second was an increase in cases towards fall 2020, and the third wave occurred in early 2021 (Shabasy, 2022). Mortality rates have reduced in

subsequent waves, but the magnitude of the death toll still weighs heavily on the world. Figure 4 exhibits the positive test percent for a standard seasonal trend of a coronavirus. Coronavirus OC43 causes acute respiratory symptoms, like SARS-CoV-2, but demonstrates more generalizable information on coronaviruses. SARS-CoV-2 is a very unique and atypical scenario.

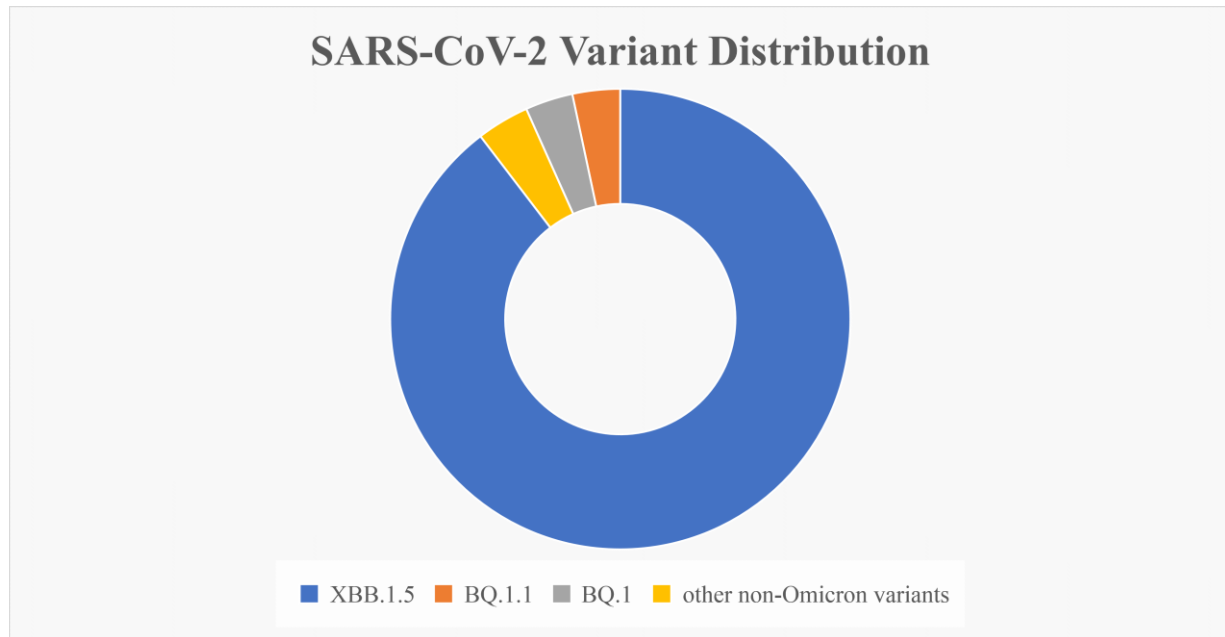
Figure 4: Percent Positive PCR Detection of Coronavirus OC43





variant of concern as of March 2023 and is responsible for over 90% of current caseload (CDC) (Figure 5).

Figure 5: March 2023 SARS-CoV-2 Variant Distribution



Droplets and respiratory secretions are the main route of transmission (Li, 2020). Symptoms include fever, malaise, cough, and general flu-like symptoms (Wang, 2020). More severe presentations can include acute respiratory distress syndrome (ARDS), multiple organ failure, and death (Huang, 2020). Older adults and those with underlying conditions, such as heart disease or diabetes, are particularly at an increased risk of transmission and severity of symptoms. There is little known about long term or chronic effects following a COVID-19 infection. SARS and MERS followed a similar trend of having sequelae long after recovery (Mehandru, 2022). Though the impact of COVID-19 sequelae is suspected to be larger, as millions report persistence of symptoms post infection compared to the SARS and MERS epidemics. Symptoms of "Long COVID" include brain fog, fatigue, joint pain, anxiety,

depression, palpitations, and other more serious cardiac symptoms (Raman, 2022). Very little is known about the cardiac implications of COVID-19. Massive systemic inflammation, known as a cytokine storm, and multiorgan dysfunction result from a COVID-19 infection. It is thought that these aspects of infection may lead to cardiac sequelae. Additionally, treatments for COVID-19 may have unknown interactions with those already on cardiovascular medication (Long, 2020). As COVID-19 sequelae continue to unfold, the world will likely see deaths attributed to COVID-19 well into the future.

#### *Dose Response & Shedding*

Numerous shedding routes have been researched in the aim of slowing down the spread of the COVID-19 pandemic. In serology negative patients, oral and rectal swabs continued to test positive for SARS-CoV-2 RNA. The trend identified that oral swabs were more commonly positive at an early stage in the infection, while rectal swabs tested positive at later stages in infection or recovery. These results imply that SARS-CoV-2 RNA remains in the GI tract and can shed through feces long after a case tests negative via oral test kits (Zhang, 2020). This is a dangerous implication to have cases shedding after recovery of symptoms. A summary of additional shedding properties is found in Table 1.

#### *Wastewater-based Epidemiology*

Wastewater data has been used to track viral spread of SARS-CoV-2 in communities during the pandemic. Up to 32% of COVID-19 cases can be asymptomatic or subclinical. Symptomatic and asymptomatic COVID-19 cases alike can shed viral particles in urine and feces (Mainardi, 2021). There is still less data on SARS-CoV-2 in wastewater than other pathogens of similar characteristics. Researchers are actively collecting more data on interactions of SARS-CoV-2

contagiousness and wastewater survivability (Kitajima et al., 2020). Promising data has been shown linking case trends with viral particle detection in wastewater. A study by Peccia et al. reported seeing an increase in viral RNA concentrations 0-2 days prior to an increase in percent positive tests during the COVID-19 pandemic (Peccia et al., 2020). The shedding pathways of SARS-CoV-2 have been heavily studied to determine by which methods and in what volumes viral particles are being contributed to wastewater facilities. It was reported that sputum actually contributed to the viral RNA quantity found in wastewater. The original belief was that fecal matter was the sole/major contributor of viral RNA into the wastewater (Li et al., 2022). Due to the public health emergency, there was a dramatic increase in research conducted regarding SARS-CoV-2. There is currently still a large number of researchers focusing on SARS-CoV-2.

Table 1: Shedding Properties

Pathogen	Infectious Dose 50	Incubation Period	Average Contagious Period	Asymptomatic	Average Shedding Volume	References
Adenovirus	6.6 viral particles	2-14 days	0-2 weeks after recovery	55%	No data available	Yezli et al., 2016   Galanti, 2019
Norovirus	As low as 18 viral particles	12-48 HOURS	0-2 weeks after recovery	Up to 30%	Billions for duration of infection	Teunis et al., 2015
SARS- CoV-2	280 viral particles	1-4 days	5-20 days	32%	No data available	Li et al., 2022

Table 2: Basic Characteristics of Pathogens

Pathogen	Variants of Concern	Transmission	Genetic archetype	Seasonality	References
Adenovirus	C type 1 & 2	Person to person (droplet)	Non-enveloped, DNA	December to March	Yezli, 2016
Norovirus	GII.4	Person to person (fecal-oral)	Non-enveloped, RNA	November to April	Doerfler, 1996   Robilotti et al., 2015   Miura et al., 2018   Jahne et al., 2020
SARS-CoV-2	Omicron (XBB.1.5)	Person to person (airborne & droplet)	Enveloped, positive strand, RNA	Seasonality is yet to be determined	CDC, 2022   Li et al., 2022

## DISCUSSION

Wastewater-based epidemiology is especially suited for monitoring enterically transmitted viruses. This type of infection can be difficult to study true prevalence given asymptomatic or subclinical infections. Even among the symptomatic cases, these individuals may not seek medical care or diagnosis for a few days of vomiting and diarrhea. The rather high burden of norovirus is likely underestimated due to these conditions. Wastewater-based epidemiology seeks to close the gap between recorded case load and actual burden.

Norovirus causes a large case burden and an estimated \$2 billion in productivity loss and healthcare expenses (CDC). Additionally, prevention is key in norovirus as there is no defined treatment or vaccine. Norovirus has an extremely low infectious dose and the capability to produce high shedding volumes (Robilotti et al., 2015). The shedding volume of billions of particles makes it extremely abundant in wastewater monitoring (Jahne et al., 2020). On average up to 30% of cases are estimated to be asymptomatic (Miura et al., 2018). Asymptomatic individuals shed the virus in an amount that is not significantly different from symptomatic individuals (Teunis et al., 2015). The symptoms of norovirus makes cases more efficient at contributing to the transmission, however asymptomatic individuals have the potential to produce and shed viral particles in similar volumes as symptomatic individuals. High shedding volume in the 30% of asymptomatic cases has the potential to contribute substantially to the caseload.

The strong seasonal pattern (Gaythorpe et al., 2017) of outbreaks seen in Figures 2 and 3 indicate that wastewater should be monitored year-round for norovirus particles. This is to detect any unseasonable outbreaks, which can be dangerous. It is also important to monitor continuously to determine how trends are progressing year to year. Norovirus is a good candidate for detection in

wastewater because of high caseload, high public health impact, high shedding volumes, highly contagious  $R_0$  (2) (Gaythorpe et al., 2017; Robiloti et al., 2015). and hardness of the viral capsid in the environment (Hellmér et al., 2014).

The spread of SARS-CoV-2 has been tracked via wastewater-based epidemiology for the duration of the pandemic (Mainardi, 2021). This pandemic has accelerated interest in the field of wastewater-based epidemiology. Asymptomatic COVID-19 cases have been found to shed viral particles at nearly the same quantities as symptomatic cases (Mainardi, 2021). Asymptomatic individuals can spread SARS-CoV-2, among other pathogens, without knowing they are contagious. It is reported that up to 32% of COVID-19 cases are asymptomatic or subclinical (CDC). This shedding pattern in combination with a basic reproductive number ranging from 2 to 4 creates a prime environment for a highly contagious pathogen.

As the pandemic progressed, many individuals complied with testing and vaccination requirements. At-home test kits created a large gap in case reporting, as many individuals would not self-report a positive at-home test. Wastewater monitoring fills the gap caused by at-home test kits to maintain surveillance on a more accurate caseload. Wastewater monitoring will be a crucial resource as SARS-CoV-2 becomes endemic.

SARS-CoV-2 would be an ideal candidate for wastewater monitoring due to the extreme public health interest currently surrounding it. Similar to SARS and MERS, this pathogen may be a good example for how the United States can prepare for other pathogens with pandemic potential. Wastewater-based epidemiology would be a great asset to use during a pandemic and has been heavily utilized during the COVID-19 pandemic. Additionally, the highly contagious nature of this pathogen, high caseload, and high shedding volumes are all pathogen characteristics that make SARS-CoV-2 a high priority for monitoring consideration.

Pathogens need to be ranked to the best of the researchers' abilities on which is the pathogen of highest public health concern. Figure 6 compiles the percent positive PCR detections graphs of norovirus, adenovirus, CoV OC43, and respiratory syncytial virus (RSV). Norovirus, adenovirus, and CoV OC43 were discussed in this thesis and RSV was added for this comparison due to its public health significance. RSV is discussed further as a future research possibility. Coronavirus OC43 is used in Figure 6 to represent a typical coronavirus in place of SARS-CoV-2, as mentioned previously. SARS-CoV-2 is a unique situation and should be placed at the highest priority for wastewater monitoring due to the pandemic it caused.

Coronavirus OC43 (CoV OC43) would be placed in a low priority based on Figure 6. Norovirus follows its seasonal trend and remains at a higher positive test percent than RSV, CoV OC43, and adenovirus for most of the year shown. RSV is mentioned in future research because of the timeframe of two months where the percentage of positive tests increases dramatically and surpasses the other viruses depicted. It is important to take Figure 6 into consideration with the other Figures and other available data when considering the priority of wastewater monitoring.



Figure 6: Comparison of Norovirus, Coronavirus OC43, Adenovirus and RSV percent positive tests

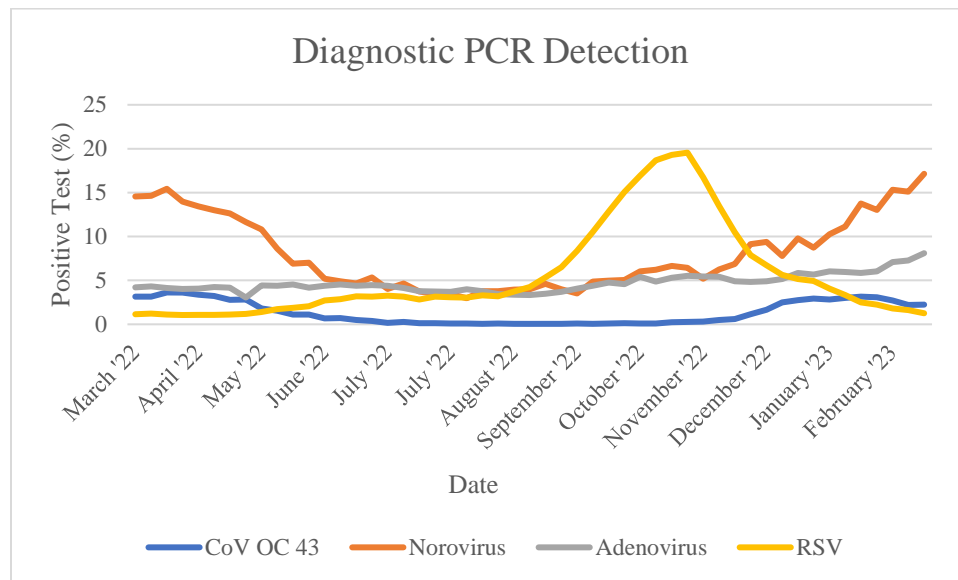


Figure 1 depicts the concerning trend of adenovirus better than Figure 6. Adenovirus percent positive tests for a one-year period can be seen increasing and not following a typical seasonal pattern. Second to SARS-CoV-2, adenovirus should be placed next in order or priority for wastewater monitoring. Adenoviruses are abundantly found in wastewater facilities and particles can survive for long periods of time in hostile environments.

Adenovirus is a non-enveloped, double-stranded linear DNA virus (Doerfler, 1996). DNA is more stable than RNA, so adenoviruses may be more resistant to disinfection processes and inhospitable environments (Dhingra et al., 2019). The clinically significant adenovirus species C is a high priority candidate for wastewater monitoring because it is responsible for more than half of adenovirus infections in immune compromised individuals (Dhingra et al., 2019), adenoviruses cause 2 to 5% of the world's respiratory infections (Akello et al., 2020), and is highly prevalent in children (Doerfler, 1996). Adenoviruses are extremely contagious with  $R_0$  as

high as 5.1 in one study (Guo et al., 2020) and low infectious dose at 6.6 particles (Yezli et al., 2011). Adenovirus is considered an emerging pathogen as the percentage of positive tests is increasing (Figure 1) due to the genetic diversity and high variation rate (Gholipour et al., 2022).

The various adenovirus strains generally cause two types of symptoms that occur (respiratory and gastrointestinal). Adenoviruses can be primarily shed through these two routes: respiratory droplets and fecal-oral. According to Teunis et al., mode of transmission is an important variable that affects the dose-response relationship (Teunis et al., 2016). This claim insinuates the dose-response relationship is different if an individual is exposed via the fecal-oral route versus respiratory droplets.

Adenovirus type C DNA can enter latency, and asymptomatic shedding can occur in feces for years after infection (Kosulin et al., 2016). The ability to enter latency and induce an asymptomatic state in an individual is a concerning ability with up to 55% of cases already being asymptomatic (Galanti, 2019). This means there are possibly large quantities of asymptomatic individuals contributing to the spread of adenoviruses. Wastewater would be able to highlight the true burden of adenoviruses.

Adenoviruses have been found to be abundant in many different environmental samples (Teunis et al., 2016). It was the most frequently detected enteric virus in wastewater samples, followed by norovirus. The survival time of adenoviruses in a wastewater environment has been reported up to 132 days (Gholipour et al., 2022). High resistance to environment because of the capsid structure (Teunis et al., 2016) and asymptomatic case burden contribute to the abundance of adenovirus particles detected in wastewater. Adenovirus is capable of surviving the wastewater environment in large quantities, and therefore, should be between SARS-CoV-2 and norovirus in ranking the monitoring priority.

## CONCLUSION

### *Strengths and Limitations*

A very unique and important aspect of wastewater-based research is that it accounts for asymptomatic or undiagnosed case load. Wastewater-based epidemiology can get an extremely accurate estimate to true caseload of a disease once more back-estimation research is complete. This work is one of the first to highlight pathogen characteristics in wastewater-based monitoring criteria. It will lay the groundwork for the wastewater monitoring framework intended to include a variety of pathogens.

Ideally, all pathogens of concern would be monitored in the wastewater, however that is not feasible with standard community resources. This is why a framework aiding community leaders in the decision of which pathogens to monitor is necessary. This thesis covers three pathogens of high public health importance, but public health importance will change over time. This framework will need to be continually updated with the most current information. The information available at this point in time is limited regarding the specifics of dose-response relationships of pathogens in the context of wastewater. This will always be the case with the emergence, reemergence, and evolution of pathogens.

An important factor in wastewater research is that it is specific to the region the facility serves. This distinct regional data allow for prevention strategies to be developed and targeted to the specific community served by the wastewater facility. The ultimate goal of this research is to lower the incidence, morbidity, and identify regional disparities of these viruses. Identification of regional disparities will need knowledge of viral serotype and community health factors to discover which communities may be disproportionately experiencing disease burden and why.

Viral shedding patterns, including viral loads, in wastewater will lead to an earlier understanding of current cases. Time is an invaluable resource in the fight against infectious diseases. With earlier notification of possible outbreaks, researchers and policymakers can work together to reduce community transmission.

### *Anticipated Research*

After pathogen characteristics are fully defined for the wastewater monitoring framework, the next step is to define community parameters. Certain aspects of a community will influence the decision on which pathogen(s) to monitor in wastewater facilities. One aspect that has a large impact is population density. Additionally, wastewater facilities are not standardized across the United States. Rural communities that used septic tanks over community wastewater programs may end up being marginalized by this method of research. Demographics will play an important role in wastewater monitoring criteria as certain pathogens disproportionately affect different groups. It is anticipated that this framework will extend beyond the three viruses highlighted in this thesis.

Respiratory syncytial virus (RSV), for example, predominantly affects children under the age of two (Bergeron et al., 2021). Respiratory Syncytial Virus (RSV) is transmitted person-to-person via respiratory droplets. The age group most commonly affected (<2 years old) presents with coughing, sneezing, fever, and rhinorrhea, but can quickly become a case of respiratory distress (Bergeron et al., 2021). The mortality of RSV is low; however, the morbidity is responsible for millions of children going to the doctor and millions of dollars in medical care costs (Eiland, 2009). The basic reproductive number has been estimated to be as high as 4.5 in previous simulations (Reis & Shaman, 2018). RSV has been detected in settled solids of wastewater

(Hughes et al., 2022). Wastewater monitoring for RSV could lead to a significant reduction in the disease burden for RSV.

Natural disasters or extreme weather events are an important lens to view wastewater-based epidemiology. Following catastrophes, communities are at a heightened risk for outbreaks of disease. Population displacement, access to clean water, secured wastewater facilities, and access to healthcare during a crisis are all driving risk factors for infectious outbreaks in communities after a disaster (Watson, 2007). This present work will inform wastewater monitoring frameworks that can be implemented to prepare a community prior to natural disasters.

As previously mentioned, QMRAs are an effective tool to predict human health impacts associated with exposure to pathogens (Haas et al., 1999). Likewise, a reverse QMRA can be applied to estimate the number of infections in a community based on pathogen occurrence in an environmental source, such as microbial particles circulating in a wastewater system. Back-estimation research will need to advance to appropriately interpret wastewater-based epidemiology. Often an afterthought, back-estimation is a method to determine how many viral particles is lost to analytical methods. Additionally, back-estimation may be the way to establish a link between case counts and viral particle quantities in wastewater. The information emphasized in this study will be used to inform a future QMRA. The QMRA will then translate the viral wastewater data to interpretable and meaningful public health information.

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