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4990

Feasibility of Collecting Pathogens in Wastewater During Outbreaks

CALIFORNIA Water Boards
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REGIONAL WATER QUALITY CONTROL BOARDS

Feasibility of Collecting Pathogens in Wastewater During Outbreaks

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Abstract and Benefits

Abstract:

This research seeks to link gastrointestinal illnesses in communities to the concentrations of the causal pathogens in the community wastewater. We focus on three pathogens that are critical for potable reuse, namely human norovirus, human adenovirus, and *Cryptosporidium* spp. Specifically, the major goals of this work were to 1) review the readily available public health data on gastrointestinal disease prevalence and outbreaks in the state of California, 2) describe the state of the literature on linking public health data with actual prevalence in a community, 3) characterize pathogen shedding rates and establish shedding distributions expected for the three pathogens, and 4) develop models that link disease prevalence, pathogen shedding rates, and wastewater concentrations. Ultimately, this feasibility study identifies the major information gaps that limit the feasibility of predicting wastewater concentrations from prevalence and vice versa.

Benefits:

- This research examines the feasibility of linking the wastewater concentrations of *Cryptosporidium* spp., human norovirus, and human adenovirus to illness prevalence in communities. This is important for identifying the highest pathogen concentrations in untreated wastewater and also for applying wastewater epidemiology to predict community prevalence.
- This research reviews the literature on fecal shedding studies. This data is critical for linking wastewater concentrations to community prevalence and vice versa.
- The results identify the major sources of community illness data and records major gaps that are necessary for understanding illness levels and dynamics.
- We use the fecal shedding data identified with literature searches and community prevalence/outbreak data identified in illness databases and primary literature and use models to predict expected worst-case wastewater concentrations.
- Ultimately, the information generated in this report is compared with wastewater concentrations reported in Pecson et al. 2021.

Keywords: Wastewater-based epidemiology, *Cryptosporidium parvum*, human norovirus, human adenovirus, fecal shedding.

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Acronyms and Abbreviations

CDPH	California Department of Public Health
DPR	Direct potable reuse
HuNoV	Human norovirus
HAdV	Human adenovirus
NLN	California Norovirus Laboratory Network
NNDSS	National Notifiable Diseases Surveillance System
QMRA	Quantitative microbial risk assessment
SSS	Surveillance and Statistics Section

Executive Summary

This feasibility report was focused on linking the concentration of pathogens in wastewater with infections in a community. The report reviews public health reports in California and the literature on gastrointestinal illness prevalence and fecal shedding. Ultimately, this information is integrated into a model to predict wastewater concentrations given community prevalence and expected fecal shedding rates. Overall results from this report suggest that worst-case human norovirus (HuNoV) concentrations are expected in California wastewaters in December and January, worst-case *Cryptosporidium* concentrations are expected in August, and there is insufficient human adenovirus (HAdV) reporting to predict when during the year wastewater concentrations are expected to be highest. The developed ShinyApp model predicts wastewater concentrations for HuNoV, HAdV, and *Cryptosporidium* given an infection prevalence in a community. It is anticipated that the predicted concentrations will better align with expected illness prevalence values as fecal shedding distributions are better defined through additional studies. The variability in the predicted concentrations could be greatly reduced with better studies aimed at characterizing fecal shedding patterns in different populations. Enhancing the communication between water utilities and public health partners would be mutually beneficial. For the public health partners, this could lead to an enhanced understanding of gastrointestinal illness prevalence in a community based on wastewater measurements. For the water utilities, timely information on outbreaks in a community could initiate a sampling campaign to capture high levels of pathogens in wastewater.

CHAPTER 1

Introduction

1.1 Overview

An accurate understanding of pathogen concentrations in wastewater is important for developing criteria for wastewater treatment. This is particularly critical for wastewater that is used as a potable water source. In the case of developing criteria for direct potable reuse (DPR), the level of treatment necessary to ensure protection of public health is of utmost concern. Quantitative microbial risk assessments (QMRA) are conducted to determine the pathogen concentrations in finished water that result in an acceptable level of human health risk. This information, combined with the starting concentrations of pathogens in untreated wastewater, are then applied to determine the $\log_{10}$ reductions needed to treat raw wastewater to finished drinking water. Without an accurate understanding of the dynamics and ranges of pathogens in untreated wastewater, the $\log_{10}$ reduction credits may be too conservative, resulting in costly and unnecessary treatments. Likewise, $\log_{10}$ reduction credits could also be insufficient to effectively protect human health.

In developing the criteria for DPR, the California State Water Resources Control Board (SWB) recommended additional research focused on understanding pathogen concentrations in untreated wastewater. Until now, the major approach to characterizing pathogen concentrations in wastewater has been directly measuring pathogen concentrations in wastewater. The California indirect potable reuse guidelines require 12-$\log_{10}$ enteric virus, 10-$\log_{10}$ *Cryptosporidium*, and 10-$\log_{10}$ *Giardia* reductions (12-10-10), and these were established based on direct wastewater measurements. One drawback of wastewater measurements is that they are time-consuming and expensive. Consequently, studies that measure pathogen concentrations in wastewater are usually limited in the number of locations, frequencies of samples, and the number of pathogens that can be quantified.

If models were developed that could accurately predict pathogen concentrations in wastewater under different infection prevalence levels in communities, worst-case wastewater concentrations could be predicted for different communities. This feasibility report is therefore focused on predicting pathogen concentrations in municipal wastewater using records of prevalence and outbreaks in combination with data on pathogen shedding. With an understanding of where and when illnesses are expected to be elevated in California, future sampling campaigns could be designed to capture worst-case pathogen concentrations in raw wastewater.

This feasibility study starts with a review of past efforts to link wastewater concentrations and illnesses in communities (Chapter 2). Chapter 3 reviews the publicly available data on gastrointestinal illnesses in California as well as the scientific literature on endemic illness rates in communities. Fecal shedding rates of gastrointestinal illnesses are reviewed in Chapter 4. In Chapter 5, the data collected in Chapters 2-4 are combined to predict wastewater concentrations of gastrointestinal pathogens with a ShinyApp mass balance model, which has an easy user interface. Finally, the report concludes with recommendations on how this information can be applied for DPR. Several research gaps are identified that would aid future efforts to link wastewater concentrations and community infections.

1.2 Pathogens of Interest

The focus of this work was on the pathogens *Cryptosporidium*, spp., human norovirus (HuNoV), and human adenovirus (HAdV), because these pathogens were measured in the partner project *Pathogen Monitoring in Untreated Wastewater* (Pecson et al. 2021) and their importance in DPR projects (Chahal et al. 2016).

1.2.1 *Cryptosporidium* Spp.

There are various species of the protozoan genus *Cryptosporidium* that infect humans, including *C. parvum, C. hominis,* and *C. meleagridis,* (Sunnotel et al. 2006). Infection with *Cryptosporidium* spp. can lead to cryptosporidiosis, a gastrointestinal illness with the most common symptom being diarrhea (CDC 2019a). *Cryptosporidium* infects patients through fecal-oral transmission. Once ingested, oocysts release sporozoites which adhere and invade intestinal epithelial cells where they mature and develop within their life cycle, infecting neighboring cells in the process. Asexual replication leads to mature zygotes which further develop into oocysts of two types, thick-walled oocysts which shed with feces, and thin-walled oocysts which carry out auto-infection in the host (Sunnotel et al. 2006).

1.2.2 Human Norovirus (HuNoV)

HuNoV, a non-enveloped virus belonging to the *Caliciviridae* family, can cause gastrointestinal illness in infected individuals. Symptoms include diarrhea and vomiting, typically lasting up to 48 hours. Three HuNoV genogroups are known to infect humans (i.e., GI, GII, and GIV), although the vast majority of cases in recent decades are associated with GII viruses, with the GII.4 genotype being most common (de Graaf et. al 2016).

1.2.3 Human Adenovirus (HAdV)

Over 100 serotypes belonging to seven subgroups of HAdV have been identified as causing disease in humans (Rafie et al. 2021). Infection by HAdV can result in a wide variety of diseases, including gastroenteritis, respiratory disease, and conjunctivitis (Table 1-1). HAdV serotypes 40 and 41 from subgroup F are most commonly associated with gastrointestinal illness and have therefore been the primary focus in DPR risk assessments and proposed guidance. As a result, HAdV subgroup F is the primary focus of this study, although fecal shedding data for other HAdV subgroups was collected when available.

Table 1-1. HAdV-Associated Illnesses by Subgroup and Serotype.

Source: Adapted from Ghebremedhin 2014.

Subgroup	Serotype	Type of infection
A	12, 18, 31	gastrointestinal, respiratory, urinary
B, type 1	3, 7, 16, 21	keratoconjunctivitis, gastrointestinal, respiratory, urinary
B, type 2	11, 14, 34, 35	gastrointestinal, respiratory, urinary
C	1, 2, 5, 6	respiratory, gastrointestinal including hepatitis, urinary
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49	keratoconjunctivitis, gastrointestinal
E	4	keratoconjunctivitis, respiratory
F	**40, 41**	**gastrointestinal**
G	52	gastrointestinal

CHAPTER 2

Previous Studies Linking Wastewater Concentrations with Community Disease

Numerous studies have measured human pathogens in wastewater, but these studies have typically focused on using those concentrations to evaluate public health risks associated with wastewater exposure and reuse. Prior to the COVID-19 pandemic, there had been relatively few studies specifically focused on linking pathogens in wastewater to illnesses in a community. The largest area of research and applications in this area of linking illnesses with pathogen presence in wastewater had been on poliovirus circulation.

2.1 Poliovirus Wastewater Surveillance

Environmental poliovirus surveillance has been applied for decades to detect the re-emergence of poliovirus in regions where it was previously eradicated. Only 0.1-1% of poliovirus infections are symptomatic (Conyn-van Spaendonck et al. 2001; Hovi et al. 1986); therefore, an outbreak can be well-underway before it is detected through traditional clinic-based surveillance. Modeling efforts that link community poliovirus infections with wastewater concentrations have illustrated that in many scenarios, sewage surveillance is more sensitive (Ranta et al. 2001) than clinic-based surveillance. The model predicted that even at low sampling frequencies, wastewater surveillance can detect as few as a single infected individual in a population of 10,000. When poliovirus is detected in sewage, clean-up vaccination efforts can be initiated to avoid major outbreaks.

Most poliovirus surveillance programs focus on detection rather than quantification. One study in the Netherlands did incorporate wastewater poliovirus quantification in their study in order to understand the sensitivity of wastewater surveillance (Lodder et al. 2012). Specifically, they challenged elderly adults with monovalent oral live attenuated poliovirus vaccine (type 1 or 3) in a population that had been previously vaccinated with the inactivated poliovirus vaccine. The live attenuated poliovirus vaccine results in poliovirus infection of the recipient's gastrointestinal tract and therefore poliovirus virions produced during infection are released to the sewage system. The researchers challenged 228 participants who remained in a sewershed that served a population of approximately 37,000 during the study. They regularly collected stool samples from volunteers after the vaccine challenge to quantify virus concentrations in the stool. They simultaneously collected wastewater samples from the community, concentrated those samples 500X, and quantified wastewater virus concentrations by plaque assay. Based on their results, they concluded that they could detect as little as one infected individual in a population of several tens of thousands. They concluded that linking the wastewater concentrations to the number of individuals infected by poliovirus is complicated due to shedding dynamics through the course of infection and also due to the greatly varied shedding rates between immune and naive populations. They noted that models which aimed to predict pathogen prevalence based on wastewater concentrations would need to include the percentage of immune (i.e., vaccinated) individuals and the mean levels of viruses shed of naive and immune individuals. Interestingly, the researchers mentioned there is a potential value of sewage surveillance for severe acute respiratory syndrome (SARS) virus and other respiratory viruses, noting that these viruses are often excreted in feces.

2.2 Wastewater Surveillance of Other Gastrointestinal Pathogens

Outside of poliovirus and before the COVID-19 pandemic, limited studies measured pathogens in wastewater with the specific intent of linking the observations to a better understanding of illnesses in a community (Kokkinos et al. 2011; Zhou et al. 2014). Of those publications that did exist, some were only interested in the presence or absence of the pathogen or the relative concentrations rather than absolute concentrations (Kokkinos et al. 2011). For example, wastewater was used to understand the human astrovirus genotypes circulating in Shandong, China (Zhou et al. 2014) and the HuNoV genotypes and strains circulating in a community in Japan (Kazama et al. 2017).

One of the few pre-COVID-19 studies that tried to calculate the prevalence of infections in a community based on wastewater concentrations was focused on a community in Sweden (Hellmér et al. 2014). The study tracked eight human viruses including HuNoV, astrovirus, rotavirus, HAdV, Aichi virus, parechovirus, hepatitis A virus (HAV), and hepatitis E virus. Pooled weekly samples were collected bi-weekly from January and March 2013 and concentrated 5000x. Virus concentrations in samples were then quantified by qPCR. Also during the study, 970 fecal samples from patients with gastrointestinal illness were analyzed for HuNoV, sapovirus, rotavirus, astrovirus, and HAdV. To calculate the disease prevalence in the community from wastewater, the study used a major, and likely flawed, assumption that individuals infected with HuNoV, HAV, enteroviruses, and HAdV excrete 10^{11} viral genomes per day. This assumption was based on a mini-review by Bosch (1998) that stated humans excrete between 10^{7} and 10^{11} norovirus, HAV, enterovirus, and HAdV particles per day (Bosch 1998). They used this maximum value of 10^{11} virus particles/day to estimate the fewest number of individuals who were infected in the sewershed, based on the following relationship:

$$N_{infected} = \{C_i / 10^{11}/(\text{volume wastewater at plant per day})\}$$

Where: *N = the number of infected individuals in the sewershed*
C = the concentration of HuNoV in gc/L

Of note from the study results, there was an outbreak of HAV during the time that samples were collected. Five of the reported cases came from individuals living within the sewershed and during this time HAV was detected in the wastewater. HuNoV, sapovirus, rotavirus, astrovirus, and HAdV were detected in every wastewater sample, even during weeks when no positive patient samples were reported.

HuNoV concentrations in wastewater during the study were between 10^{4} and 10^{5} gc/L (Figure 2-1). With a 10% method recovery taken into account and the 10^{11} virus genomes per day shedding rate, they estimated that between 71 and 1200 people were infected by HuNoV during this timeframe. They noted that the peak in virus concentrations in wastewater occurred 2-4 weeks before the peak occurred in diagnosed patients with HuNoV GII (Figure 2-1). However, as there were only weekly samples analyzed, this report of the wastewater as a leading indicator is likely not statistically significant. It is worth noting that while the concentration of HuNoV GII increased by more than an order of magnitude from the first sample to the outbreak peak, the diagnosed cases increased by only ~3x during the same course of the outbreak (Figure 2-1).

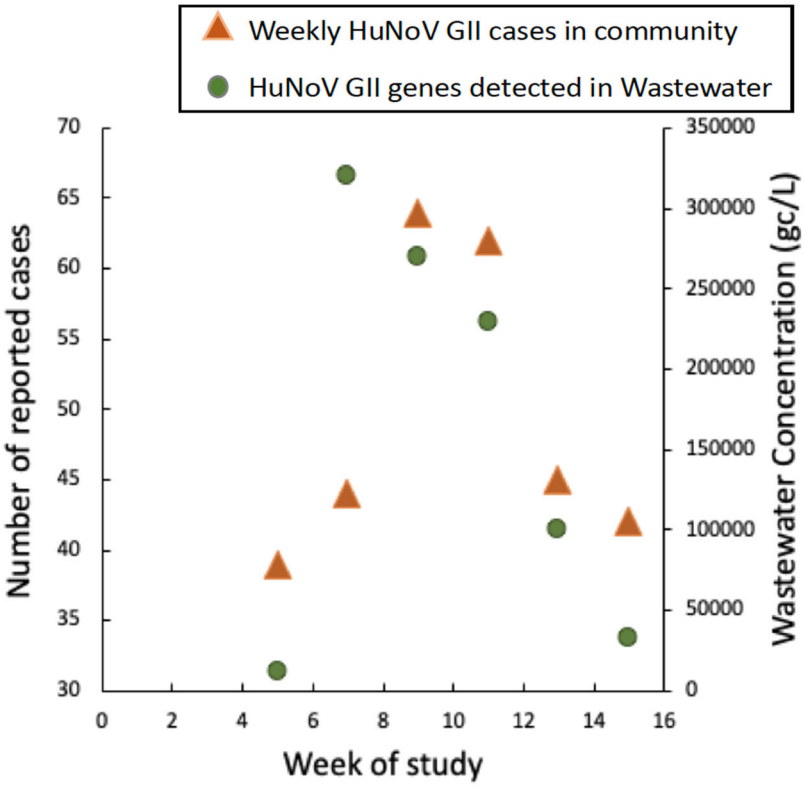


Figure 2-1. Weekly Reported HuNoV GII Cases Compared to HuNoV GII Gene Concentrations in Wastewater.

Data showing number of weekly HuNoV GII cases reported in community (orange triangles) and concentration of HuNoV GII gene detected in wastewater samples over the same weeks (green circles). *Source:* Data from Hellmér et al. 2014.

Two studies were identified in the literature review that did not attempt to use measured wastewater concentrations to predict the number of illnesses in a community but did study how wastewater concentrations correlated with cases in a community.

A study of HuNoV in Irish wastewater (Rajko-Nenow et al. 2013) quantified HuNoV GI and GII in composite wastewater influent samples from a plant serving a population of approximately 92,000. Samples were collected on a weekly basis for thirteen weeks between January and March and analyzed for HuNoV G1 and GII. Stool samples were simultaneously analyzed from symptomatic patients collected throughout Ireland during the same period and analyzed for the presence of HuNoV RNA. They used a membrane filter adsorption-elution method to concentrate the wastewater 90x. They reported that the highest concentration of HuNoV GII measured in the thirteen collected wastewater samples occurred one week before the peak of reported illnesses, again suggesting that wastewater could be a leading indicator of HuNoV illnesses in the community. Similar to the Hellmér et al. (2014) study, however, the limited number of wastewater data points precluded a possible correlation between HuNoV wastewater concentrations and HuNoV illnesses. Interestingly, the peak of reported GII cases was 171 in a single week and the peak for GI cases was four. The wastewater concentrations, however, were similar between the two genotypes with the peak for GII being $2.2x10^6$ gc/L and GI being $5.59x10^5$ gc/L.

A more recent study on HuNoV in wastewater (Kazama et al. 2017) aimed to address the major data limitations in the Hellmér et al. (2014) and Rajko-Nenow et al. (2013) studies and identify statistical

correlations between the concentrations of HuNoV in wastewater and reported illnesses. Influent samples were collected weekly from the wastewater treatment plant of a small Japanese town with approximately 14,000 inhabitants. They measured HuNoV GI and GII concentrations over a three-year period and collected governmental data on the reported gastroenteritis cases in an area that included the town and throughout Japan. They conducted a cross-correlation analysis between the number of gastrointestinal illnesses reported either 1) in the small district (10 clinics) or 2) across all of Japan (3000 clinics) and the $\log_{10}$-transformed HuNoV GI and GII sewage concentrations. They reported a significant correlation between gastrointestinal cases in the district and HuNoV GII wastewater concentrations (Figure 2-2) with a 0-week lag time having the greatest correlation. In contrast, a statistically significant correlation was not found between gastrointestinal illnesses and HuNoV GI concentrations. They attributed this to the lower number of reported HuNoV GI cases during the study timeframe (14 GI cases versus 92 GII cases). These results for HuNoV GI and GII are similar to the Hellmér et al. (2014) study results and suggest that HuNoV GI is either excreted at higher levels, that people with HuNoV GI visit clinics less frequently, or that diagnostic screening methods do not capture GI as well as GII. Ultimately, any of these scenarios could result in fewer reported cases of GI compared to GII.

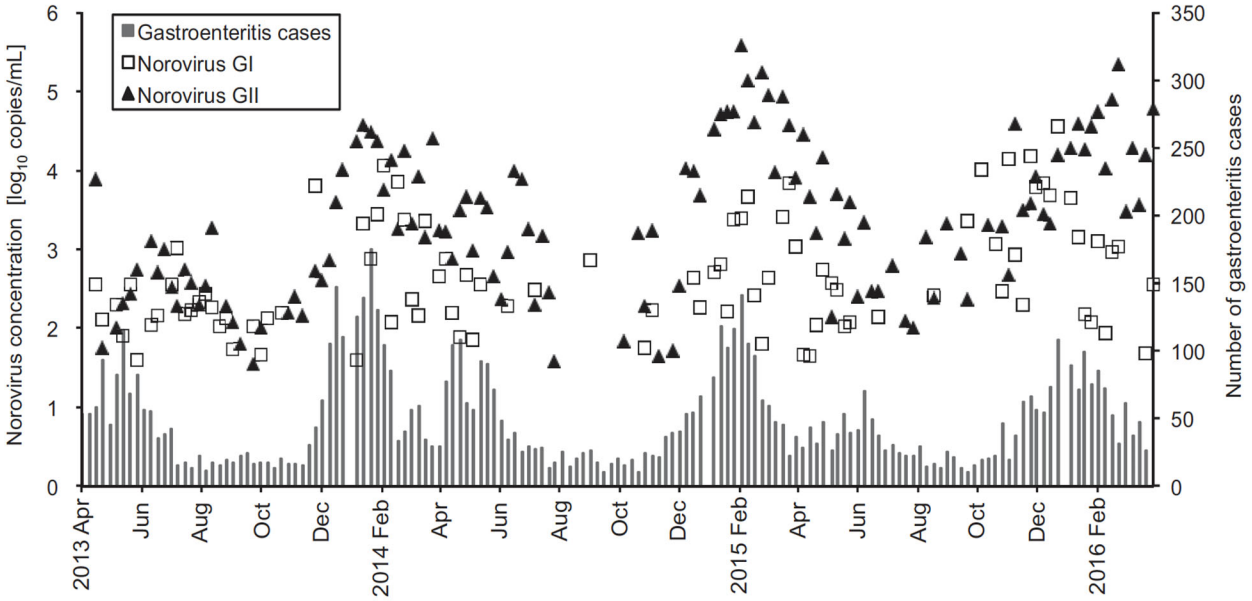


Figure 2-2. Wastewater Concentrations for HuNoV GI and GII in Japanese Wastewater Treatment Plant Influent and Corresponding Gastrointestinal Illnesses Reported in the Larger District during the Same Time.

Source: Kazama et al. 2017.

CHAPTER 3

Disease Surveillance

Developing models that link gastrointestinal illnesses with wastewater concentrations requires an understanding of disease case prevalence in communities. Even without absolute values of case prevalence, trends in case prevalence might help identify where and when elevated concentrations of pathogens in wastewater would be expected. This in turn could inform future wastewater sampling campaigns to better capture peak pathogen concentrations in raw wastewater, representing the worst-case wastewater pathogen levels that could be present in reuse settings.

The overall goals of this research on disease surveillance were several-fold. First, we aimed to understand the landscape of publicly available data on gastrointestinal illness outbreaks and disease case prevalence in the state of California. Second, we aimed to gather the available data and present it based on the location, time, and the number of outbreaks/reported illnesses. With these data we hoped to inform when and where samples should be collected in order to obtain the highest wastewater concentrations of *Cryptosporidium*, HuNoV, and HAdV. The reported illness data is expected to greatly underrepresent the prevalence of illness in a community. Therefore, our third aim was to conduct a literature review on studies of gastrointestinal illness prevalence in communities. The information gathered through these efforts was ultimately applied to the models developed in Chapter 5 to link wastewater concentrations with absolute illness prevalence.

3.1 Approach

We studied public health data available on gastrointestinal illnesses in the state of California to estimate when and where samples should be collected to obtain the highest wastewater concentrations of *Cryptosporidium*, HuNoV, and HAdV. This data was also considered in identifying appropriate prevalence values to input into the model.

Federal, state, and county health departments were investigated as sources of public health data. For all pathogens, inquiries were made to obtain illness numbers (as opposed to outbreak numbers), both monthly and yearly, at the state and county level, over all possible years. Scientific literature was reviewed to estimate prevalence of asymptomatic, symptomatic, and unreported cases. Each of the three pathogens was considered separately due to distinct differences in public health data reporting.

3.2 Cryptosporidiosis

Cryptosporidiosis is both a reportable and notifiable disease. *Cryptosporidium* spp. is the only one of the three pathogens examined here that falls in this category. Reportable means that according to California Code of Regulations, cryptosporidiosis must be reported within one day of identification to the local health officer (CCR 2020). Laboratory diagnostics for cryptosporidiosis are time consuming and require experienced technicians for its detection by microscopy, immunological methods, antigen detection methods, histology, or molecular methods (Khurana and Chaudhary 2018). Reporting is mandatory for reportable diseases, while notifiable diseases can voluntarily be reported to aid in national monitoring.

3.2.1 Databases Investigated for Cryptosporidiosis

Both federal and state sources for public health data were investigated (Table 3-1). Cryptosporidiosis data theoretically should contain the most public health data for the pathogens investigated because it is a reportable and notifiable disease. Data reporting is mandatory at the state level. The California

Department of Public Health (CDPH) data sources tended to have more data availability for California than the federal data repositories.

Table 3-1. Potential Data Sources for Cryptosporidiosis.

Network	Summary
California sources	
California Department of Public Health	Summary reports available on CDPH website. Data reported as monthly totals for the state and yearly totals by county. Additional data can be requested by filling out a CDPH public health records request (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Monthly-Summary-Reports-of-Selected-General-Communicable-Diseases-in-CA.aspx).
CDPH Public Health Records Request System	Data requests answered by Surveillance and Statistics Section of CDPH (https://cdph.govqa.us/WEBAPP/_rs/(S(zim23nktwdjiyvbff3aag4lz))/SupportHome.aspx?sSessionID=73145234249C[QP[U[GJGMEIJYEDVPWMWSJPMWIE).
California Health and Human Services	Freely available spreadsheet for reportable illnesses (yearly totals for 2001-2018 at county level). Downloaded July 2020 (https://data.chhs.ca.gov/contact).
National sources	
U.S. Department of Health and Human Services, Office of the Chief Data Officer	Same spreadsheet as posted on CHHS was downloaded July 2020 (https://healthdata.gov/dataset/Infectious-Diseases-by-Disease-County-Year-and-Sex/t6yk-dy7f).
National Outbreak Reporting System	Incomplete dataset: Only 2 outbreaks and 6 illnesses were reported to NORS in CA over 1998-2017 for cryptosporidiosis (https://www.cdc.gov/nors/index.html).
National notifiable diseases surveillance system (NNDSS)	Some federal cryptosporidiosis data is available in various reports found in **CDC stacks collections**, **WONDER**, and **NNDSS summary reports**. These reports provide snapshots of federal monitoring rather than a complete record of CA illnesses (https://wwwn.cdc.gov/nndss/infectious-tables.html)
CryptoNet	Molecular tracking system used to keep track of species, genotype, and subtype information since 2010. This database focuses on molecular information rather than accounting of all reported illness numbers. (https://www.cdc.gov/parasites/crypto/cryptonet.html)
Sub-state level sources	
County public health departments	Each county public health department provided reports of illness numbers in their own formats on their websites. Data from San Diego and San Francisco counties were similar to those available from CDPH (yearly and monthly total illness numbers, annual communicable disease reports, and periodic reports showing multi-year data)

Although cryptosporidiosis data must be reported within 1 day of identification, that data is not available for external use until they have been reviewed and released, and after data is reported the number of cases is orders of magnitude lower than published prevalence vales of symptomatic infection (discussed further in Sections 3.2.3 and 3.2.4). The CDPH Surveillance and Statistics Section (SSS) of the Infectious Disease Branch regularly reports the data on their website. For example, the Jan-June 2020 report was available on the website by the end of August 2020. The data obtained through CDPH SSS is an under-representation of what exists in the community, especially for datasets reported by location and month. CDPH uses a "low cell suppression" protocol for de-identification processes that involves forcing cells with cases less than 11 to zero for monthly data for smaller counties. (Data De-Identification Guidelines (DDG) by the CA Department of Healthcare Services (DHCS, n.d.).

The following datasets were used for this report to determine when and where cryptosporidiosis illnesses were reported.

1. CA Health and Human Services Open Data Portal (CHHS, n.d.)
    - **Date range: 2001-2018**
    - **County level?**: Yes, yearly case counts for male, female, and both sexes by county, with population data
    - **Monthly level?**: No
    - **Note**: This dataset originally had case data for the more counties than available in dataset #2, although it is no longer available.
2. Data request to CDPH SSS (for monthly data by county)
    - **Date range:** 1994-2019
    - **Monthly level?**: Yes, monthly case counts for each county
    - **County level?**: Yes, county level case counts for each month
    - **Data source**: Formal data request to CDPH Surveillance and Statistics Section, California Department of Public Health
    - **Note**: Many counties listed as zero in this dataset despite having case data in dataset 1 due to de-identification process for small counties (DHCS, n.d.)
3. Monthly data by year for total state
    - **Date range:** 2001-2019
    - **Monthly level?:** Yes, monthly case data by year for entire state
    - **County level?**: No, only used state totals. Didn't use the totals for each year by county because that data was more convenient in dataset 1 above.
    - **Data source**: From multiple reports
        - 2011-2018 (CDPH 2020b): https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/YearlySummariesofSelectedCommDiseasesinCA.pdf
        - 2001-2010 (CDPH Yearly Summaries of Selected General Communicable Diseases in California, 2001-2010, 2015): https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/YearlySummaryReportsofSelectedGenCommDiseasesinCA2001-2010.pdf
        - 2019 (CDPH 2020a): https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/2019Year-endIDBCaseCountsbyMonthandLHJ.pdf
4. 2019-2020 data
    - CDPH, 2021b. Provisional Quarterly Summary Report of Selected California Reportable Diseases January-December 2020
    - Monthly totals for the entire state for January thru June 2020.

- Requested additional data from CDPH, but they will not release the data until the next report is posted.

3.2.2 When and Where Cryptosporidiosis Is Most Frequently Reported

The purpose of the following data analysis was to predict where and when concentrations in wastewater would be highest based on reported cases in counties and over time.

3.2.2.1 Reported Cryptosporidiosis Cases by Month

Based on the pooled data for cryptosporidiosis across the state of California, cryptosporidiosis cases were reported most often in August and least often in February over the timeframe of 2001-2019 (Figure 3-1). Specifically, an average and standard deviation of 20 ± 7 cases were reported in February and 54 ± 23 in August for the state of California. This suggests that on average, *Cryptosporidium* levels in California wastewater would be highest in August. A similar trend is observed nation-wide, with more cryptosporidiosis cases observed in August (Gharpure 2019).

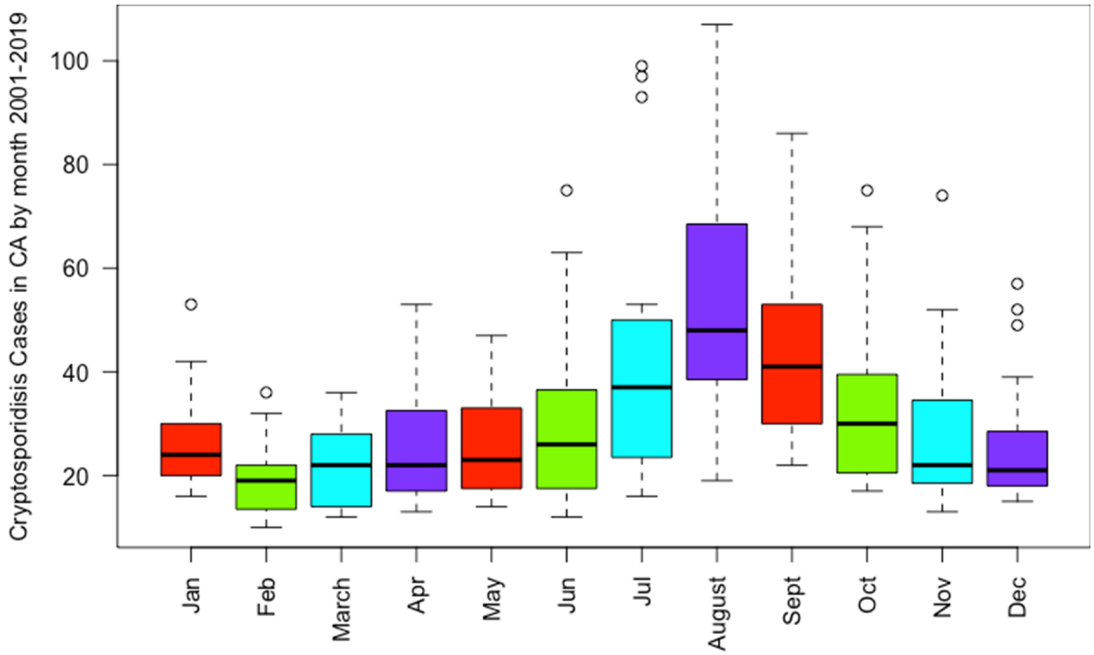


Figure 3-1. Reported Cryptosporidiosis Illnesses by Month in California.
Source: Data from CDPH 2020b and CDPH 2020a.

At the county level, this trend was not always observed. Higher average case values per 100,000 people were visible in late summer in the county level data for Los Angeles and Orange county, but the trend was not clear in San Francisco, Fresno, Marin, or Santa Clara (Figure 3-2). Larger sample sizes would be necessary to confirm trends for most counties. Not only are case numbers very low for most counties, but the de-identification process for the monthly data involves suppressing low cells (Scott 2016). For counties with less than 250,000 people, CDPH replaces the illness numbers with zero when less than 11 cases are reported in that month. and could not be distinguished from actual reports of no cases. Therefore, the monthly county-level data underestimates even the very low number of reported cases. The state-wide totals did not meet the criteria to require suppressing of low cells, so this dataset had larger numbers of cases overall than the monthly county level data. We note that this case number data is normalized by population in an attempt to predict where wastewater concentrations might be highest. Due to the suppression of low cells, counties with less than 250,000 people could only be analyzed using the yearly data rather than the monthly data.

3.2.2.2 Reported Yearly Cryptosporidiosis Cases by County

To understand where in the state reported cases are highest, we pooled county level data by year and over several years. On a yearly basis, cryptosporidiosis case data reported by county suggests that the highest yearly case averages (over approximately 3.5 per 100,000 people) include Inyo, Humboldt, Marin, San Francisco, San Luis Obispo, San Mateo, Santa Barbara, and Siskiyou counties. Overall, the highest yearly case data occurs in Inyo county, a county with a population of 18,500 in 2018 (Figures 3-3 and 3-4). Other counties with relatively high annual case values include Humboldt and San Luis Obispo counties. Based on this preliminary and incomplete data set, future studies might seek high wastewater levels by sampling in these counties.

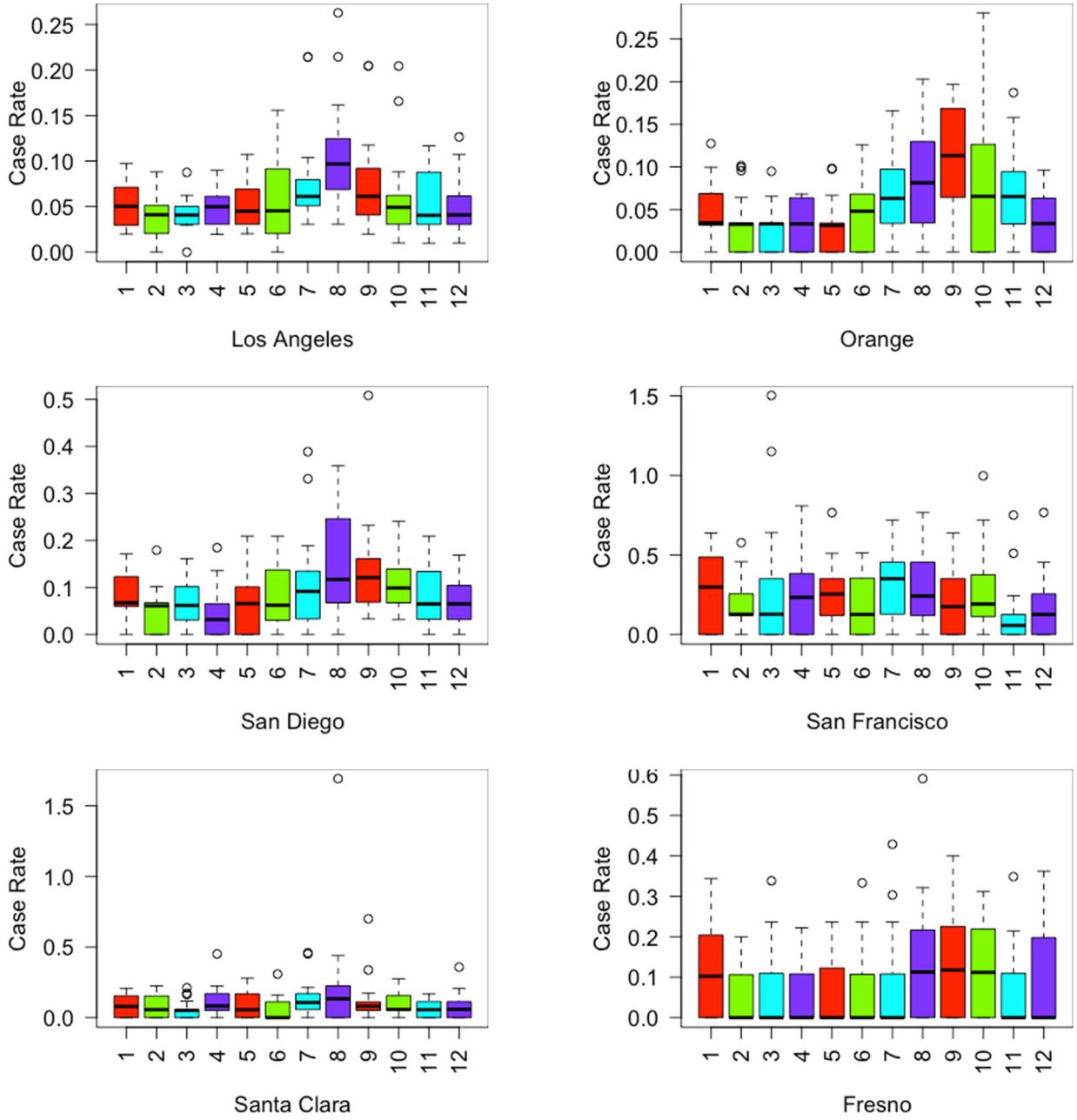


Figure 3-2. Reported Cryptosporidiosis Cases by Month per 100,000 People in California Counties over 2001-2018.

Source: Data courtesy of CDPH Surveillance and Statistics Section.

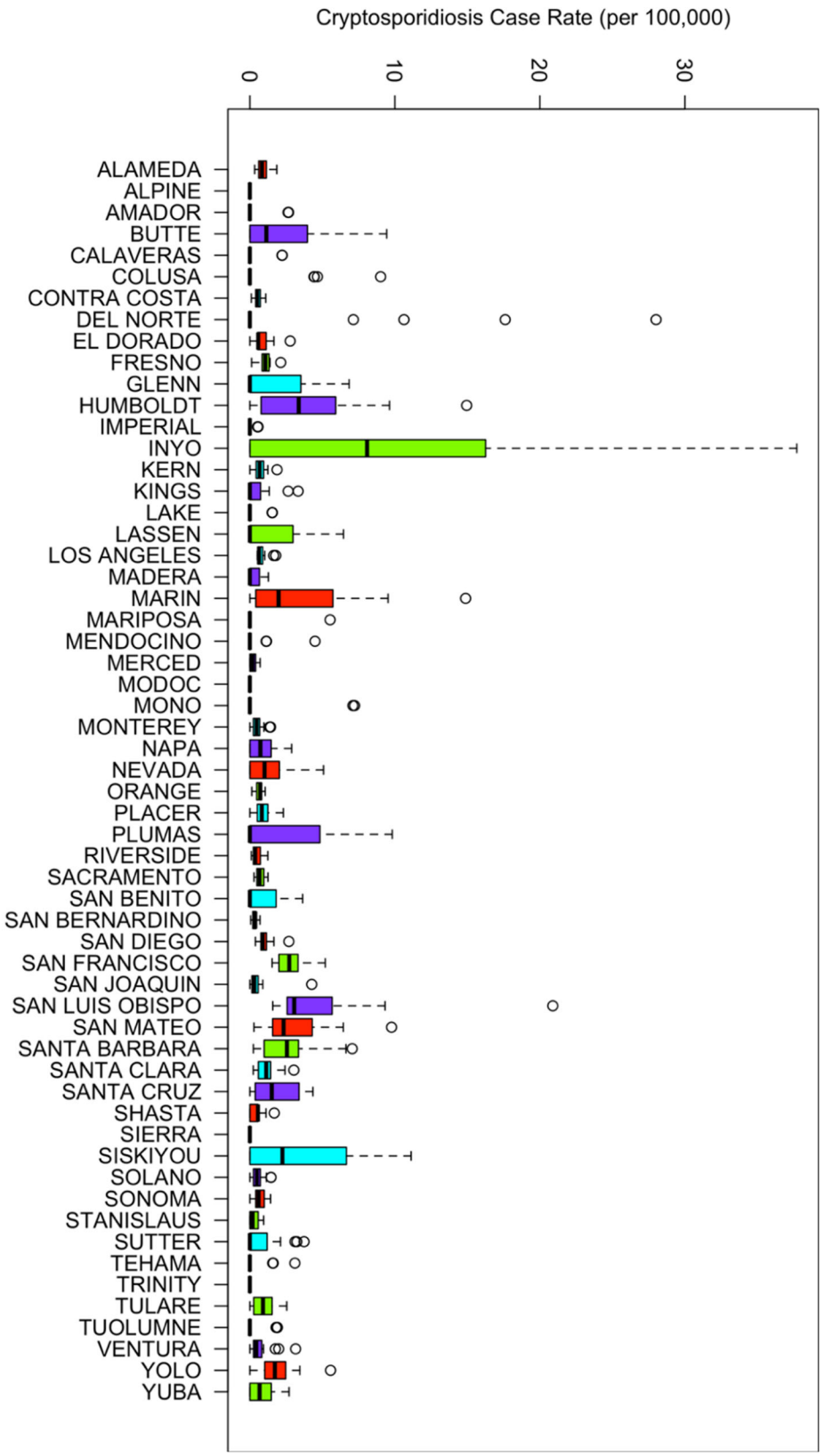


Figure 3-3. Reported Cryptosporidiosis Yearly Cases in California Counties 2001-2018.
Source: Data from CHHS, n.d.

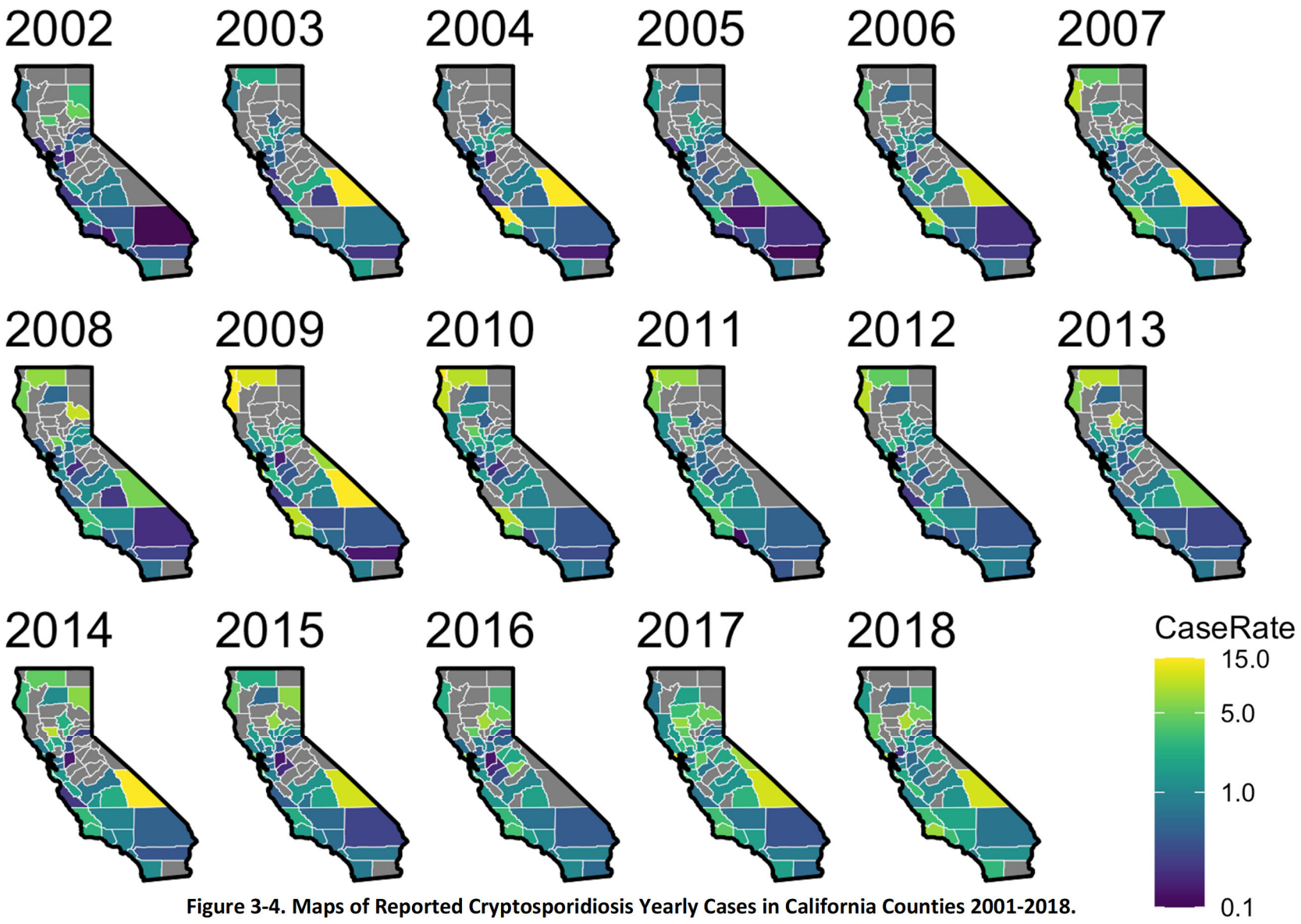


Figure 3-4. Maps of Reported Cryptosporidiosis Yearly Cases in California Counties 2001-2018.
Source: Data from CHHS, n.d.

Due to 1) the high numbers of people who are infected by cryptosporidiosis but do not get tested and 2) the removal of county data with low case values prior to data being posted on these public databases, the case data, and especially the case data by month and county, greatly underrepresent cases. This is especially true for small counties where the low case value numbers have been removed prior to the state sharing the data. Plotting the monthly data by county demonstrates this issue (Figure 3-5). In Figure 3-5, the counties colored gray have no data or zero cases reported. We therefore recommend using the yearly cases by county instead of the monthly case data by county to identify where wastewater concentrations are expected to be highest.

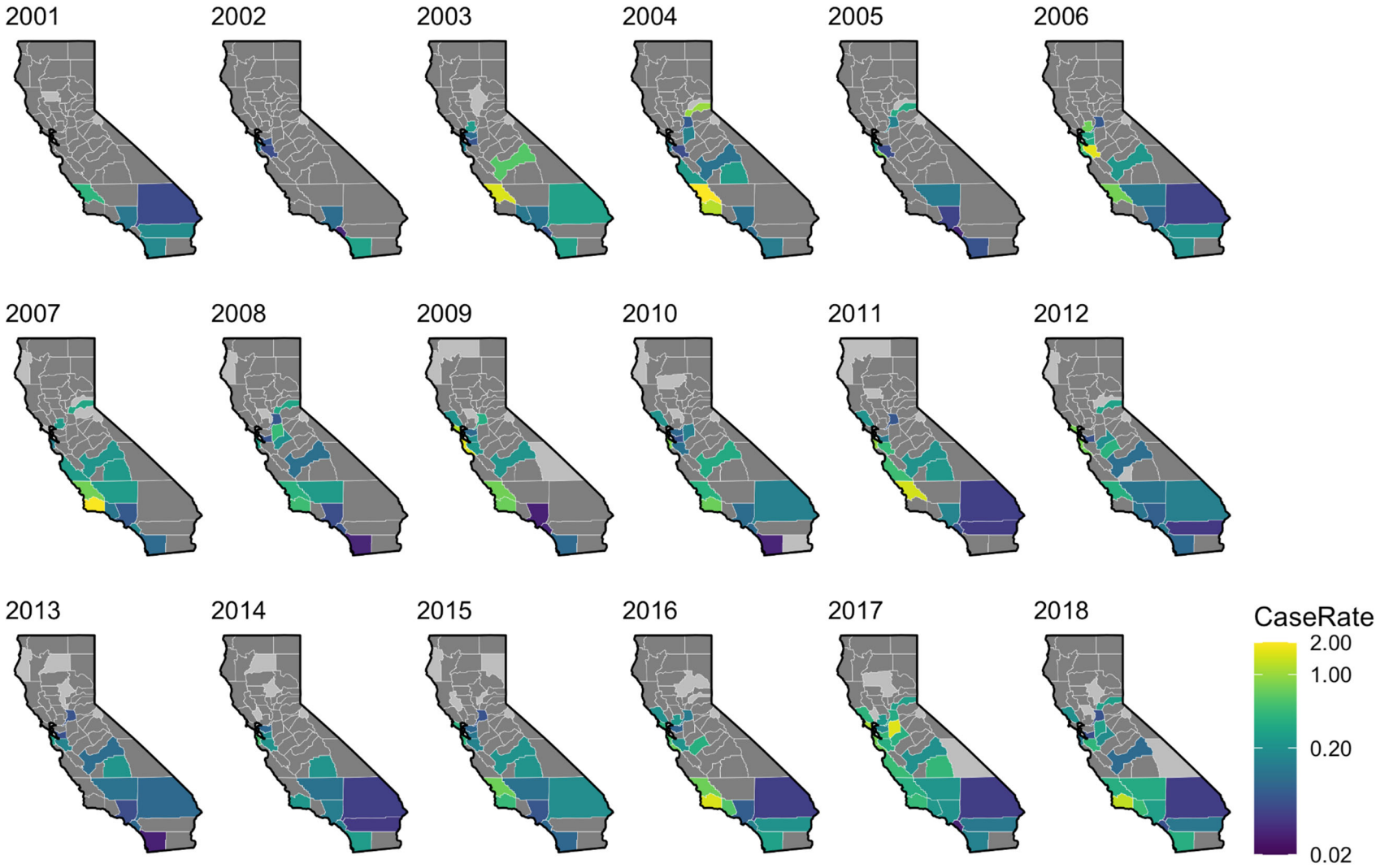


Figure 3-5a. Maps of Cryptosporidiosis Cases in August.
Source: Data courtesy of CDPH Surveillance and Statistics Section.

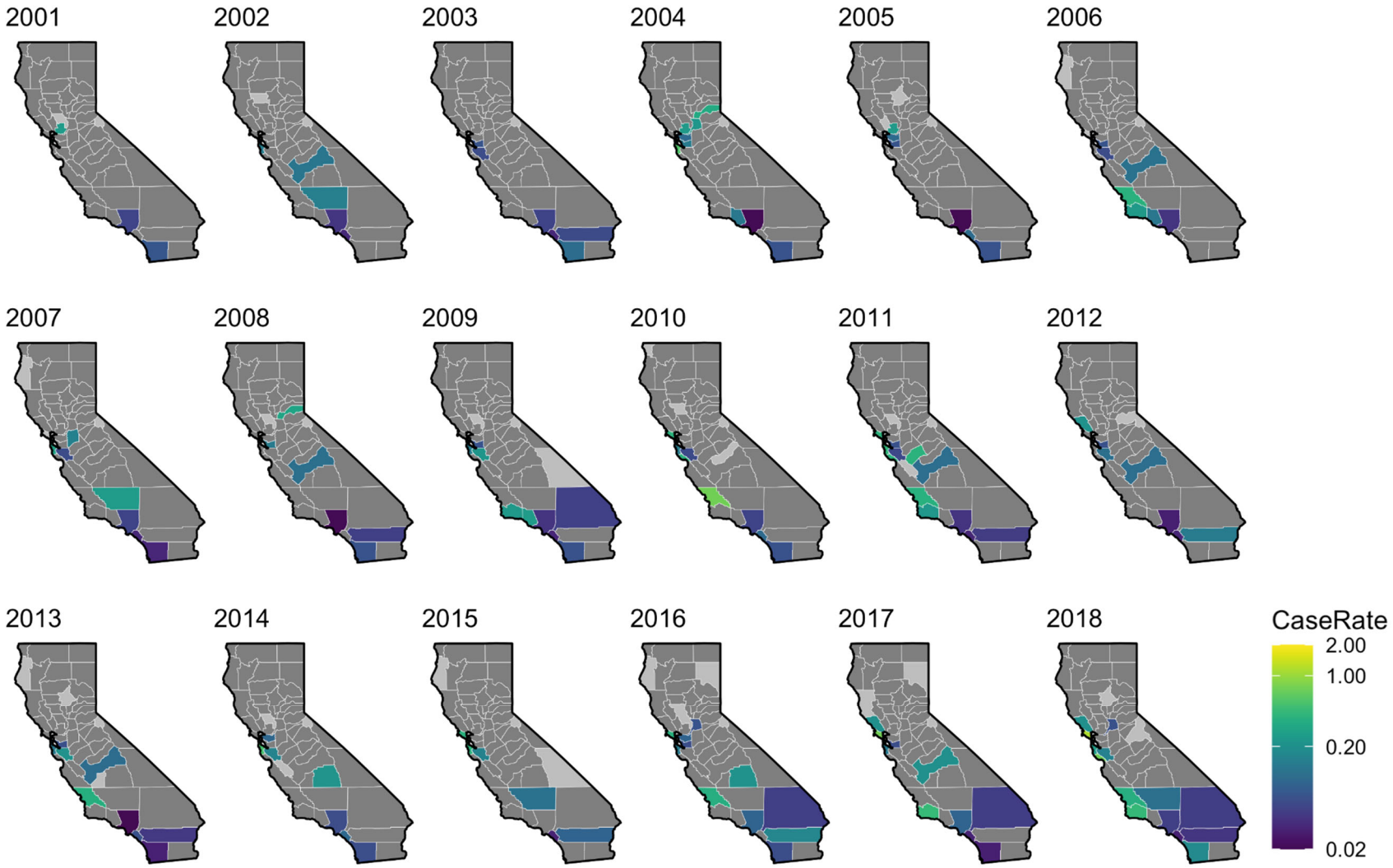


Figure 3-5b. Maps of Cryptosporidiosis Cases in February.
Source: Data courtesy of CDPH Surveillance and Statistics Section.

3.2.2.3 Implications for Wastewater Surveillance of *Cryptosporidium*

In a typical year, the data suggests the highest number of cases of cryptosporidiosis cases are likely to occur in August. High cases have been observed in Inyo, Humboldt, Marin, San Francisco, San Luis Obispo, San Mateo, Santa Barbara, and Siskiyou counties. While keeping in mind that this dataset is limited based on the overall low numbers of reported cases, we recommend sampling in these counties during the month of August to capture wastewater *Cryptosporidium* spp. levels on the high end of the distribution curve. An additional aspect that was not considered here is the fraction of the population that is connected to sewer systems. Future work should integrate the percentage of the populations in these counties that are connected to municipal wastewater treatment plants.

3.2.3 Prevalence of *Cryptosporidium* Infections in the Community

The cases reported to the county and state vastly underestimate the prevalence of cryptosporidiosis in the community. Furthermore, many of those who are infected by *Cryptosporidium* spp. do not exhibit symptoms (i.e., asymptomatic). In order to estimate wastewater concentrations, accurate values for community prevalence of *Cryptosporidium* spp. infections during both endemic and epidemic scenarios are needed. We therefore conducted a brief literature review on academic studies that measure or predict prevalence. We then use these data to link wastewater concentrations to prevalence with our models in Chapter 5.

According to a meta-analysis of 13 clinical studies from Nordic countries involving male and female adults (Hörman et al. 2004), the prevalence of cryptosporidiosis cases in the general Nordic population is estimated as 0.99% in the asymptomatic population and 2.91% in the symptomatic population. In their study, a symptomatic individual referred to someone with at least one of the following symptoms: vomiting, gastroenteritis or diarrhea, abdominal pain, cramps, or discomfort. To go from these numbers to overall prevalence in a community, one needs to know the fraction of the population that is symptomatic. In the general population 11.6% of the population is expected to have gastroenteritis in any given month, according to 52,840 surveys given over 1996-2003 (Jones et al. 2007; Herikstad et al. 2002). Consequently, 88.4% of the population is asymptomatic. Based on these numbers, the overall prevalence of *Cryptosporidium* spp. infections in any given month is estimated to be

0.0099*0.884 = 0.88% of entire population has asymptomatic cryptosporidiosis in any given month

0.0291*0.116 = 0.34% of entire population has symptomatic cryptosporidiosis

Based on this analysis of the literature, and assuming that the California prevalence is similar to the prevalence in Nordic countries, we estimate that the average prevalence for *Cryptosporidium* spp. infections is 1.21% over the entire population for any given month.

A breakdown of prevalence of acute diarrheal disease by age group demonstrates higher prevalence in children ages <5 (Table 3-2; Jones et al. 2007) thus, the estimates for the population actively contributing to wastewater rather than diapers may be lower than reported. Note, that prevalence of diarrheal disease that impaired daily activities or lasted >1 day was estimated at 5.1%, while prevalence of any diarrhea not linked to a chronic illness was reported at 7.7% (Jones et al. 2007). Prevalence estimates for Cryptosporidium spp. infections were taken from a meta-analysis that only included studies on adults involving both genders from Nordic countries (Hörman et al. 2004).

Table 3-2. Acute Diarrheal Illness Prevalence.

Data represents surveys from people that did not report an underlying chronic diarrheal disease, and only includes those with impairment of daily activities from three or more loose stools or >1 day of duration.

Source: Jones et al. 2007.

	Cycle 1 ($n=8645$)		Cycle 2 ($n=12\,395$)		Cycle 3 ($n=14\,139$)		Cycle 4 ($n=15\,578$)		Cycles 1–4 ($n=50\,757$)	
	%	(±95% CI)	%	(±95% CI)	%	(±95% CI)	%	(±95% CI)	%	(±95% CI)
Acute diarrhoeal illness	5·1	(0·6)	4·5	(0·5)	5·0	(0·5)	5·2	(0·5)	5·1	(0·3)
Sex										
Male†	4·7	(0·8)	4·3	(0·7)	4·4	(0·8)	4·5	(0·6)	4·5	(0·4)
Female	5·5	(0·8)	4·7	(0·7)	5·6	(0·7)	6·0	(0·7)‡	5·5	(0·4)‡
Age (yr)										
<5	8·3	(2·9)‡	7·3	(2·8)	8·5	(2·9)	10·3	(3·0)‡	8·8	(1·5)‡
5–17	3·8	(1·3)	3·9	(1·2)	4·7	(1·7)	4·2	(1·2)	4·5	(0·8)‡
18–35	6·3	(1·2)	5·0	(1·0)	5·9	(1·0)	6·2	(1·0)	5·9	(0·6)
36–54†	5·4	(1·0)	5·3	(1·0)	5·3	(0·9)	5·5	(0·8)	5·4	(0·5)
55–64	2·9	(1·3)‡	2·4	(1·2)‡	2·7	(0·9)‡	3·6	(1·0)‡	3·0	(0·6)‡
>65	2·7	(1·0)‡	2·2	(0·9)‡	2·5	(0·9)‡	2·4	(0·7)‡	2·4	(0·4)‡
Race										
White†	5·1	(0·7)	4·7	(0·6)	5·3	(0·6)	5·5	(0·5)	5·3	(0·3)
Black	4·6	(1·7)	3·2	(1·4)	3·3	(1·1)‡	4·1	(1·5)	3·7	(0·8)‡
Hispanic	6·3	(2·6)	4·0	(1·8)	5·5	(2·4)	4·9	(1·6)	5·2	(1·1)
Residence										
Urban†	5·1	(0·9)	4·1	(0·8)	4·5	(0·8)	4·7	(0·7)	4·6	(0·4)
Suburban/town	5·4	(0·9)	4·7	(0·7)	4·9	(0·7)	5·3	(0·7)	5·1	(0·4)
Rural	4·0	(1·5)	4·7	(1·4)	6·0	(1·5)	6·4	(1·4)‡	5·7	(0·8)‡
Medically insured†										
Yes	5·2	(0·6)	4·5	(0·6)	4·9	(0·5)	5·1	(0·5)	5·0	(0·3)
No	5·6	(2·2)	5·4	(2·1)	6·4	(2·0)	6·7	(1·8)	6·3	(0·1)‡
Education (among those >25 yr old)										
Less than high school	3·7	(2·1)	2·9	(1·6)	3·9	(1·5)	4·5	(1·8)	3·8	(0·9)‡
High School graduate†	4·5	(0·8)	4·6	(0·9)	5·1	(0·8)	5·1	(0·7)	4·9	(0·4)
College graduate	5·8	(1·2)	5·3	(1·1)	4·9	(1·1)	4·8	(0·8)	5·1	(0·5)

† Reference group for statistical comparison.

‡ Statistically different from comparison group within that cycle, $P<0·05$.

Another meta-analysis found a prevalence value of 0.6-4.3% for North America, but this included studies that involved children (Fayer and Ungar 1986). Many of the studies focused on day-care attendees and hospital patients with diarrhea, but the inclusion of children may explain the higher range of prevalence as symptomatic cryptosporidiosis is much more common in young children, with one study showing a prevalence of 5.2% among children 13-24 months versus 2% among children 48-60 months in Kenya (Gatei et al. 2006). Also, day-cares are high-risk areas for Cryptosporidium transmission (Vandenberg et al. 2012).

3.2.4 Needs and Recommendations for Understanding *Cryptosporidium* Infections in the State of California

A symptomatic *Cryptosporidium* spp. infection value of 0.34% in the entire population should correlate to 340 cases per 100,000 individuals. This equates to approximately 130,000 people per 39.78 million in the California population having symptomatic *Cryptosporidium* spp. infection in any given month. For comparison with historical reported data, in 2019 there was an average of 62 cases reported per month across the entire state of California. Illness reporting, even for conditions that are required to be reported according to state regulation, appears to be insufficient for estimating prevalence of disease when compared to meta-analysis of published clinical studies that test both asymptomatic and symptomatic adults. Reported cases of cryptosporidiosis are more than three orders of magnitude lower than prevalence estimates from meta-analysis of clinical studies. It is therefore not recommended to use reported illness numbers to compute expected wastewater concentrations. It is worth noting that *Cryptosporidium* spp. is the only one of the three pathogens included in this feasibility study for which reporting is required by regulation and should therefore have the highest quality reported data of the three pathogens. As the prevalence data collected with the systematic reviews was not specific for the state of California, a future study probing the values of symptomatic and asymptomatic cases in the state would be valuable for efforts linking prevalence values to wastewater concentrations.

3.3 HuNoV-Associated Illness

HuNoV does not cause a pathogen-specific reportable disease. In other words, reporting cases to the state is not required. Outbreaks of any disease, however, must be reported immediately according to California Code of Regulations (CCR 2020; Khurana and Chaudhary 2018). Thus, if multiple cases of gastroenteritis are observed, they must be reported to local health officers. Furthermore, foodborne and waterborne disease outbreaks are notifiable at the federal level. This means that data can be voluntarily reported to CDC to support monitoring efforts nationwide (CDC, n.d.a.). Thus, in contrast to cryptosporidiosis, HuNoV infections are tracked in California and by the CDC as outbreaks rather than cases. An outbreak is defined as a common exposure that causes two or more illnesses

3.3.1 Databases Investigated for HuNoV

Both federal and state sources for public health data were investigated for HuNoV case data (Table 3-3).

Table 3-3. Potential Data Sources for HuNoV Illnesses.

Network	Description
National Data Sources	
National Outbreak Reporting System (NORS)	A federal database containing 895 outbreaks and 17,200 illnesses reported in CA over 1998-2017 for norovirus. For each outbreak data includes year, month, number of illnesses, etiology, and confirmed vs suspected. (https://www.cdc.gov/nors/index.html)
Norovirus laboratory network (NLN)	Reports produced triannually on norovirus outbreaks in California, with numbers of outbreaks by county, but not illnesses. (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/CA-NLN-Report-Archive.aspx)
Calicinet	A federal database with genetic information from public labs. Data is only available to participating health labs.
Norovirus Sentinel Testing and Tracking (NoroSTAT)	A federal program that uses NORS data to create reports about norovirus. CA data insufficient. (https://www.cdc.gov/norovirus/reporting/norostat/data.html)
National Notifiable Diseases Surveillance System (NNDSS)	Foodborne and waterborne outbreaks are federally notifiable (https://wwwn.cdc.gov/nndss/infectious-tables.html) CDC Morbidity and Mortality Weekly Reports (MMWR) contained information regarding norovirus in US: Near Real-Time Surveillance of U.S. Norovirus Outbreaks by the Norovirus Sentinel Testing and Tracking Network — United States, August 2009–July 2015
California Data Sources	
CDPH public health records request system	A form is available to request CDPH records. Responses to forms took weeks to months, stating no records exist relating a general request for norovirus data. Follow up questions pointing to specific programs and specific reports posted on CDPH websites, were slightly more successful, but yielded little information beyond CDPH reports published online. (https://cdph.govqa.us/WEBAPP/_rs/(S(ge4zfzvjy2ngiyveohm4yz5m))/RequestLogin.aspx?sSessionID=&rqst=1&target=YpURA3m6cNU+N1K9kEqQhqz8yC2ZLKNdSdB4wnowVJ5S8CGTBp2GIItHg4/I0pUM8Jvp1Aad4YheCcTrA795fG9P3xL5LmB/wFQjiIoSWN7tLnJa+Bm/oEirHbO2IQAI)
Sub-state Level Data Sources	
County health departments	Each county health department has varying amounts of information on their websites regarding the pathogens investigated. Contacting county health departments also yielded additional information.

The most fruitful datasets were obtained from the NORS database and the California Norovirus Laboratory Network (NLN) triannual reports. The federal NORS database yielded a spreadsheet with statewide California data on HuNoV outbreaks and illnesses for 1999-2017 at the monthly level. Data visualization on the website is available as well as excel download of the data, including 893 lines of California data with information by year, month, number of illnesses, etiological agent causing illness (i.e., HuNoV, HuNoV genogroup I, HuNoV genogroup II, HuNoV unknown, others), and etiological agent status as either confirmed or suspected.

The NLN triannual reports from 2017-present provided the numbers of outbreaks rather than total case numbers, and conversations with VRDL staff indicated that they did not have illness numbers available to share. Outbreak numbers were reported as cumulative totals in four-month blocks and shown on maps by region. NLN was established in 2017, so this source of data is not available for previous years.

3.3.2 When and Where HuNoV Infection Is Most Frequently Reported

3.3.2.1 Reported HuNoV Illnesses by Month

NORS statewide data combined from 1999-2017 suggests that the lowest average number of illnesses occurs in August and the highest number occurs in December/January (Figure 3-6). Breaking down the data by confirmed etiology also suggests lower numbers of illnesses in August than January (Figure 3-7). Data from San Diego county also suggests outbreaks more often occur in December-January (Figure 3-8). These limited data on HuNoV outbreaks suggest that wastewater concentrations would be highest in December and January and lowest in August. Future efforts aimed at measuring HuNoV wastewater concentrations on the high end of the distribution should therefore focus on samples collected in December and January.

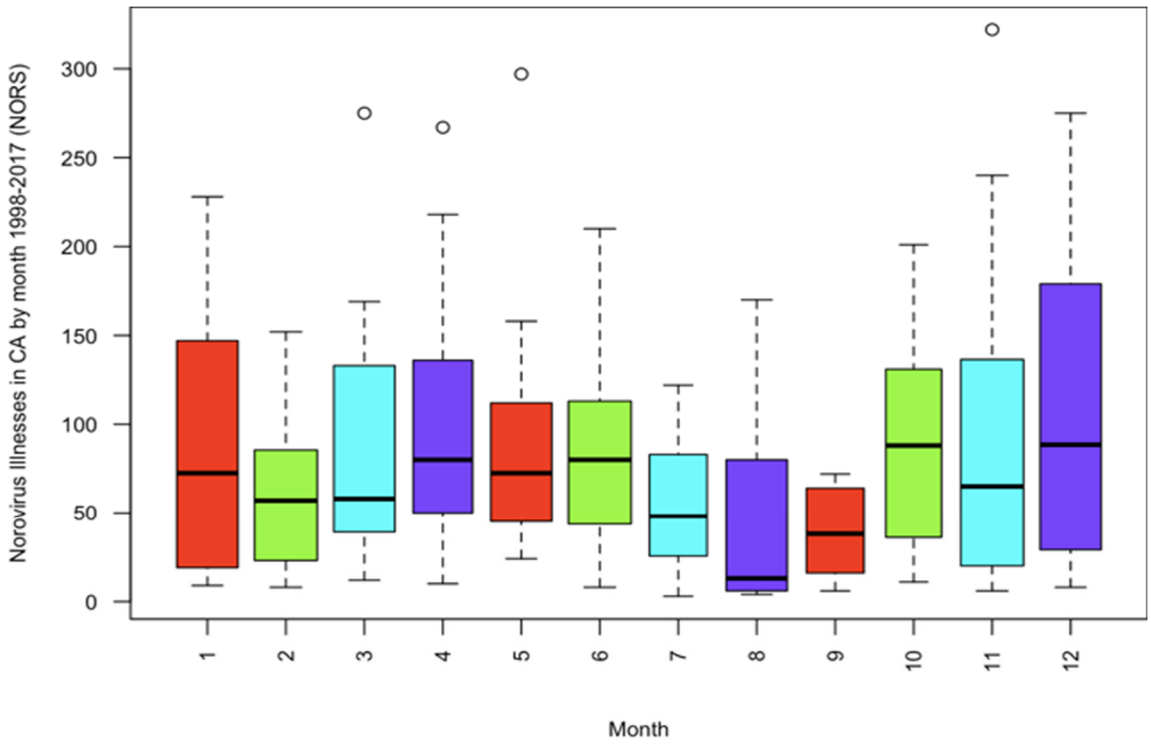


Figure 3-6. HuNoV Illnesses by Month.
Statewide NORS data from 1999-2017 for all etiology combined, both suspected and confirmed cases.

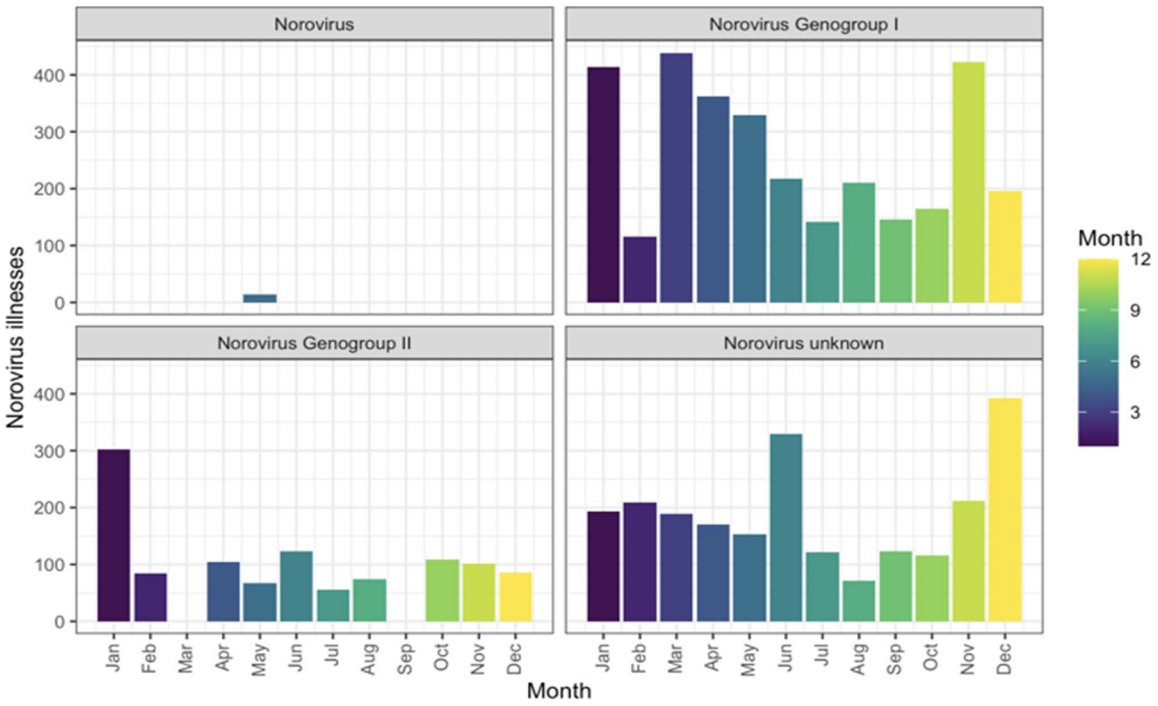
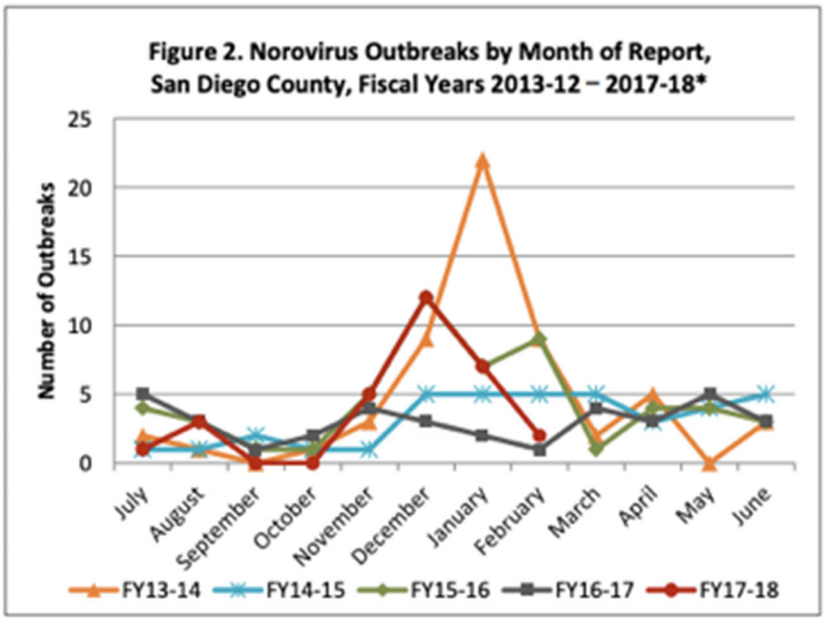


Figure 3-7. Breakdown of HuNoV Illnesses by Etiology and Month.
Statewide NORS data from 1999-2017 for confirmed cases by etiology.



*2017-18 data are year-to-date. Data are provisional and subject to change as additional information becomes available. Data are presented using fiscal years (the San Diego County fiscal year is July-June) due to the seasonal nature of norovirus outbreaks. Data current as of 2/15/2018.

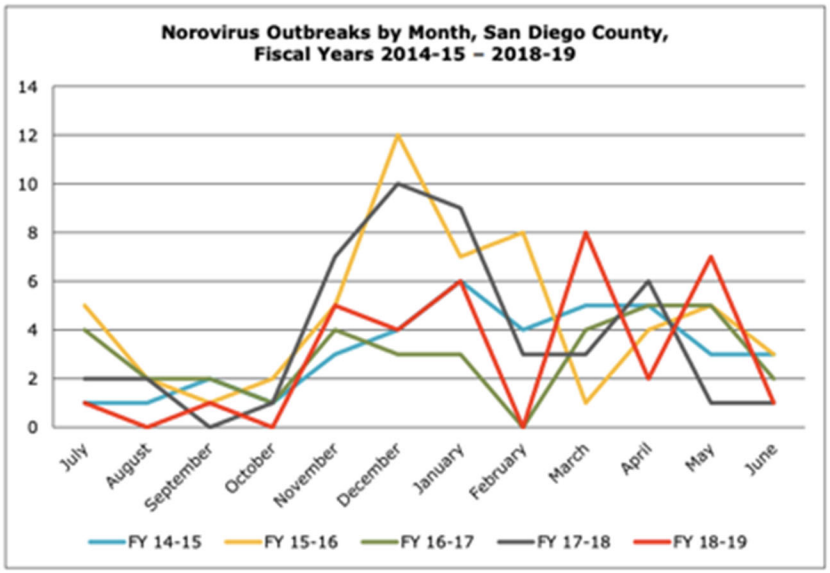


Figure 3-8. HuNoV Outbreaks in San Diego County by Month of Report.
*Source: S*an Diego County 2018.

3.3.2.2 Reported Cases of HuNoV-Associated Illness by County

HuNoV illness numbers were not available at the county level by month. According to NLN triannual reports from CDPH, Los Angeles county had at least one HuNoV outbreak reported every four months over Oct 2017-Sept 2019, and also had the most outbreaks of any other county at 31 total reported outbreaks (Figure 3-9). Staff from VRDL indicated they had no record of the number of illnesses per outbreak to allow us to normalize these numbers to population. Contra Costa, Alameda, Santa Cruz, Orange, and San Diego counties reported outbreaks in five out of the six triannual reports over Oct 2017-Sept 2019 (Table 3-4). Over ten outbreaks were reported during this time in Butte, Contra Costa,

Orange, and San Diego counties. Note that the number of outbreaks reported by San Diego County on their county website is far greater than the number reported by the NLN triannual reports, suggesting differences in reporting format or errors in reporting.

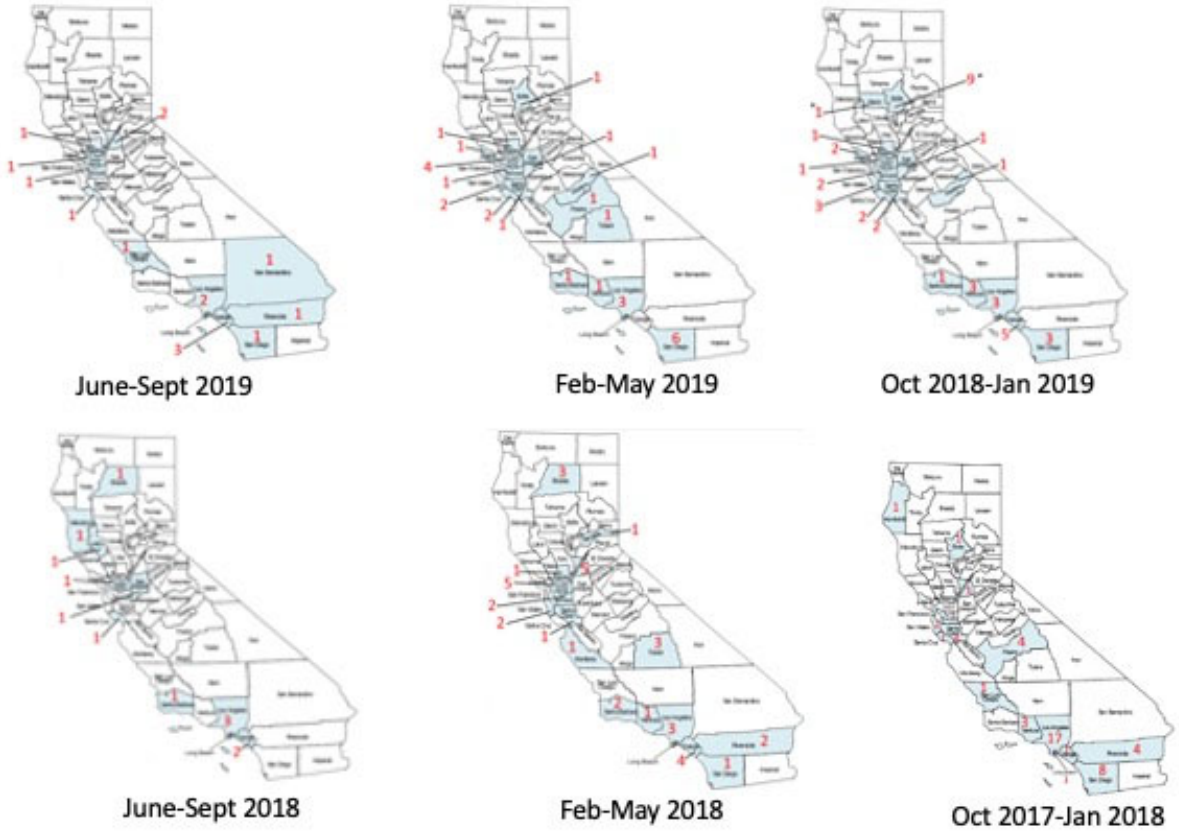


Figure 3-9. HuNoV Outbreaks.
Source: CDPH, n.d.a.

Table 3-4. HuNoV Outbreak.

Source: Data from CDPH, n.d.a.

County	Number of NLN triannual reports at least 1 outbreak occurred in (out of 6)	Total number of outbreaks Oct 2017-Sept 2019
Humboldt	1	1
Shasta	2	4
Mendocino	1	1
Lake	1	1
Butte	3	11
Glenn	1	1
Nevada	1	1
Sacramento	3	8
Solano	4	4
Marin	2	3
Contra Costa	5	12
San Joaquin	3	3
Alameda	5	9
San Mateo	4	9
Santa Clara	4	8
Santa Cruz	5	7
Madera	2	2
Fresno	2	5
Monterey	1	1
Tulare	2	4
San Luis Obispo	2	2
San Bernardino	1	1
Santa Barbara	4	5
Ventura	4	8
Los Angeles	6	31
Orange	5	15
Riverside	3	7
San Diego	5	19

3.3.2.3 Implications for Wastewater Surveillance of HuNoV

Reported HuNoV data from NORS and CDPH suggest that in a typical year the most illnesses occur in December or January. Outbreaks are most likely in Los Angeles county, as well as in Contra Costa, Orange, San Diego, Alameda, Santa Cruz, and Butte counties. Thus, wastewater surveillance programs are expected to find the highest occurrence of HuNoV in wastewater in December or January in Los Angeles, Contra Costa, Orange, San Diego, Alameda, Santa Cruz, or Butte counties.

3.3.3 Prevalence of HuNoV in the Community

There are limited studies on HuNoV prevalence in the United States. According to two published meta-analyses (Figure 3-10), prevalence of HuNoV cases is estimated as 4% in the asymptomatic, North-American population (95% CI 1-16%; Qi et al. 2018) and 20% in the symptomatic population with gastroenteritis in developed countries (95% CI 17-22%; Ahmed et al. 2014). In any given month, 11.6% of the population has been estimated to have acute gastroenteritis, according to 52,840 surveys given over 1996-2003 (Jones et al. 2007).

Based on this data, the overall prevalence for H in any given month is estimated to be:

0.04*0.884*100% = 3.5% of total population having asymptomatic norovirus in any given month

0.20*0.116*100% = 2.3% of entire population having symptomatic norovirus

Combined, this means that the overall prevalence for HuNoV infection is approximately 5.8% over the entire population for any given month.

Note that a prevalence of 2.3% symptomatic individuals out of the entire population should correlate to 2,300 cases per 100,000 people or 920,000 symptomatic people per 39.78 million in CA in any given month. In 2017 there were an average of 46 HuNoV illnesses reported to NORS per month across the entire state of California. This means that the number of reported HuNoV illnesses are over four orders of magnitude lower than the number of illnesses that are estimated using prevalence studies.

One potential source of error in these numbers is that a high proportion of studies analyzed in the meta-analyses mentioned above were focused on young children aged < 5. Children had similar prevalence for symptomatic illness but higher asymptomatic HuNoV prevalence (Figure 3-10). In addition, many children in this age group do not contribute to wastewater. This may cause the asymptomatic prevalence value to be higher than reality for the general population that contributes to wastewater. There was not sufficient data in the HuNoV prevalence meta-analyses to break down the subgroup regions by age, but globally prevalence for asymptomatic children ages < 5 has been reported at double that of adults (Qi et al. 2018).

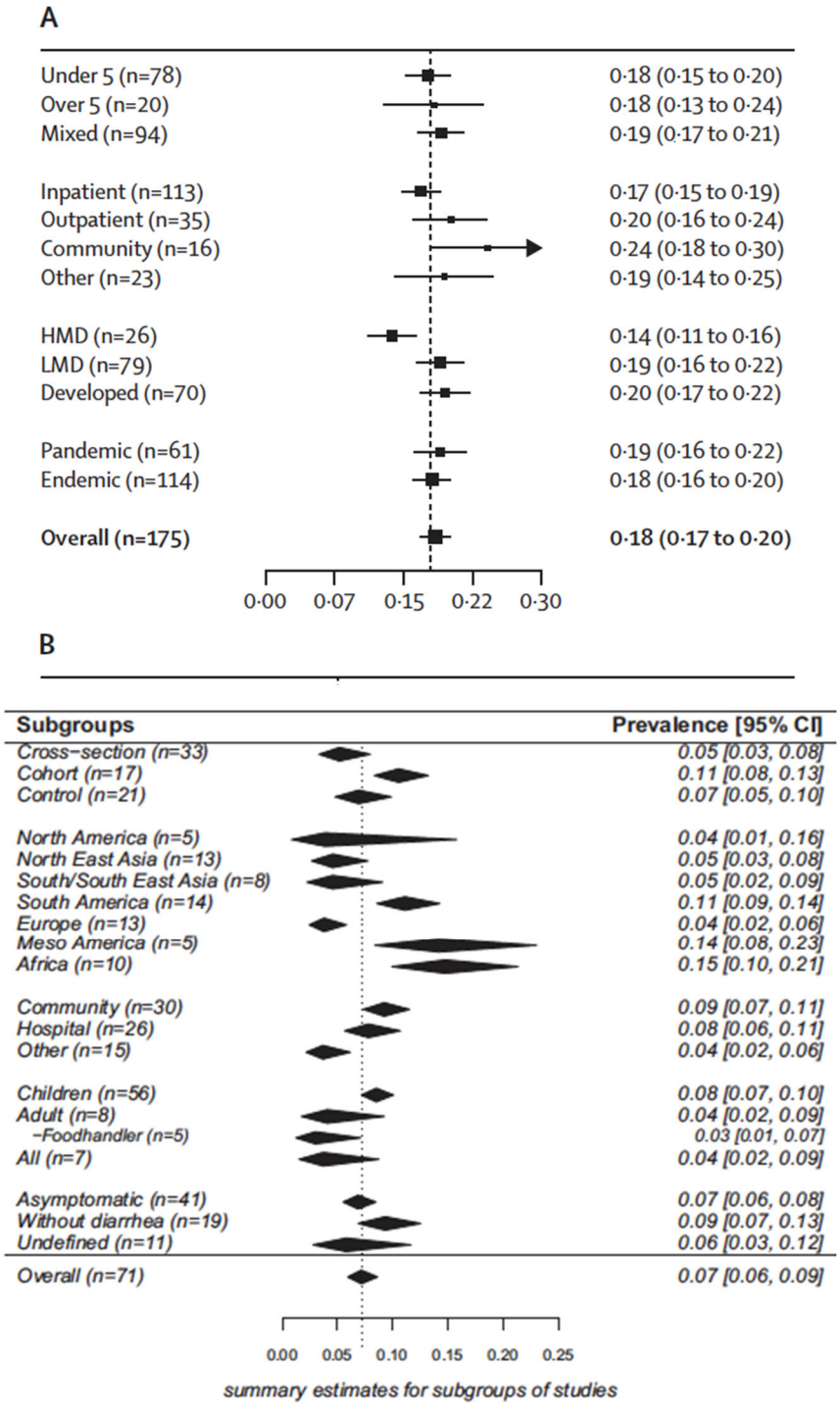


Figure 3-10. Published Meta-analyses on the Prevalence of HuNoV Infections.
Source: (a) Ahmed et al. 2014; (b) Qi et al. 2018.

Fraction of patients with acute gastroenteritis that test positive for norovirus by PCR. The number of studies included in the meta-analysis for each subgroup is indicated by the n value. Abbreviations: High-mortality developing (HMD), Low-mortality developing (LMD) (Ahmed et al. 2014). B) The fraction of asymptomatic individuals that tested positive for norovirus, where the polygon width shows the confidence interval for that subgroup, and the n value indicates the number of studies compiled for that subgroup within the meta-analysis (Qi et al. 2018)

3.3.4 Needs and Recommendations for Understanding HuNoV Infections in the State of California

HuNoV illness is not listed on the federal or state list of illnesses that require reporting; however, regulation does require that gastroenteritis outbreaks caused by HuNoV be reported to the local health officer. Nevertheless, reported illnesses available through CDPH NLN reports and through NORS are incredibly low compared to prevalence values calculated by meta-analysis of clinical studies. Literature meta-analysis provides HuNoV prevalence values that are roughly four orders of magnitude higher than HuNoV illnesses reported to NORS. It is not recommended to use reported illness numbers to compute expected wastewater concentrations. A future study on HuNoV prevalence in the state of California could confirm that the conclusions made in the meta-analyses for developed countries correspond with prevalence in California.

Our review of California public health records suggests that wastewater concentrations in average California wastewater would be highest in December and January. A wastewater based epidemiology project focused on HuNoV would benefit from increased wastewater measurements during that time period.

3.4 HAdV-Associated Illness

As HAdV causes a range of different illnesses depending on the HAdV subgroup, HAdV infections are identified through several avenues, including most commonly through surveillance of gastrointestinal illness, respiratory illness, or disease outbreaks. Fecal shedding of HAdV has been observed for different HAdV serotypes, regardless of the disease caused. Respiratory illness, illness associated with urinary tract infection, and keratoconjunctivitis caused by HAdV have been correlated with fecal shedding as well as gastrointestinal illness (Bonot et al. 2014; Mena and Gerba 2009).

Respiratory illnesses caused by HAdV are frequently detected through influenza surveillance efforts. Influenza is both a reportable disease (i.e., reporting to the local health department is required) and a federally notifiable disease (i.e., data can be voluntarily reported to the CDC). As a result, the respiratory illnesses caused by HAdV and identified during influenza surveillance are often reported in influenza surveillance datasets or reports.

Gastrointestinal illness due to HAdV can periodically be identified through tracking of HuNoV infection and other outbreaks resulting in gastrointestinal illness. Again, although HAdV does not cause a pathogen-specific reportable disease, outbreaks of ANY disease must be reported immediately according to California Code of Regulations (CCR 2020), and foodborne and waterborne disease outbreaks are also notifiable at the federal level (CDC, n.d.a.).

3.4.1 Databases Investigated for HAdV Infection

Federal, state, and county sources of data were investigated for HAdV data (Table 3-5).

Table 3-5. Potential Data Sources for HAdV Illness.

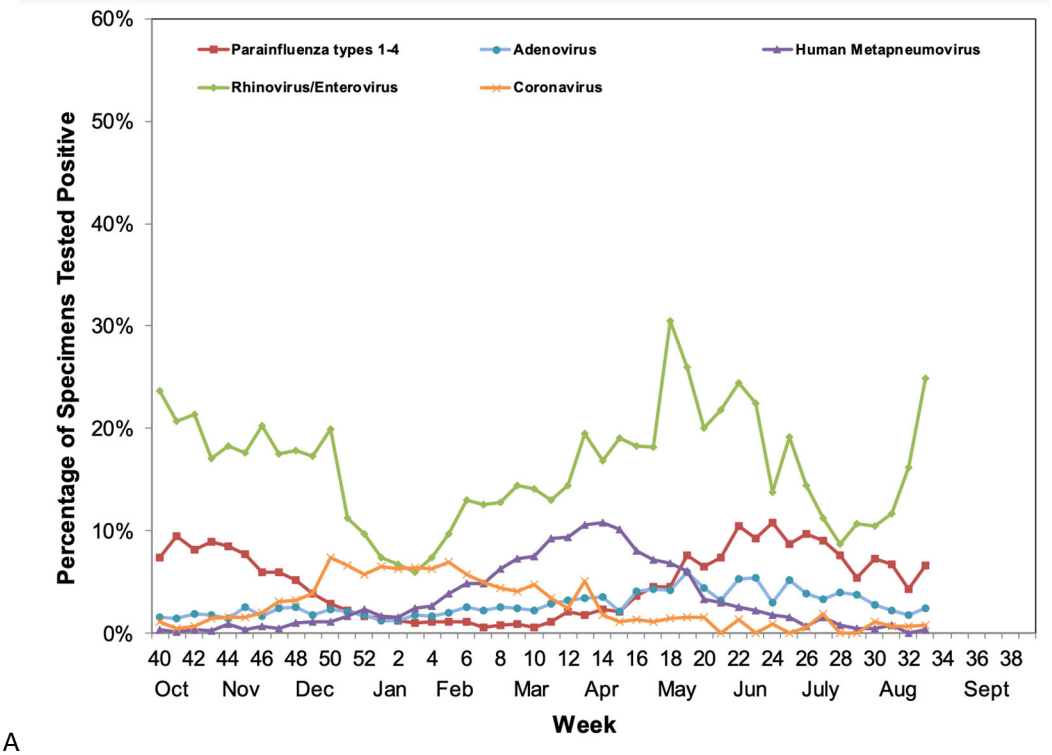
Network	Notes
CDPH Influenza and other Respiratory Virus Surveillance	Weekly respiratory adenovirus data available on CDPH website for influenza seasons 2008-2020 (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Immunization/Flu-Reports.aspx)
CDPH NLN Triannual reports	Adenovirus 40 & 41 is analyzed in stool along with norovirus and mentioned in the NLN triannual reports, but there is no adenovirus given. Conversation with VRDL indicated this is due to the incredibly small number of outbreaks reported (October 2017-September 2019)
California Health and Human Services	A freely available spreadsheet for influenza surveillance, including adenovirus data was downloaded July 2020 (https://data.chhs.ca.gov/dataset/influenza-surveillance).
California Department of Public Health	Additional data was requested by filling out a CDPH public health records request (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Monthly-Summary-Reports-of-Selected-General-Communicable-Diseases-in-CA.aspx).
U.S. Department of Health and Human Services, Office of the Chief Data Officer	Same spreadsheet as posted on CHHS was downloaded July 2020 (https://healthdata.gov/State/Influenza-Surveillance/aa6f-huzp).
National Respiratory and Enteric Virus Surveillance System (NREVSS)	Federal respiratory adenovirus data since 1989 (https://www.cdc.gov/surveillance/nrevss/index.html)
National Adenovirus Type Reporting System (NATRS)	Federal collection of data such as type from positive adenovirus samples since 2014 (https://www.cdc.gov/adenovirus/reporting-surveillance/natrs/publications.html)
National notifiable diseases surveillance system (NNDSS)	Federal data is available in various reports found in **CDC stacks collections**, **WONDER**, and **NNDSS summary reports**. For example, a nationwide report from MMWR is available titled "Human Adenovirus Surveillance — United States, 2003–2016" (https://www.cdc.gov/mmwr/volumes/66/wr/mm6639a2.htm?s_cid=mm6639a2_w)
National Center for Biotechnology Center	CDPH publishes their outbreak data and pointed to the NCBI database. For example: Outbreak of Epidemic Keratoconjunctivitis Caused by Human Adenovirus Type D53 in an Eye Care Clinic — Los Angeles County, 2017 (https://www.ncbi.nlm.nih.gov/)
County public health departments	San Diego and San Francisco county health department websites were investigated as a source of data, but adenovirus data was not found

Ultimately, the primary source of HAdV infection data was the influenza and respiratory surveillance programs, although the HAdV serotypes identified through these programs were not of primary interest to the project. VRDL NLN reports state that samples are tested for HAdV types 40 and 41, however conversation with VRDL indicates the reports do not contain HAdV types 40 and 41 -associated disease outbreak data because HAdV-associated disease outbreaks are rarely reported in the state.

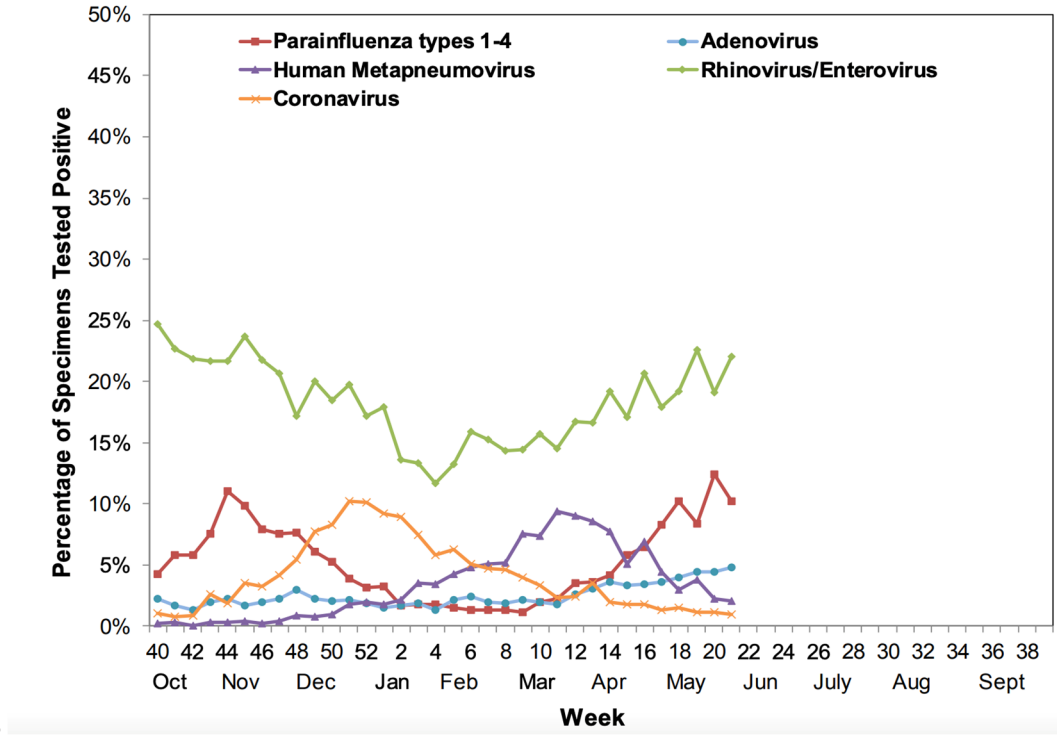
3.4.2 When and Where HAdV Infections Are Most Frequently Reported

Data reporting for HAdV infections is insufficient to answer when and where the highest concentrations of HAdV types 40 and 41 would be expected in wastewater, specifically for California. Through HuNoV disease surveillance, HAdV infections are also tracked, but HAdV-associated disease outbreaks are only extremely rarely reported according to VRDL. The lack of numbers for HAdV-associated disease in the NLN triannual reports indicates that either HAdV-associated gastroenteritis outbreaks are incredibly rare in the state of California or that HAdV-associated gastroenteritis outbreaks that do occur are not reported (VRDL reports any numbers they do have in the reports). Similar to what is observed in California, HAdV-associated gastroenteritis outbreaks are rarely reported across the U.S. From 2003 to 2016, only 2,138 HAdV reports were made to the federal National Adenovirus Type Reporting System (Binder et al. 2017). Of the data collected during this period, HAdV species B and C were the most commonly detected HAdV subgroups. These subgroups are associated with respiratory disease. Only five of the typed samples belonged to HAdVs associated with gastrointestinal disease; more specifically, the five samples were identified as belonging to HAdV serotype 41. Past work focused on HAdV prevalence in developed countries suggests enteric HAdV infections lack seasonal patterns (Binder et al. 2017; Bates et al. 1993; Khanal et al. 2018).

Data for influenza-like illness due to HAdV infection is more often reported. For example, influenza surveillance indicates the prevalence of HAdV detected in clinical samples as compared to detection of other respiratory viruses (Figure 3-11).



A



B

Figure 3-11. HAdV-Positive Clinical Samples Detected through Influenza Surveillance.
Percent of respiratory pathogen detections for adenovirus and other respiratory pathogens found through influenza surveillance at clinical sentinel laboratories from the last CDPH weekly Flu Report of the year A) 2017-2018 and B) 2018-2019.
Source: CDPH, 2021a.

3.4.3 Prevalence of HAdV Infection in the Community

Prevalence of gastroenteritis due to HAdV infection has been estimated at 1.55 – 12% globally (Mena and Gerba 2009). HAdV serotypes 40 and 41 are estimated as the cause of illness in 3 – 20% of children hospitalized with gastroenteritis in developed countries (Table 3-6; LeBaron et al. 1990; Bates et al. 1993; Mena and Gerba 2009). Notably, peak prevalence of HAdV infection is expected to occur in individuals less than two years old. This demonstrates that long-term immunity generally results from infection with HAdV 40 or 41; however, HAdV infections do still occur in adults and adolescents, sometimes without symptoms (LeBaron et al. 1990). Prevalence of infection by HAdV serotypes 40 and 41 is not well documented in the general population, likely because most infections are self-limited (Khanal et. al 2018).

Table 3-6. Enteric HAdV in Children.

Each study describes a population tested for adenovirus.

Source: Data from Mena and Gerba 2009.

Location of Study	Size of Study	% Pos. for HAdV	% Pos. for Enteric HAdV (HAdV40/HAdV41)	Study
Sweden	200 well 416 ill	1.5 13.5	0 7.9	Uhnoo et al. 1984
USA	270	6.7	1.1	Rodriguez et al. 1985
USA	372 well 538 ill		1.3 5.2	Kotloff et al. 1988
Guatemala	191 well 385 ill		4.7 (of well) 14.0 (of total)	Cruz et al. 1990
Korea	90 well 345 ill		2 9	Kim et al. 1990
Arizona	129 well 345 ill	8 8	2 2	Lew et al. 1991
Finland	248 ill	4		Ruuska and Vesikari, 1991
Sweden	50 well 100 ill	18 74	0 0	Allard et al. 1991
Argentina	766 well 180 ill	14.4 13.3	0.8 (of well) 33.0 (of total HAdV)	Mistchenko et al. 1992
England	1426 ill	17.8	16.4 of total HAdV	Bates et al. 1993
Rome	417 ill	7		Donelli et al. 1993
Brazil	79 well 67 ill	11.4 10	 43.0 of total HAdV	Harsi et al. 1995
Australia	4473 ill		3.2 of total HAdV	Grimwood et al. 1995
England	452 ill	32 (non HAdV 40/41)	22 (HAdV40) of total HAdV 46 (HAdV41) of total HAdV	Bryden et al. 1997
China	44 ill	100	58 (HAdV40); 32 (HAdV41)	Wang and Chen, 1997

3.4.4 Needs for Disease Surveillance Data

Despite the concentrations of HAdV serotypes 40 and 41 found in wastewater, sporadic illnesses or disease outbreaks caused by HAdV types 40 or 41 are rarely reported in California. The discrepancy between reported HAdV prevalence in the population and measured wastewater concentrations could be a consequence of multiple factors. Asymptomatic infection and shedding of enteric HAdV could result in elevated HAdV levels in wastewater without added reports of HAdV-associated gastroenteritis. In addition, cases of gastrointestinal illness could be self-limiting in individuals, so even if symptoms of gastroenteritis do present, illness is not serious enough for the individual to seek medical attention or report the case. More complete surveillance and prevalence data for HAdV-associated disease is needed for the general population to better explain the HAdV concentrations observed in wastewater.

3.5 Comparison to DPR2 Data

DPR2 (Pecson et al. 2021) measured wastewater data through 2020. Reported cryptosporidiosis illnesses in 2020 were higher than average historic cases in the month of January and February, but cases were reduced in April, May, and June 2020 compared to historical case numbers (Table 3-7). VRDL reported receiving no samples from 2020 to test for gastrointestinal pathogens. These data are not surprising considering the stay-at-home order issued in California March 19, 2020, due to COVID-19.

Table 3-7. Reported Cases of Cryptosporidiosis in California.
Source: Data from CDPH 2020b, CDPH 2020a, and CDPH, 2021b.

Month	2001-2019 mean	2001-2019 standard deviation	2020
Jan	27	9	42
Feb	20	7	28
March	22	8	13
Apr	25	11	12
May	27	10	13
Jun	31	17	13
Jul	43	26	
August	54	23	
Sept	43	17	
Oct	34	17	
Nov	28	15	
Dec	26	13	

CHAPTER 4

Fecal Shedding Rates

The quantity of pathogens entering the sewage system is dependent on both the number of infected people in the sewershed and the number of pathogens shed by the infected individuals. This chapter reviews published data on fecal shedding levels and dynamics for *Cryptosporidium* spp., HuNoV, and HAdV. Specifically, fecal shedding levels include the exact concentrations of pathogens measured in stool samples collected for patients infected with the pathogen of interest. Dynamics of pathogen shedding encompass the duration of shedding and the distribution of pathogen levels excreted in feces over the shedding period. This information is critical for linking community illness levels (i.e., prevalence) to wastewater concentrations. Ultimately, we use the assembled data from this chapter in models that estimate wastewater concentrations with prevalence data in Chapter 5.

Virus shedding can occur via a number of routes, including through saliva, urine, feces, and vomitus. As the focus of this feasibility study is on pathogens most relevant to DPR, we focus on virus concentrations shed in feces.

Many studies on fecal shedding rates initiate sampling when individuals present themselves to clinics with a gastrointestinal illness. In some cases, only one sample is provided and thus the study does not include temporal data. In other cases, the individual is enrolled in the study and additional samples are collected for a specific period of time after they visit the clinic with symptoms. Arguably the most informative fecal shedding studies involve presymptomatic sampling. In this way, shedding dynamics are captured from the period before an individual visits a clinic through the period in which the pathogen is shed. This is sometimes conducted by enrolling family members in the fecal study when a patient presents the illness. Another approach is infecting individuals in a controlled manner and regularly monitoring for the pathogen in feces. Either way, fecal samples are collected before patients contract the illness and early shedding dynamics are captured, even before the patient is symptomatic. Furthermore, these types of studies capture the dynamics of shedding asymptomatic patients who would be missed in studies that wait for ill patients to visit a clinic.

Research on fecal shedding is often conducted by medical researchers trying to understand the dynamics and presentation of the disease, rather than capturing the absolute quantities of infectious agents that exit the body in different forms. Consequently, many of the available shedding studies on gastrointestinal illnesses are qualitative or semiquantitative; that is, researchers look only for the presence or absence of the pathogen signal in feces. These studies might capture the length of time from the onset of the disease that the pathogen signal is measured in fecal samples. They sometimes measure the cycle threshold (CT) value reported by the qPCR instrument, which is semi-quantitative. In these cases, there may be valuable information on the relative pathogen concentrations from one sample to another, but the absolute abundances excreted are not defined. When studies utilize quantitative approaches to determine pathogen loads in the feces, they typically use molecular methods for HuNoV and HAdV. For *Cryptosporidium* spp., fecal concentrations are usually obtained by counting oocysts with fluorescence microscopy.

When environmental microbiologists measure pathogen concentrations in water, they are typically very careful to make accurate measurements due to the health implications of the results. When medical researchers study fecal shedding, they are often less concerned with reporting accurate quantities, and more concerned with identifying the etiologic agent of a patient's illness. As a result, the fecal shedding

literature often reports data without quality controls for their reported concentrations. For example, pathogen studies in wastewater frequently assess the presence of inhibitors in the sample and demonstrate that the measured quantities are not impacted by reverse transcriptase or polymerase inhibition. This is very rarely mentioned in fecal shedding studies despite the likelihood that fecal matter contains PCR inhibitors. Likewise, the absence of negative controls reported for qPCR reactions is common in fecal shedding data. The lack of these types of quality controls on quantitative measurements in fecal shedding studies reduces the confidence in the reported numbers and makes cross-study comparisons difficult.

4.1 *Cryptosporidium* Spp.

Early studies on *Cryptosporidium* spp. shedding rarely determined quantitative concentrations in feces, typically providing only positive or negative stool results. Early staining technology approaches provided limited capacity for providing quantitative data. Quantitative data, however, provides great insights into the dynamics of oocyst shedding and the duration of which it occurs. With more developed immunoassay technologies, studies have more frequently quantified fecal concentrations of oocysts, especially among acquired immunodeficiency syndrome (AIDS) patients. In the past decade, qPCR has increasingly been used to determine fecal concentrations in various stool samples. Use of immunofluorescent assays and qPCR simplify quantitative measurement compared to older methods such as Ziehl-Neelson staining, which was time-consuming and difficult. These literature values provide a range of $1.3 \times 10^2 – 9.3 \times 10^9$ oocysts shed/day per person, which will be used within the model discussed in Chapter 5. The concentration studies herein take into account all species of *Cryptosporidium.*

4.1.1 Methods for Quantifying *Cryptosporidium* Spp.

For the quantification of *Cryptosporidium* spp. Oocysts, fecal smear samples were typically collected and stored at 4C. Many studies evaluated the specimens within 24 hours of collection, however some waited up to one week before analysis (Daniels et al. 2015). For sample analysis, fecal samples were generally fixed in 10% formalin, with early studies usually performing a modified Ziehl-Neelson staining method to count the number of oocysts present (Stehr-Green et al. 1987; Shepherd et al. 1988; Jokipii and Jokipii 1986; Melo Cristino et al. 1988). Additional studies used other similar staining methods (Baxby et al. 1985). More recent studies used various immunoassays, such as immunomagnetic separation, direct immunofluorescence, or ELISA tests, to determine oocyst concentrations (Goodgame et al. 1993; Daniels et al. 2015). In more recent research, however, qPCR has been the dominant method for quantitative concentration measurements (Mary et al. 2013; Yang et al. 2014).

4.1.2 Concentrations of *Cryptosporidium* Spp. Measured in Feces

Many studies that track *Cryptosporidium* spp. oocyst shedding do not report absolute concentrations. Most available studies report their findings as *Cryptosporidium* spp. positive and negative stool samples, giving data to demonstrate oocyst shedding duration. Table 4-1 lists the few studies available that give quantitative concentrations of oocysts shed in feces. The shift to absolute quantification of *Cryptosporidium* spp. fecal concentrations in future studies will lead to better determination of the range and distribution of oocyst shedding from infected individuals. To enhance predictions of wastewater concentrations (discussed in Chapter 5), these future quantitative measurements of oocyst fecal concentrations will be needed.

Goodgame et al. (1993) identified 15 AIDS patients with oocyst-positive stool samples and no other cause of chronic diarrhea, and then instructed them to collect all stools passed within a 24- hour period and each specimen was assayed in triplicate on three separate days. 467 stool samples total from the 15

patients were collected and assayed, but three patients submitted incomplete or improperly reported specimens. There was a larger variation in excretion between patients (4- to 5-log differences) then between days of each patient (within one order of magnitude), from 0.02 x 10^6 to 1140 x 10^6 oocysts/day. The results demonstrated a fairly uniform excretion pattern over several days for individual patients, but high variability between patients.

White et al. (1994) identified 10 patients with documented HIV infection, CD4 cell count <100/mm^3, chronic diarrhea, and an oocyst-positive stool sample. Patients collected bowel movements for one or multiple 24 hour periods. Some were randomized to receive paromomycin treatment, but the data used for this report pertains only to the placebo group. Of the placebo group, a median daily shed count of 3.14x10^8 oocysts/day was reported, with a maximum of 9.3x10^9 oocysts/day. This study further reported a lack of significant daily change in oocyst concentration for AIDS patients with cryptosporidiosis.

Daniels et al. (2015) assayed 85 human fecal samples and identified 12 oocyst-positive samples. The samples are described as being from "apparently healthy" individuals. This may describe asymptomatic cases which do not show signs of diarrhea, but it is not clear. The study did not collect a 24 hour sample and instead determined the oocyst count per 10 grams of feces. Converting the reported value using the median fecal weight of 128 grams, a geometric mean of 4.1 x 10^3 oocysts/day was reported (Rose et al. 2015). These results may demonstrate the lower fecal concentrations apparent in asymptomatic cases.

Mary et al. (2013) used 10 "well-characterized" positive stool samples from the French ANOFEL *Cryptosporidium* National Network. The fecal concentrations were calculated by qPCR and provided a range of 3.8 x 10^4 – 4.5 x 10^9 oocysts/day after conversion with median daily fecal weight. Unfortunately, this study does not provide insight into the nuances of human excretion as no data on the origins of the fecal samples is reported.

Yang et al. (2014) conducted qPCR and ddPCR on 6 oocyst-positive fecal samples from an unreported source. From their reported concentrations of oocysts/gram of feces, values were adjusted with a factor of 128 grams to determine 24 hour excretion and a mean of 4.6x10^6 oocysts/24 hour period was determined. Like Mary et al. (2013), origins of the fecal samples are not reported.

Table 4-1. Reported Quantitative Fecal Concentrations of *Cryptosporidium* Spp.

Study	Sample Number	Method	Patient Info	Reported Average	Reported Range
Daniels et al. 2015	85	Immunomagnetic separation, direct immunofluorescence	Aged 6 months – 79 years, diarrhea patients, 87% rural homes	319 oocysts/10 g	10 – 4,909 oocysts/10 g
Goodgame et al. 1993	12	Quantitative IFA (Merifluor Kit)	AIDS Patients	N/A	2.0×10^4 – 1.2×10^9 oocysts/day
White *et al*. 1994	10	Quantitative IFA (Merifluor Kit)	AIDS Patients	3.14×10^8 oocysts/day (median)	0.0 – 9.3×10^9 oocysts/day (one case had a negative stool sample)
Mary *et al*. 2013	60	qPCR	Positive stool samples from French ANOFEL Cryptosporidium National Network.	N/A	300-59,000 oocysts/g (300 oocysts/g determined to be lower detection limit)
Yang *et al*. 2014	6	qPCR corrected 18S rRNA	Positive human fecal samples from unreported source	24,800.60 oocysts/g	14,529.80 – 46,886.80 oocysts/g
Yang *et al*. 2014	6	ddPCR 18S rRNA	Positive human fecal samples from unreported source	39,723.00 oocysts/g	22,813.00 – 67,708.00 oocysts/g

4.1.3 Dynamics of *Cryptosporidium* Spp. Shedding

Some studies have shown that many of patients with symptomatic cryptosporidiosis experience diarrhea, the duration of which relates to the duration of oocyst shedding (Jokipii and Jokipii 1986). Table 4-2 provides a summary of the duration of fecal shedding in immunocompetent patients. It is important to note also that it has been demonstrated that people with co-infections of HIV and *Cryptosporidium* spp. have much more prolonged periods of oocyst shedding, shedding oocysts longer than 60 days after initial observation (Tam et al. 2020).

Table 4-2. Duration of *Cryptosporidium* Spp. Oocyst Shedding in Immunocompetent Patients.

Study	Mean Shedding of Oocysts Duration (days)
Stehr-Green et al. 1987	18.8
Chappell et al. 1996	14-38
Shepherd et al. 1988	17-19
Jokipii and Jokipii 1986	<31
Melo Cristino et al. 1988	12
Baxby et al. 1985	20
Tangermann et al. 1991	25.9

4.1.4 Summary of *Cryptosporidium* Spp. Shedding

Review of current literature on the shedding of *Cryptosporidium* has shown much data demonstrating the range of fecal concentrations and the duration of which an infected patient sheds oocysts. However, qualitative data on the types of patients shedding oocysts is lacking. Many studies focus on patients with co-infections of HIV and *Cryptosporidium* as in Goodgame et al. (1993) and White et al. (1994) or list no information on fecal samples as in Mary et al. (2013) and Yang et al. (2014), limiting the ability to correlate shedding with certain demographic characteristics. Having comparisons of immunocompromised and immunocompetent patients, rural and urban residences, and ages of patients are a few examples of demographic comparisons that would increase understanding of fecal concentrations and their variability among patients.

4.2 Human Norovirus (HuNoV)

A number of studies have determined HuNoV fecal concentrations from individuals with gastroenteritis. Most research measured concentrations in fecal samples starting after patients presented with symptoms of gastrointestinal illness. Other work enrolled volunteers for human feeding trials and studied fecal concentrations of HuNoV from volunteers before ingestion of HuNoV through the entire duration of shedding. Median levels of HuNoV measured in stool ranged from 10^5 to ~ 10^{11} gene copies HuNoV/g stool, depending on the study.

4.2.1 Methods for Measuring HuNoV Shedding

HuNoV has historically been unculturable, although in recent years enteroid culture systems have successfully been used for HuNoV replication in vitro. HuNoV is therefore measured almost exclusively by PCR-based methods in fecal samples. Samples are collected either as stool samples or swabs. After collection, stool samples or swabs are sometimes stored at 4°C for short periods of time or at -70 or -80 °C for longer-term storage. Prior to analysis, stool samples and swab samples are usually suspended in phosphate-buffered saline (PBS) or tissue culture media at ~10% (w/v). After the solids are removed by centrifugation, the nucleic acids are extracted from the solution and then quantified with RT-qPCR. Genotype specific primers are typically used to differentiate HuNoV GI and HuNoV GII.

4.2.2 Concentrations of HuNoV Genomes Measured in Feces

Lai et al. (2013) studied a HuNoV-associated disease outbreak in a nursing home. Samples were collected from both nursing home employees and residents after the onset of symptoms until symptoms were absent. The mean concentration in residents was approximately 10 times higher than the concentration in the employees. In every sample, the initial concentrations were the highest. Specifically, the mean initial viral load in employees was $10^{8.33}$ and the mean initial load in residents was $10^{9.63}$. This suggests that studies that start sampling once a person presents symptoms may miss the highest portion of the human norovirus shedding curve.

Teunis et al. (2015) started sampling patients and healthcare workers in a hospital or care facility when two or more PCR-cases had been confirmed in a ward. This occurred four times in winters 2009-2011. They continued sampling weekly until patients tested negative. In total, 230 fecal samples were collected from 102 subjects, split fairly evenly between healthcare workers and patients. The peak GII.4 (i.e., serotype within the GII serogroup) concentrations reports were 10^{7} gc/g for staff and $10^{7.5}$ gc/g for patients. The results suggested that people who exhibit symptoms shed more than people who are asymptomatic, although the results were not statistically significant.

Chan et al. (2006) quantified HuNoV in the stools of 627 patients that displayed gastrointestinal symptoms, with 54 testing positive for either HuNoV GI, HuNoV GII, or both. The mean and maximum values measured in the Chan study were orders of magnitude lower than in other studies, possibly due to a lag between when symptoms started and when the single samples were collected. Interestingly, the GII loads were over 100 times higher than the GI loads.

One of the two HuNoV challenge studies identified in the literature was by Atmar et al. (2008) They inoculated 18 healthy adults in the US and 11 developed gastrointestinal illnesses. They measured fecal concentrations for 8 weeks post inoculation. The mean peak concentration measured was ~10^{11} gc/g, about one order of magnitude higher than the peak value measured in the Lai et al. (2013) study that was not designed to be a challenge study but did include longitudinal data.

In the second challenge study that was identified in the literature (Kirby et al. 2014), 15 healthy volunteers were challenged with HuNoV GI.1 and 51 were challenged with GII.2. Fecal samples were collected daily for the first 7 days post the challenge and then weekly thereafter. The peak shedding for the symptomatic GI.1 was $10^{9.1}$ and for GII.2 was $10^{6.7}$. The cumulative amount shed through the whole illness was 10^{11} for GI.1 and $10^{8.8}$ for GII.2.

Table 4-3. Reported Quantitative Fecal Concentrations of HuNoV.

Study	Strain	Sample size	Patient Info	Reported Average (gc/g)	Concentration range (gc/g)
Chan et al. 2006	GI	8	Patients with GI symptoms	8.4×10^5 (median)	2.2×10^4–2.9×10^{10}
Chan et al. 2006	GII	37	Patients with GI symptoms	3×10^8 (median)	2.5×10^4–7.7×10^{10}
Reymão et al. 2018	GII	108	Pediatric patients in Brazil (<9 years old)	3.9×10^8 (median)	6.6×10^3 - 5.4×10^{11}
Atmar et al. 2008	Not specified	16	Healthy adults (ages 18 – 50)	9.5×10^{10} (median peak)	5×10^8 –1.6×10^{12} (peak)
Lai et al. 2013	GII	33	Nursing home employees	2.1×10^8 (mean)	NA
Lai et al. 2013	GII	42	Nursing home residents	4.0×10^9	NA
Teunis et al. 2015	GII.4	40	Hospital employees	1×10^7	~10^4 - ~10^9 (peak)
Teunis et al. 2015	GII.4	80	Hospital patients	3.2×10^7	~10^4 - ~10^9 (peak)

4.2.3 Dynamics of HuNoV Shedding

In the Lai et al. 2013 study, where sampling started at symptom onset, measured fecal concentrations of HuNoV from nearly all patients immediately started to decrease. Interestingly, stool concentrations decreased in a log linear manner until sampling ended.

The Teunis et al. (2015) study included a mixture of subjects, some who were likely already infected with HuNoV and others who were pre-symptomatic. Samples were collected weekly until the stools of a patient were negative. The staff shed for a median of 20 days and the patients shed for a median of 40 days. They ultimately developed a model with the temporal measurements that estimates viral shedding for both symptomatic and asymptomatic shedders. Results indicate that symptomatic patients shed longer than asymptomatic patients, although comparisons were not statistically significant.

In the Atmar et al. (2008) challenge study, shedding peaked approximately four days after virus inoculation. In the Kirby et al. (2014) study, where both GI.1 and GII.2 were studied, GII.2 virus was shed for an average of 5 days, while GI.1 virus was shed for 17 days. Despite differences in the length of shedding, both types peaked in stool samples at 4 days post inoculation.

4.2.4 Summary of HuNoV Shedding

It is worth noting that the strains studied in the two reviewed challenge studies no longer circulate. Kirby et al. (2014) noted that it is not known if the GI and GII results are representative of GI and GII strains that currently circulate. It is worth noting that in the studies on circulating GI and GII viruses, the GII strains are excreted at higher levels and for longer periods of time. On the other hand, in the challenge studies, the GI strain is excreted at higher levels and for longer. This suggests that the differences between GI and GII may not be consistent for all strains. The combined results also illustrate the vastly different shedding dynamics and intensities between different HuNoV serotypes and strains. This complicates efforts to link HuNoV prevalence and wastewater concentrations because the relationships will depend on which virus strains are circulating and which virus strains are targeted with the PCR assays.

4.3 Human Adenovirus (HAdV)

A limited number of studies have measured absolute HAdV concentrations in fecal samples. Although HAdV subgroups F and G are most commonly associated with gastrointestinal illness, few studies focused solely on concentrations of these enteric HAdV subgroups. More commonly, HAdV concentrations in stool samples were measured for all HAdV subgroups. Studies measuring fecal concentrations of HAdV were primarily focused on immunocompromised patients. HAdV subgroup C was the predominant HAdV subgroup found in stool samples, despite subgroup C HAdV being associated with respiratory disease. A wide range of HAdV concentrations was measured in stool samples, from 10^2 gene copies/gram stool to over 10^{11} gene copies/gram stool. It is important to note that this range is limited by qPCR quantification limits on the lower end. Inhibition of PCR due to detrital material in nucleic acid extracts could also play a role in the absolute concentrations reported. Past research has evaluated the positivity of HAdV in stool samples, however a large portion of this work is qualitative, only providing information on whether or not a sample was HAdV positive or negative. Here we have collected only quantitative data on HAdV fecal concentrations.

4.3.1 Methods for Measuring HAdV Shedding

Fecal concentrations of HAdV were measured using molecular approaches (e.g., qPCR). Some studies targeted a conserved region of the HAdV genome with qPCR, while others used multiple subgroup specific targets. Studies using a broadly reactive HAdV qPCR assay frequently supplemented this approach with sequencing of a variable region of the HAdV genome to determine which subgroup and/or serotype infected the individual. Frequently, studies evaluating HAdV fecal concentrations reported HAdV levels as CT values from qPCR analysis. These data were not included here, because the CT values cannot be accurately interpreted to obtain absolute concentrations of virus in stool without more information.

4.3.2 Concentrations of HAdV Measured in Feces

Absolute concentrations of HAdV in stool samples were measured in multiple studies, and the findings from this research are summarized in Table 4-4. Work was primarily focused on fecal shedding from immunocompromised patients, and several studies evaluated HAdV stool concentrations from patients before and after stem cell transplantation. A couple studies did evaluate concentrations of HAdV in stool samples of immunocompetent children and adults. Average concentrations of HAdV were on the order of 10^4 to 10^6 gene copies/gram of feces in most studies, though in the study by Kosulin et al. (2016), transplant patients had much greater median HAdV stool concentrations, approximately 10^9 gene copies/gram stool. The range of HAdV concentrations measured in fecal samples was large, covering over nine orders of magnitude. The differences in concentrations observed in different fecal samples could be due to several reasons, including the possibility that samples were taken at different time

points over the course of HAdV infection, and therefore may not have all been taken at the peak of infection when viral titers would be expected to be highest. Differential immune responses of patients could also have played a role, whereby HAdV infection in some individuals may have been limited, resulting in low levels of viral replication, while HAdV infection and viral replication in other individuals may have been significantly more robust.

It is important to note the HAdV concentrations measured in stool samples were not restricted to HAdV serotypes 40 and 41. In fact, most studies typed HAdV-positive samples, and among the typed stool samples, HAdV subgroups B and C were most commonly identified, regardless of the location of the study or patient background.

Table 4-4. Reported Fecal Concentrations of HAdV.

Study	Subgroup	Sample Number	Method	Patient Info	Average concentration (gc/g)	Concentration range (gc/g)
Berciaud *et al.* 2012	57 typed samples – B (n = 15), C (n = 36), D (n = 1), F (n = 5)	98	qPCR of conserved genome region for HAdV loads in stool; sequencing for HAdV typing	Immunocompetent and immunocompromised; children (n = 73), adults (n = 24)	~10^6 (median)	5 x 10^2 - > 10^{10}
Lion *et al.* 2010	45 samples - A (11%), B (4%), C (75%), D (7%), F (2%); 37 typed samples – C02 (43%), C01 (38%), A12 (8%), A31 (5%), B3 (2%), B16 (2%), D19 (2%)	138	qPCR – six assays for subgroups A-F	Stem cell transplant patients	Peak ranged from 2 x 10^7 – 10^{11}	1 x 10^2- 1 x 10^{11}
Srinivasan et al. 2015	Not specified	39	qPCR	Stem cell transplant pediatric patients	5.2-$\log_{10}$ (median)	2-$\log_{10}$ – 11.3-$\log_{10}$
Jeulin et al. 2011	Predominantly serogroup B	> 17	qPCR,	Stem cell transplant patients, children, and adults	5.47-$\log_{10}$ (mean)	Not given
Vetter et al. 2015	qPCR primers target HAdV subgroup C, but no typing conducted	48	qPCR	Random sample of individuals (ages 20 – 50) in Brazil without diarrhea	1.43 x 10^4 (summer average); 4.01 x 10^3 (winter average)	4.04 x 10^2 – 6.72 x 10^5
Kosulin et al. 2016	HAdV	84	qPCR of conserved genome region for HAdV loads in stool; PCR of positive samples	Stem cell transplant pediatric patients	10^9	10^2 – 10^{11}

4.3.3 Dynamics of HAdV Shedding

None of the studies in Table 4-4 that quantified HAdV concentrations in fecal samples evaluated the duration of HAdV shedding. A Peruvian study monitored a group of 20 HIV-positive patients with male-to-male sexual contact for HAdV shedding using rectal swabs (Curlin et al. 2010). In this group of individuals, HAdV shedding of different serotypes occurred in bursts over the monitoring period for 15 of the 20 subjects, and the time-frame of detectable HAdV in rectal swabs from a single episode of infection varied from days to over five weeks in certain cases. Viral loads during these bursts of infection generally followed a bell shaped curve, with peak loads observed in the center of the HAdV detection period. Another study monitored stool samples from stem cell transplant patients for HAdV twice weekly from before transplantation through >100 days post-transplantation in certain cases (Jeulin et al. 2011). In multiple patients, stool samples were qPCR positive for HAdV for 100 or more days, suggesting chronic infection. It is important to note that the groups of individuals monitored in these studies may have exhibited prolonged infection compared to healthy individuals, and these findings should therefore not be overinterpreted when evaluating shedding dynamics for the general population. More data on the dynamics of HAdV fecal shedding is needed to identify the period of shedding and the viral loads expected from HAdV infection in the general population.

4.3.4 Summary of HAdV Shedding

Studies evaluating HAdV concentrations in fecal samples were largely focused on individuals with compromised immune systems. From the couple of studies that did evaluate fecal concentrations among immunocompetent individuals, the average HAdV concentrations measured did not appear to differ drastically from immunocompromised patients in most cases. Nonetheless, more research is needed to better understand HAdV levels in stool from immunocompetent populations experiencing gastroenteritis or asymptomatic infection.

In addition, the work conducted on HAdV concentrations in feces focused broadly on all HAdV subgroups, although species or strain identification was conducted in some cases. Notably, HAdV belonging to subgroup C was detected most prevalently in stool samples. While HAdV subgroup C is associated with respiratory illness, the fact that HAdV subgroup C was detected in a large portion of HAdV positive stool samples indicates this virus subgroup may be present in wastewater. The lack of data for fecal shedding of HAdV subgroup F, a focus of DPR guidance and risk assessment, presents a significant limitation in accurately predicting wastewater concentrations using the approach outlined in Chapter 5.

CHAPTER 5

Modeling Wastewater Concentrations

5.1 Objectives

The literature reviews described above provided expected ranges of infection prevalence values and fecal shedding concentrations for the three target pathogens. With this data, an epidemiological model was developed to link wastewater influent concentrations of pathogenic particles with community infection prevalence.

The model allows one to estimate the prevalence of infections within a 24-hour timeframe. Inputs of the model include various details on the location desired to model. Other parameters related to the illness can be modified to suit the outbreak simulation. We note that the basic mass balance model is a first-step to linking prevalence, shedding rates, and wastewater concentrations. The model will need to be revised as more information becomes available on shedding rates and infection prevalence.

5.2 Theory

To link wastewater pathogen concentration and community prevalence, a mass balance was conducted. Inputs to the model include the location of the simulation and the pathogen being simulated. A ShinyApp reactive model was created in R to allow for user-friendly instant simulation (WEM, n.d.). The R code of the ShinyApp Model is provided in Appendix A.

5.2.1 Inputs

Various parameters are input by the user based on the location they are intending to simulate (Figure 5-1). These inputs include the service population of the wastewater treatment plant, the daily wastewater influent flowrate, and the estimated range of infection prevalence values for the area.

The model also allows for the input of fractional factors to further expand the extrapolation capacity of the model. These include a factor for the fraction of cases reported out of total cases to account for underreporting and a factor for the fraction of infected cases who shed pathogens at all.

5.2.1.1 Fecal Concentrations

A literature review described in Chapter 4 provided quantitative fecal concentrations for individuals shedding each of the pathogens. Estimations of shedding distributions were developed from the various studies detailed in Chapter 4. The pathogen fecal concentrations in individuals were assumed to follow a log-normal distribution, using the means and ranges found in literature (Tables 4-1, 4-3, 4-4). The individual shedding distributions differ between the pathogens. Adenovirus, for example, exhibits different shedding concentrations between asymptomatic and symptomatic cases (Vetter et al. 2015; Berciaud et al. 2012). Norovirus and *Cryptosporidium* have no statistically significant difference in shedding concentrations between symptomatic and asymptomatic cases (Newman et al. 2016; Teunis et al. 2015; Samie et al. 2006). Approximate distributions of fecal concentrations were determined by agglomeration of literature data which can be seen in tables in Chapter 4. Norovirus demonstrates a large difference in shedding rates between GI and GII strains, as much as 100-fold (Chan et al. 2006). The model here uses GII concentrations as it is much more common in infection (Chan et al. 2006; Huhti et al. 2011). Conversion of units from gene copies shed/g feces to gene copies shed/24 hours differs for asymptomatic and symptomatic cases. The three pathogens studied all have diarrhea as a main symptom, so the daily fecal mass used for unit conversion needs to correlate to the presence or absence

of symptoms (CDC 2019b; Uhnoo et al. 1984). 128g feces/day is used for asymptomatic cases, and 796 g feces/day is used for symptomatic cases with the assumption that all symptomatic cases experience diarrhea (Rose et al. 2015; Wierdsma et al. 2011). Figure 5-2 shows a typical individual distribution of fecal shedding rates for adenovirus.

Originally, the distribution of these individual shedding rates were applied to the model, however this creates unrealistic scenarios of community shedding rates. For instance, if the distribution of individual fecal concentrations is used as an input in the model with x iterations (Figure 5-2), then some iterations will apply the extreme high and low concentrations as the shedding rate for *all* infected persons. In reality, this would not happen. Ultimately this creates estimated wastewater concentrations that spread several orders of magnitude. To address this issue, a mean population shedding rate distribution was created (Figure 5-3). Specifically, the model will produce various individual shedding distributions based on literature data for fecal shedding like Figure 5-2 and calculate the mean of each one. This collection of mean shedding rates will then form its own distribution like Figure 5-3, which is channeled into the model calculations. The bimodal distribution apparent in Figure 5-2 is a result of the differing shedding concentrations between asymptomatic cases and symptomatic cases, with means around 10^6 gene copies/24 hours and 10^9 gene copies/24 hours, respectively. Each iteration takes a random proportion of asymptomatic cases within a range determined from literature. For adenovirus, this range is approximately between 35-50% (Van et al. 1992; Hebbelstrup Jensen et al. 2019). For *Cryptosporidium*, this range is approximately between 70-85%, from both calculations in Section 3.2.3 and literature (Wang et al. 2002). For norovirus, this range is approximately between 25-50% (Miura et. al 2018).

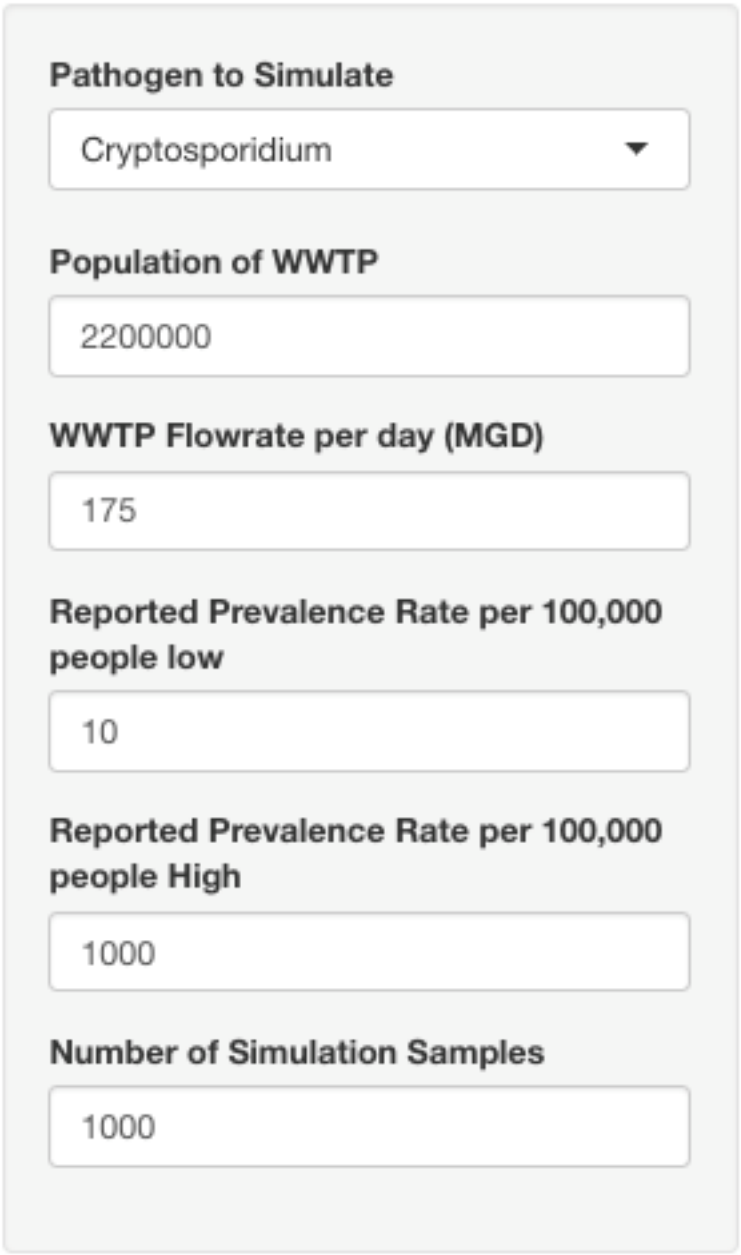


Figure 5-1. Input Interface of the ShinyApp Model.
Source: WEM, n.d.

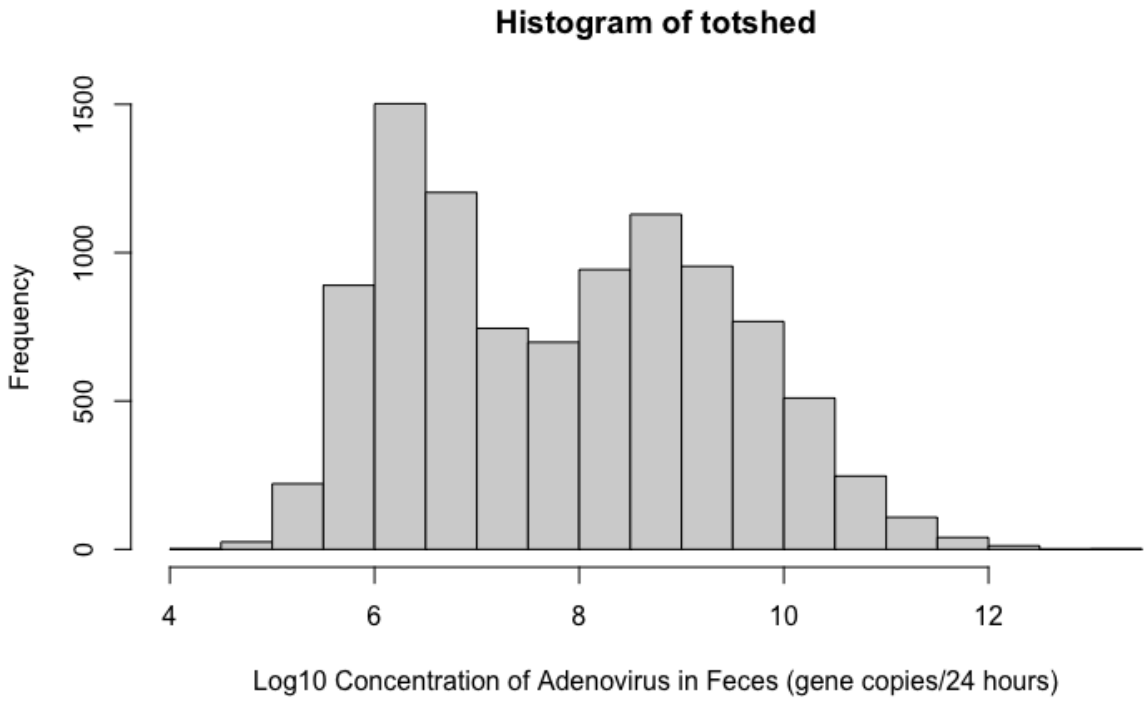


Figure 5-2. Example Individual Fecal Concentration Distribution for Adenovirus.

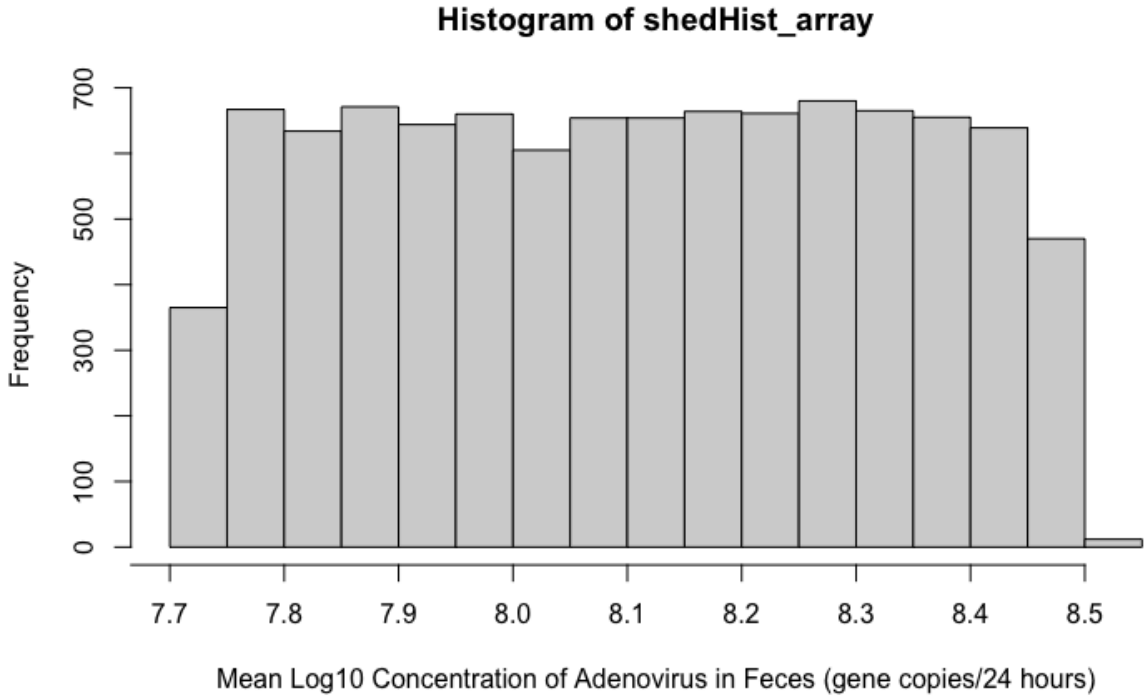


Figure 5-3. Example Population Mean Fecal Concentration Distribution for Adenovirus.

5.2.2 Calculations

Once the parameters are entered, the ShinyApp will automatically re-analyze the model and produce a graph. The calculation behind this modeling is a mass balance based on the following relationship.

$$C_{WW} = \frac{f_{prev}}{100{,}000} * 10^{C_{shed}} * \frac{Pop}{Q_{WW}} * 0.264172 \quad \text{(Equation 5-1)}$$

Where:

C_{WW}	=	Concentration of gene copies/oocysts in WW influent (gc/L)
f_{prev}	=	Prevalence of cases per 100,000 people
100000	=	Factor to normalize f_{prev}
C_{shed}	=	Log concentration of gene copies/oocysts shed by 1 person (log gc/day)
Pop	=	Population of WWTP
Q_{WW}	=	Volumetric flowrate of WWTP (gal/day)
0.264172	=	Conversion for gal to liter (gal/L)

If the input parameter for the fecal shedding rate is in gc or oocysts per day, then:

$$C_{Shed} = C_{Input} \quad \text{(Equation 5-2)}$$

Where:

C_{Input}	=	Log_{10} concentration of gene copies/oocysts shed by 1 person (log gc/day)

If the input parameter for the fecal shedding rate is in gc or oocysts per gram of feces, then:

$$10^{C_{Shed}} = 10^{C_{Input}} * 128 \quad \text{(Equation 5-3)}$$

$$10^{C_{Shed}} = 10^{C_{Input}} * 796 \quad \text{(Equation 5-4)}$$

Where:

C_{Input}	=	Log concentration of gene copies/oocysts shed by 1 person (log gc/g)
128	=	Median fecal wet mass excreted per person in one day (g/day) (Rose et al. 2015)
796	=	Median diarrheal fecal wet mass excreted per person in one day (g/day) (Wierdsma et al. 2011)

Conversion of asymptomatic concentrations are conducted with Equation 5-3, while symptomatic concentrations are converted with Equation 5-4.

5.2.3 Outputs

The ShinyApp model outputs a scatter plot with a regression line like one shown in Figure 5-4.

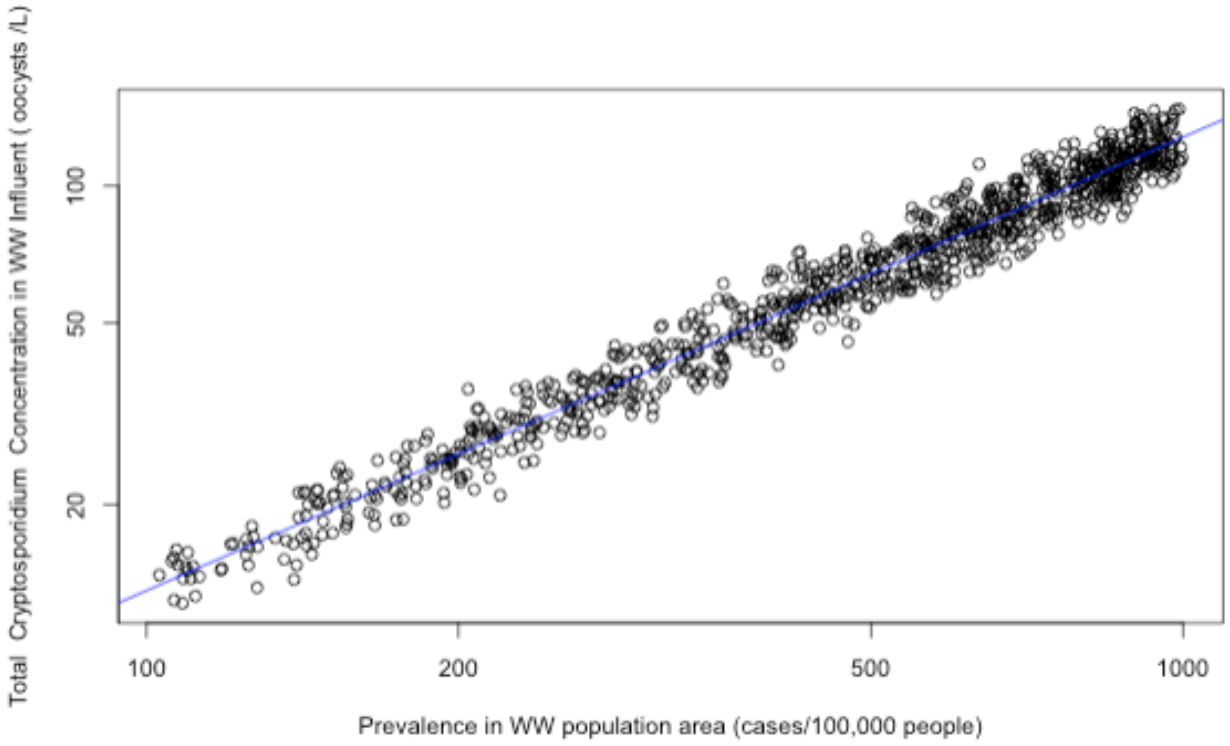


Figure 5-4. Example Output of the ShinyApp Model.
Source: WEM, n.d.

5.3 Incorporation of DPR2 Data into Model

In order to assess the critical inputs of the model, the model was run using wastewater influent concentration data measured in DPR2 (Pecson et al. 2021; Table 5-1).

Table 5-1. Wastewater Concentrations Measured in Pecson et al. 2021.

Pathogen	Quantification Method	Mean Wastewater Concentration (log_{10} organisms/L)^2	Standard Deviation Wastewater Concentration (log_{10} organisms/L)^2
Cryptosporidium spp.	Microscopy (EPA 1693)	1.7	0.4
Adenovirus	Molecular (qPCR)	4.9	1.5
Norovirus GII	Molecular (EPA 1615)	4.5	1.1

Specifically, the Pecson et al. (2021) wastewater concentration values were used to calculate the estimated prevalence range based on the mass balance described in Equation 5-5.

$$f_{prev} = C_{wwDPR2} * \frac{100000}{C_{shed} * Pop} * \frac{Q_{ww}}{0.264172} \qquad \text{(Equation 5-5)}$$

Where:

f_{prev}	=	Prevalence of cases per 100,000 people
C_{shed}	=	Concentration of gene copies/oocysts shed by 1 person (gc/day)
C_{WWDPR2}	=	Concentration of gene copies/oocysts in WW influent from Pecson et al. (2021) (gc/L)
Q_{WW}	=	Volumetric flowrate of WWTP (gal/day)
Pop	=	Population of WWTP
100000	=	Factor to normalize f_{prev}
0.264172	=	Conversion for gal to liter (gal/L)

A random log-normal distribution was generated using the mean and standard deviations reported in Pecson et al. (2021). Values used for this analysis are provided in Table 5-1. The resulting prevalence range was compared to literature prevalence values for California (Table 5-2).

This model does not consider the decay of genetic material through the sewage network and assumes that the signals of all excreted organisms reach the wastewater treatment plant. Recent research on SARS-CoV-2 RNA suggests that there is minimal decay in virus RNA concentrations measured at the temperatures and time frames that are relevant for sewage systems (Ahmed et al. 2020). Further investigation into decay rate of the three target pathogens of this report is necessary to confirm this assumption.

Table 5-2. Estimated Prevalence Ranges for Each Pathogen Based on Pecson et al. (2021) Wastewater Concentrations and Equation 5-5.

	Quantile Prevalence (cases/100,000 people)				
Pathogen	**0%**	**5%**	**50%**	**95%**	**100%**
Cryptosporidium spp.	2.57	14.0	167	1840	18800
Adenovirus	2.45×10^{-3}	0.880	65.5	5430	4.70×10^{5}*
Norovirus GII	5.00×10^{-6}	6.36×10^{-4}	3.31×10^{-2}	2.00	31.0

[*]Non-real values for prevalence (>100%)

In order to not include outliers, the 5%-95% quantile range of the calculated prevalence values is used for this testing. Table 5-3 compares the prevalence values estimated with the wastewater concentrations from Pecson et al. (2021) and prevalence ranges obtained from the literature review in Chapter 3.

Table 5-3. Comparison of Calculated Prevalence Values and Literature.

Pathogen	Calculated Prevalence Low (%)	Calculated Prevalence High (%)	Literature Prevalence Low (%)	Literature Prevalence High (%)
Cryptosporidium spp.	1.40×10^{-2}	1.84	0.6	4.3
Adenovirus	8.80×10^{-4}	5.43	1.55	12
Norovirus GII	6.36×10^{-7}	2.00×10^{-3}	1	16

There are several possible reasons for the discrepancies between the estimated prevalence values and the background community prevalence values reported in the literature. First, there are very limited studies on shedding rates (Chapter 4) and that is the key parameter linking prevalence and wastewater concentrations. It is very possible that the shedding rate distributions used here are significantly different than the actual shedding distributions of infected individuals. As mentioned in Chapter 4, studies on shedding rates rarely incorporate important quality control criteria when they quantify pathogens in fecal matter. Another possible explanation for the difference in estimated and reported prevalence data is that these illnesses are common in children and hospital patients and the feces of these individuals are often not included in a community's wastewater. Consequently, wastewater concentrations would be lower than expected based on the community's prevalence. Another possible explanation for why the norovirus predictions are low based on wastewater concentrations may be due to the impact that the SARS-CoV-2 pandemic has had on communicable illnesses. Public health data made available by the CDC, as well as correspondence with the California Department of Public Health, confirms that norovirus outbreaks were significantly lower in 2020 than in other years, with only two outbreaks reported from March 2020 to December 2020 in the entire United States. By comparison, there were 86 HuNoV outbreaks reported in January 2020 alone (CDC, n.d.b.). The prevalence values from literature, which were based on "normal" years, are likely significantly higher than the true prevalence of these pathogens during the COVID-19 pandemic. This is supported by Pecson et al. (2021) reporting that measured norovirus wastewater concentrations were significantly lower than literature values of norovirus concentrations in non-pandemic years. If wastewater concentrations decrease, then prevalence would be the only other variable that would likely decrease, as fecal concentrations should be consistent.

5.4 Model Implementation

Following the test of the model on predicting prevalence with Pecson et al. (2021) data, we used the model to predict wastewater concentrations with a range of prevalence. Figure 5-5 shows the input parameters for the example simulation. The example simulation presented here is for *Cryptosporidium* spp. and HuNoV concentrations in the influent of San Diego's main wastewater treatment plant. Input parameters include the population served by the plant, the average influent flow rate, and a range of prevalence expected based on prevalence data from Chapter 3.

Figure 5-6 and 5-8 shows the graphical output of the ShinyApp. The model user can zoom in and out of the model based on the prevalence range input.

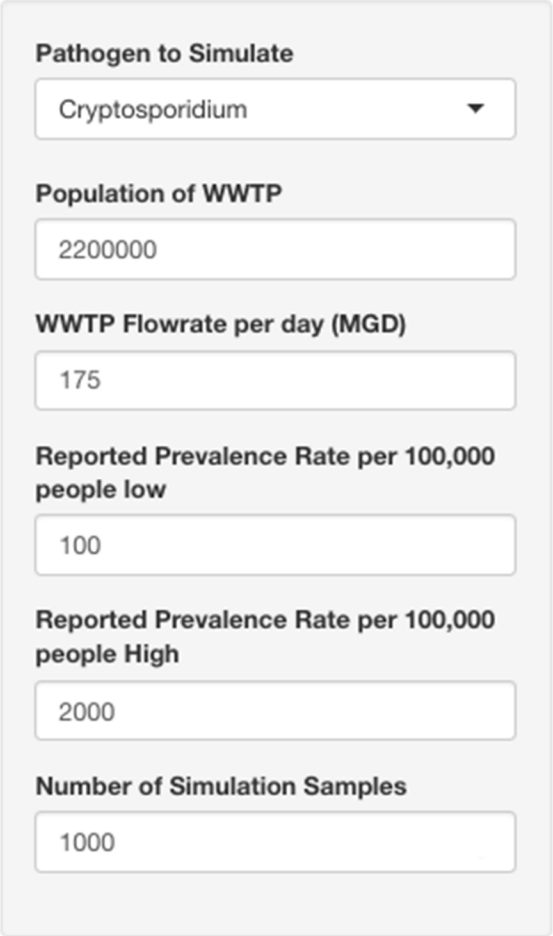


Figure 5-5. ShinyApp Inputs for *Cryptosporidium* Simulation.

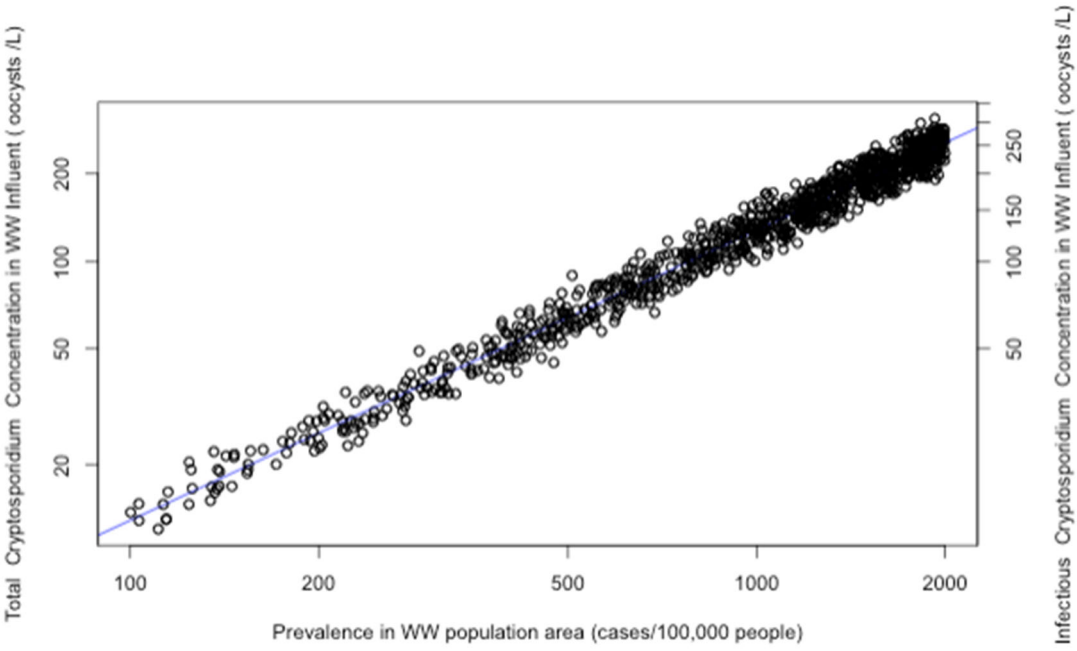


Figure 5-6. Scatter Output of the ShinyApp Model Based on Inputs from Table 5-3.

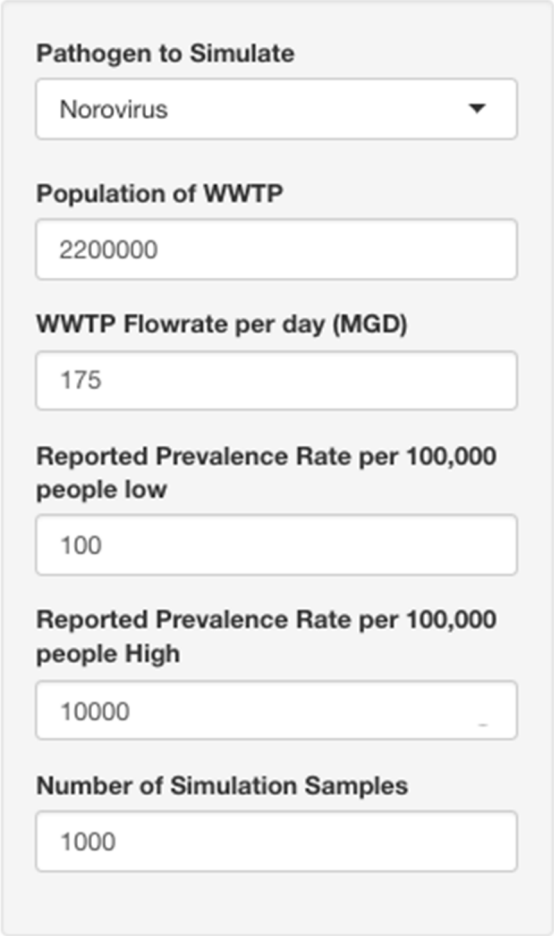


Figure 5-7. ShinyApp Inputs for HuNoV Simulation.

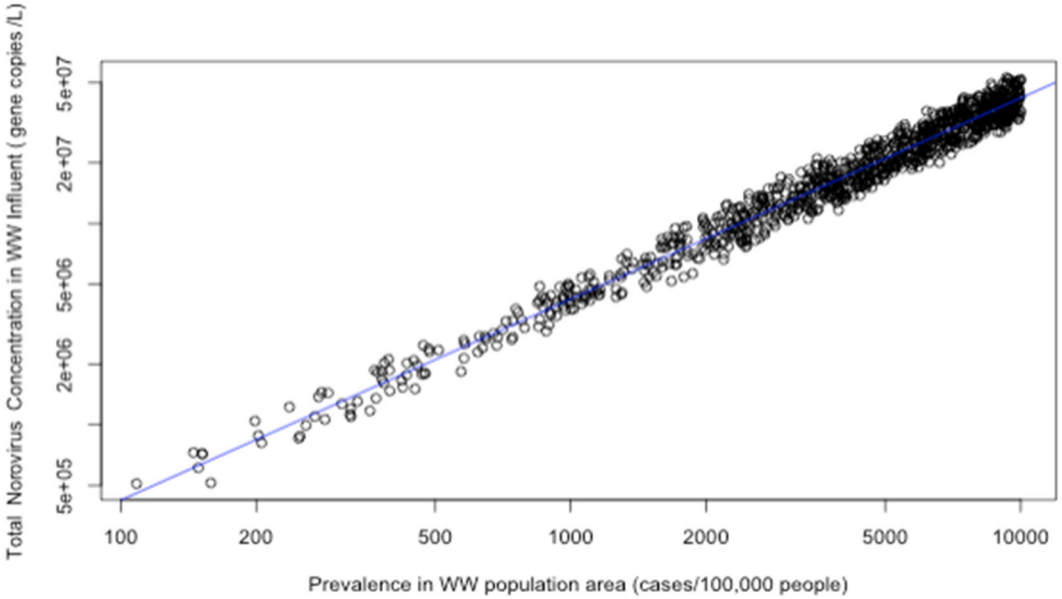


Figure 5-8. Scatter Output of the ShinyApp Model Based on Inputs from Figure 5-7.

5.5 Evaluating Confidence in Model

The three key driving variables of the model are fecal concentrations, wastewater concentrations, and prevalence. There is a varying level of confidence in the accuracy of each parameter for each pathogen, and Table 5-4 semi-quantitatively determines the variables that need most attention and further exploration. Criteria for qualitative assessment of data confidence include: the number of papers published, the sample sizes of the studies, whether the studies are clinical or population-based, the demographics of the patients (immunocompetency, symptoms, etc.), and the location of the study. Each variable is assigned a color denoting its confidence level based on the parameters mentioned, with green being fairly high confidence, red indicating a strong lack of confident data, and yellow indicating need for more data, but some relevant data is available.

5.5.1 Fecal Concentration Data

Fecal shedding concentrations are generally the parameter with the least confidence, and it is recommended for there to be much more population-based studies for all three pathogens. Tables of the studies can be found in Chapter 4.

Quantitative fecal concentration data for *Cryptosporidium* is quite underdeveloped. Studies such as Goodgame et al. (1993) and White et al. (1994) have low sample numbers and both focus on shedding in immunocompromised patients. Mary et al. (2013) and Yang et al. (2014) both report fecal concentration from uncharacterized fecal samples, with minimal to no information on the patients from which the samples come from. Mary et al. has a high sample size (60), while Yang et al. is quite a small sample pool (6) with a very tight range of concentration values. Daniels et al. (2015) has both a large samples size (85) and a large range of demographics (aged 6 months - 79 years with diarrhea), but the reported concentrations are significantly lower by several orders of magnitude than all other studies mentioned above, making it difficult to determine which data sets are most reliable. Due to these reasons, this parameter is assigned red.

Much of the available quantitative data on fecal concentrations of adenovirus is focused on stem cell transplant patients, as seen in (Lion et al. 2010; Srinivasan et al. 2015; Jeulin et al. 2011; Kosulin et al. 2016). These studies provide shedding rates for a highly immunocompromised clinical population that does not represent population fecal concentrations that would be seen in a wastewater treatment plant catchment zone. In addition, this project mainly focuses on adenoviruses type F, which represents a small portion of these studies (0-5%). Vetter et al. 2015 provides a large sample size (50) of random sampling of individuals without diarrhea, providing an asymptomatic fecal shedding concentration range. Berciaud et al. 2012 had both a large sample size (98) and assayed both immunocompetent and immunocompromised patients. The study was done in a clinical setting, but it demonstrated no significant difference in shedding between immunocompetent and immunocompromised shedding, possibly verifying the potential for clinical stem cell patient data to apply to the population. For the reasons above, this parameter was designated yellow as there is a couple decent studies, but more are needed to solidify.

There are several studies on quantitative fecal shedding of norovirus GII strains, using localized outbreaks in hospitals and nursing homes with sample sizes ranging from 33-108 patients and similar medians and ranges of fecal concentrations. The fecal concentration data for norovirus is the most comprehensive of the three pathogens, but further study into the population shedding dynamics from a wider sample size would improve confidence in norovirus fecal concentrations. This parameter has therefore been assigned green.

5.5.2 Wastewater Concentration Data

In the context of this report, wastewater concentration data is dependent on the findings of Pecson et al. (2021). As discussed in Pecson et al. (2021), wastewater concentration data for *Cryptosporidium* and adenovirus are comparable to historical findings, suggesting that the COVID-19 pandemic did not significantly affect "normal" prevalence and that the Pecson et al. (2021) data is valid for input into the model under "normal" circumstances for these two pathogens. However, norovirus concentrations were shown to be significantly lower than historical data, suggesting that prevalence is significantly affected as shown in outbreak data mentioned in Section 5.3. Due to this discrepancy, it is difficult to use Pecson et al. (2021) norovirus concentrations in the model alongside literature values for prevalence for comparison. As such, norovirus wastewater concentrations were designated yellow, while the other two were designated green.

5.5.3 Prevalence Data

As discussed in Section 3.2.3, *Cryptosporidium* prevalence data relies upon two meta-analyses by (Hörman et al. 2004) and (Fayer and Ungar 1986) that use 13 and 14 studies, respectively. Hörman et al. (2004) provided a tight range of prevalence from about 1-2% while Fayer and Ungar (1986) provides a non-outbreak range for North America between 0.6-4.3%. Hörman et al. limited the meta-analysis to only adults while Fayer and Ungar (1986) included studies involving children. These two meta-analyses provide an estimate of prevalence for *Cryptosporidium*, but there is a need for increased case reporting, as the prevalence determined from government department data from locality to national level is significantly lower than these estimates. *Cryptosporidium* prevalence has been designated yellow as the amount of studies included in the meta-analyses is quite low.

As described in Section 3.4.3, adenovirus induced-gastroenteritis prevalence values range from 1.55-12% globally according to Mena and Gerba (2009), with LeBaron et al. (1990) estimating that the 40/41 serotypes account for 5-20% of hospitalizations for diarrhea in developed countries. However, Khanal et. al (2018) reports that prevalence of infection for serotypes 40/41, including asymptomatic, is not well documented in the general population due to the self-limiting nature of the infection. Also, high amounts of infantile infection affects the contribution of shed pathogen material to wastewater due to diaper wearing as discussed in Section 5.6.3. True population prevalence of adenovirus is yet to be well-described, resulting in the assignment of red for this parameter.

There are limited studies on prevalence of norovirus in the US, as described in Section 3.3.3. Two meta-analyses were used in this report that provide a summary of a range of possible prevalence values. The meta-analyses had a high number of studies included, 175 in one and 71 in the other. This high amount of studies used to estimate potential prevalence of norovirus inspires more confidence than the limited number of studies used in *Cryptosporidium* meta-analyses. However, like *Cryptosporidium* and adenovirus, lack of reported cases to government institutions at all levels makes it difficult to confirm the estimates from the meta-analyses, as reported norovirus prevalence values are four orders of magnitude lower than would be expected based on the prevalence estimates. For this reason, prevalence of norovirus is designated yellow.

Table 5-4. Summary of Parameter Confidence.

Pathogen	Fecal Concentrations	Wastewater Concentrations	Prevalence
Cryptosporidium spp.			
Adenovirus			
Norovirus GII			

5.6 Additional Variables to Be Explored in Future Model Versions

The following sections introduce variables that could improve the model accuracy. These would require a broader literature review to be implemented as well as additional studies on human shedding dynamics and pathogen fate in wastewater.

5.6.1 Outbreak Shedding Rates

Over the course of an infection, a patient will shed pathogen particles at varying rates. Generally, early and late infection times involve lower shedding rates, with heightened shedding rates during the middle of the infection. The current model assumes the mean shedding rate of infected persons and does not account for the timing of infection. This can cause incorrect estimations by the model during outbreaks. The model provides estimations of wastewater concentrations based on a 24-hour time frame. This assumes that although people will be shedding particles at different rates over the course of infection, the mean value of shedding for cases will account for the variation. However, in the case of an outbreak, shedding curves may align. So, at one point most cases could be aligned on their peak shedding period. The model would thus overestimate the number of infected individuals as the true mean shedding would be higher due to overlap in infection period. Incorporation of the dynamics of pathogen shedding and outbreak scenarios could improve the model, but a better understanding of the time-resolved shedding rates through the course of the infections would be necessary.

5.6.2 Fecal Mass Data

The model presented above uses the total amount of virus shed per day as an input. As most studies report the number of organisms shed per mass of feces, the conversion from fecal concentration to fecal rate requires assumptions about the amount of feces shed per day. Fecal mass among populations has many contributing factors that make it difficult to choose an accurate average value. The diet of a population, specifically fiber intake, greatly varies the daily average fecal mass of a population (Rose et al. 2015). Fiber intake has been correlated to income classification of a population, as higher income populations tend to have lower fiber intake leading to lower daily fecal mass. More information on population-specific fecal mass would bolster the accuracy of the model.

5.6.3 Age of Infection

Cryptosporidium spp. and adenovirus 40/41 are most prevalent in children <23 months (Liu et al. 2016). The determination of wastewater oocyst concentrations in the model does not take into account the amount of cases that may shed their feces in diapers and thus do not shed into the sewage systems. Children typically stop using diapers in the range of 18-30 months old (University of Utah Health 2016). A child in diapers may not contribute oocysts to the area's wastewater stream, and as a result, lower wastewater concentrations may be measured than would be predicted by the model for the same

infection prevalence value. Incorporating data that reports the percentage of diaper-wearing cases would further bolster the model's efficacy in accurately predicting epidemiological occurrences of the pathogen. In addition, further data on the amount of cloth diapers used would fine-tune the diaper factor as the use of cloth diapers as opposed to disposable diapers would still contribute to wastewater concentrations.

5.6.4 Pathogen Partitioning to Wastewater Solids

Another important source of variability that should be investigated is the phase in which wastewater is measured. Pecson et al. (2021) and the model developed in this report focus on measurements in wastewater influent. Some methods that measure pathogens in wastewater first remove the solids and then concentrate and enumerate the pathogens in the liquid phage. Other methods extract nucleic acids and or oocysts from the combined liquid and solid sample. Viruses tend to associate strongly with solids. The K_{id} values for bacteriophage MS2 and the mouse coronavirus MHV in wastewater influent are 270 and 1500 mL/g (Ye et al. 2016), respectively, and primary wastewater treatment removes an estimated 50-70% of total suspended solids (Chahal et al. 2016). In order to compare the concentration predictions from the model with wastewater concentrations measured in the literature, the partitioning between liquids and solids could be defined in the model and this would align with the method used to measure the wastewater concentrations.

CHAPTER 6

Recommendations

There is a direct relationship between pathogen levels in wastewater and gastrointestinal pathogen infection levels in a community. Mathematical relationships should therefore be possible to predict wastewater concentrations based on different community prevalence values, given that accurate fecal shedding rates and pathogen decay rates are available for the calculations. In this feasibility study, we have reviewed the state-of-knowledge on *Cryptosporidium* sp., HAdV, and HuNoV infection prevalence in communities and fecal shedding rates, and used the resulting data to predict concentrations in communities. We assumed that the decay of the pathogen gene or oocyst concentrations through the sewage systems was negligible. The wastewater concentrations that are predicted with the model for each pathogen are broad, covering several orders of magnitude for a specific community infection prevalence. Based on the reviews of public health data, prevalence studies, and fecal shedding data, a number of research areas that would aid future efforts to predict expected wastewater concentrations under different prevalence conditions are provided in the following sections. The results of the feasibility study are also applied to recommend when and where wastewater in California would be expected to have the highest concentrations.

6.1 Measuring Worst Case Pathogen Concentrations in Wastewater

A main goal of this project was to estimate prevalence during worst case scenario outbreaks of the three target pathogens. For each pathogen, there are certain conditions which present targets for measuring worst case pathogen concentrations in wastewater. For *Cryptosporidium*, data from Chapter 3 shows that cryptosporidiosis infection is most common in August within California, a similar trend is observed nationwide as well. Limited data makes it difficult to observe a regional trend by county thus far; additional wastewater surveillance and increased case reporting would be necessary to link local illness prevalence and wastewater concentration dynamics. However, wastewater surveillance in the counties with higher reported prevalence, like Inyo, Siskiyou, and San Luis Obispo counties, during the late summer may be reasonable locations to begin. As mentioned in Chapter 3, HuNoV data from NORS and CDPH show a high number of HuNoV-related illness in December and January. Unlike *Cryptosporidium*, illness reporting by county and month was not available. However, outbreak reporting from VRDL and some individual county health departments suggest Los Angeles, Contra Costa, Orange, San Diego, Alameda, Santa Cruz, and Butte county are good locations for further wastewater surveillance, specifically during the months of December and January. HAdV reporting in California is very insufficient, as HAdV-related outbreaks are rarely reported to the VRDL, a trend similarly observed for the US. Most commonly reported HAdV-related illness is of species B and C, typically associated with respiratory disease not gastroenteritis. This lack of available data and past work suggesting lack of seasonal disease patterns prevents formation of a clear recommendation as to the best place and time of year to survey for wastewater concentrations.

6.2 Major Research Gaps

6.2.1 Fecal Shedding

Available fecal concentration data provides a range of data points that are useful for this report, but the distribution of shedding concentrations needs further research. The possible fecal concentrations of pathogen materials has a range of many orders of magnitude from patient-to-patient. Knowing the likelihood of a patient to shed a certain concentration of pathogen material would aid in understanding

the nature of surveying outbreaks through wastewater. This project has assumed a log normal distribution of shedding concentrations, but further research will be needed to confirm this.

6.2.2 Prevalence

In general, the gastrointestinal illness data is sparse and better reporting would aid in understanding wastewater pathogen concentrations under endemic and epidemic conditions. In the absence of improved state-wide or country-wide reporting, scientific studies to better understand prevalence in communities could help fill this gap in understanding.

Mass balances can be conducted to predict community prevalence of diseases using wastewater concentration or to predict worst case wastewater concentrations with prevalence information. To test and calibrate these models for CA, however, prevalence studies of both symptomatic and asymptomatic illnesses in CA communities is critical. Without these studies, it is very difficult to show that the models are accurate. There is ongoing active research in this area for SARS-CoV-2, and similar studies could focus on measuring prevalence for HuNoV, *Cryptosporidium*, and adenovirus infections in California communities.

6.3 Modeling

6.3.1 Improvements of the Model

As discussed in Section 5.5, there are many variables applicable to the developed model that can be explored further to improve the implementation of the model. Even as the model exists in its current state, a larger breadth of reference data would be needed for its improvement. Namely, more quantitative fecal concentration data to reference. If more quantitative fecal concentration data was available among a broader population of patients, a more accurate distribution of concentrations could be implemented in the model. For this model we assume that “Super Shedders,” or those who shed a very high amount of pathogen, are accounted for within the normal distribution of fecal shedding rates. However, this may not be the case and fecal concentrations might actually demonstrate a bimodal or multimodal distribution, with instances of “Super Shedders” being much higher than just the extremes of a unimodal distribution. If so, this would alter the calculation of fecal concentration within the model.

In future iterations, an incorporation of wastewater phase would widen the applicability of the model. The current model calculates expected wastewater concentrations in raw wastewater influent, but testing in this capacity may not always be feasible. Samples may need to be taken from other stages of the wastewater treatment process, such as after primary treatment, and the concentration of pathogens in these samples may vary. As wastewater passes through the treatment process, pathogen particles can remain in the solid phase, resulting in lower concentration measurements taken post-raw wastewater. Input by the model user on the origin of the wastewater samples to be taken and incorporation of the corresponding mass balance required for samples other than raw wastewater influent would provide a more useful model.

Demographic data on prevalence of the pathogens, namely age, would also further improve the model. As mentioned previously, much of these pathogens present disease in very young children who may not even contribute to wastewater if in diapers. The predicted prevalence within the model for a measured wastewater concentration would therefore be underestimated. Incorporation of a factor which accounts for the percentage of diaper-wearing infections is recommended.

6.3.2 Use of Model Results

The purpose of the model is to provide a probabilistic estimate of the correlation between wastewater concentrations of pathogen material and the prevalence of infection within the wastewater treatment plant population. The trend line of the model provides the most likely correlation between the two variables. So, if there is an expected prevalence range of infection within the community, the model user would be able to input the expected range and output the range of possible wastewater concentrations of the pathogen material. Going forward, this model could be used in coordination with public health surveillance. As improved fecal shedding and more data on illness prevalence in CA becomes available, the accuracy of the model and its applications in water reuse will increase.

6.4 Partnerships between Water Utility and Public Health Agencies

The application of the model could be increased by enhanced communication between public health partners and water utilities. This would be particularly valuable with increase gastrointestinal illness surveillance. Communication between public health partners and water utilities was reviewed in the Expert Panel report where they identified data sources and potential partners to improve public health surveillance (Olivieri et al. 2016). The report identifies several approaches to improving communication, including regular meetings between public health and water utility representatives to establish relationships and to develop procedures for joint investigations on public health alerts. A Public Health Assessment Interview Form was developed in that effort to increase interactions between the water sector and public health officials. The data generated in this feasibility report along with the preliminary model developed here for gastrointestinal illnesses could serve as an additional tool to help establish relationships and development joint protocols.

One issue that will need to be resolved is that the publicly available data on cases and outbreaks is at the county level rather than the sewershed level. Consequently, the measurements made at a wastewater treatment plant do not often “match” with the publicly available case and outbreak data. Generating new reports at the sewershed level take time and effort on the part of the public health agencies and can therefore be difficult to obtain. In the future, developing a straightforward mechanism for generating sewershed case and outbreak data for the utilities, researchers, and public would greatly enhance the potential for closer collaborations between public health and water utilities and researchers.

With improved communication, public health officials would benefit from timely wastewater data for predicting trends and potentially prevalence of gastrointestinal illnesses in communities. Likewise, water utilities would benefit from timely outbreak data to predict increases in wastewater concentrations. Sampling campaigns could be initiated when the model predicts high wastewater concentrations with public health data on prevalence.

APPENDIX A

ShinyApp Code

dpr3shinycode

```
library(shiny)
library(ggplot2)

# Define UI for application
ui <- fluidPage(

    # Application title
    titlePanel("Wastewater Epidemiological Model"),

    # Sidebar
    sidebarLayout(
        sidebarPanel(

            #Start with type of pathogen for simulation
            selectInput("Pathogen", "Pathogen to Simulate",
c("Cryptosporidium","Adenovirus","Norovirus")),

            #Population that the WWTP serves
            numericInput("Pop", "Population of WWTP", 2200000),

            #Daily flowrate to the WWTP
            numericInput("WWTPflowrate", "WWTP Flowrate per day (MGD)",
                         175),

            #Minimum expected prevalence reporting
            numericInput("PrevRateLow", "Reported Prevalence Rate per 100,000
people low",
                         10),

            #Maximum expected prevalence reporting
            numericInput("PrevRateHigh", "Reported Prevalence Rate per
100,000 people High",
                         1000),

            #Number of simulation samples
            numericInput("SampleNos", "Number of Simulation Samples",
                        1000, max = 10000)
        ),

        # Make panels for plots
        mainPanel(
           tabsetPanel(type = "tabs",
```

```
                    tabPanel("Plot",
                             plotOutput("Plot")),
                    tabPanel("Fecal Concentration Distributions",
                             plotOutput("Crypt"),
                             plotOutput("Adeno"),
                             plotOutput("Noro")))

        )
    )
)

# Define server logic
server <- function(input, output) {

  output$Plot <- renderPlot({

    #Axes Labels-----------------------------------------
            ylabel1<- " Concentration in WW Influent ("
            ylabel2<- "/L)"
            ylabel3<- "Infectious "
            ylabel4<- "Total "
            if (input$Pathogen == "Cryptosporidium") {
              unit <- "oocysts"
              rownum <- 1
            } else if (input$Pathogen == "Adenovirus"){
              unit <- "gene copies"
              rownum <- 2
            } else {
              unit <- "gene copies"
              rownum <- 3
          }
    #-------------------------------------------------

        # Column 1 -> Cryptosporidium
        # Column 2 -> Adenovirus
        # Column 3 -> Norovirus
        pathogenvalues <- matrix(c(
                                   4.30,  6,   8.5,   # (1) regular shed mean

                                   0.96, 1.2,  0.8,    # (2) regular shed SD

                                   4.30, 4.2,  8.5,    # (3) asymptomatic
shed mean

                                   0.96, 0.54, 0.8,    # (4) asymptomatic
shed SD

                                   0.7,  0.35, 0.25,   # (5) asymptomatic
percent low
```

```
                                    0.85, 0.50, 0.50),  # (6) asymptomatic
percent high

                                  3)

        shedHist <- function (x,y,z){ #x = which pathogen
                                      #y = percent of asymptomatic cases
                                      #z = sample number
          regshed <- rnorm((z-
y),pathogenvalues[x,1],pathogenvalues[x,2])+log10(796)
          #796g avg weight of diarrhea
          asymptomaticshed <-
rnorm(y,pathogenvalues[x,3],pathogenvalues[x,4])+log10(128)
          #128g avg weight of healthy feces
          totshed <- c(regshed,asymptomaticshed)
        }

        n <- 1
        samps <- input$SampleNos[1]
        shedHist_array <- c(1:samps)

        #generating random distribution
        while(n < (samps+1)) {
          asymptomaticpercent <-
samps*(runif(1,pathogenvalues[rownum,5],pathogenvalues[rownum,6]))
          shedHist_output <- shedHist(rownum, asymptomaticpercent,samps)
          shedHist_mean <- mean(shedHist_output)
          shedHist_array[n]= shedHist_mean
          n=n+1;
        }

        #Uniform distribution of numbers in prevalence rate range
        f_prev <-
runif(input$SampleNos[1],input$PrevRateLow,input$PrevRateHigh)

        #From log form to numeral
        C_shed <- 10^shedHist_array

        #Calculating mass balance of concentration of gene copies in
wastewater influent based off report equation
        C_ww <-
f_prev*C_shed/(input$WWTPflowrate*1000000)*input$Pop/100000*0.264172


      #Creates Scatter Plot of gene copies vs. prevalence
      plot(f_prev,
         C_ww, log = "xy",
         xlab = "Prevalence in WW population area (cases/100,000 people)",
```

```
         ylab = paste(ylabel4,input$Pathogen,ylabel1,unit,ylabel2))

      #Creates a linear regression based on plotted data
      regline <- lm(log10(C_ww)~log10(f_prev))
      abline(regline, col = "blue")

      #Generates fecal concentration distributions
      asympCrypto <- samps*(runif(1,pathogenvalues[1,5],pathogenvalues[1,6]))
      shedCrypto <- shedHist(1, asympCrypto,samps)
      asympAdeno <- samps*(runif(1,pathogenvalues[2,5],pathogenvalues[2,6]))
      shedAdeno <- shedHist(2, asympAdeno,samps)
      asympNoro <- samps*(runif(1,pathogenvalues[3,5],pathogenvalues[3,6]))
      shedNoro <- shedHist(3, asympNoro,samps)

      #Outputs fecal concentration distributions
      output$Crypt <- renderPlot({
        hist(shedCrypto, breaks = 20, main = "Histogram of Cryptosporidium
Fecal Concentrations",
             xlab = "Log10 Oocysts Shed/24h")
      })
      output$Adeno <- renderPlot({
        hist(shedAdeno, breaks = 20, main = "Histogram of Adenovirus Fecal
Concentrations",
             xlab = "Log10 Gene Copies Shed/24h")
      })
      output$Noro <- renderPlot({
        hist(shedNoro, breaks = 20, main = "Histogram of Norovirus Fecal
Concentrations",
             xlab = "Log10 Gene Copies Shed/24h")
      })

    })

}

# Run the application
shinyApp(ui = ui, server = server)
```

Shiny applications not supported in static R Markdown documents.

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