Enumerating asymptomatic COVID-19 cases and estimating SARS-CoV-2 fecal shedding rates via wastewater-based epidemiology

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Running title: Enumerating SARS-CoV-2 Fecal Shedding and Asymptomatic Cases via Wastewater

Highlights

- Wastewater-based epidemiology with clinical testing monitored SARS-CoV-2 in dorms.

- 79.2% of SARS-CoV-2 infections were asymptomatic, and 20.8% were symptomatic.

- Clinical and wastewater data aggregated to estimate SARS-CoV-2 fecal shedding rate.

- Mean fecal shedding rate based on the N1 gene was 6.84 ± 0.77 $\log_{10}$ gc/g-feces.

- Mean fecal shedding rate based on the N2 gene was 7.74 ± 0.53 $\log_{10}$ gc/g-feces.

Graphical Abstract

Campus Dorms | Fecal Shedding | Clinical Testing |

Asymptomatic

Symptomatic
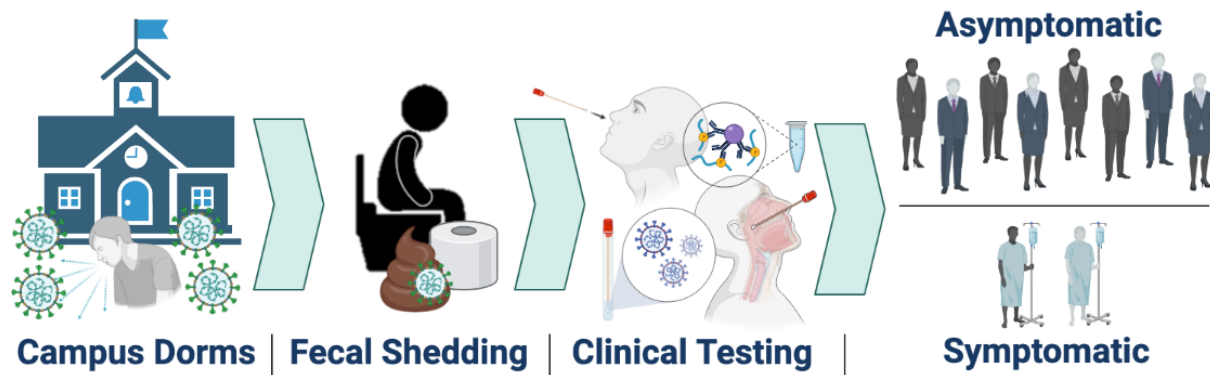
1   **Abstract**

2   Wastewater-based epidemiology (WBE) was utilized to monitor SARS-CoV-2 RNA in

3   sewage collected from manholes specific to individual student dormitories (dorms) at the

4   University of Arizona in the fall semester of 2020, which led to successful identification and

5   reduction of transmission events. Positive wastewater samples triggered clinical testing of almost

6   all residents within that dorm; thus, SARS-CoV-2 infected individuals were identified regardless

7   of symptom expression. This current study examined clinical testing data to determine the

8   abundance of asymptomatic versus symptomatic cases in these defined communities. Nasal and

9   nasopharyngeal swab samples processed via antigen and PCR tests indicated that 79.2% of

10   SARS-CoV-2 infections were asymptomatic, and only 20.8% of positive cases reported COVID-

11   19 symptoms at the time of testing. Clinical data was paired with corresponding wastewater virus

12   concentrations, which enabled calculation of viral shedding rates in feces per infected person(s).

13   Mean shedding rates averaged from positive wastewater samples across all dorms were 6.84 $\pm$

14   0.77 $\log_{10}$ genome copies per gram of feces (gc/g-feces) based on the N1 gene and 7.74 $\pm$ 0.53

15   $\log_{10}$ gc/g-feces based on the N2 gene. Quantification of SARS-CoV-2 fecal shedding rates from

16   infected persons has been the critical missing component necessary for WBE models to measure

17   and predict SARS-CoV-2 infection prevalence in communities. The findings from this study can

18   be utilized to create models that can be used to inform public health prevention and response

19   actions.

20

21   **Keywords:** SARS-CoV-2, fecal shedding, asymptomatic, SARS-CoV-2 infection, public health

22   response, wastewater-based epidemiology, COVID-19, dormitories

23

1. Introduction

SARS-CoV-2 infections may not end with current vaccination efforts. Some scientists conjecture that the virus' evolution may require long-term monitoring and more intensive public health measures to control transmission of new variants. Thus, additional public health surveillance strategies can and should be implemented to prevent and respond to outbreaks; wastewater based epidemiology (WBE) may be one of those tools.

Human wastewater (i.e., sewage) may be among the first indications of aggregated, population-based SARS-CoV-2 infections in a community (Medema et al., 2020b), followed by healthcare facility reported number of infections, hospitalizations, and ultimately deaths. WBE is an effective, critical tool that has been demonstrated to monitor concentrations of SARS-CoV-2 RNA in wastewater as an indicator to survey the SARS-CoV-2 infection prevalence at a population level (Medema et al., 2020b). The results from these surveys can be used to track community infection dynamics (Peccia et al., 2020) and guide targeted public health response actions (Betancourt et al., 2021). Therefore, fecal shedding of SARS-CoV-2 may be utilized as a biomarker to estimate SARS-CoV-2 infections in a population..

Shedding estimates of SARS-CoV-2 from infected individuals have strong implications for the effectiveness of WBE to determine disease prevalence and guide public health interventions. Although SARS-CoV-2 has been shown to shed in the upper respiratory tract, the lower respiratory tract, feces, urine and serum (Cevik et al., 2020), WBE leverages RNA shedding of SARS-CoV-2 in feces. Infected persons shed virus into sewage via feces (Cevik et al., 2020), or urine (Brönimann et al., 2020), both of which can then be used to monitor population-level infection changes. Although SARS-CoV-2 has been isolated in urine of a COVID-19 patient (Sun et al., 2020), the incidence of the virus in urine is reported to be low

47   (Brönimann et al., 2020; Morone et al., 2020); whereas, intact SARS-CoV-2 virus have been

48   isolated from feces (Wang et al., 2020; Xiao et al., 2020; Zhang et al., n.d.). Further, viral RNA

49   has been recovered even in the absence of intact virus isolation from stool samples (Wölfel et al.,

50   2020), highlighting the sensitivity of molecular methods applied for WBE.

51   During the early stages of the pandemic in the Netherlands, SARS-CoV-2 was detected in

52   sewage six days before initial cases were reported (Medema et al., 2020a). Mulitple jurisdictions

53   around the world have monitored concentrations of SARS-CoV-2 RNA in sewage samples to

54   quantify the total number of infected persons in the community that excreted the virus into

55   wastewater (Ahmed et al., 2020; Chavarria-Miró et al., 2021; Curtis et al., 2020). More recently,

56   the University of Arizona (UArizona) used WBE, dovetailed with targeted clinical testing, to

57   prevent COVID-19 outbreaks in student dormitories (dorms). In particular, 91 wastewater

58   samples containing SARS-CoV-2 RNA provided early-warning that at least one infected

59   individual was present in the community (Betancourt et al., 2021). The UArizona case study

60   highlighted the effectiveness of WBE to detect the presence of SARS-CoV-2 infected individuals

61   in a defined community and to ultimately contain outbreaks by triggering public health response

62   actions. Although WBE has been utilized to raise the alert of SARS-CoV-2 presence with a

63   dichotomous threshold, quantifying the number of SARS-CoV-2 infected individuals cannot be

64   done without accurate fecal shedding rate estimates.

65   This research aims to estimate SARS-CoV-2 fecal shedding rates in infected persons by

66   aggregating data from positive wastewater samples with numbers of known symptomatic and

67   asymptomatic individuals in defined communities. To that end, the Campus Re-entry study at the

68   UArizona in Fall 2020 (Betancourt et al., 2021) provided a unique setting to calculate mean

69   shedding rates for defined communities. Specifically, 13 dorms served as a case study to test the

6

70   utility of WBE to monitor SARS-CoV-2 levels in sewage and initiate public health action based

71   upon those results. Positive SARS-CoV-2 detection in wastewater samples from any given dorm

72   led to point prevalence antigen and/or PCR testing for the vast majority of dormitory residents

73   regardless of symptomatic expression; thus, the numbers of symptomatic and asymptomatic

74   individuals was known. This current study determines the proportion of COVID-19 infections

75   that were asymptomatic versus symptomatic and pairs these clinical data with wastewater results

76   to extrapolate the fecal shedding rate of SARS-CoV-2 in infected students. Findings from this

77   study will inform how WBE can be utilized for public health prevention and response actions.

78   **2. Methods**

79   *2.1. Dormitory Sites*

80   In total, 13 dorms (Dorm A–M) were monitored throughout the Fall 2020 Semester

81   (August 17–November 20). Data from the final weeks of classes and exams following the

82   Thanksgiving break (November 21–December 17) are not included in this analysis, as students

83   were advised to complete the semester virtually and not return to campus. Each wastewater

84   sample was collected from sewer manholes specific to each dorm's effluent prior to convergence

85   with other sewer pipelines; thus, all wastewater samples were specific to the defined

86   communities living in each dorm. However, two sets of dorms (Dorm E and F; Dorm H and I)

87   required sampling from a single sewer manhole distal from the mixing of wastewater from the

88   two dorms but proximal to convergence with other pipelines. The dorms in each set are located

89   directly side-by-side and were considered a 'combined dorm' for all public health response

90   actions (i.e., clinical testing interventions) and data analysis. Dorms varied in infrastructure and

91   resident occupancy (Table 1). Due to the pandemic, UArizona limited dorm room occupancy to a

92   maximum of two.

93   *2.2. Wastewater Sampling and Analysis*

94   Wastewater samples from each dorm were collected between 9:00 and 10:30 am, then

95   analyzed for SARS-CoV-2 RNA at least twice per week. One set of dorms was monitored on

96   Monday/Wednesday/Friday (Dorms A, B, C, D, G, L), while a second set was monitored

97   Tuesday/Thursday/Saturday (Dorms E-F, H-I, J, K, M). Grab samples were collected inside

98   sewer manholes with sterile Nalgene® bottles that were affixed to extension poles, which

99   allowed collection at depths of 5 to 15 feet. Velocity (ft^3/s) of wastewater within the sewer was

100   measured using a Global Water FP 211 flow probe (Global Water, College Station, TX).

101   Multiple readings of the minimum and maximum flow rates (gallons per minute) over a one-

102   minute period were averaged and recorded. Flow rates were calculated using Manning's equation

103   for partially full pipes (Akgiray, 2005). For all wastewater samples, the wastewater's height and

104   depth was estimated to be approximately two inches, based upon the height of the fluid on the

105   propellor sensor. Note, this assumption was necessary since exact measurements were not

106   possible inside sewer manholes. The depth of the wastewater was never above the propellor

107   sensor and a velocity reading could not be recorded if wastewater height was not near the top of

108   the propellor (2-inch height) but was nonetheless considered reasonable. Statistical methods were

109   used to estimate flow rates for samples for which velocity could not be measured due to site

110   obstruction or the flow probe not being available (See Section 2.6).

111   Grab samples collected at the same time of each sampling day were considered adequate

112   based on a prior multiple sampling event. In that case, multiple samples were collected at a

113   particular site over a 30-minute period, and analysis indicated virtually identical virus

114   concentrations (Betancourt et al., 2021). This suggest that virus particles disperse upon entering

115   the piping/sewer and remain in the sewer for extended periods of time rather than being removed

116   by plug flow (Manor et al., 2014). Samples were stored on ice and travel time to WEST was 30

117   minutes or less from the dorms. Wastewater processing and analysis for SARS-CoV-2 RNA

118   followed procedures previously described (Betancourt et al., 2021). Samples were tested for the

119   virus using the United States (U.S.) Centers for Disease Control and Prevention (CDC) RT-PCR

120   assays that target regions of the nCoV nucleocapsid gene (N1 and N2; Table S1) (Research use

121   only kit, Integrated DNA Technologies, Coralville, IA). Real-time PCR for the simultaneous

122   detection of N1 and N2 genes was performed on a LightCycler 480 Instrument II (Roche

123   Diagnostics) with the LightCycler® Multiplex RNA Virus Master (Roche Diagnostics) (Table

124   S2).

125   *2.3. Clinical Testing Data*

126   Positive detection of SARS-CoV-2 RNA (via N1 and N2 gene regions) in wastewater

127   was utilized as a leading indicator of the presence of SARS-CoV-2 infections within the dorm

128   communities. Results were immediately communicated to the UA Task Force and Campus Re-

129   Entry Working Groups, which planned and conducted clinical testing of residents as a response

130   action(s), as previously described (Betancourt et al., 2021).

131   Clinical tests for COVID-19 diagnosis were performed by antigen testing via nasal swab

132   samples and RT-PCR via nasopharyngeal swab samples (Betancourt et al., 2021). The analytical

133   performance characteristics for the antigen test were 96% sensitivity and 100% specificity, as

134   provided by the manufacturer (Sofia SARS Antigen FIA, Quidel, San Diego, CA, USA).

135   Performance of the RT-PCR tests were determined by the University of Arizona Genetics Core

136   for Clinical Services (CDC 2019-nCoV RT-PCR Diagnostic Panel) with a limit of detection at

137   150 viral copies/reaction, or 30 viral copies/µl of sample.

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138   Clinical testing data was obtained from the University Campus Health Services (CHS)

139   and the Test All Test Smart (TATS) program. Symptomatic and asymptomatic cases in the study

140   were tracked based on location and program where clinical tests were conducted. Individuals

141   with COVID-19 symptoms who sought clinical testing were tested through CHS. Clinical testing

142   of non-self-reporting (i.e., asymptomatic and sub-clinical) students and employees was

143   conducted through the TATS program that runs several testing locations on campus and

144   mobilizes pop-up sites as needed (i.e., targeted clinical testing at dorms with positive wastewater

145   detection of SARS-CoV-2). A negative antigen test at CHS, where patients were exhibiting

146   symptoms, was followed by PCR testing to confirm results; data was de-duplicated in situations

147   when both testing methods were used to confirm test results (Betancourt et al., 2021). To ensure

148   compliance with the Human Subjects Protection Program (HSPP), the use of clinical data was

149   reviewed and approved by a UArizona Institutional Review Board (IRB).

150   *2.4. Alignment of wastewater and clinical data*

151   To estimate the number of SARS-CoV-2 infected individuals who contributed to a single

152   positive wastewater sample, a 6-day range of clinical data was considered. Positive clinical cases

153   from the day before, day-of, and four days after sampling were included in the count of infected

154   individuals contributing to viral shedding. The rationale for this approach is:

155   1) Residual virus—shed from individuals testing positive for COVID-19 the day prior to

156   sampling—may be detected in wastewater the following day. Previous sampling indicates that

157   virus can persist for extended periods of time and be detected even when an estimated 1000

158   gallons of wastewater has continued to flow through a sewer system (Betancourt et al., 2021).

159   2) Individuals infected with SARS-CoV-2 may shed virus into wastewater prior to

160   showing symptoms and/or being identified as a clinical case. The viral load of SARS-CoV-2

161   appears to peak in infected persons within the first week of infection (Cevik et al., 2020) and the

162   median incubation period for COVID-19 is estimated to be approximately five days (Lauer et al.,

163   2020).

164   Following a positive wastewater sample, clinical testing was performed on nearly all

165   residents living in the dorm via the TATS program (Betancourt et al., 2021). Individuals that

166   tested positive were removed from the dorm and transferred into isolation. However, some

167   individuals in the early stages of infection may have tested negative on the day of wastewater

168   testing, but tested positive after reaching peak viral load of SARS-CoV-2 a few days later. It is

169   possible that these individuals were shedding virus in feces even though they tested negative via

170   initial TATS conducted within 24 hours of a positive wastewater signal. Additionally, the full

171   dorm community was not always available on the first day of targeted testing and were tested a

172   day or more after a positive wastewater detection. Therefore, clinical data for 4-days following

173   positive wastewater samples was included in the count of infected individuals since these

174   persons likely shed and contributed virus to the wastewater samples for several days.

175   The majority (68 out of 81) of positive wastewater samples were associated with new

176   reported cases of infections within this 6-day period.

177   2.5. *Viral Shedding Rate Estimation*

178   The fecal shedding rate of SARS-CoV-2 RNA per gram of feces from an infected

179   individual was enumerated based on known concentrations of viral RNA in positive wastewater

180   samples and the fact that the total numbers of infected persons contributing to the total virus load

181   in the samples were also known. Equations from previous reports using viral loads in wastewater

182   to estimate the number of infected persons in a community (Ahmed et al., 2020; Chavarria-Miró

183   et al., 2021; Curtis et al., 2020) were modified so that the fecal shedding rate of viral RNA per

11

184 infected person could be quantified. The fecal shedding rate (FS) in terms of genome copies per

185 gram-feces (gc/g-feces) was calculated as follows:

186   (Eq. 1) $$FS = \frac{(VC*Q*f*h)}{G*I}$$

187 where VC is the virus concentration (genome copies/L) in the wastewater sample, Q is the flow

188 rate (gpm) of wastewater in the sewer manhole at time of sample collection, f is the conversion

189 factor between gallons and liters, h is the conversion factor between minutes and days, G is the

190 typical mass of stool produced per person per day (Curtis et al., 2020; Rose et al., 2015), and I is

191 total number of infected persons contributing to the wastewater sample based on the 6-day range

192 for clinical data (see Section 2.5). Confidence intervals were calculated based on the standard

193 deviations between sample calculations.

194   Fecal shedding rates based on N1 and N2 genes were calculated independently.

195 *2.6. Statistical Analysis*

196   Statistical analyses were performed in Microsoft Excel (version 16.47.1, 2021) and R

197 studio (R Studio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston,

198 MA. *http://www.rstudio.com/*). Multiple imputation for measurement error (MIME) was

199 performed to estimate flow rates for missing data when the flow probe was unavailable, as

200 previously described with modifications (Canales et al., 2018).

201 **3. Results**

202 *3.1. Asymptomatic and Symptomatic Clinical COVID-19 Cases*

203   Asymptomatic and symptomatic cases of COVID-19 were differentiated based on clinical

204 testing program – TATS for asymptomatic and CHS for symptomatic (see Section 2.4). For all

205 dates in the study period, a total of 711 clinical cases were reported among the 13 unique dorms.

206 Clinical data was combined for dorms with converged sewage systems (Dorm E-F and Dorm H-

207   I). Across all dorms, 148 symptomatic cases and 563 asymptomatic cases were reported; thus,

208   79.2% of SARS-CoV-2 infections were asymptomatic and only 20.8% were symptomatic (Table

209   2).

210   Examining specific dorm communities, reported clinical cases ranged from zero in Dorm

211   L to 171 in Dorm D (Table 2). Dorms G and M had the fewest reported infections, as well as the

212   highest and lowest asymptomatic rates. With limited data (two infections in Dorm G and five

213   infections in Dorm M), the asymptomatic rate was calculated as 100% for Dorm G and 20% for

214   Dorm M. Combined Dorm E & F was the only dorm to report equal number of symptomatic and

215   asymptomatic cases (Table 2). Seven of the eleven monitored communities had asymptomatic

216   rates between 70 – 85% (Table 2). Two dorms (E & F and M) had asymptomatic rates at 50% or

217   below, while Dorm G was the only to report 100% of new cases as asymptomatic (Table 2).

218   *3.2. Virus Fecal Shedding Estimation*

219   In total, 81 samples in the study period were positive for SARS-CoV-2 N1 and/or N2

220   gene(s) and 238 samples resulted in no detection of the virus (Table S3). Clinical and wastewater

221   data were aggregated (see Section 2.5) to calculate the N1 and N2 viral shedding rate per

222   infected person (see Section 2.6). Only 13 positive wastewater samples were omitted from viral

223   shedding calculations due to zero reported cases of infection within the 6-day range for clinical

224   data (Table S3). Negative wastewater samples were not included in viral shedding estimations

225   due to no detection of SARS-CoV-2 N1/N2 genes. Also, negative samples did not trigger a

226   response action to conduct clinical testing on residents; thus, there is limited clinical data for the

227   days that wastewater was negative. Overall, 68 total positive wastewater samples were aligned

228   with clinical data to extrapolate the fecal shedding rates of SARS-CoV-2 N1 and/or N2 genes.

229   The average N1 shedding rate per infected person was calculated to be 6.84 ± 0.77 $\log_{10}$

230   gc/g-feces, considering all positive wastewater samples (n=60) across all dorms (Table 3). The

231   median was 6.65 $\log_{10}$ gc/g-feces with a full range 5.74 - 9.76 $\log_{10}$ gc/g-feces (Table 3). Within

232   specific defined communities, Dorm C had the highest average N1 shedding rate at 7.26 ± 0.14

233   (n=2), while Dorm A had the lowest average at 6.45 ± 0.60 $\log_{10}$ gc/g-feces (n=13). The widest

234   range of N1 shedding rates was found in Dorm J with a minimum at 5.98 and a maximum at 9.76

235    $\log_{10}$ gc/g-feces.

236   The average N2 shedding rate per infected person was calculated to be 7.74 ± 0.53 $\log_{10}$

237   gc/g-feces across all positive wastewater samples (n=32) from all dorms (Table 3). The median

238   was 7.60 with a full range from $6.74 - 8.60$ $\log_{10}$ gc/g-feces (Table 3). For specific communities,

239   combined Dorm H & I had the highest average N2 shedding rate at 8.21 ± 0.55 (n=2), while

240   Dorm A had the lowest average at 7.21 ± 0.35 (n=5) $\log_{10}$ gc/g-feces. The widest range of N2

241   shedding rates was found in Dorm K with a minimum at 7.32 and a maximum at 8.28 $\log_{10}$ gc/g-

242   feces.

243   With just one positive wastewater sample (n=1) each, Dorms C and D had the lowest

244   number of estimates for N2 viral shedding rates (Table 3). For Dorm D, the N1 shedding rate

245   could not be calculated due to zero wastewater samples being positive (i.e., non-detect) for the

246   N1 gene target (Table S3D). Although Dorm D (8.35 $\log_{10}$ gc/g-feces) reported a higher N2

247   shedding rate than Dorm H & I (8.21 $\log_{10}$ gc/g-feces), it was not reported as a higher average

248   due to only a single sample (n=1). Combined Dorm E & F also had a low number of samples

249   with positive detection for N1 (n = 2) and none for N2 (Table 3; Table S3E); thus, the shedding

250   rate for this combined dorm community could not be calculated for N2.

14

251 **4. Discussion**

252 *4.1 Estimation of Asymptomatic Cases as a Percent of Total Cases*

253   The total number of asymptomatic and symptomatic cases of COVID-19 were discerned

254 in this study based on clinical testing programs – TATS for asymptomatic and CHS for

255 symptomatic (see Section 2.4). Of the total 711 cases of COVID-19 that were reported via

256 positive antigen and/or PCR clinical tests, the vast majority (79.2%) did not self-report and were

257 therefore considered asymptomatic. We should note that this finding may be overestimated in the

258 case that mildly symptomatic individuals did not self-report, which would categorize them as

259 asymptomatic if tested and found positive via TATS. This high rate of asymptomatic cases may

260 be due to the younger and perhaps healthier population surveyed in this study. However, the

261 finding suggests that the number of people infected with SARs-CoV-2 in the U.S. may be vastly

262 underestimated, which could have downstream effects for determining the immune population

263 and herd immunity thresholds.

264 *4.2 Viral Shedding Estimation*

265   Fecal shedding rates of SARS-CoV-2 RNA were estimated from dorm wastewater

266 samples using a modified equation from previous reports (Ahmed et al., 2020; Chavarria-Miró et

267 al., 2021; Curtis et al., 2020). This calculation accounts for the number of infected people within

268 a defined community, the amount of fecal material excreted, the shedding rate of infected

269 individuals, the flow rate of the wastewater, and the viral load within the wastewater. Of these

270 parameters, the fecal shedding rate of SARS-CoV-2 from infected individuals is the least known.

271 Until this research, shedding rate estimations were based upon limited results that indicated a

272 large variance across a small number of individuals (Wölfel et al., 2020). Shedding rates were

273 further extrapolated from the 90th percentile of this limited dataset and used to calculate the

274   number of infected persons contributing to viral loads in wastewater (Ahmed et al., 2020;

275   Chavarria-Miró et al., 2021; Curtis et al., 2020). However, models and predictions based on these

276   percentile shedding rates are likely to be inaccurate without pairing wastewater results with

277   clinical data.

278   In this current study, wastewater from student dorms with known defined communities

279   was sampled and assayed for SARS-CoV-2 RNA. Wastewater samples that tested positive for

280   viral RNA triggered targeted clinical testing of almost all residents within the specific dorm.

281   Consequently, fecal shedding rates could be calculated since virus wastewater concentrations and

282   the corresponding numbers of infected dorm residents were both known. These shedding rate

283   estimations are reasonable since each dorm was a theoretically closed population, where

284   nonresidents were prohibited from entering dorms, and individuals who were identified as

285   positive were isolated within other facilities. This also prevented recounting of positive

286   individuals and additional shedding on days subsequent to being counted as an infected

287   individual, since persistent shedding occurs over multiple days (Cevik et al., 2020; Gupta et al.,

288   2020). Therefore, the shedding rates calculated from the dorms represent incident infections

289   only.

290   For estimating the number of individuals shedding and contributing to the viral load in

291   wastewater samples, a 6-day range of clinical data was considered (See Section 2.5). Then, fecal

292   shedding rates of SARS-CoV-2 RNA were extrapolated from wastewater viral loads in samples

293   specific to UArizona dorms in which the exact number of infected persons was known. This

294   estimation is based on a calculation that accounts for the number of infected persons within a

295   defined community; the amount of fecal material excreted per person; the fecal shedding rate of

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296   viral RNA from infected individuals; the flow rate of the wastewater and the viral load within the

297   wastewater (See Section 2.6).

298   The aggregation of wastewater and clinical data enabled quantification of SARS-CoV-2

299   fecal shedding rates per infected person. Mean shedding rates averaged over all dorms with

300   positive wastewaters were 6.84 ± 0.77 $\log_{10}$ gc/g-feces based on the N1 gene and 7.74 ± 0.53

301   log10 gc/g-feces based on the N2 gene (Table 3). Statistical parameters included in Table 3

302   include number of samples (N), average values (Avg), standard deviation (Std dev), median

303   (Med), minimum (Min), and maximum (Max). These statistics are important to establish the

304   precision of the calculated shedding rates since many factors influence shedding rates. The

305   calculated rates based on the incidence of both N1 and N2 are fairly consistent with reasonable

306   calculated standard deviations. It is also important to note that mean rates are based on relatively

307   large numbers of calculations: n = 60 (N1) and n = 32 (N2). Notably, N2 gene values were

308   approximately 1-log greater than the N1 gene in feces.

309   While this paper develops population-level shedding rates, individual shedding rates may

310   vary based on multiple factors such as sex, age, co-morbidities, socioeconomic status, and the

311   severity of symptoms and duration of infection. The duration of shedding appears to be affected

312   by the severity of the COVID-19 disease: asymptomatic (6 days) < mild symptoms (10 days) <

313   moderate symptoms (12 days) < serious (14 days) < critical (32 days) (Chen et al., 2020).

314   Recent reports also suggest that asymptomatic individuals had a shorter duration of viral

315   shedding than pre-symptomatic individuals (Hue et al., 2020).

316   Our data assumes early infection shedding rates because those people would be identified

317   within the 6-day period and removed from the dorm and brought into an isolated living quarter.

318   However, it is possible that some individuals may have started and/or continued shedding viral

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319   RNA in feces outside of the assumed 6-day period. The shedding rate may also be slightly

320   overestimated based on case capture definitions, as although the vast majority of individuals in

321   each dorm were tested after positive wastewater sample results, a minority of residents were not

322   tested. Shedding rates in this study are based on young university students who reside in

323   dormitories and do not necessarily approximate the general population. Nevertheless, a recent

324   study estimated the total number of active shedders from SARS-CoV-2 RNA levels in

325   wastewater from a large metropolitan area similarly demonstrating that shedding resulted from a

326   high proportion of asymptomatic individuals (Chavarria-Miró et al., 2021).

327   Some studies have reported similar initial shedding rates from symptomatic and

328   asymptomatic infections (Lavezzo et al., 2020; Van Vinh Chau et al., 2020); whereas, other

329   studies have found lower viral loads in asymptomatic cases (Han et al., 2020; Zhou et al., 2020).

330   Also, some reports indicate that only approximately 50% of COVID-19 patients shed viral RNA

331   in stool samples (Gupta et al., 2020; Medema et al., 2020a), suggesting the possibility that not all

332   infected individuals shed viral RNA in feces. Regardless of these considerations, viral load in

333   wastewater in this study were based on all individuals (symptomatic and asymptomatic)

334   contributing to the sewage system. Therefore, fecal shedding estimates are general for defined

335   communities of mixed clinical cases, while considering the high proportion of asymptomatic to

336   symptomatic reported cases.

337   Lastly, it is important to note that no fecal samples were collected from individual

338   COVID-19 patients directly. Therefore, results in this study are specific to wastewater from

339   defined communities and is not intended to inform clinical data. Data and results from this study

340   should be compared with clinical research and evaluations.

341   *4.3 Concluding Remarks*

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342   This study may have significant implications for public health. The fecal shedding rate of

343   SARS-CoV-2 RNA derived in this study can be utilized to estimate the total number of

344   infections in a community based on wastewater viral concentrations. Knowledge of disease

345   prevalence, especially as a leading indicator, can be used to assist communities in efficient

346   resource allocation to prevent and contain COVID-19 outbreaks. This study also provides further

347   understanding for the total number of cases that are symptomatic versus asymptomatic. Moving

348   forward, the number of reported cases can provide context for estimating the number of cases

349   that were asymptomatic and/or unreported. This, in turn, could have implications for

350   understanding the proportion of individuals that has been exposed to COVID-19 and for

351   understanding progress towards immunity within a community.

352   **Acknowledgements**

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Table 1: Meta-data for student dormitories.

Dorm	Fall 2020 Occupancy[a]	Capacity	Room Type	Bathrooms
A	611	722	Single, Double	Community, All-Gender
B	342	400	Single, Double	Community
C	123	181	Single, Double, Triple[b]	Community
D	623	731	Single, Double, Suite-Style	Suite
E	206	300	Single, Double	Community, All-Gender
F	56	60	Single	Community
G	231	300	Apartments	In-Room (1 per bedroom)
H	195	238	Single, Double	Community, All-Gender
I	181	238	Single, Double	Community, All-Gender
J	424	482	Single, Double	Community
K	328	369	Double	Community, All-Gender
L	76	106	Single, Double, Triple[b]	Community, Sink-in-Room
M	132	152	Single, Double	Community

[a] Occupancy varied throughout the Fall 2020 semester, so the highest numbers of residents at any given time throughout the semester are shown.

[b] Due to the pandemic, UArizona limited rooms to a maximum of two occupants. No triple-occupant rooms were offered during the time of this analysis.

Table 2: Clinical positives for COVID-19 in wastewater-monitored dorms, 8/17/20-11/20/20

Dorm	Clinical Positives	Symptomatic (CHS)		Asymptomatic (TATS)[a]	
		Total	Percent[b]	Total	Percent[b]
A	164	35	21.3%	129	78.7%
B	92	16	17.4%	76	82.6%
C	10	3	30.0%	7	70.0%
D	171	34	19.9%	137	80.1%
E & F	16	8	50.0%	8	50.0%
G	2	0	0.0%	2	100.0%
H & I	74	17	23.0%	57	77.0%
J	111	16	14.4%	95	85.6%
K	66	15	22.7%	51	77.3%
L	0	0	-	0	-
M	5	4	80.0%	1	20.0%
Total	711	148	20.8%	563	79.2%

[a] Asymptomatic cases may also include mildly symptomatic individuals that did not self-report.

[b] Percent of total new clinical positive cases of infection.

Table 3: SARS-CoV-2 Viral Shedding Per Infected Person Extrapolated from UArizona Dorm WBE

Dorm	N1 Avg.	Stdev	Med.	Min.	Max.	n	N2 Avg.	Stdev	Med.	Min.	Max.	n
A	6.45	0.60	6.36	5.78	7.66	13	7.21	0.35	7.33	6.74	7.52	5
B	6.99	0.49	7.21	6.21	7.40	5	7.51	0.33	7.51	7.27	7.74	2
C	7.26	0.14	7.26	7.16	7.35	2	8.05	-	-	-	-	1
D[a]	-	-	-	-	-	-	8.35	-	-	-	-	1
E & F[b]	6.65	0.12	6.65	6.57	6.73	2	-	-	-	-	-	-
H & I	6.74	0.68	6.65	5.74	7.71	12	8.21	0.55	8.21	7.82	8.60	2
J	6.99	0.96	6.78	5.98	9.76	14	7.43	0.29	7.54	7.01	7.93	10
K	7.08	0.89	6.68	5.80	8.28	12	8.13	0.46	8.28	7.32	8.59	11
Total	6.84	0.77	6.65	5.74	9.76	60	7.74	0.53	7.60	6.74	8.60	32

All values are presented in $\log_{10}$ genome copies per gram of feces ($\log_{10}$ gc/g-feces), except for n (number of samples). Avg., average;

Stdev, standard deviation; Med., median; Min., minimum; Max., maximum; n, number of samples. (-), no data.

[a] N1 shedding rate could not be calculated from Dorm D due to no positive wastewater sample corroborating with a new reported case(s) of

infection or clinical positive test.

[b] N2 shedding rate could not be calculated from Dorm E & F due to no positive wastewater sample corroborating with a new reported

case(s) of infection or clinical positive test.