

Wastewater Surveillance at North Carolina Agricultural and Technical State University progress report

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1. Introduction

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The current study aims to assess the prevalence for SARS-CoV-2 genome fragments and genetic mutations in wastewater on the campus of North Carolina Agricultural and Technical State University dormitories. This study was designed to trace the virus' spread in a year-long analysis across the campus by (i) Determining the presence of SARS-CoV-2 RNA in wastewater effluent from dormitories across the NC A&T campus (ii) Compare wastewater sampling strategies: Grab vs Composite sampling (iii) Compare SARS-CoV-2 RNA levels detected in wastewater to COVID-19 positive clinical cases and (iv) Identify SARS-CoV-2 variants detected in wastewater samples.

2. Materials & Methods
2.1.Sampling Sites

The study was conducted at manholes associated with 12 dormitories across NCAT campus (Aggie Village 1, Aggie Village 3, Aggie Village 4, Aggie Village 6, Aggie Village E, Barbee Hall, Cooper Hall, Haley Hall, Holland Hall, Morrison Hall, Morrow Hall, and Pride Hall) shown in Figure 1. The selected dorms housed 3434 students in Spring 2021 and 4976 students in Fall 2021. To decrease transmission, the institution required students who tested positive for SARS-CoV-2 to self-isolate in a quarantine dormitory (Haley Hall ~average 10 students per month) and employed the wastewater-based surveillance (WBS) as a secondary screening tool for SARS-CoV-2 spread on campus.

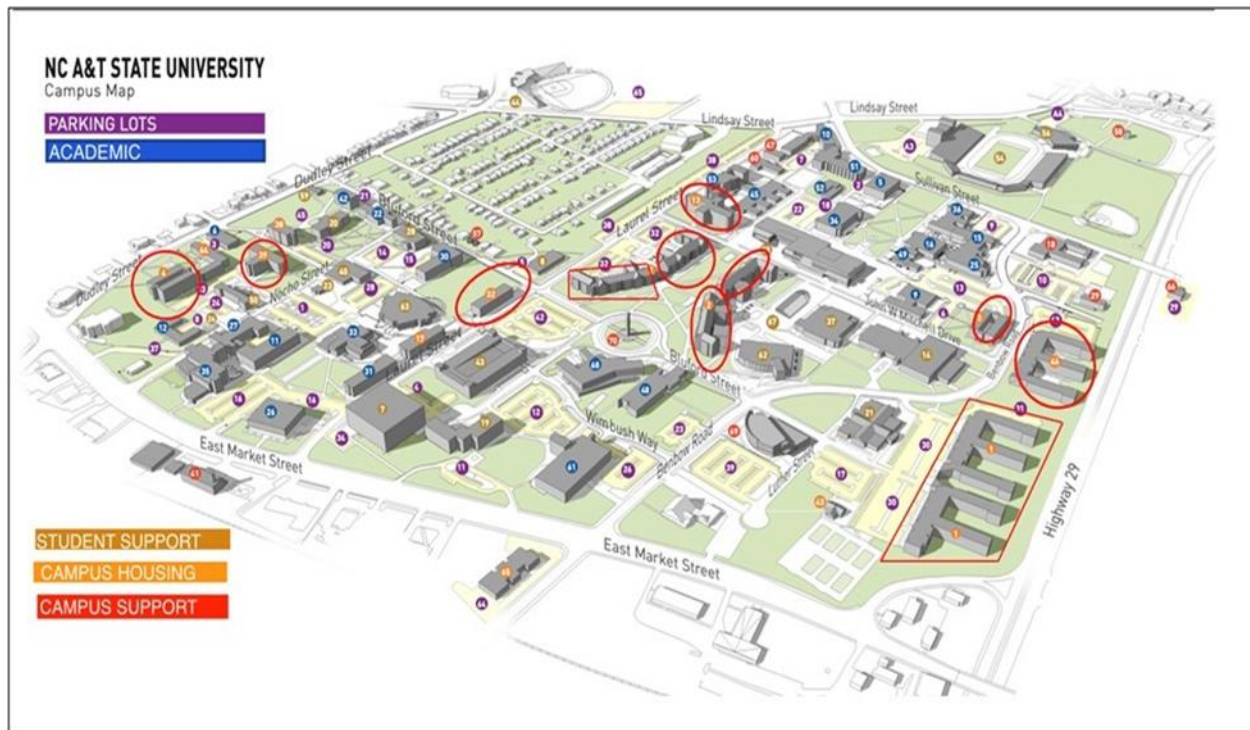


Figure 1. Campus map of North Carolina A&T State University, sampling sites marked in red (Campus Map of North Carolina A&T State University, 2021)

2.2. Sample Collection

Samples of wastewater were collected from January to December 2021 utilizing wastewater streaming from campus buildings (Figure 2). Sampling frequency occurred two days per week in the morning (Tuesday and Thursday) due to the human waste (feces) being more abundant in wastewater early in the morning than at other times of the day (Heaton et al., 1992). SARS-CoV-2 persists in fecal specimens approximately 10 days longer in contrast to the respiratory specimens (Chiu et al., 2020; Sims & Kasprzyk-Hordern, 2020). Moreover, after symptom emergence, the viral concentration in the feces rapidly increases, and shedding persists until the concentration decreases below the limit of detection (LoD) (Balboa et al., 2021). Therefore, based on these parameters, manholes placed outside the dormitories were chosen as the sampling sites with untreated wastewater. The number of persons tested for SARS-CoV-2 on campus was compared to SARS-CoV-2 levels in the wastewater to estimate the number of RNA copies per person in total sewage water. Using the following equation (1), the number of covid cases per building were calculated to encourage students to get tested (Hellmér et al., 2014).

$$\text{No. of infected individuals} = \frac{\text{No. of RNA copies per liter of wastewater}}{\text{Contributions of RNA copies per person total sewage water}}$$

Grab sample: were collected from six manholes using two different methods: installed lines equipped with an integrated suction cup into a sterile collection container using a manual pump. For the remaining manholes, samples were collected using a sterile water bottle tied to a rope. In

addition, small autosamplers from ISCO GLS were installed in the campus's top three high-risk dormitories (due to higher population density) to gather samples over time. During the spring semester, composite samples were collected as well at the same frequency in the top three risk dormitories. The autosampler container usually accommodates 9500 ml of wastewater, but was only able to collect 7500 ml to prevent overflow. At each collecting point (day), the wastewater was transferred to a sterile container, and the operation was restarted. Through the accumulation of samples over time, SARS-CoV-2 levels were monitored (Curtis et al., 2020; Walsh et al., 2020).

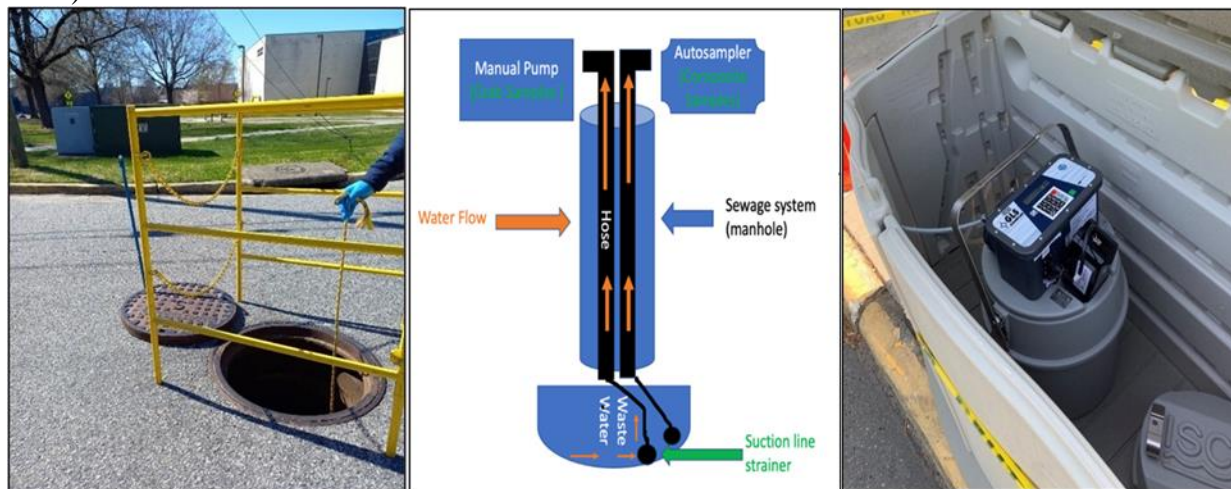


Figure 2. showing the set-up for grab and composite sampling strategies. Grab setup with a direct flow from the manholes (left) was collected using a collection bottle while composite autosampler (right) collected water continuously.

3. RESULTS & DISCUSSION

3.1. Clinical COVID-19 Positive Cases in 2021

To determine the number of COVID-19 positive persons present in the dormitories, data was collected from the Student Health Center of NCAT campus during 2021 (Spring-Summer- Fall) as shown in Figure 3. An increase in positive cases towards the end of the Spring semester in April (314 cases) and early in the Fall semester in August (312) were recorded, compared to only a few cases (23) reported in summer months. Overall, clinical testing at the NCAT health center found a significant number of COVID-19 cases in the months of January, March, April, and August. COVID-19 positive cases were extremely low (23 cases) throughout the Summer months (May-July). Higher number of cases were reported in Spring 2021 due to the vaccines still being rolled out and the university launched its vaccination drive during the Spring 2021 semester; in conjunction with the CDC guidelines. As compared to Spring semester, lower numbers of clinical cases in the fall semester were observed on campus and the outbreaks were not as severe because of the vaccination roll out. In the fall semester, an improvement in the vaccination rates was seen, with 5,625 of 13,000 students (43.2%) receiving vaccinations according to the North Carolina A&T State University health center. During this time, a correlation between the number of SARS-CoV-2 viral RNA copies and the number of clinical

cases was also detected, which prompted the University to increase clinical testing in high-risk areas, allowing asymptomatic and symptomatic COVID-19 students to be quarantined to mitigate the transmission of the virus.

Additionally, NCAT campus clinical cases were compared to those in Guilford County (Greensboro) and the state of North Carolina. A consistent spike in clinical cases were observed across January and August in NC, Guilford County and NCAT campus (Figure 4). However, a surprise surge in clinical cases was observed on campus in April as opposed to NC state and Guilford County while a surge in December was observed in NC state and Guilford County while not many cases were reported on campus (due to thanksgiving break extended over winter). Multiple universities have solely or in collaboration with their respective state governments have utilized WBE for reporting community level-based transmission and surveillance. While some of these studies have reported similar detection and diagnosis methods (Betancourt et al., 2021; Gibas et al., 2021), a holistic university level dashboard could help provide a thorough comparison and assessment of results between these universities and for internal administrative purposes would be ideal for integrating the wastewater results with dormitory-level aggregate reporting of positive tests, close contacts, and clinical symptom reports in the future.

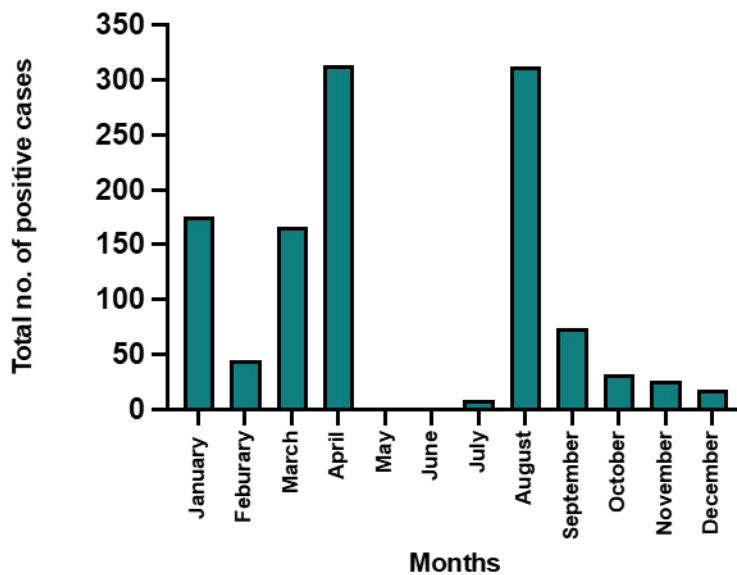


Figure 3. Clinical data (total number of positive cases) in Spring-Summer-Fall 2021 for North Carolina A&T State University

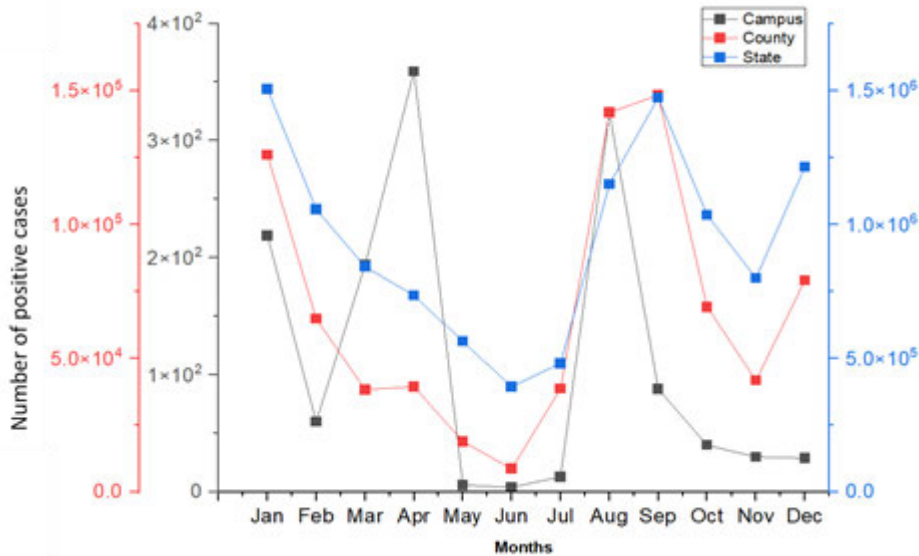


Figure 4. Total number of positive Covid-19 cases in North Carolina (NC) state, Guilford County, NCAT campus in 2021 (Jan-Dec).

3.2. Virus Concentration in wastewater

Higher viral RNA copies of SARS-CoV-2 for the N1 gene were detected during the Spring semester as compared to the Fall and Summer semesters in 2021. The average number of viral RNA copies detected in the Spring was 10^7 log RNA copies/L with an average C_T value of 26.80 for three replicates (Figure 5a, d). The lowest average levels of 10^5 log RNA copies/L were detected on campus during Summer semester (Figure 5b, e). The average C_T value for the Fall semester was approximately 35; suggesting that the number of RNA copies detected was lower in the Fall than in the Spring semester. (Figure 5 c, f). For better clarity, implementing questionnaires in these dormitories could help quantify more infections and their correlations with the RNA copies detected each day could provide us with more robust data in the future (Gibas et al., 2021). Thus, by computing and plotting the total concentration detected daily, it is possible to examine the spread of the virus in a given area for every new infection arising. In addition, using an indigenous wastewater virus such as PMMoV that could serve as an internal control for normalizing, may correct for any differences resulting from RT-PCR as shown in other studies (Feng et al., 2021; Graham et al., 2021; Wolfe et al., 2021). Quantifying the infectious virions from wastewater samples, although missing in our study analysis, could also help evaluate the infection rate of SARS-CoV-2 and indicate the natural decay of the virus.

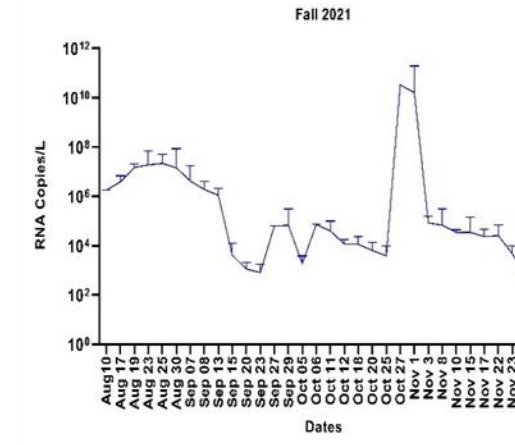
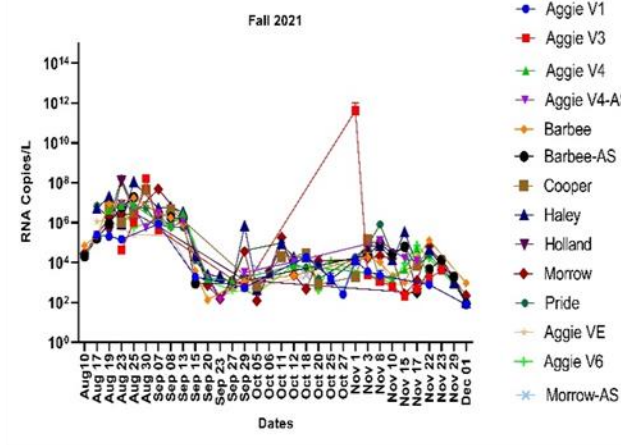
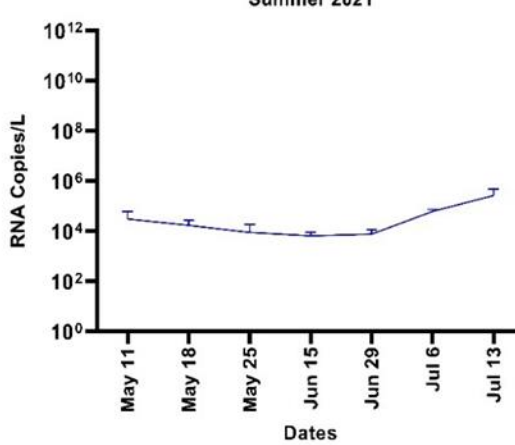
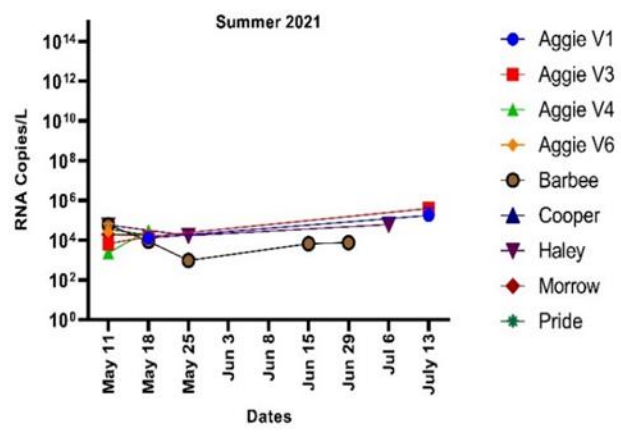
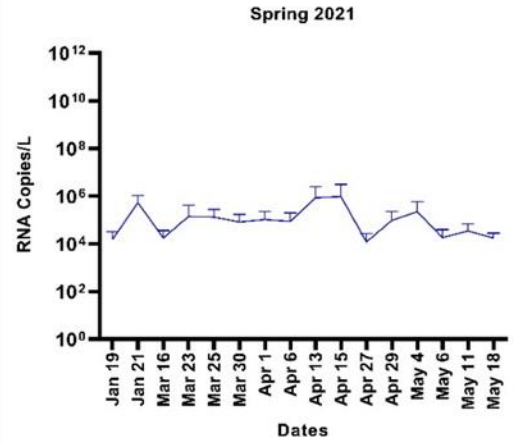
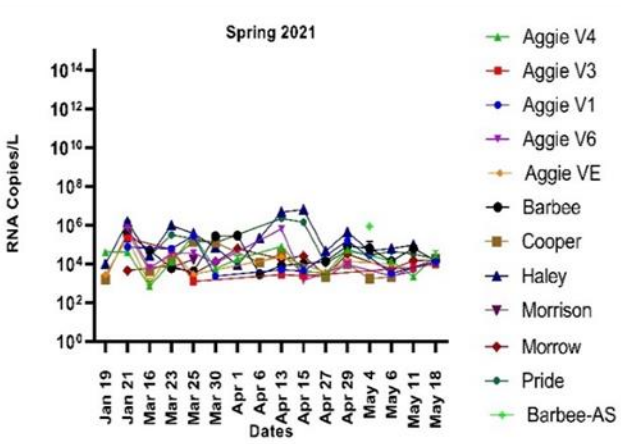


Figure 5. Graphs showing RNA copies/L for all dormitories across campus for Spring-Summer-

Fall semesters 2021. Highest level of RNA was detected in Spring as compared to Summer/Fall. The left panel (a-c) shows the RNA copies for each dormitory while the right panel (d-f) represents the trend of average RNA concentration during each semester.

