Sewage Testing to Predict COVID-19 Outbreaks for the Coast Guard at Land and Sea

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ABSTRACT

The COVID-19 pandemic had a major impact on the capability of the Coast Guard to operate its missions. High density residential situations such as training dormitories or vessels, together with a high prevalence of asymptomatic COVID-19 infections greatly increase the risk of rampant infection. Identified cases must be quickly removed from the population to prevent an outbreak. The Coast Guard has adopted rapid tests such as the antigen test which have greatly reduced turn-around time and cost, but they remain costly in terms of administration time and require professional interpretation. Sewage testing provides an inexpensive alternative to screening large numbers of individuals for asymptomatic infections; a single test can indicate an infected individual in a population providing justification for subsequent screening. It can also inform decision makers when all infected individuals have been removed from a population. To demonstrate the feasibility of sewage testing for the Coast Guard, we assessed the sewage at four dormitories at Training Center Cape May, the US Coast Guard's recruit training center, weekly for 1.5 months and contrasted viral sewage levels with diagnosed cases at that location. Data was concomitantly assessed by laboratories at the University of Connecticut and the Coast Guard Academy. Levels of virus in the sewage correlated well with identified cases; dormitories lacking infections (James Hall) showed no levels of SARS-CoV-2 in the sewage, whereas the dormitory with the most cases (Sexton Hall) showed the most viral sewage load. Pilot studies assessing viral load in the Coast Guard Cutter EAGLE showed similar results. As a result, the Coast Guard is building two laboratories capable of performing sewage analysis for training centers on the west and east coast, and seven individuals have trained in the technique at Coast Guard Academy's laboratory. Pilot studies have demonstrated similar efficacy on CGC Eagle, and future work will focus on assessment of sewage from Coast Guard Cutters.

INTRODUCTION

Coast Guard units are at high risk for COVID-19 infection due to close living conditions at land and sea. To reduce the risk of outbreaks, Coast Guard units are regularly screened using nasal swabs for PCR and antigen testing. While these methods are highly effective, they are costly and labor intensive. Recent studies demonstrate wastewater surveillance for SARS-CoV-2 (COVID-19) can identify the virus shed by individuals 4 to 10 days before a person exhibits symptoms (Larsen and Wigginton, 2020). Sewage testing is a low cost and non-invasive alternative method of testing populations By identifying emerging outbreaks of COVID-19 (SARS-CoV-2) before individuals become symptomatic, Coast Guard commands can preemptively adjust medical testing to identify and remove positive individuals from close living conditions.

To determine the correlation of sewage COVID levels with positive cases, we analyzed two Coast Guard installations: USCGA and Training Center Cape May. Samples were taken from two sewage access locations that carry sewage from two separate annexes of Chase Hall. The four dorms at Cape May were sampled separately at four different access points. Samples were analyzed at the Coast Guard Academy and the University of Connecticut using identical techniques. Data was analyzed and contrasted against medically diagnosed cases to determine their correlation with the goal of predicting cases based on sewage viral load.

A secondary goal of this project was to develop methodology to test black water sewage from Coast Guard cutters. Cutter sewage is significantly denser than the sewage analyzed in Chase Hall and Cape May and requires protocol modifications prior to assessment. The surveillance data from cutters will influence Coast Guard command decisions regarding liberty, testing frequency policies, and decision-making about crew readiness prior to getting underway.

METHODS

Sampling of sewage: Sewage effluent was sampled from open well sewage lines at two locations outside of Chase Hall (U.S. Coast Guard Academy, Fig 1A) and at one location for each of the four dormitories at TRACEN Cape May. Absorptive material was placed in the sewage effluent stream for 24 hours (Fig 1B) and was subsequently stored in a plastic bag at 4C prior to analysis. Bovine Syncytial Respiratory Virus (BRSV) vaccine was added to each sample as a process control, and the liquid was extracted. Virus was concentrated using Ceres Nanotrap Viral Capture magnetic particles according to manufacturer's instructions (Fig 1C and 1D).

Nucleic Acid Extraction and Analysis: RNA was extracted using the Qiagen Viral RNA kit according to manufacturer protocol. Real time quantitative reverse transcription polymerase chain reaction was performed to assess the N and E genes of SARS-CoV-2, BRSV, and pepper mottle virus (PPMoV), a biomarker that indicates the quantity of human feces present in the sewage sample (see references). Expression of N and E genes was quantified relative to PPMoV. Higher relative expression indicates greater presence of virus in the sample.

Figure 1. Method of sewage sample retrieval and extraction.
A. Samples are placed in raw sewage manholes.
B. Absorbent material is placed into the manhole.
C. During extraction, virus particles are absorbed onto magnetic Nanotrap beads and a neodymium magnet is used to purify virus from the remainder of the sewage sample.
D. Following this step, RNA is extracted by the Qiagen Viral kit as per manufacturer's instructions.

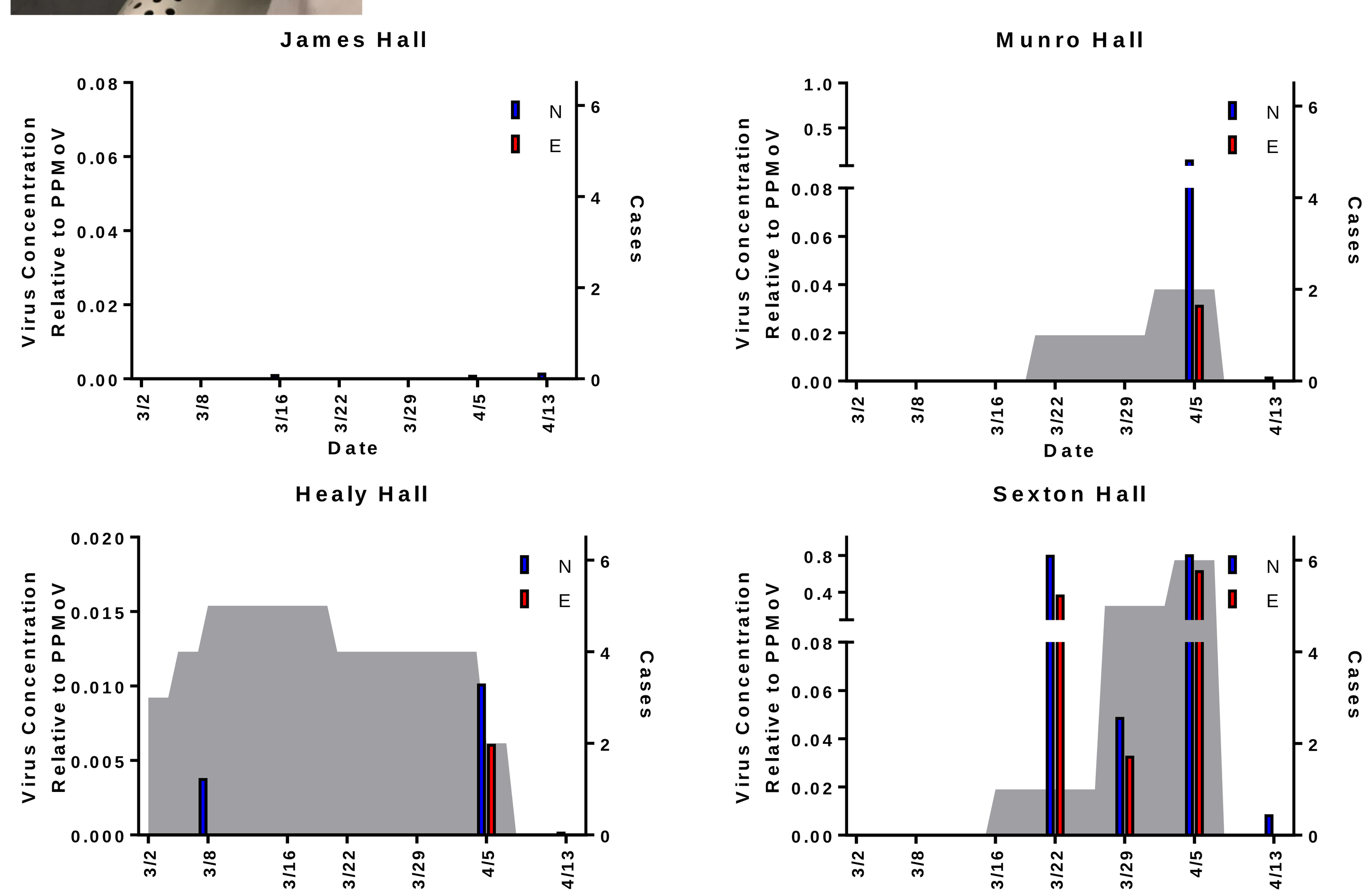

Figure 2. Correlation of Relative Virus Concentrations with Identified Cases at TRACEN Cape May Performed by the University of Connecticut MARS Laboratory. Sewage samples were retrieved after a 24 hour period on the dates indicated on the x axis. Relative amount of SARS-CoV-2 genes N and E were calculated through comparison to human fecal indicator PPMoV. Positive cases identified by the clinic are shown on the right y axis and gray background and are removed as they move to new barracks or recover after 14 days.

RESULTS

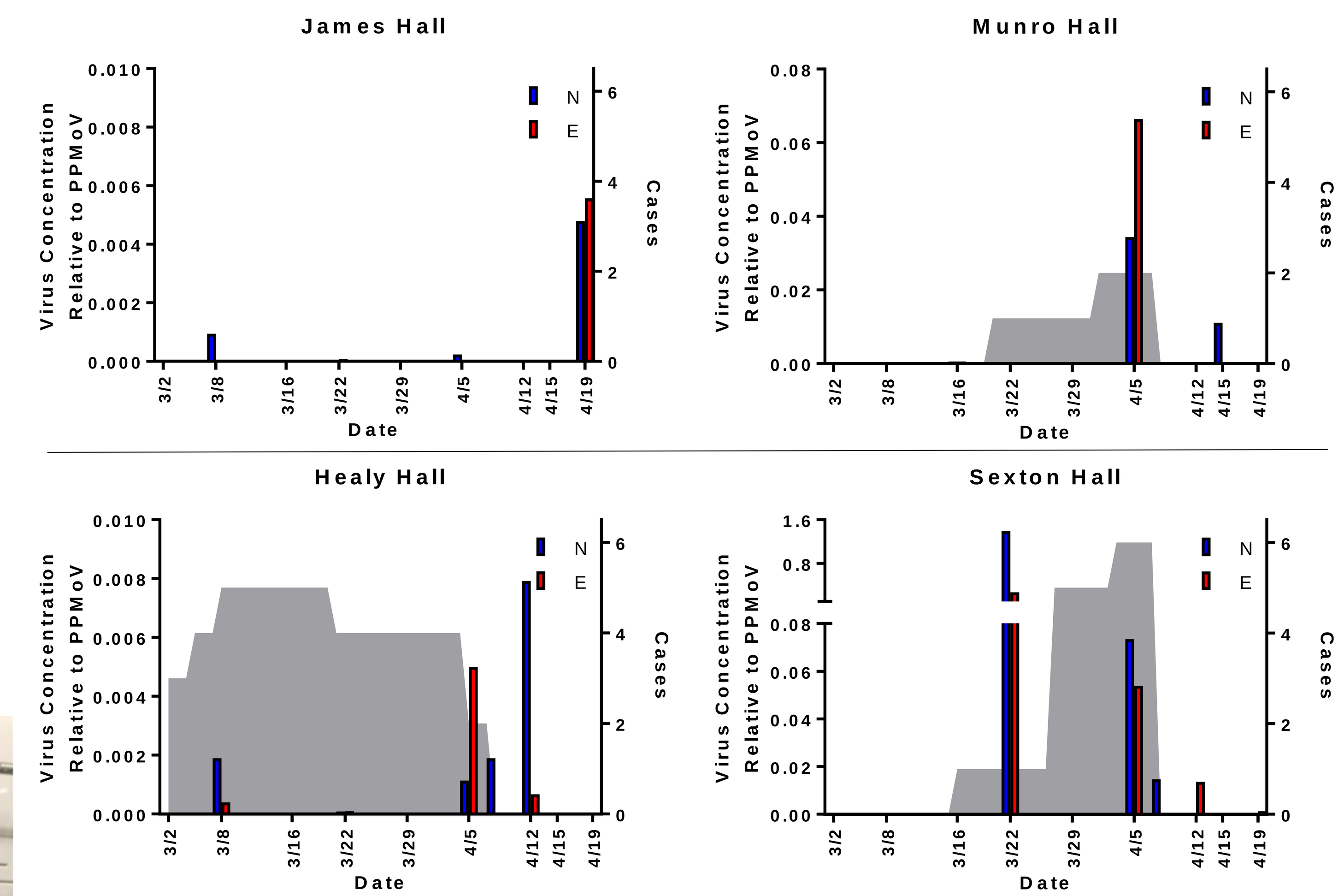

Figure 3. Correlation of Relative Virus Concentrations with Identified Cases at TRACEN Cape May Performed by the Coast Guard Academy Sewage Surveillance Laboratory. Sewage samples were retrieved after a 24 hour period on the dates indicated on the x axis. Relative amount of SARS-CoV-2 genes N and E were calculated through comparison to human fecal indicator PPMoV. Positive cases identified by the clinic are shown on the right y axis and gray background and are removed as they move to new barracks or recover after 14 days. Diagnosed cases only known to 4/11/21.

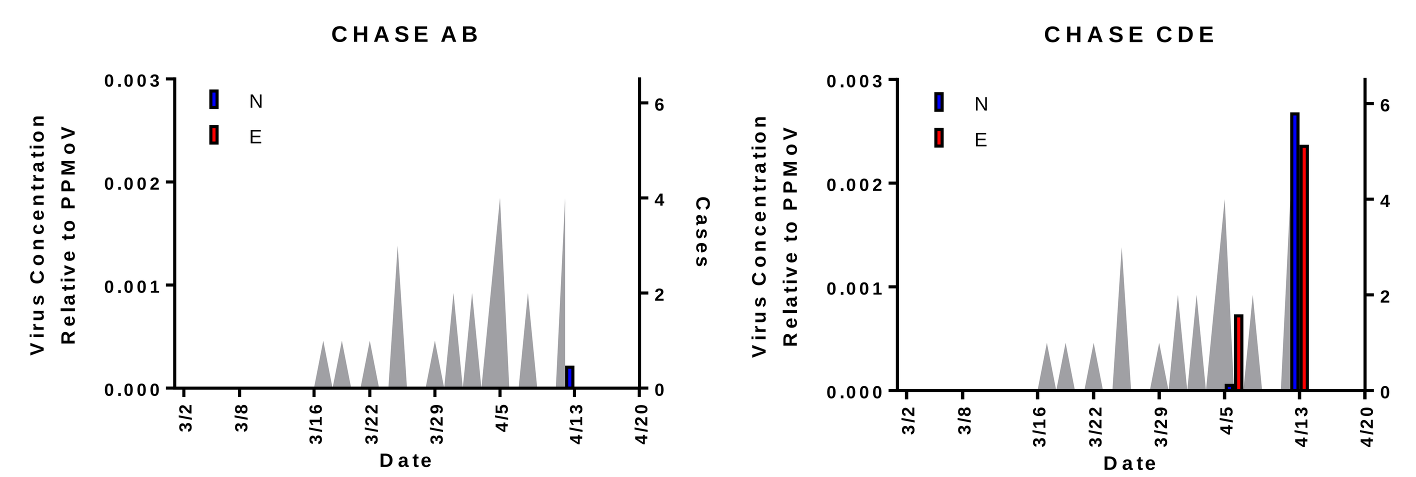

Figure 4. Correlation of Relative Virus Concentrations with Identified Cases at the U.S. Coast Guard Academy Performed by the CGA Sewage Surveillance Laboratory. Sewage samples were retrieved after a 24 hour period on the dates indicated on the x axis. Relative amount of SARS-CoV-2 genes N and E were calculated through comparison to human fecal indicator PPMoV. Positive cases identified by the clinic are shown on the y axis and gray background and are immediately removed upon isolation.

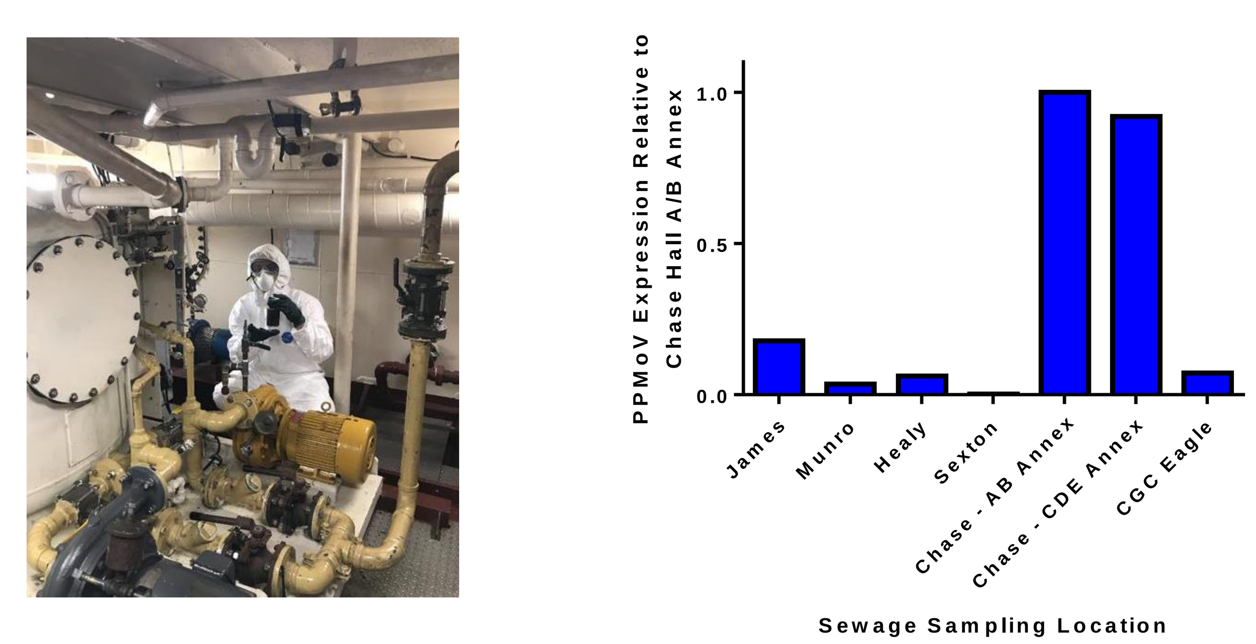

Figure 5. Assessment of SARS-CoV-2 Virus at U.S.C.G.C. Eagle. A technique for assessment of black water sewage was developed in our laboratory. No viral genes were detected in Eagle's sewage, consistent with the last case being diagnosed in early January. Human fecal gene expression was similar to that observed in other samples indicating proper RNA extraction and amplification.

IMPACT

➢Sewage COVID-19 levels correlated with diagnosed COVID-19 cases.

➢The Coast Guard is establishing two additional PCR laboratories to test patient and sewage samples at Coast Guard units throughout the country.

➢Six reservists were trained and are currently establishing the PCR laboratory at Coast Guard TRACEN Petaluma, California. A second lab is being prepared at TRACEN Cape May.

➢Developed method for analyzing black water sewage from Coast Guard Cutters.

➢For commands that are mostly vaccinated, sewage testing could replace routine testing of asymptomatic individuals and be used to assess breakthrough infections.

➢Substantial cost savings: one sewage sample is $33, whereas individual COVID-19 medical tests range from $100-130.

FUTURE DIRECTIONS

➢ Sewage isolates may be sequenced to determine the presence of variants.

➢ Establish baselines of SARS-CoV-2 genes in blackwater sewage from Coast Guard Cutters.

➢ Apply sewage surveillance technique to assessing other viruses such as influenza and viral meningitis.

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ACKNOWLEDGEMENTS

The authors thank Dr. Kendra Maas of the University of Connecticut for technical expertise and guidance in development of the Coast Guard Sewage Surveillance Program. The authors thank Coast Guard HSWL personnel for their generous contributions, MST2 Rhee for his valuable assistance in the laboratory, and the crew of EAGLE for providing sewage samples.