Wastewater-based Biosurveillance of Pathogens Causing Illness in US Military Training Centers

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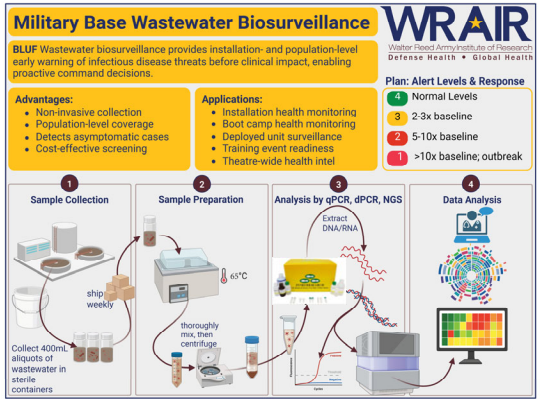
Background and Methods



A pilot wastewater-based biosurveillance study is being conducted at Fort Sill, Oklahoma, and Fort Benning, Georgia, with the aim to demonstrate the feasibility of monitoring major respiratory viral pathogens in wastewater from Army basic trainee barracks to inform Force Health Protection strategies.

- Respiratory infections account for a significant proportion of military hospitalizations for infectious diseases, highlighting the need for effective surveillance strategies (1). Many military populations, particularly new recruits congregating at military bases for basic combat training represent a prime population with intense risk of infectious disease spread (2).
- Wastewater-based biosurveillance offers a valuable One Health approach to monitor infectious disease transmission for high-density settings like basic training. This approach analyzes sewage samples for the presence of viral and microbial DNA and RNA shed by infected individuals, even those who are asymptomatic, thereby providing an anonymous and population-level assessment of infections (3,4).

BRIEF METHODS Single-timepoint grab wastewater samples are collected weekly from the influent stream at area-specific lift/pump stations. Water is heat inactivated prior to concentrated via centrifugation and 50 mL extracted with the Zymo Quick DNA/RNA water kit. Three respiratory pathogen targets (SARS-CoV-2, Influenza A/B, RSV) are quantified using reverse transcription quantitative PCR (RT-qPCR) with the Wastewater Surveillance Panel v1 from GT-Molecular. Pepper mild mottle virus (PMMoV) is measured as a fecal normalization control (5). Viral concentrations are reported as genomic copies per liter of wastewater, derived from standard curves run concurrently with each assay batch. Pathogen signals are normalized to PMMoV to control for variation in fecal strength across samples, further accounting for dilution effects from rainfall, industrial discharge, and variable population contributions. Log10-transformed normalization [log10(pathogen) - log10(PMMoV)] is the default method, consistent with CDC/NWSS pipelines. Two rolling baselines are computed: a short-term (4-week) and long-term (8-week) rolling window. Each baseline calculates the mean and standard deviation of normalized pathogen signal from eligible prior timepoints. The most recent timepoint is excluded from all baseline calculations to prevent self-reference bias. The cumulative baseline includes all prior timepoints (excluding the most recent), providing a long-term reference for pathogen activity across the entire surveillance period.

Results

Fort Sill, OK surveillance began in early 2026 demonstrating low-level detection of SARS-CoV-2.

- A lift station serving the trainee barracks allows for the monitoring of PPMoV (human fecal control) and respiratory viruses (SARS-CoV2, RSV, and FluA/B) by RT-qPCR. SARS-CoV was detected at low levels during monitoring but was not sustained over multiple weeks. **(Panel A).**
- A second collection point serving an on base office block not adjacent to the trainee barracks was discontinued due no detectable PPMoV (data not shown).

Beginning May 2026, surveillance at Fort Benning, GA resulted in low-level, but sustained SARS-CoV-2 detection exclusively from the Trainee Barracks.

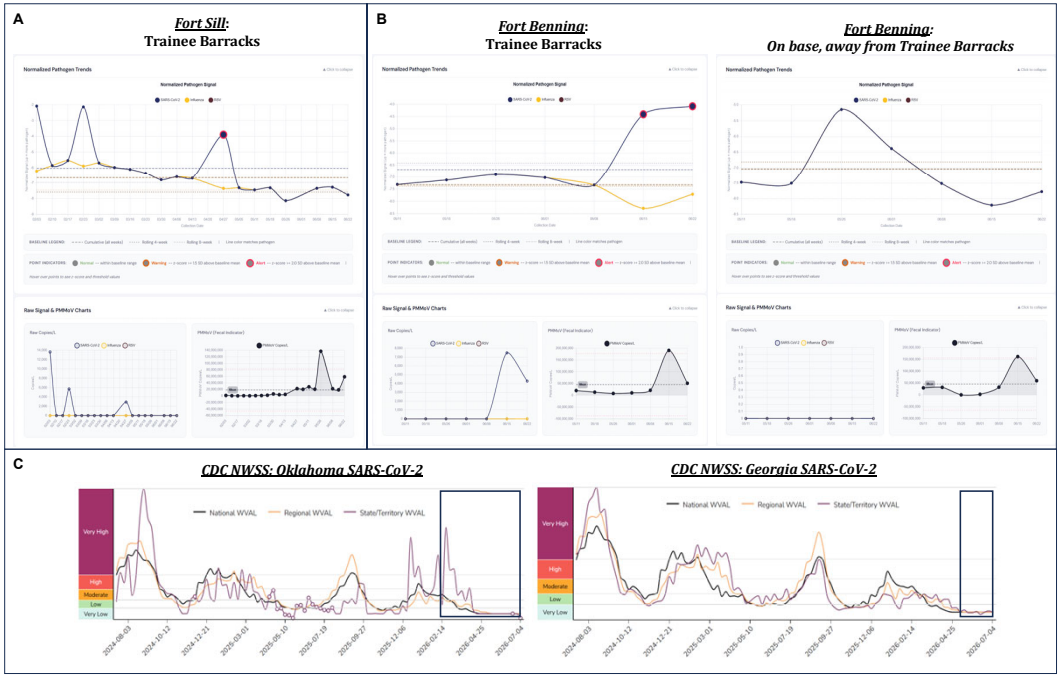
- A lift station serving the trainee barracks at Fort Benning, GA allows for the monitoring of PPMoV (human fecal control) and respiratory viruses (SARS-CoV2, RSV, and FluA/B) by RT-qPCR. SARS-CoV-2 has been detected and sustained at a low level (**Panel B, Left**).
- PPMoV was detected consistently at the site away from the barracks at Ft. Benning while respiratory viruses were not detected. This site allows for assessment of respiratory virus presence on base exclusive of the trainees (**Panel B, Right**).

Sites represent unique surveillance opportunities.

1. Oklahoma state testing is sporadic and does not include the county in which Fort Sill is located (**Panel C, left**, CDC NWSS).
2. While Georgia state surveillance includes in the county where Fort Benning is located, our enrolled sites support the targeted assessment of both trainee and on base populations (**Panel C, right**, CDC NWSS).
3. Dark box overlays in **Panel C** represents the time period of overlap surveillance activities between the pilot and CDC NWSS activities.

Results are reported to each site weekly allowing for rapid implementation of force health protection measures when warranted.

- Example data visualization graphical output from HTML dashboards are presented in **Panel A and B.**



Discussion

- This pilot study is a continued effort successfully monitoring for respiratory viruses in wastewater in military training environment. Our long-term goals are (1) enhance the surveillance to include comprehensive panel of targets, including emerging infection threats, pathogens of site specific interest, antimicrobial resistance (AMR), and possibly illicit drugs; (2) incorporate advanced next-generation sequencing and bioinformatics to further genomic surveillance on pathogens and pathogenesis genes, and (3) establish wastewater surveillance programs in Army Training Center at other military training bases.
- Wastewater based biosurveillance reflects community-level pathogen circulation and cannot identify individual cases. Signal variability may arise from specific sewer system characteristics, industrial inputs, weather events, and population mobility. Results are best evaluated alongside clinical surveillance data if available.
- Consistent, weekly sampling and analysis supports the establishment of baselines, assessment of alert levels, and generation of a report to inform countermeasure deployment. Weekly sampling, combined with sickcall data for respiratory viruses will demonstrate the use of wastewater-based biosurveillance for Force Health Protection.
- Weekly sample processing followed by data communication as raw data, normalized data, and a sites-pecific HTML dashboard facilitates the fine-tuning of reports with input from sites for ease of interpretation and future implementation of appropriate interventions.
- Not all sites are lift/sewer points on bases are candidates for surveillance efforts, as was seen with the office space away from trainee barracks at Fort Sill. This site demonstrated the ease of identifying fecal input in wastewater via RT-qPCR, allowing for the efficient use of time and resources by our collection partners.
- Fine scale wastewater based biosurveillance at the level of the individual pump or lift station facilitates targeted monitoring of specific populations on base. Even with a targeted small-scale population served by lift stations or sewer pumps, our study demonstrates surveillance methods for fecal input and respiratory viruses are suitable.

Conclusions

While current levels of respiratory viruses are low, our pilot successfully established a wastewater-based biosurveillance program at military training centers documenting baseline measurements of fecal indicators required for appropriate contextualization of identified increases in respiratory viruses.

- In the absence of alternative surveillance modalities, wastewater based biosurveillance adds value with no clinical requirements for testing. The resultant data is a frontline defense for Force Health Protection. This impact will be the strongest other testing modalities are limited.

Acknowledgment

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