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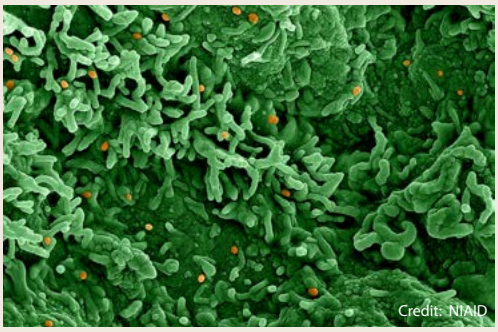


In this issue:

Reportable Medical Events, Military Health System Facilities, Week 13, Ending April 1, 2023

THE RETURN OF REPORTABLE MEDICAL EVENTS SUMMARIES TO MSMR

The *MSMR* is pleased to announce the return of a recurring feature, a summary of reportable medical events (RMEs) affecting DOD service members and other MHS beneficiaries. Sentinel RME summaries were a regular feature of *MSMR* until 2010. The *MSMR* editors welcome reader comments on the format and content of these summaries as we seek to expand the usefulness of the journal.

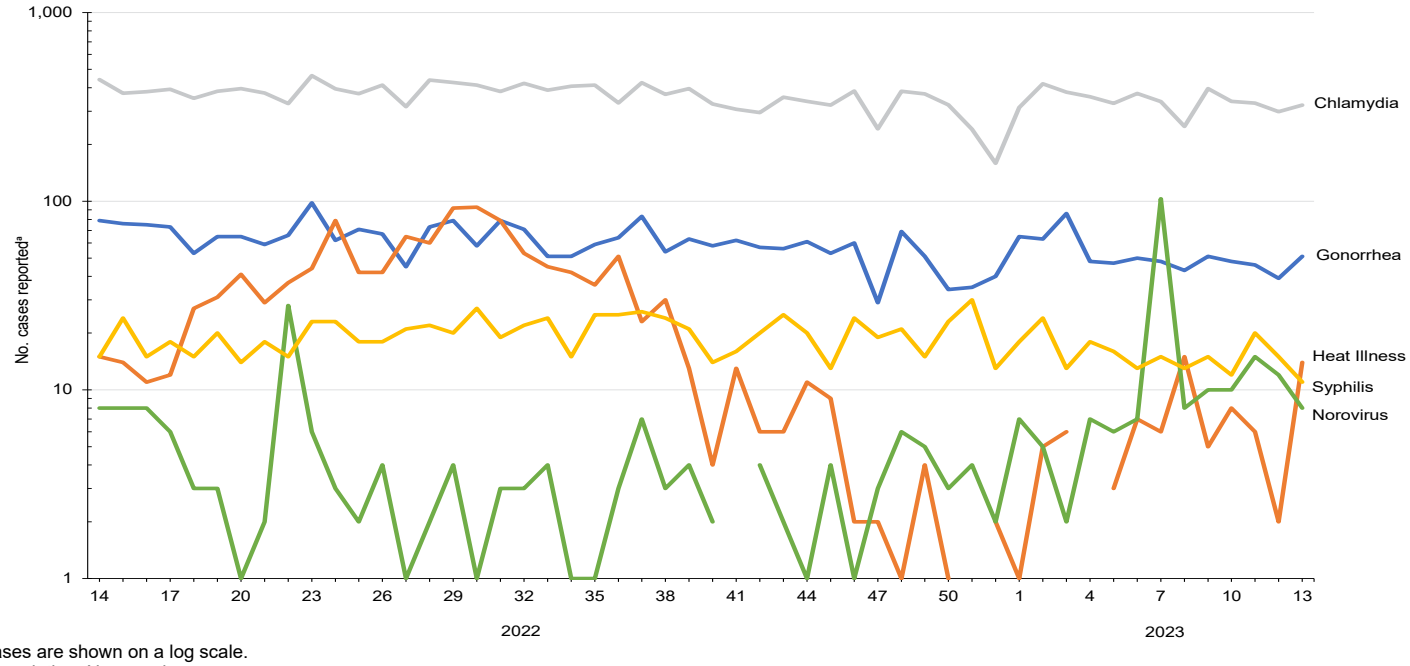
Reportable Medical Events (RMEs) are documented in the Disease Reporting System internet (DRSi) by health care providers and public health officials throughout the Military Health System (MHS). The DRSi collects reports on over 70 different RMEs, including infectious and non-infectious conditions, outbreak reports, STI risk surveys, and tuberculosis contact investigations. These reports are reviewed by each service's public health surveillance hub, which serves as an active primary prevention component to identify other service members at risk, assess need for post-exposure screening and prophylaxis, or inform other actions to protect and assure public health. Primary prevention (reducing disease occurrence) is the most effective method for preserving the medical readiness of the force.

Routine monitoring, evaluation, and publication of RMEs provide an important data resource for both policymakers and commanders, to guide their efforts for controlling and preventing diseases with potential measurable impacts on public health and force readiness—strategic, operational, and tactical. RMEs were chosen by consensus and recommendations from each service, which evaluated lists of nationally-notifiable diseases from the Centers for Disease Control and Prevention, position statements from the Council of State and Territorial Epidemiologists, and other events identified as significant military health threats meriting added surveillance. A complete list of RMEs is available in the 2022 *Armed Forces Reportable Medical Events Guidelines and Case Definitions*.[1]

The data presented in the Table not only list the most recent case counts but reveal trends of incidence for the past 2 months, year-to-date, and throughout the preceding year. Data reported in the Table are considered provisional and do not represent conclusive evidence until case reports are fully validated.

The most recent data on the 5 most frequent RMEs among total active component cases, as reported per week during the preceding year, are depicted in the Top 5 RME Trends by Calendar Week graph. COVID-19 is excluded from the graph due to 2023 changes in reporting and case definitions.

TOP 5 RME TRENDS FOR PRECEDING YEAR BY CALENDAR WEEK



[a]Cases are shown on a log scale.
Abbreviation: No., number.
Note: There were 0 heat illness cases in week 51 of 2022 and week 4 of 2023. There were 0 norovirus cases in week 41 of 2022.

TABLE. Reportable Medical Events, Military Health System Facilities, Week 13, Ending April 1, 2023[a]

Reportable Medical Event[b]	Active Component[c]					MHS Beneficiaries[d]
	Feb	Mar	YTD 2023	YTD 2022	Total, 2022	Mar
	no.	no.	no.	no.	no.	no.
Amebiasis	0	1	2	2	13	0
Arboviral Diseases, Neuroinvasive and Non-Neuroinvasive	0	0	0	0	1	0
Brucellosis	0	0	0	0	2	0
COVID-19[f]	4,143	2,967	14,079	116,597	209,917	1,174
Campylobacteriosis	13	23	54	47	229	16
Chikungunya Virus Disease	0	0	0	1	1	0
Chlamydia trachomatis	1,333	1,512	4,433	5,028	19,359	230
Cholera	0	1	1	0	2	0
Coccidioidomycosis	0	1	4	3	13	-
Cold Weather Injuries[e]	42	16	73	103	151	0
Cryptosporidiosis	4	6	16	10	46	2
Cyclosporiasis	0	0	0	0	10	0
Dengue Virus Infection	0	1	1	0	1	0
E. coli, Shiga Toxin-Producing	2	2	4	4	67	3
Ehrlichiosis/Anaplasmosis	0	0	0	0	3	0
Giardiasis	7	4	14	17	70	3
Gonorrhea	191	208	680	878	3,296	38
Haemophilus influenza,[e] invasive	0	0	0	1	1	1
Hantavirus Disease	0	0	0	0	1	0
Heat Illness[e]	29	35	78	44	1,211	-
Hepatitis A			2	4	16	0
Hepatitis B	14	13	35	33	116	7
Hepatitis C	6	6	20	9	56	8
Influenza-associated Hospitalization[g]	0	0	4	8	148	2
Lead Poisoning, Pediatric[h]	-	-	-	-	-	2
Legionellosis	0	0	1	1	4	2
Leishmaniasis	0	0	1	0	1	0
Leptospirosis	1	0	2	0	1	1
Lyme Disease	6	2	13	13	64	2
Malaria	2	0	6	2	26	1
Meningococcal Disease	0	1	1	0	2	0
Mpox	0	0	0	0	93	0
Mumps	0	0	0	0	0	1
Norovirus	126	48	200	60	219	52
Pertussis	0	0	1	1	10	0
Post-Exposure Prophylaxis Against Rabies	42	44	125	106	503	25
Q Fever	0	0	1	1	3	0
Rubella		2	2	2	3	1
Salmonellosis	3	9	13	6	122	6
Schistosomiasis	0	0	0	0	1	0
Severe Acute Respiratory Syndrome (SARS)	5	4	13	1	1	0
Shigellosis	5	4	12	5	33	3
Spotted Fever Rickettsiosis	57	66	203	4	70	1
Syphilis (All)	0	0	1	253	1,035	10
Toxic Shock Syndrome	0	0	1	0	0	0
Trypanosomiasis	0	0	1	0	1	0
Tuberculosis	0	1	1	2	10	2
Typhus Fever	0	1	1	1	1	0
Varicella	0	0	1	2	16	9
Total case counts	6,031	4,978	20,100	123,249	236,949	1,602

Abbreviations: RME, reportable medical event; MHS, Military Health System; YTD, year-to-date; no., number.
[a] RMEs reported through DRSi as of April 13, 2023 are included in this report. RMEs were classified by date of diagnosis, or where unavailable, date of onset. Monthly comparisons are displayed for the periods February 1-28, 2023 and March 1-31, 2023. Year-to-date comparison is displayed for the period January 1-March 31, 2023 for MHS facilities. Previous year counts are provided as: previous YTD, January 1-31 March, 2022; total 2022, January 1-December 31, 2022.
[b] RME categories with 0 reported cases among active component service members and MHS beneficiaries for the time periods covered were not included in this report.
[c] Services included in this report include: Air Force, Army, Coast Guard, Navy, Marine Corps, and Space Force, including personnel classified as FMP 20 with Duty Status of AD, Recruit, or Cadet in DRSi.
[d] Beneficiaries included: individuals classified as FMP 20 with Duty Status of Retired and individuals with all other FMPs except 98 and 99. Civilians, contractors, and foreign nationals were excluded from these counts.
[e] Only reportable for active component service members.
[f] Only cases resulting in hospitalization or death after case definition update on May 4, 2023.
[g] Influenza-associated Hospitalization is reportable only for individuals aged 65 years or younger.
[h] Pediatric Lead Poisoning is reportable only for children aged 6 years or younger.

Enhanced Mpox Outbreak Case Detection Among MHS Beneficiaries Through Use of ESSENCE (Electronic Surveillance System for the Early Notification of Community-based Epidemics)

Sasha A. McGee, PhD, MPH; Jamaal A. Russell, DrPH, MPH; Maura Metcalf-Kelly, MPH

Early awareness of cases of infectious disease facilitates timely implementation of control measures and policies to prevent disease or reduce spread. To support force health protection, the Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE)[1] collects near real-time biosurveillance data globally on U.S. military personnel. ESSENCE systematically queries millions of health encounters to detect records of potential public health importance. Statistical algorithms detect unusual increases, alerting public health staff to findings that may warrant investigation.[1] ESSENCE receives several types of data including outpatient health encounter records from U.S. Military Health System (MHS) facilities, pharmacy prescriptions, laboratory results, and radiology reports. This report describes how ESSENCE contributed to case detection from the onset of the mpox outbreak.

The U.S. government declared mpox a national public health emergency on August 4, 2022.[2] Nearly 3 months earlier, on May 13, 2022, the World Health Organization (WHO) was notified of a cluster of 3 mpox cases from a single household in the United Kingdom. These cases were suspected to be locally acquired; 2 days later 4 additional cases were reported by sexual health clinics, all among male patients who have sex with men.[3] By May 21, 2022, locally acquired cases of this zoonotic disease had been confirmed in 11 other nonendemic countries including the U.S.[4]

To detect specific health events, queries in ESSENCE are typically constructed with chief complaint keywords or codes from the International Classification of Diseases 10th Revision, Clinical Modification (ICD-10-CM) linked by logical operators (e.g., "and", "or"). An initial mpox query was developed on May 20, based on known symptoms and specific criteria for discharge diagnosis ICD-10-CM codes and chief complaint keywords **(Table 1)**.

While ESSENCE is designed to support surveillance and outbreak detection, the system's performance should be evaluated when utilized during a public health response. This report describes how ESSENCE was used to monitor the mpox outbreak, assesses the system's performance to detect confirmed/probable cases among MHS beneficiaries during May–August 2022, and describes approaches for improving system performance and utilization.

Methods

The initial mpox ESSENCE query was modified 5 days after its creation, to align with the WHO case definition for suspected cases (lowest threshold to meet the case criteria), followed by a minor change to additionally capture the keyword "monkey" without "pox" due to frequent misspellings **(Table 1)**.[5] Potential mpox cases included all health encounters that met the query criteria. Encounters with a discharge diagnosis ICD-10-CM code for diaper rash or hand, foot, and mouth disease, a chief complaint mentioning monkey bars, or a missing Electronic Data Interchange Personal Identifier (EDI-PI) were excluded as potential cases.

To assess the performance of the final version of the ESSENCE mpox query results, potential cases identified from May 1 through August 31, 2022 were compared to a validated master case list maintained by the Armed Forces Health Surveillance Division (AFHSD) Integrated Biosurveillance (IB) Branch. Epidemiologists at AFHSD IB created the master case list by compiling information about potential cases from various sources including the ESSENCE mpox query, reportable medical events (RMEs) from the Disease Reporting System internet (DRSi),[6] service-specific reporting to AFHSD, notifications from stakeholders who received weekly case count reports, as well as direct informal communications. AFHSD IB epidemiologists contacted the appropriate Defense Center for Public Health (DCPH) to inform them of potential cases and request additional information. To validate the master case list, AFHSD IB epidemiologists reviewed electronic medical records of potential cases using Armed Forces Health Longitudinal Technology Application (AHLTA) to gather epidemiological, laboratory, and clinical information. CDC case definitions were used to classify potential cases as confirmed, probable, suspect, or not a case.[7] Starting in July, the master case list was cross-checked daily with Defense Health Agency (DHA) Composite Health Care System and MHS GENESIS medical records in which mpox was recorded as a diagnosis, using reports provided by DHA administrative personnel. Results from 2 simpler, alternative queries, B04 ICD-10-CM code (alternative query 1 [AQ1]) and B04 ICD-10-CM code (AQ2) or mention of "monkey" in the chief complaint, were also compared with the mpox master case list to evaluate potential approaches to improve case detection. These queries were not implemented during the mpox outbreak but were retrospectively reviewed.

Results

A total of 3,067 unique encounters, from 2,750 unique individuals, as determined by EDI-PIs, were detected between May 1 and August 31, 2022.

TABLE 1. ICD-10-CM Codes and Chief Complaint Keywords Used in Versions of the ESSENCE Mpox Query

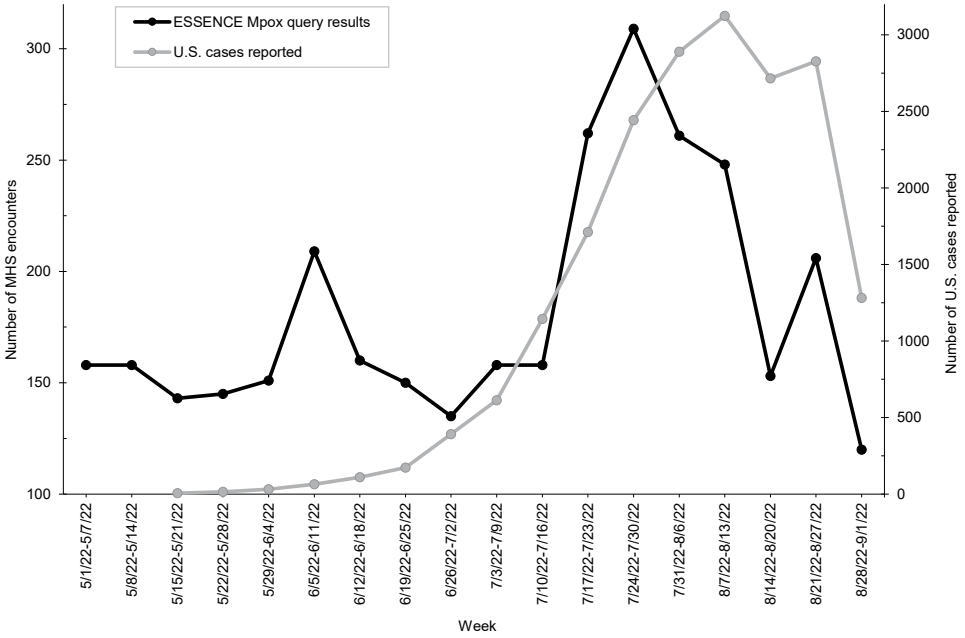
Initial version of ESSENSE mpox query (May 20-24, 2023)			Final version of ESSENCE mpox query (May 25, 2023)	
	Code/Keyword	Description	Code/Keyword	Description
Criteria 1				
One of the following ICD-10 codes/chief complaint keywords	B08	Other viral infections characterized by skin and mucous membrane lesions, not elsewhere classified	B08	
	B08.[0,2,3,4,5,6,7] and all subcodes	Other orthopoxvirus infections	B08.0	
	B09	Unspecified viral infection characterized by skin and mucous membrane lesions	B08.09	Other orthopoxvirus infections
	I88	Nonspecific lymphadenitis	B09	
	I88.[0,8, or 9] and all subcodes	Nonspecific mesenteric lymphadenitis, Other nonspecific lymphadenitis, Non-specific lymphadenitis, unspecified	*R21*	Rash and other nonspecific skin eruption
	L04 and all subcodes	Acute lymphadenitis	L98.9	
	L98.9	Disorder of the skin and subcutaneous tissue, unspecified	*Rash (and not "crash" or "no rash")*	
	R59 and all subcodes	Enlarged lymph nodes		
AND			AND	
One of the following ICD-10 codes/chief complaint keywords	R50	Fever of other and unknown origin	I88	
	R50.9	Fever, unspecified	I88.[0,8, or 9] and all sub-codes	
	R51 and all subcodes	Headache	L04 and all subcodes	
	R53.1	Weakness	*M54 and all subcodes*	Dorsalgia
	R53.8	Other malaise and fatigue	*M79.1*	Myalgia
			R50	
			R50.9	
			R51 and all subcodes	Headache
			R52	Pain, unspecified
			R53.1	
			R59 and all subcodes	Enlarged lymph nodes
			Swollen lymph node	
			Fever (and not "no fever")	
			Body ache (or "bodyache")	
Criteria 2				
One of the following ICD-10 codes/chief complaint keywords	B04	Monkeypox	B04	
	Monkeypox		Monkeypox (or "monkey pox")	
			Orthopox	
			Monkey	

Abbreviations: ICD-10-CM, International Classification of Diseases, 10th Revision, Clinical Modification; ESSENCE, Electronic Surveillance System for Early Notification of Community-based Epidemics.

ESSENCE mpox surveillance of MHS beneficiaries was initiated earlier, on May 1, than U.S. mpox case reporting, on May 17 **(Figure 1)**. The peak in the total number of health encounters returned by the mpox query preceded the peak in reported U.S. cases by 2 weeks. The first confirmed case of mpox among MHS beneficiaries was detected as a potential case by the ESSENCE mpox query 5 days prior to laboratory confirmation **(Figure 2)**. The patient, an active duty service member in Germany, developed symptoms in late May, and reported no travel within the previous 90 days.

Table 2 summarizes the ESSENCE mpox query results by validated, master list case status and compares its results to those from the alternative queries (AQs). The ESSENCE mpox query detected 70.1% of confirmed/probable mpox cases based on a review of medical records for 2,750

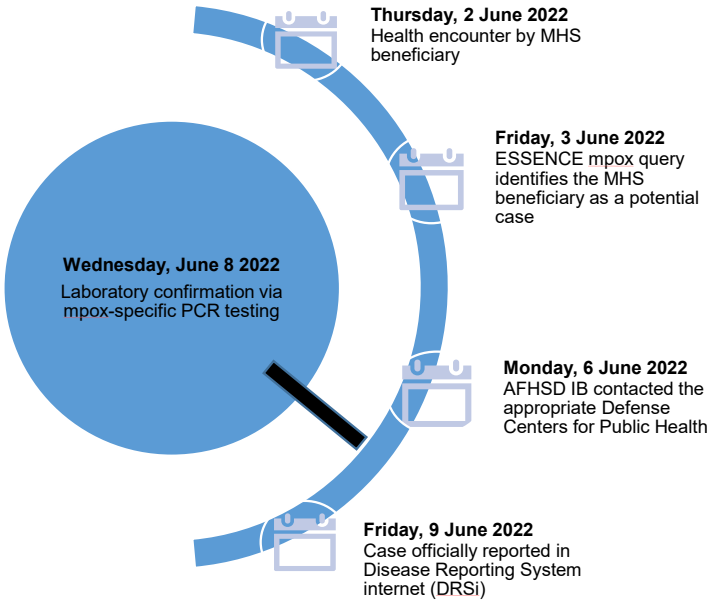
FIGURE 1. Comparison of MHS Health Encounters Identified by ESSENCE and Reported U.S. Mpox Case Trends,[a] May 2022-August 2022



Abbreviations: MHS, Military Health System; ESSENCE, Electronic Surveillance System for the Early Notification of Community-based Epidemics.

[a] Data reported to the Centers for Disease Control and Prevention as of September 7, 2022; reporting began May 17, 2022. https://www.cdc.gov/poxvirus/monkeypox/response/2022/mpx-trends.html

FIGURE 2. Timeline of the Identification of First Mpox Case Among MHS Beneficiaries



Abbreviations: MHS, Military Health System; AFHSD, Armed Forces Health Surveillance Division; IB, Integrated Biosurveillance Branch.

individuals. AQ1 and AQ2 reduced the number of potential cases requiring medical record review (64 and 259, respectively), while still detecting 63.2% and 70.1% of confirmed/probable cases, respectively. The positive predictive value (PPV) of the ESSENCE mpox query was 2.2% (61/2,750). AQ2 had a higher PPV, at 23.5% (61/259), with equal sensitivity. AQ1 had the highest PPV, 85.9% (55/64), but was less sensitive, detecting 6 fewer confirmed/probable cases.

Discussion

The time series plot of ESSENCE mpox query encounter counts closely tracked the U.S. outbreak peak late in the summer of 2022. The CDC's U.S. data included MHS beneficiary case data, as mpox is a nationally notifiable condition as well as a DOD RME. ESSENCE detected the first confirmed mpox case among MHS beneficiaries 5 days prior to laboratory confirmation and 6 days prior to reporting in DRSi. Detection was facilitated by the inclusion of the ICD-10-CM code for mpox (B04) in the discharge diagnosis prior to laboratory confirmation. Practitioners may use this code broadly to indicate a clinically suspected or confirmed case, exposure, or testing.

The development and usefulness of queries to detect health events of interest is challenging when associated signs or symptoms are nonspecific or shared with other health events (i.e., many potential ICD-10-CM codes), or the code(s) do not yet exist (e.g., novel diseases, unknown etiology), or are not unique. Many potential cases detected by the mpox query were ultimately ruled out based on medical record review, or subsequent test results or alternate diagnoses (i.e., not a case) despite initial clinical suspicion. Depending upon the health event, the latter group may still be useful for public health detection.

Reviewing the medical records for 2,750 potential cases detected by the mpox query was time-intensive (5-10 minutes per record) and completing a review the same day as detection became unfeasible for a single, dedicated reviewer. The cost, in both time and effort, could make the approach impractical in many outbreak scenarios or not worth the benefit, given that opting for AQ2 would have reduced the number of records for review by more than 10-fold, without sacrificing identification of true positive cases.

ESSENCE queries can easily be modified as knowledge of the disease

TABLE 2. Confirmed/Probable and Suspected Mpox Cases Among MHS Beneficiaries, May 1–August 31, 2022

Case status	Master case list[a]		Final version of ESSENCE mpox query			Alternative query 1: mpox ICD-10-CM code (B04)			Alternative query 2: B04 ICD-10-CM code or mention of "monkey" in chief complaint		
	No.	% of Total (n=181)	No.	% of Case Status Total (per Master Case List)	% of Total EDI-PIs	No.	% of Case Status Total (per Master Case List)	% of Total EDI-PIs	No.	% of Case Status Total (per Master Case List)	% of Total EDI-PIs
Confirmed/probable[b]	87	48.1	61	70.1	2.2	55	63.2	85.9	61	70.1	23.6
Suspected[c]	22	12.2	11	50.0	0.4	4	18.2	6.3	10	45.5	3.9
Not a case[d]	72	39.8	39	54.2	1.4	4	5.6	6.3	29	40.3	11.2
Total EDI-PIs reviewed[e]	--	--	2,750	--	100.0	64	--	100	259	--	100.0

Abbreviations: MHS, Military Health System; ESSENCE, Electronic Surveillance System for the Early Notification of Community-based Epidemics; ICD-10-CM, International Classification of Diseases 10th Revision, Clinical Modification; EDI-PI, Electronic Data Interchange Personal Identifier.

[a] Excludes cases with unknown EDI-PIs (n=12).

[b] Persons with DNA detected from a Non-variola Orthopoxvirus Generic Real-Time PCR Test or antibodies detected from an orthopoxvirus (or poxvirus) serology test.

[c] Persons with signs and symptoms consistent with mpox, for whom laboratory testing had not yet been conducted or test results were pending.

[d] Suspected cases subsequently ruled out based on laboratory test results.

[e] EDI-PIs are a unique identifier assigned to each person with a record in the Defense Eligibility and Enrollment Record System database, including all military personnel, family members, employees, and most contractors, to idenfity the individual in all interactions with DOD. These values are not column totals, but the number of unique EDI-PIs returned in the ESSENCE query results; medical record review results determined whether an EDI-PI identified in the query was added to the master case list.

epidemiology evolves, transmission patterns change, and case definitions are updated. Adjustments to the query, to reduce potential cases requiring review, must be carefully evaluated, as it could lead to confirmed cases being missed, as the AQ1 results demonstrate. Over the course of the outbreak the number of encounters with chief complaints mentioning "monkeypox" increased as patients requested educational information or were offered or received the vaccine, especially when it became more widely available. The ESSENCE mpox query was subsequently modified by removing symptom keywords and including negation terms to exclude vaccination-related encounters and persons without exposure or symptoms seeking medical education. When the modified query was executed for the analysis period, it returned 430 unique encounters (246 unique EDI-PIs; PPV=20.2%) with a sensitivity of 100%.

The desired tolerance for the completeness of case capture should consider human resources, surveillance goals, and characteristics of the health event (e.g., incidence, disease epidemiology), among other factors. In contrast to the results of this study, a recent study of case definitions to identify COVID-19-related encounters among MHS beneficiaries found that inclusion of a greater number of ICD-10-CM codes increased sensitivity at the cost of specificity.[8]

The analyses in this report had several limitations. The mpox query likely underestimated the detection of potential cases and therefore missed detection of some confirmed/probable cases (i.e., decreased sensitivity) for several reasons. Data from MTFs using MHS GENESIS were initially received in ESSENCE following a lag of a month; beginning on July 28, 2022, data were received daily beginning with July 23 encounters, but were incomplete (approximately 10% of the expected number were received). The sensitivity of the mpox detection query is dependent upon the completeness and accuracy of the chief complaint and ICD-10-CM codes entered in the medical records. ESSENCE does not capture health encounters or testing for MHS beneficiaries at non-MHS facilities, which could mean that some confirmed/probable cases among MHS beneficiaries are missed (i.e., decreased sensitivity). The master case list likely underestimated the true number of confirmed/probable cases, as health encounter notes and test results for MHS beneficiaries visiting non-MHS facilities were often not available in AHLTA. The population most at risk of mpox infection, men who have sex with men, may be more likely to seek care outside MHS, which would lead to a true case rate higher than stated in the AFHSD master list. MHS GENESIS medical records were not reviewed, so some cases may have gone undetected and led to an underestimation of the true incidence. Once test results became available, some cases classified as suspected in this analysis may have subsequently been classified as confirmed/probable, or not a case, which may have led to underestimation of the sensitivities of confirmed/probable case detection queries.

During the mpox outbreak ESSENCE biosurveillance was centralized, with AFHSD IB conducting all aspects of query development, data review and extraction, and electronic medical record review for all MHS beneficiaries. More efficient biosurveillance utilizing ESSENCE would be decentralized, incorporating the 2 designated ESSENCE users required at each MTF[9] to conduct ongoing data review and initiate public health investigations. These MTF ESSENCE users' awareness of local events and collaboration with local public health and clinical staff for record reviews would expand resources for timely follow-up of potential cases. In a decentralized approach

AFHSD IB would refine queries, receive and compile critical findings reported by local users or DCPH, and maintain and report the master case list results to key stakeholders. Valuable insights and refinements on the specific process could be informed by pilot programs between AFHSD and a DCPH or several MTFs. Advancing towards a decentralized MHS-wide approach would improve the effectiveness of the ESSENCE biosurveillance process, by employing the situational awareness of those closest to the location of a public health event, in collaboration with strategic oversight and technical support provided by AFHSD staff.

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Portable RT-PCR and MinION Nanopore Sequencing as a Proof-of-Concept SARS-CoV-2 Biosurveillance in Wastewater

Oksana M. Pavlyuk, PhD; Jacque Engler, MSc; Craig Strapple; Blake W. Stamps, PhD; Michael T. Dietrich, PhD (Lt Col, USAF)

In this study, wastewater samples collected from a participating sentinel site were initially screened for the presence or absence of SARS-CoV-2 RNA using portable RT-PCR, with positive samples sequenced using a handheld MinION nanopore sequencing device. Genomic biosurveillance of SARS-CoV-2 and its variants within wastewater has been established as an early warning system of infectious disease spread in a given catchment area, due to good correlation between spikes in viral levels detected in wastewater coincident with increases in COVID-19 incidence rates.[1-3] Moreover, viral titers detected in a single wastewater sample are reflective of pre-symptomatic, asymptomatic, and post-symptomatic cases, making wastewater-based epidemiology (WBE) a cost-effective, non-invasive public health surveillance method complementary to clinical diagnostic testing. The results of this study demonstrate the utility of population-scale SARS-CoV-2 epidemiology for insights into the viral evolution and transmission dynamics associated with specific SARS-CoV-2 variants that are necessary for effective strategies of containment and timely deployment of appropriate countermeasures.

What are the new findings?

In this study, 2 different portable nucleic acid detection technologies, RT-PCR and MinION Mk1C nanopore sequencing, identified SARS-CoV-2 variants in wastewater collected at Tyndall AFB during a 2-month surveillance period. This highly multiplexed approach circumvented signal dropout associated with the detection of newly emerging SARS-CoV-2 variants, significantly reducing time for sample-to-pathogen identification, for improved infectious disease surveillance.

What is the impact on readiness and force health protection?

Based on the biosurveillance data obtained through portable detection technologies, viral levels in wastewater monitored at military installations can signify SARS-CoV-2 spatio-temporal dynamics in relation to population density as well as other crucial variables. Wastewater biosurveillance can contribute to data-informed policy decisions such as force health protection condition (HPCON) level adjustments.

Wastewater-based epidemiology serves as an early warning system of surges in viral levels in a geographically-defined catchment area that has been utilized to monitor infectious disease emergence and spread, including COVID19.[4-8] Although primary transmission of SARS-CoV-2 is through the respiratory aerosol,[9,10] other routes of transmission are possible[11,12] as a result of viral shedding due to broad tissue tropism of SARS-CoV-2, including the gastrointestinal (GI) tract.[13-15] SARS-CoV-2 lineages were detected in wastewater throughout the COVID19 pandemic, making wastewater-based epidemiology a valuable tool in understanding and tracking SARS-CoV-2 transmission dynamics at a population level.[16-21]

Methods

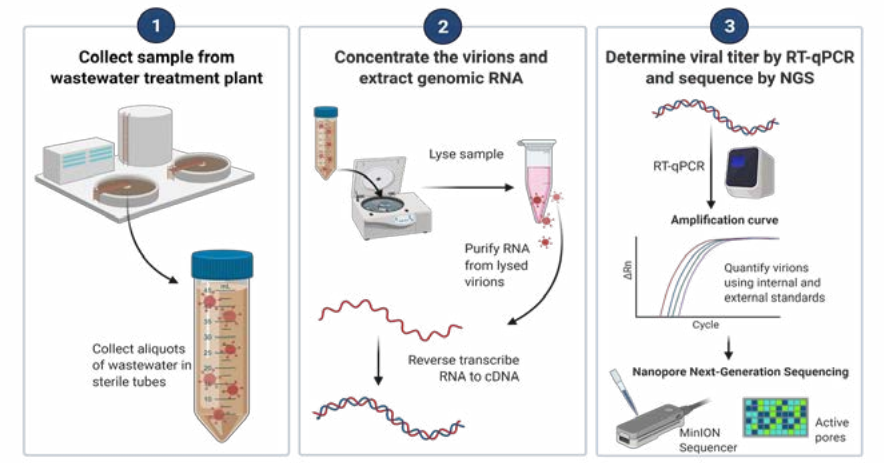
Sample collection from wastewater lift station and initial processing

Site selection for this study was based on reported positive COVID-19 clinical cases. Wastewater influent, prior to any treatment, was collected weekly by an autosampler at a lift station servicing Tyndall AFB in May-June of 2021. During the 8-week study, 50mL composite wastewater samples collected over 24 hours, at 2 hour intervals, were processed for SARS-CoV-2 detection. Each sample was pasteurized at 58° C for 30 minutes to inactivate any virus before further sample handling **(Figure 1)**.

Concentration of virions and genomic RNA extraction

Viral particles were concentrated using 700µL of magnetic nanobeads (Ceres Nano) by incubation at room temperature for 20 minutes, magnetic nanobead collection, followed by supernatant discarding. Viral particles were lyzed and RNA extracted using M1 Sample Prep kit (Biomeme). SARS-CoV-2 virions were lyzed through incubation in lysis buffer for 10 minutes at room temperature, followed by genomic RNA binding to syringe resin, and sequential resin washing to remove protein and salt contaminants. Purified RNA was eluted in 0.2mL buffer and concentration determined with Qubit (ThermoFisher).

FIGURE 1. Epidemiology Workflow for Detection of SARS-CoV-2 Virions in Wastewater to Determine the Scale of SARS-CoV-2 Infection in a Defined Catchment Area[a]



[a]Composite wastewater samples collected over a 24-hour period using an autosampler were screened for SARS-CoV-2 RNA using multiplex RT-PCR targeting a conserved Orf1ab region and a variable spike gene. The remaining total nucleic acid extract was used for MiniON nanopore sequencing using a whole genome PCR tilting approach.

Viral titer determination by RT-PCR and MinION sequencing

RT-PCR assay (Biomeme) was performed using SARS-CoV-2 panel targeting Orf1ab gene and S gene. Briefly, 25-100ng of extracted RNA template was added to the lyophilized qPCR mix containing master mix, reverse transcriptase and DNA polymerase enzymes, primers and probes (20µL total reaction volume) under the specific cycling conditions: cDNA synthesis (55 °C for 2 min), polymerase activation (95 °C for 60 sec), PCR (45 cycles of denaturation at 95 °C for 1 sec, and annealing/extension at 60° C for 20 sec). For the SARS-CoV-2 variant assignment, a proprietary variant panel targeted variant-specific mutations and the modified cycling condition of annealing/extension at 62° C for 20 seconds.

To prepare the sequencing library, cDNA synthesis was performed using random hexamers (NEB S1330S) and SuperScript IV reverse transcriptase (ThermoFisher Scientific 18090010), followed by PCR amplification using primers generating 1200 base pairs (bp) amplicons spanning 29 kilobases (kb) SARS-CoV-2 genome in a tiled fashion with a 20 bp overlap. Odd-numbered primer pairs (FWD+REV primer pairs 1,3,5…29) were pooled in equimolar concentration (Primer Pool 1), while even-numbered primer pairs (FWD+REV pairs 2,4,6...28) were grouped in a separate primer pool (Primer Pool 2). Each sample was amplified twice using primer pools 1 and 2 and Q5 Hot Start High-Fidelity Master Mix (NEB M0494), followed by combining amplicons generated with each primer pool for every sample. Following Agencourt AMPure XP beads clean-up (Beckman Coulter) and Qubit quantification, end-repair and dA-tailing of the amplified cDNA samples used NEBNext Ultra II End Repair/dA-Tailing Module (NEB E7546). Barcode ligation used Native Barcoding Expansion (Oxford Nanopore EXP-NBD104), followed by adapter ligation using Ligation Sequencing Kit (Oxford Nanopore SQK-LSK109) to the pooled barcoded samples (equimolar concentration of 100 fM). After the final AMPure XP beads clean-up and Qubit quantification, the sequencing library was loaded on a primed R9.4.1 flow cell (Oxford Nanopore FLO-MIN106D) and sequenced for 8 hours.

Bioinformatics pipeline for sequence analysis and variant calling

Primary data acquisition used MinKNOW operating software (Oxford Nanopore Technologies), while base-calling employed Guppy (Oxford Nanopore Technologies). Processed reads were mapped against SARS-CoV-2 reference (NC_045512.2, Wuhan-Hu-1) with Minimap 2. SAMtools sorted aligned BAM files for coverage data and a consensus sequence.

Results

Portable multiplex RT-PCR pre-screening resulted in identification of SARS-CoV-2 positive samples

A primary sample screening used multiplex RT-PCR assay with 2 different primers-probe sets targeting SARS-CoV-2 Orf1ab and S-gene. Additional primers-probe set targeting MS2 bacteriophage provided an internal process control. RT-PCR analysis for each sample was systematically performed in triplicate using water as a no-template control. Overall, 6 of the 8 samples tested positive for SARS-CoV-2, evidenced by a positive signal in the red (S-gene) and green (Orf1ab) channels, as well as the corresponding amplification plots and Cq values **(Figure 2a)**, which allowed initial sample assessment for the presence or absence of SARS-CoV-2 RNA. RNA process control (RPC) signal corresponding to MS2 bacteriophage RNA (amber channel) allowed a semi-quantitative estimation of SARS-CoV-2 titer relative to the MS2 phage particles based on corresponding Cq values. Overall, 4 samples contained higher levels of genome copies/mL relative to the internal control **(Figures 2b-c)**, which was consistent with high levels of COVID-19 cases reported at the installation and throughout Florida during the sampling period.

MinION nanopore sequencing using whole genome PCR tiling approach resulted in a comprehensive mutational profiling of SARS-CoV-2 variants

Wastewater samples that tested positive for SARS-CoV-2 RNA were sequenced by tiled amplicon sequencing, while lineage assignment and genome coverage assessment with ARTIC+NextClade bioinformatics pipeline identified distinct SARS-CoV-2 variants in all 6 wastewater samples **(Figure 3)**. In comparison to the previous ARTIC SARS-CoV-2 sequencing workflow that generated 400 bp amplicons, fewer primer pairs generating longer amplicons resulted in good genome coverage and unambiguous lineage assignment while maintaining good overall sequencing yield and read quality **(Table)**. A comprehensive mutational profile of SARS-CoV-2 isolates was obtained by whole genome sequencing

FIGURE 2a. Portable Multiplex RT-PCR for Wastewater Sample Screening and SARS-CoV-2 Detection[a]

[a]RNA extracted from wastewater was used as a template (25-50ng per 20uL reaction) for RT-PCR using assays targeting open reading frame 1a and 1b (1ab, green) and spike protein gene (S, red). Assay targeting MS-2 phage (amber) were used as internal RNA Process Control (RPC).

FIGURE 2b. SARS-CoV-2 Genetic Biomarker Levels in Wastewater Samples

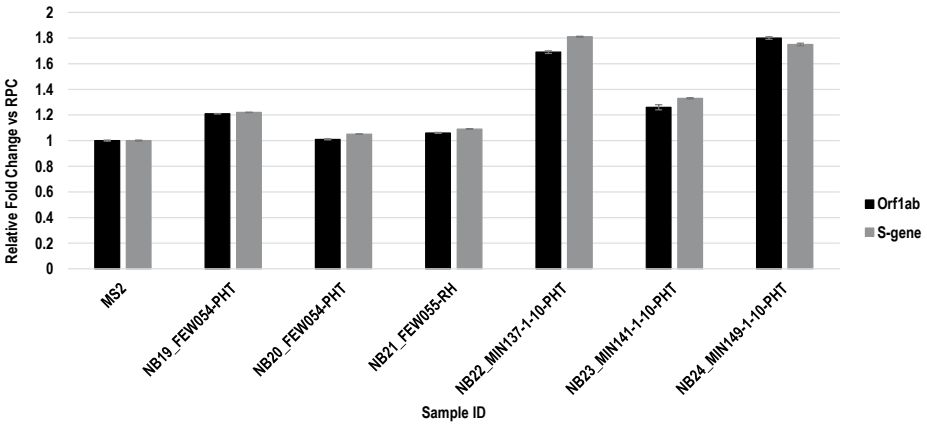
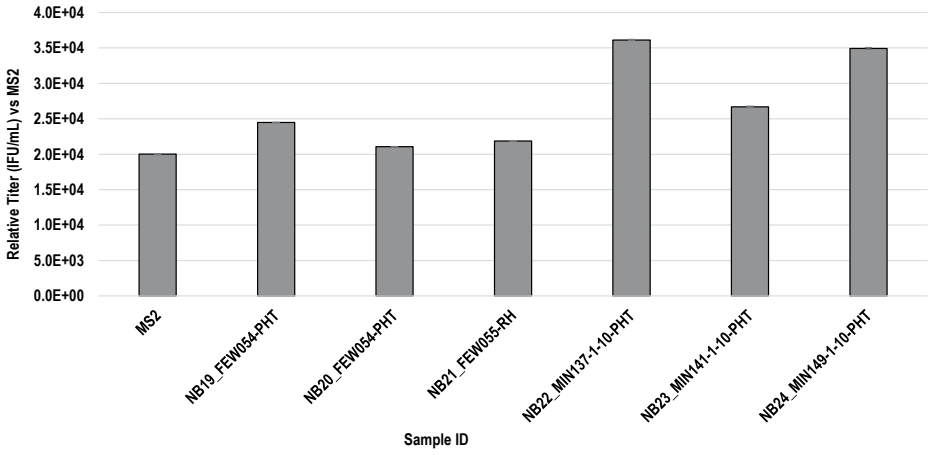


FIGURE 2c. SARS-CoV-2 Concentration in Wastewater Samples



with varying degrees of nucleotide substitutions, but no deletions or insertions. Lineage-defining amino acid substitutions in the Spike protein were generated using NextClade bioinformatics analysis and included previously uncharacterized mutations **(Figure 3)**. Interestingly, in addition to lineage-defining mutations, additional mutations both in the Spike protein as well as other genomic regions were identified within variants with a defined lineage, e.g. B.1.1.7 (Alpha), thus reflecting the complexity of wastewater samples and genetically divergent SARS-CoV-2 isolates.

SARS-CoV-2 Delta specific RT-PCR assay confirmed lineage assignment by targeting variant defining mutations in the S-gene

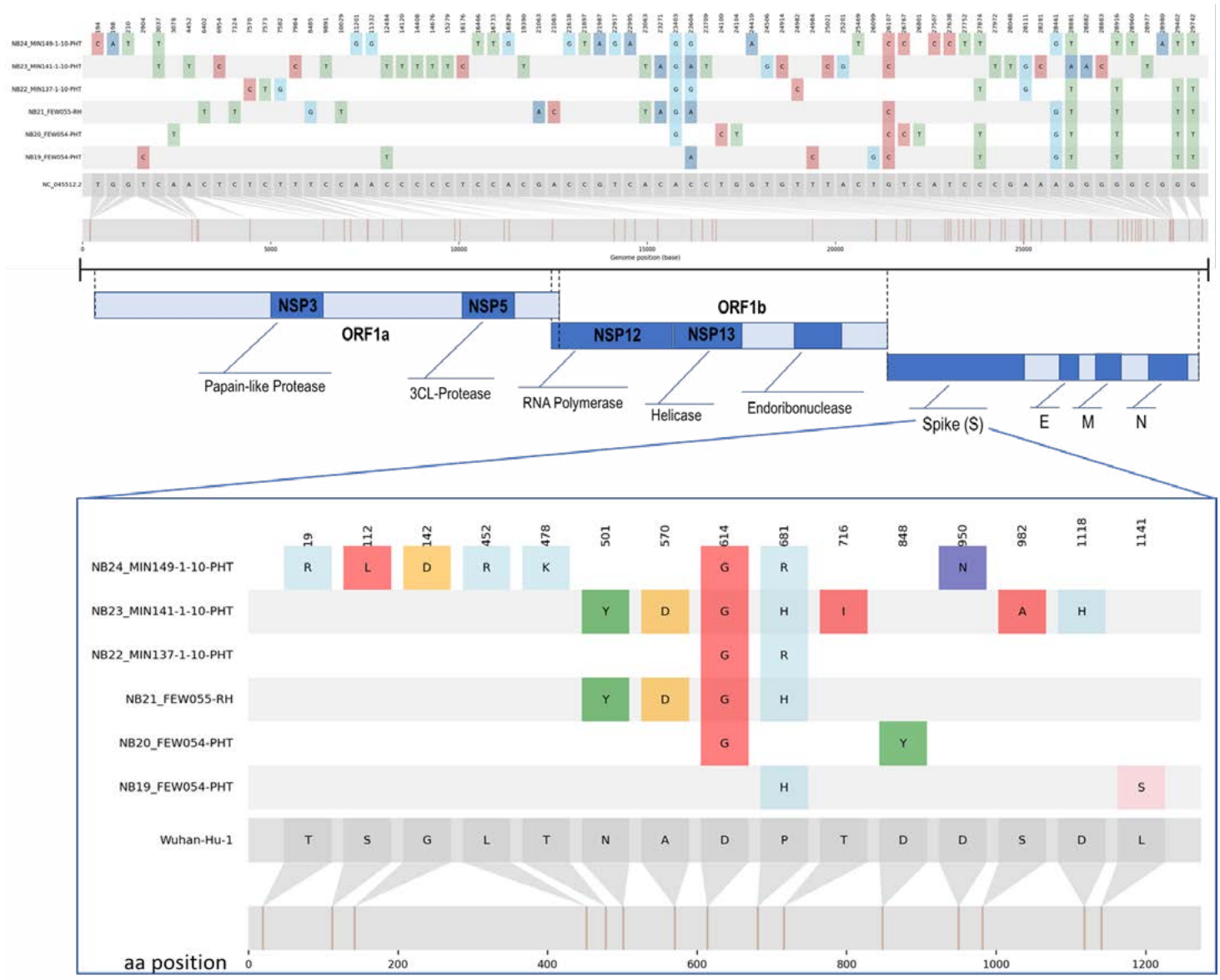
Lineage assignment of SARS-CoV-2 variants determined by MinION sequencing was confirmed using a Delta RT-PCR panel of assays targeting mutations specific to the Delta variant **(Figure 4)**. When the wastewater sample NB24_MIN149-1-10-PHT containing SARS-CoV-2 Delta variant, determined by MinION sequencing, was assayed with Delta-specific RT-PCR triplex, the observed signals in the green and amber channels targeting 681R and 452R mutations, respectively, combined with a lack of signal in the red channel targeting deletion at position 156, confirmed unique mutations previously identified with MinION sequencing.

Discussion

In this study, 8 composite wastewater samples were collected, using an autosampler for a 24-hour preiod each week, from a wastewater lift station servicing a military installation. Each sample was screened for SARS-CoV-2 RNA using multiplex RT-PCR targeting a conserved Orf1ab region and variable Spike gene. This method resulted in unambiguous identification of 6 SARS-CoV-2-positive samples, using very low input RNA.

Relatively high SARS-CoV-2 viral titers detected in the wastewater provided good sequencing output, as evidenced by the number of sequencing reads obtained from each sample that tested positive.

FIGURE 3. SARS-CoV-2 Whole Genome Sequencing (WGS) and Variant ID Using Portable MinION Nanopore Platform[a]



[a] RNA from wastewater samples previously tested positive by RT-PCR for SARS-CoV-2 was used as template for cDNA synthesis and sequencing library preparation. Whole genome amplification was accomplished using Midnight primers spanning SARS-CoV-2 genome in a tiled fashion generating 1200 bp amplicons with a 20 bp overlap. Followed by barcoding and sequencing adapter addition. Sequencing was performed on a MinION Mk1B device using R9.4.1 flow cell.
Lineage defining amino acid (aa) mutations in the Spike protein relative to the Wuhan-Hu-1 reference are indicated in the inset.

TABLE. MinION Mk1B Sequencing Output and Genome Coverage

SampleID	N50 (bp)	Read counts (kb)	% Genome coverage	Pango lineage	WHO label
NB24-MIN149-1-10-PHT	1,182	943	85.88	B.1.617.2	Delta
NB23-MIN141-1-10-PHT	1,179	690	80.70	B.1.1.7	Alpha
NB22-MIN137-1-10-PHT	1,171	800	59.80	B.1	
NB21-FEW055-RH	1,149	420	61.88	B.1.1.7	Alpha
NB20-FEW054-PHT	1,143	400	64.62	B.1	
NB19-FEW054-PHT	1,159	550	59.68	B	

Abbreviations: bp, base pair; kb, kilobases; WHO, World Health Organization.

The RNA material was of sufficient quantity and purity for whole genome tiled amplicon sequencing using long-read nanopore sequencing, allowing several assays for comprehensive SARS-CoV-2 characterization from a single sample source.

Based on this proof-of-concept study, the combination of portable RT-PCR and MinION sequencing for nucleic acid detection can reduce the time for sampling-to-pathogen identification from days to hours (under 10 hours from wastewater sample concentration to SARS-CoV-2 variant identification in the current study) if sampling at the source and circumventing sample transport to a research facility. This method potentially avoids informational loss due to viral instability in the absence of the host, or RNA instability in the absence of the virus.

Lineage ID assignment with ARTIC+Nextclade bioinformatics pipeline allowed identification of variants of concern including Delta and Alpha of the Wuhan-Hu-1 strain originally isolated at the onset of the pandemic. Similarly, 5 of 6 isolates contained P681 mutation in the furin cleavage site (P681 RRAR) at the S1/S2 junction of the Spike protein. P681 mutation renders furin cleavage site less acidic and increases recognition and cleavage efficiency by the furin enzyme, resulting in greater SARS-CoV-2 infectivity. The other notable Spike protein mutations identified with MinION nanopore sequencing included N501Y and L452R, located in the receptor-binding domain and thought to be responsible for more efficient RBD-ACE2 binding and increased viral infectivity.[22-27]

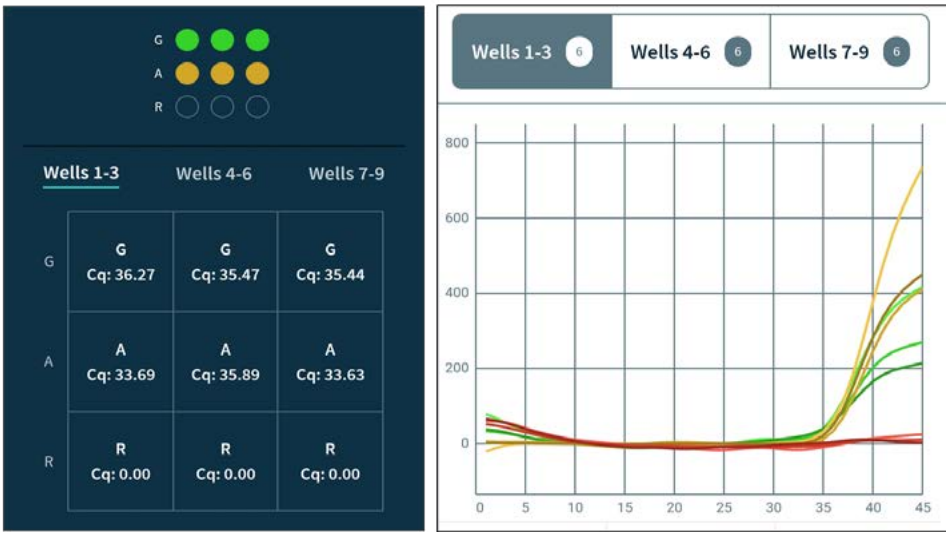
The presence of S-gene mutations identified with MinION nanopore sequencing was confirmed by a variant-specific RT-PCR assay targeting lineage-defining mutations in the gene-encoding Spike protein. A Delta variant panel targeting 452R, 681R, and 156/157del mutations in the Spike protein that confirmed variant ID assignment was found consistent with the sequencing data, as evidenced by the amplification of the regions encoding the 452R and 681R mutations and absence of signal corresponding to the 156/157 deletion. Interestingly, whole-genome sequencing revealed additional non-lineage-defining mutations in the Spike protein, specifically 112L, indicating the utility of whole-genome sequencing in the SARS-CoV-2 variant tracking and newly emerging variant evolution. It is unclear whether 112L mutation is the consequence of genetic viral diversification from animal reservoirs or human intrahost-dependent viral recombination, as the corresponding clinical SARS-CoV-2 specimens were not available for comparison. Notably, 112L Spike protein mutation is not found in any other variants of concern and has not been linked to any of the evolutionary advantageous viral phenotype traits such as higher infectivity and transmission, more effective replication and potentially increased disease severity due to higher viral burden, or more efficient evasion of host defenses.

At the height of the pandemic, and during specific variants of concern surges such as Delta or Omicron, on average 5-10% of the symptomatic population was tested daily through PCR-based methods or antigen tests, depending on the size of the installation and corresponding population size. This strategy is both cost-prohibitive and labor-intensive for extended periods of time. In contrast, wastewater SARS-CoV-2 levels can be monitored continuously, as demonstrated by this study, on a weekly basis and cost-effectively. A single wastewater sample can be assayed with 1 test per sample to capture a majority of the population, including symptomatic and asymptomatic cases. It is cost prohibitive to continue randomly testing up to 10% of the population when numbers of new cases approach baseline, but continuous monitoring of SARS-CoV-2 levels in wastewater can provide early indicators of SARS-CoV-2 resurgence due emergences of new variants of concern.

Some of the potential limitations of this wastewater biosurveillance include the inability to capture 100% of the population, particularly for more targeted (e.g., building-level) biosurveillance, as an entire population cannot effectively contribute to a sewer shed, particularly from any given building on a sampling day. Wastewater surveillance sustainability at DOD installations will depend upon the ability to expand beyond COVID-19 to include additional pathogens of public health concern. Initial training of bioenvironmental engineering staff is required to make portable WBE feasible.

Overall, portable wastewater SARS-CoV-2 biosurveillance is a sustainable, highly informative, and effective methodology for continuous population monitoring because it can be implemented at the outbreak source, facilitating outbreak containment by providing public health

FIGURE 4. Multiplex RT-PCR for SARS-CoV-2 Variant Resolution at the Lineage Level[a]



[a]Following nanopore sequencing, remaining RNA extracted from wastewater samples was used as template (25ng per 20uL reaction).

For RT-PCR using assays targeting Delta-specific mutations in the Spike gene: 681R (green channel), 452R (amber) and 156/157del (red).

responders with actionable, data-driven information for health force protection more expeditiously.

This study demonstrates the utility of portable RT-PCR and nanopore sequencing platforms for rapid SARS-CoV-2 detection and variant identification at the lineage level, effectively applying this highly multiplexed technology for genomic biosurveillance and public health response to current and future pandemics. Nanopore sequencing in conjunction with variant-specific RT-PCR was validated as an effective method for rapid identification and tracking of the distribution and prevalence of SARS-CoV-2 variants at military installations, which can lack clinical laboratory capabilities while experiencing potential outbreak clusters for newly emerging variants of concern.

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Disclaimer: The views expressed are those of the authors and do not reflect the official guidance or position of the United States Government, the Department of Defense, nor the United States Air Force.

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Increasing Incidence Rates of Eosinophilic Esophagitis in Active Component Service Members, U.S. Armed Forces, 2009–2021

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Eosinophilic esophagitis (EoE) is characterized by symptoms associated with dysfunction of the esophagus due to chronic mucosal eosinophilia and inflammation.[1,2] Environmental and food allergens, enhanced type 2 helper T cell (Th2) activity, genetic predisposition, impaired esophageal epithelial barrier, and potential for fibrosis have all been implicated in EoE.[1,3] Predominant symptoms in adults include dysphagia and food impaction, with 1 systematic review indicating symptom prevalence ranging from 29-100% and 25-100% respectively.[1,4] Treatment is focused on the triad of dietary modifications, medications, and dilation to control symptoms and restore normal esophageal function.[1]

The reported prevalence of EoE has been increasing worldwide, with a recent meta-analysis estimating 34.2 cases per 100,000 persons.[5] Subgroup analysis of North American adults reveals similar rates, at 31.9 cases per 100,000 adults, with individual studies on U.S. adults ranging from 9.45 to 58.9 cases per 100,000.[5] The incidence of EoE has been increasing over the past several decades, and while this phenomena is at least partially due to increased awareness and interest in the condition, some studies report that the rate of EoE has disproportionately risen with the increased rate of biopsies during the same study periods, suggesting a true increase in EoE.[6,7]

EoE is more common in men, those of White race/ethnicity, and those with atopic disease.[8] It can present at any age, but the majority of cases occur among children, adolescents, and adults under 50 years.[8] Because the majority of the active component military is comprised of White men under 50 years, EoE may be an important contributor to the burden of disease in this population. This study examines the incidence of EoE among active component service members (ACSM) to characterize the disease impact on this population, and evaluate change in the incidence over the study period. EoE is disqualifying for accession into military service due to potential for uncontrolled symptoms or food impactions in austere or medically limited environments; consequently, new service members should not have a diagnosis of EoE.[9]

Methods

The surveillance period covered January 1, 2009 through December 31, 2021. The surveillance population included all ACSM of the Army, Navy, Air Force, and Marine Corps. The data used to determine incident cases of EoE were derived from the Defense Medical Surveillance System (DMSS), which documents both ambulatory encounters and hospitalizations of active component members of the U.S. Armed Forces in fixed military and civilian (if reimbursed through the Military Health System) clinics and hospitals.

An incident case of EoE was defined by 2 outpatient medical or Theater Medical Data Store (TMDS) encounters within 365 days of each other or 1 hospitalization with a diagnosis of EoE in any diagnostic position (ICD-9: 530.13, ICD-10: K20.0). The incident date was defined as the date of the first hospitalization or outpatient medical encounter that included a defining diagnosis of EoE. ICD codes were used to define cases because histologic data were not available and previous studies indicated high specificity of the ICD codes.[10,11]

Results

The 7,592 incident cases of EoE among ACSM during the 2009 to 2021 surveillance period resulted in an overall incidence rate of 43.5 cases per 100,000 person years (p-yrs). Crude (i.e., unadjusted) incidence rates for selected covariates in 2021 are shown in **Table 1**. In 2021, the incidence rate of EoE among men was more than twice than the rate among women. The rate of EoE increased with each older age category, with the highest rates in those over the age of 40. Among racial/ethnic groups, the highest rate of EoE was among non-Hispanic Whites. Service members of the Air Force had the highest rate, and those in the Marine Corps had the lowest, while the rate among officers was almost twice the rate for enlisted members. Pilots and aircrew had the highest rates among occupation groups, followed by health care workers.

Incidence rates steadily climbed throughout the surveillance period, from 21.2 cases per 100,000 in 2009 to 62.4 cases per 100,000 p-yrs in 2021 **(Figure 1)**. Rates among the 2 oldest age categories increased at a faster rate than service members in their 20s **(Figure 1)**. Incidence rates among men increased at a higher rate compared to rates among women **(Figure 2)**. In addition, the incidence rate increased most notably among non-Hispanic Whites during the surveillance period **(Figure 3)**.

Discussion

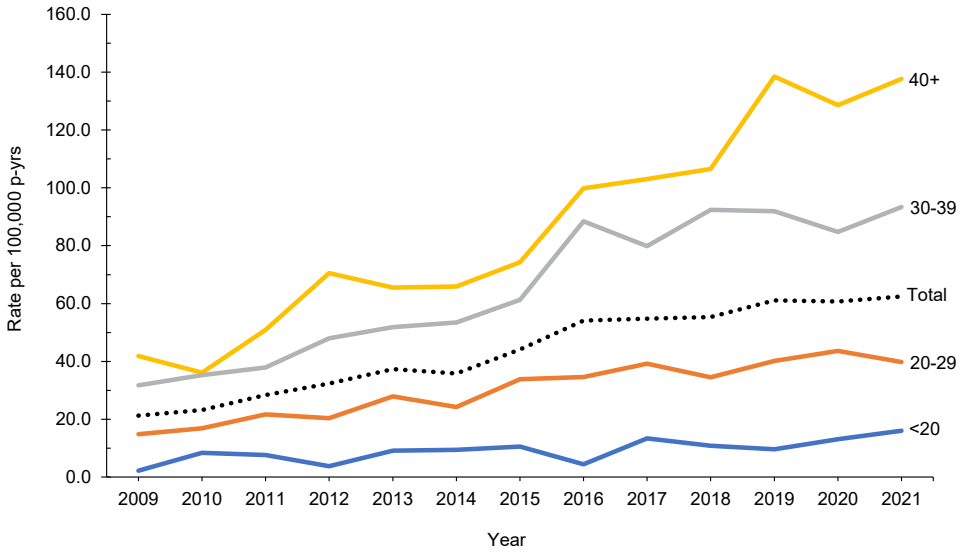
The results of this study show a steady increase in the incidence of EoE among ACSM between 2009 and 2021. Previously published literature has indicated that the incidence and prevalence of other Th2 type allergic and atopic diseases are increasing among ACSMs.[12] Although there is variability in methodology, multiple studies demonstrate the increasing incidence and prevalence of EoE in a variety of populations studied worldwide.[13-15] The increasing trend

TABLE 1. Incidence Rates of Eosinophilic Esophagitis in Active Component Service Members, 2021

	No.	Rate[a]
Total	831	62.4
Sex		
Male	763	69.4
Female	68	29.5
Age group, y		
<20	15	16.0
20-29	294	39.8
30-39	346	93.4
40+	176	137.7
Racial/ethnic group		
Non-hispanic White	643	88.5
Non-hispanic Black	45	20.9
Hispanic	75	31.8
Other/unknown	68	44.3
Military rank		
Enlisted	595	54.3
Officer	236	100.8
Branch of service		
Army	286	59.7
Navy	200	58.4
Air Force	250	76.0
Marine Corps	95	52.8
Primary occupational category		
Combat-specific[b]	95	51.5
Motor transport	13	31.9
Pilot/air crew	55	117.6
Repair/engineering	266	67.3
Communications/ intelligence	169	59.4
Health care	95	85.0
Other	138	51.6

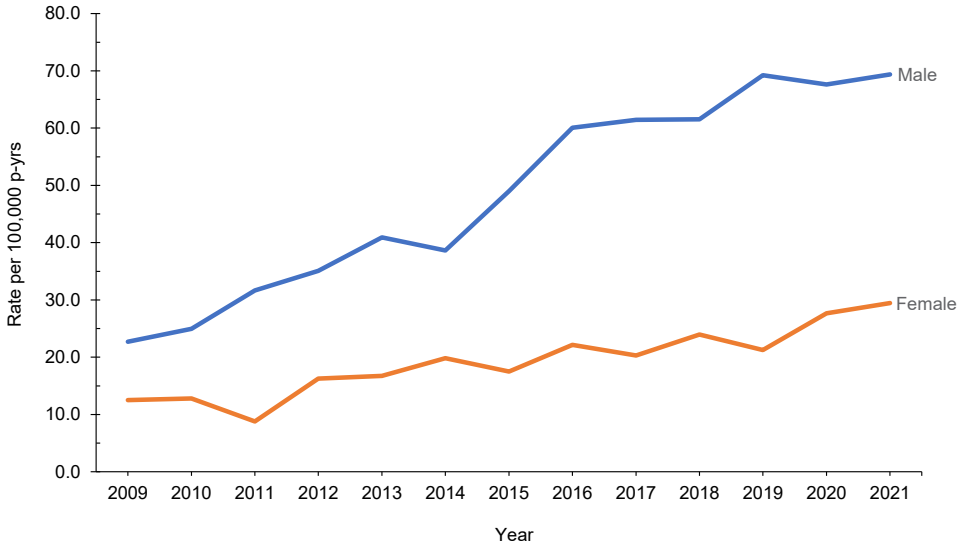
Abbreviation: No., number.
[a] Rate per 100,000 person-years.
[b] Includes infantry, artillery, combat engineering.

FIGURE 1. Incidence of Eosinophilic Esophagitis in Active Component Service Members by Age Group, 2009-2021



Abbreviation: P-yrs, person-years.

FIGURE 2. Incidence of Eosinophilic Esophagitis in Active Component Service Members by Sex, 2009-2021



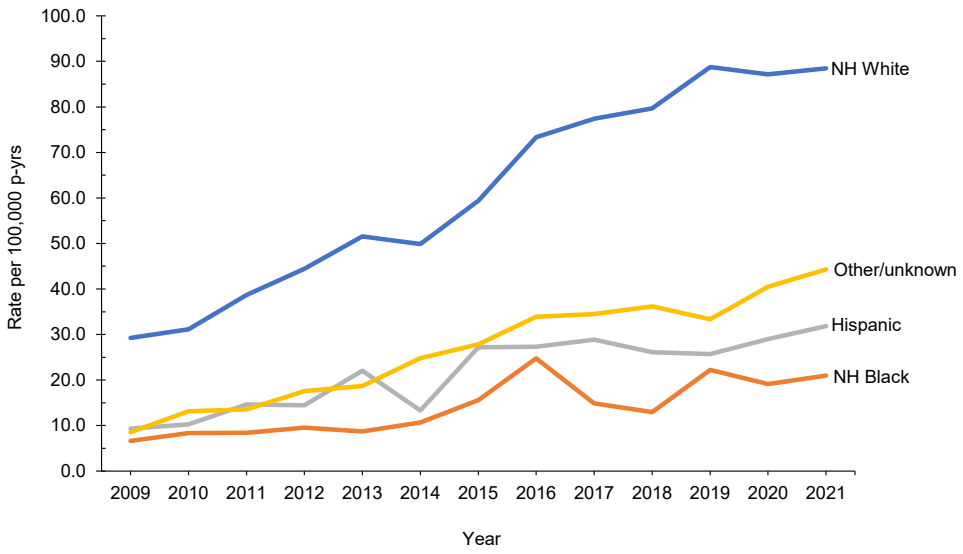
Abbreviation: P-yrs, person-years.

is largely unexplained and likely not due to increase disease recognition alone.[6,7,14]

Although the incidence rates presented in this study are in accordance with findings from some studies in the U.S. adult population with similar methodology, these rates are higher compared to other studies in the U.S. and Europe that used more stringent case definitions, such as pathologist-validated reports.[13,15-17] The demographic risk factor results of this study are consistent with published literature, with higher incidence rates of EoE in men, non-Hispanic Whites, and adults in their 30s and 40s.[18] ACSMs are predominantly younger (55% are in their 20s), White (69%), and male (83%), which may account for the higher rates in this study compared to others. With universal health care in the military, more members may have access to care than in other U.S. studies, which could also account for the increased rates identified in this study. Military members may seek medical care towards the end of the career to prevent career-ending medical evaluations, which could contribute to the highest rates within the oldest age range. This study did not attempt to identify specific occupational, environmental, or demographic risk factors among ACSM, which would require additional analyses.

As with all studies that utilize administrative data, this study is reliant upon the coding of medical encounters. Incident cases in this study may have been ruled out later by biopsy, which would have overestimated incidence rates. A full review of pathology

FIGURE 3. Incidence of Eosinophilic Esophagitis in Active Component Service Members by Racial/Ethnic Group, 2009-2021



Abbreviation: P-yrs, person-years.

reports or chart reviews would need to be conducted to validate all cases. A prior study, however, found that the use of the ICD-9 code (530.13) is very specific for EoE (99% specificity), which would suggest that most cases identified by ICD codes are true cases.[10]

The incidence of EoE among ACSM should continue to be monitored, as the rate did not appear to plateau during the surveillance period despite a reduction during the first 21 months of the COVID-19 pandemic, when medical care was less available for non-urgent conditions. As more ACSM are diagnosed with EoE, complications from treatment and need for periodic endoscopic interventions will increase. Management options are currently limited, with only 1 FDA-approved biologic, dupilumab, for the treatment of EoE—and ongoing biologic therapy is disqualifying for military service. Other treatments include proton pump inhibitors and topical "swallowed" corticosteroids (asthma inhalers) that are used off-label for EoE.[19] Strictures require endoscopic dilation, which has associated risks of anesthesia and procedural complications. More studies are needed to evaluate specific and modifiable exposures such as pollutants, diet, infectious diseases, and environmental allergens that may be associated with EoE.[20-22]

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