Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Major Article

SARS-CoV-2 genomic surveillance as an evidence-based infection control approach in an offshore petroleum employee population

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Background: Industrial hygienists (IH) in the oil and gas business instituted an extraordinary number of safety protocols to limit spread of SARS-CoV-2 onto offshore platforms in the Gulf of Mexico. We used genomic surveillance to provide actionable information concerning the efficacy of their efforts.
Methods: Over 6 months, employees at a single company were serology and PCR tested during a 1-5 day predeployment quarantine and when postdeployment symptoms were reported. From each positive test (n = 49), SARS-CoV-2 genomes were sequenced. Phylogenetic analysis was used to investigate the epidemiology of transmissions.
Results: Genomic surveillance confirmed 2 viral strains were infecting 18 offshore workers. Genomic data combined with epidemiological data suggested that a change in quarantine protocols contributed to these outbreaks. A pre-deployment outbreak involved a WHO variant of interest (Theta) that had infected 4 international workers. Two additional predeployment clusters of infections were identified.
Conclusions: Our findings support that IH quarantine/testing protocols limited viral transmissions, halted offshore outbreaks, and stopped the spread of a variant of interest. The study demonstrates how genomic data can be used to understand viral transmission dynamics in employee populations and evaluate safety protocols in the offshore oil and gas industry.

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has presented many industries with new and complex challenges, particularly for industrial hygiene (IH) and occupational health and safety (OHS) professionals who assess and manage the risk of SARS-CoV-2 spread in the workplace.[1] SARS-CoV-2 is an especially pernicious pathogen, as the transmission is primarily airborne, asymptomatic individuals can transmit the virus, and the duration of infectiousness varies among individuals and viral variants.[2-5] Studies suggest that the shortest and longest incubation period is estimated to be 2 and 14 days respectively. The median incubation period is estimated to be 4.91-7.54 days.[6,7] Individuals with long incubation periods occur more often in the elderly, are less symptomatic, and are less likely to transmit the virus.[8] It is estimated that 59% of infections come from asymptomatic transmissions, 35% from pre-symptomatic, and 24% from individuals that never develop symptoms.[3]

These factors pose uniquely challenging issues for the oil and gas industry, where SARS-CoV-2 infections have major consequences for workers on offshore platforms. Confined workspaces enable the virus to quickly spread among employees, who are highly mobile and travel internationally.[9] Increased infection rates may result in skeleton crews managing complex and dangerous machinery, which intensifies the risk for on-the-job injuries or chemical spills.[10] Furthermore, flights for remote medical evacuation and diagnostic

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testing crews greatly increase the risks of injury[11,12] and the financial burden on the industry.[13] Given the essential nature of the oil and gas industry, and the significant financial and safety interests in limiting spread and mitigating outbreaks, the impact of COVID-19 on offshore platforms could have a devastating effect if left unabated.

Pathogen sequencing (WGS) can modernize the work of IH and OHS professionals by providing objective information about how pathogens spread in the workplace. Because WGS objectively shows when transmission occurs, it can elucidate the effectiveness of methods used to reduce viral transmission including airflow systems, social distancing, and contactless delivery systems. Furthermore, WGS provides information concerning the impact of different viral variants on pathogenesis, transmissibility, and vaccine efficacy. The COVID-19 pandemic has been particularly transformative by driving home the power of WGS, and genomic surveillance is increasingly used in infection control and prevention in hospitals as well as by public health entities.[14-21] However, WGS has yet to be widely adopted by other high-risk industries where there is potential to improve workplace safety.

In early 2021, an oil and gas company with offshore platforms in the Gulf of Mexico enlisted the services of a molecular biology research lab to implement genomic surveillance of SARS-CoV-2. The IH department of the company already employed extensive COVID-19 prevention protocols which were designed to identify individuals infected with SARS-CoV-2 prior to deployment to offshore platforms, reduce transmissions to co-workers and their families, and lessen the risk of depression or death. Pre-deployment procedures included multi-day hotel-based quarantines, surface disinfection, and serological and PCR-based testing prior to worker deployment. However, even with these protective measures, an offshore COVID-19 outbreak involving 18 workers led the IH department to initiate genomic surveillance to determine if a source or sources could be identified. Subsequently, the IH department implemented WGS of SARS-CoV-2 as a standard operating procedure for ongoing monitoring of pre- and post-deployment employee safety policies during the evolving pandemic. Here we report our findings during 5 months of observation.

METHODS

Employee population and quarantines

Employees and contractors for the company generally resided across Gulf Coast communities; however, some employees resided in a variety of national and international locations. Unsupervised quarantines were initiated early in the pandemic to reduce the interactions of employees with the broader population. Also, as PCR tests are more accurate 2-3 days-postinfection, a short quarantine would improve the likelihood that COVID-19 positive individuals would be identified prior to offshore deployment. Quarantines took place at 1 of 4 hotels where employees were instructed to remain in their rooms except for mealtimes. Food was catered from outside restaurants and employees had the option to eat with other employees outside or go back to their rooms. Presumably some interaction with hotel staff occurred.

At the start of this study, the length of quarantine at Hotel B was 2 weeks. The length of the quarantine at Hotel A had recently changed to only 1 day following a negative serology and PCR test. Testing protocols were administered prior to deployment for the majority of the population, however, there were occasions that necessitated individuals deployed to rigs without tests, for example, healthcare workers or individuals responding to other emergency situations. This study involved individuals that deployed to 1 of 4 offshore platforms. The employee population base incorporated approximately 300-400 individuals across 4 platforms. The information we obtained for most individuals included quarantine hotel, platform designation, and in some cases, contracting company. During the 2019-2020 pandemic, employees typically deployed for 3 weeks.

Data management and industrial hygiene protocols

A case management system was used to document cases, symptoms, and actions taken in association with any COVID-19 positive case. Prior to deployment offshore, employees were quarantined at 1 of several hotels for up to 7 days. During that time, employees were given a serology test (see details below), which detects antibodies to the virus, and a reverse-transcription quantitative PCR testing (RT-qPCR) test, which detects viral RNA associated with an active infection (Fig 1). The timing of these tests varied as the pandemic evolved. If an employee was serology-negative and PCR-negative, they exited quarantine and were deployed offshore. If an employee had a previous infection but had finished a home-based self-quarantine, they were typically serology-positive and PCR-negative, so they were cleared for deployment.[22] Serology-negative and PCR-positive employees were isolated, interviewed to identify other employees that they may have come into contact with, and given follow-up care instructions prior to sending home. The leftover testing sample in viral transport media (VTM) was shipped for sequencing.

Once deployed to the offshore platform, employees who presented with COVID-19 symptoms were isolated and a nasopharyngeal swab in VTM was shipped for RT-qPCR testing. If positive, diagnostic teams were flown to the platform and the entire available population was retested. All positive cases were medevacked from the platform for a 10-day inland quarantine. Some individuals could have been missed during offshore testing efforts due to the nature of the industry and travel across platforms in the employee and contractor population.

Serology and quantitative PCR testing

Serology tests were used to identify the presence of IgG and/or IgM antibodies. The Rapid ICT POS (Aytu BioScience) serology test was used, which is performed via a finger stick, provides results in 15 minutes. Nasopharyngeal swabs were collected in VTM and shipped overnight to a diagnostic testing laboratory. The diagnostic laboratory performed RT-qPCR on extracted viral RNA using FDA Emergency Use Authorization (EUA) approved assays for SARS-CoV-2 S, N and Orf1ab genes, based on Applied Biosystems TaqMan 2019 nCOV assays, and a human control using the manufacturer's protocol. In some cases, BioFire Respiratory 2.1 (RP2.1) Panel was also used for SARS-CoV-2 detection.

Study samples

Left-over VTM samples that were positive for SARS-CoV-2 were de-identified and sent to BioInfoExperts for further studies (http://www.bioinfox.com). The total number of samples received included the initial 19 pre- and postdeployment cases from the first suspected outbreak, as well as an additional 30 samples from individuals who tested positive over the next 5 months. Some of these samples were from individuals who had previously tested positive, completed a quarantine, and tested positive again. These samples were classified as "remnant positives," which are likely non-infectious;[23] however, in 2 of these cases we were able to generate a full viral genome. Metadata collected for each de-identified employee included hotel-quarantine start date, testing date and result, and date and location of platform deployment. This study was performed under WCG IRB #1-1455346-1.

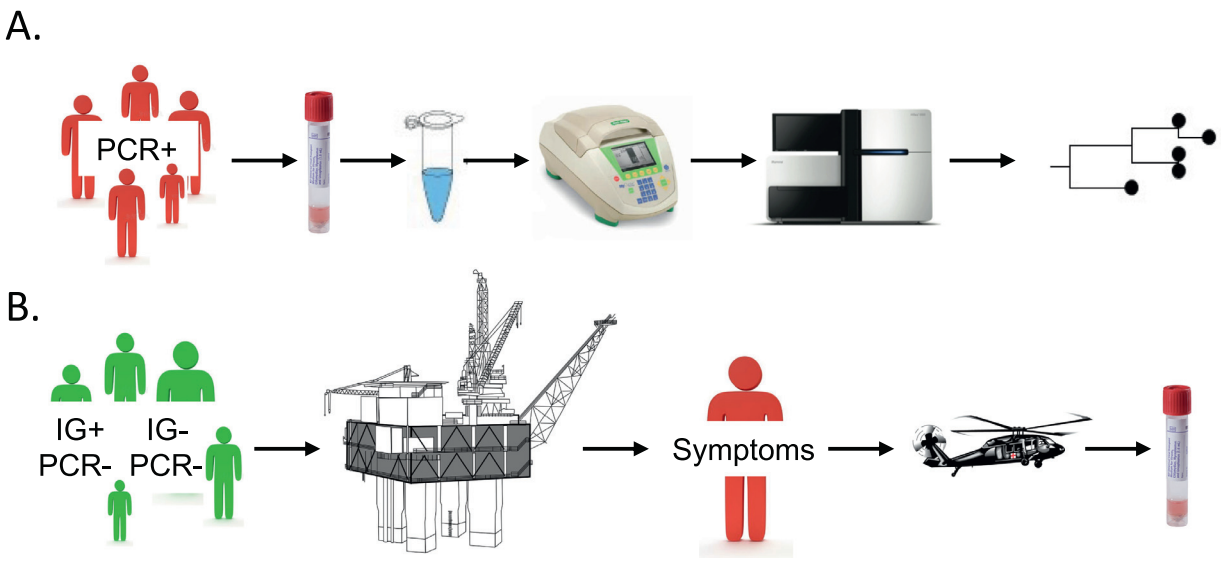

Fig 1. Predeployment quarantine of employees, routine serology, and rtPCR monitors for new SARS-CoV-2 infections in employee population. (A) All PCR+ cases are considered active infections and procedures are immediately initiated to avoid consequences of infection. Leftover nasopharyngeal samples in VTM are shipped to molecular biology lab where they are lysed and viral RNA isolated. SARS-CoV-2 genomes are amplified using the ARTIC PCR protocol and a whole genome sequence is generated. The results are uploaded into a HIPPA-compliant cloud-based server where the Pango lineage is assigned, and genetic distance analysis is performed. IH personnel can log on to a secure portal to review information about potential outbreaks. Turn-around time from sample to report is typically 5-10 days postsampling. (B) During quarantine, individuals who are antibody positive and PCR negative are typically those that had reported a previous infection, completed quarantine, and are no longer shedding virus; those that are antibody negative and PCR negative are not considered infected. In either case, these employees are allowed to deploy offshore. Should symptoms appear on the platform, they are immediately retested, and if positive, they are medevacked off the platform and the leftover nasopharyngeal sample in VTM is shipped to molecular biology lab for whole genome sequencing and analysis.

SARS-CoV-2 amplicon sequencing

SARS-CoV-2 sequencing methods were based on the ARTIC network nCoV-2019 V3 primer scheme using 2 multiplexed primer pools to create overlapping 400 bp amplicon fragments in 2 PCR reactions. A detailed version of this protocol can be found here: https://andersen-lab.com/secrets/protocols/. Briefly, viral RNA was extracted using the Quick-RNA Viral Kit (Zymo Research) according to the manufacturer's instructions using 400uL VTM from the nasopharyngeal swabs. SARS-CoV-2 RNA was reverse transcribed with SuperScript IV (Invitrogen). The virus cDNA was amplified in 2 multiplexed PCR reactions (1 reaction per ARTIC network primer pool) using Q5 DNA High-fidelity Polymerase (New England Biolabs). Following an AMPureXP bead (Beckman Coulter) purification of the combined PCR products, the amplicons were diluted, and libraries were prepared using NEBNext Ultra II DNA Library Prep Kits (New England Biolabs). The libraries were purified with AMPureXP beads and quantified using the Qubit High Sensitivity DNA assay kit (Invitrogen) and Tapestation D5000 tape (Agilent). Libraries were normalized and pooled in equimolar amounts at 2 nM. The 2 nM library pool was sequenced with the Illumina MiSeq using a MiSeq reagent kit V3 600 cycles (Illumina).

Data analysis

Raw data from 49 samples were processed on the FoxSeq v.4.0 (http:/foxseqllc.com) analytical pipeline, which automates the following steps: for each sample, raw reads were filtered using *Trimmomatic*[24] based on read length and mapped to the SARS-CoV-2 reference genome (NC045512.2/Wuhan-Hu-1/2019) using Bowtie2.[25] Mapping quality statistics were generated using *picard* (http://broadinstitute.github.io/picard). Bases were called for each position using *bcftools mpileup* (https://github.com/samtools/bcftools) and filtered if the depth was <50 and/or the frequency of either the reference or the alternative allele was <80%. A consensus sequence was generated for each sample using *bcftools consensus*. Pango lineages[26] were assigned using Pangolin 3.06 with the PangoLEARN algorithm (https://github.com/cov-lineages/pangolin). Genetic distances were calculated using *dnadist* in the ape package in R. Results from analytical pipeline, including sequence quality, pangolin lineage, and distance clustering was delivered automatically in a web-based password-protected portal, which provided evidence of outbreaks to IH personnel in <2 weeks. For outbreak confirmation, maximum-likelihood phylogenetic trees were inferred using IQTREE v2[27] and visualized using FigTree v1.5.

RESULTS

COVID-19 positive cases

Early in 2020, despite testing and quarantine measures, an individual on a deep water reported the onset of COVID-19 symptoms (Day 0) and 1 day later (Day 1), a second individual on the same platform also presented with COVID-19 symptoms. Over a 4-day post-onset period (Day 1 – Day 4), a total of 19 of their offshore co-workers tested PCR-positive for SARS-CoV-2. In order to understand if the infections came from an identifiable source, IH implemented whole genome sequencing as a standard operating procedure for PCR-positive workers. Over a total of 5 months, 30 additional positive samples were collected and sequenced, including another expected transmission among 4 individuals that had travelled together from the Philippians. We used whole genome sequencing and phylogenetic analysis to identify the epidemiological history of these infections.

Genome sequences

We generated near-full length SARS-CoV-2 genome sequences for all PCR-positive samples (n = 49). On average, the mean coverage for all genomes was ~13K, with >97% of all sites having at least 20x coverage.

Genomic epidemiology

To identify genetically related infections, we inferred a phylogenetic tree of high-coverage viral genomes. We defined an "Outbreak" as: (1) sequences that grouped together on the tree with high support (>70%); (2) sequences were separated by <2 mutations on average; and 3) outbreaks contained >2 individuals. We also classified sequences into Pango lineages, which is a widely used naming system for describing geographic origin and spread of SARS-CoV-2 variants.[28]

We found evidence for 3 potential distinct and genetically related outbreaks (Fig 2). Outbreak #1 consisted of 5 individuals with infections assigned to Pango lineage B.1.234. Outbreak #2 consisted of 13 individuals, all of whose infections were assigned to Pango lineage B.1.2. Every individual in these two outbreaks were already deployed to a platform. Outbreak #3 consisted of 4 individuals assigned to the relatively rare lineage, P.3. All 4 individuals in Outbreak #3 were identified and treated before leaving the hotel (predeployment).

There were 2 other clusters of sequences in the tree that did not meet the definition of an outbreak. Cluster #1 consisted of 3 sequences although 2 of them were from the same person sampled 15 days after the first sample was taken (remnant positive). Cluster #2 contained 4 sequences from 3 people, all of which were assigned to lineage B.1.1.519. However, the genetic distance among sequences was >2 mutations.

Outbreak timelines

We examined the quarantine and testing history of cases, and together with the genetic information, we constructed a timeline of the early platform outbreaks (Outbreak #1 an #2), which were identified over 4 days (Fig 3).

Outbreak #1 contained the initial person who reported the onset of COVID-19 symptoms (Day 0). This outbreak consisted of 5 individuals who had quarantined at Hotel A, all that were deployed to the same platform. While the deployment dates varied, their pre-deployment testing routine was the same: a RT-qPCR and a serology test was administered during quarantine, and if they were PCR-negative (typically results have a <24-hour turn-around), they were deployed 1 day later. Three individuals, including the index case, were deployed only 3 days prior to the first onset of symptoms. Three of these 5 individuals eventually reported symptoms on the offshore platform (BIE018, BIE025, and BIE024) and the others were asymptomatic at the time of testing.

Outbreak #2 consisted of 13 individuals on the same platform as outbreak #1. Of these, 6 individuals quarantined at Hotel A, where dates of testing and deployment varied; however, the procedure always involved a negative serology and PCR test. The other 7 people in Outbreak #2 quarantined at Hotel B, where the

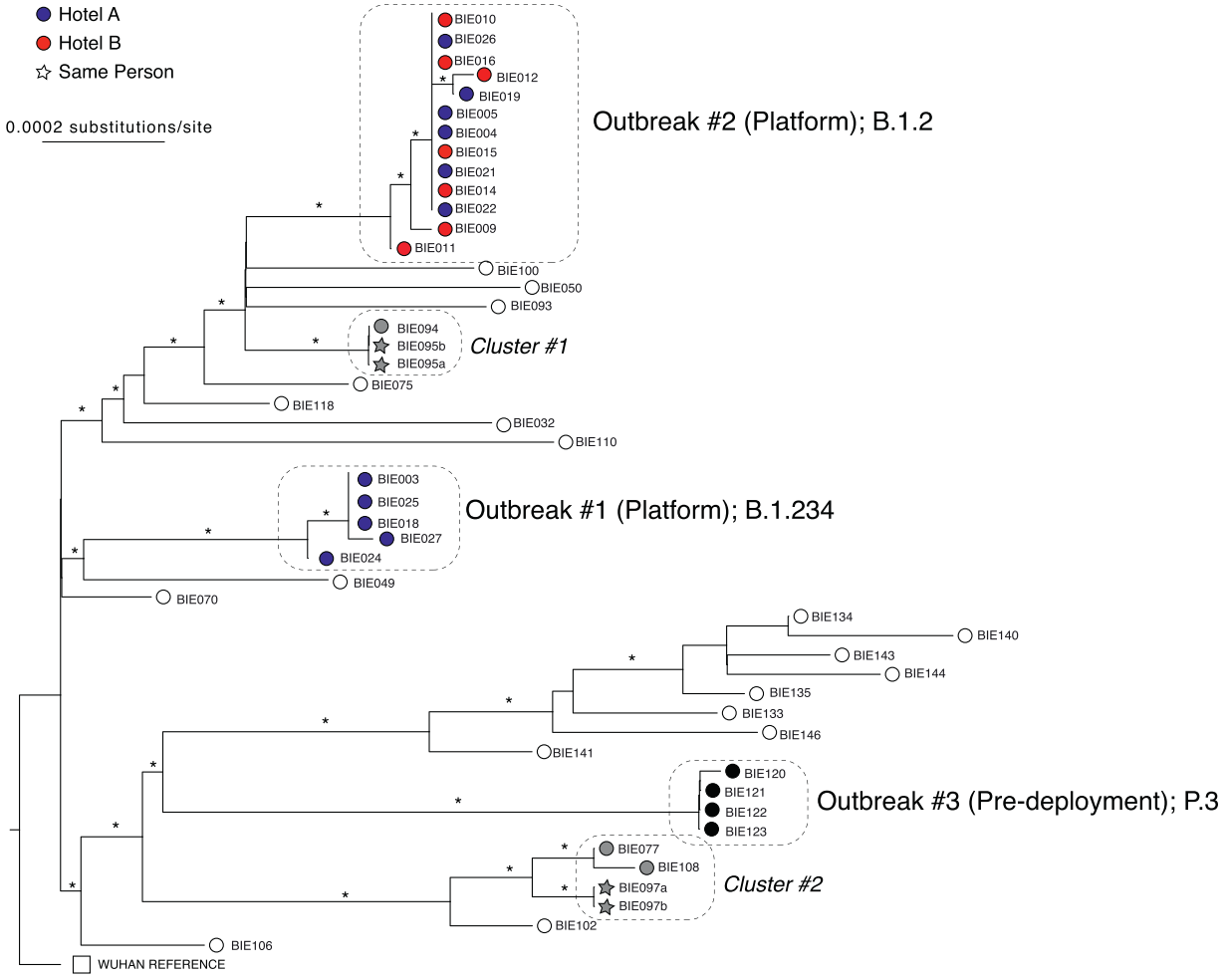

Fig 2. Maximum likelihood phylogenetic tree of 49 SARS-CoV-2 sequences. Dotted boxes denote groups of related sequences. Filled circles indicate sequences that were part of an outbreak. Sequences from individuals quarantined at Hotel A and B are colored blue and red, respectively. Branches are scaled by number of mutations. Sequences from the same individual are indicated with a star. The tree is rooted by the reference sequence, which is one of the earliest sampled viruses from Wuhan (square). Asterisks indicate branch support >70%.

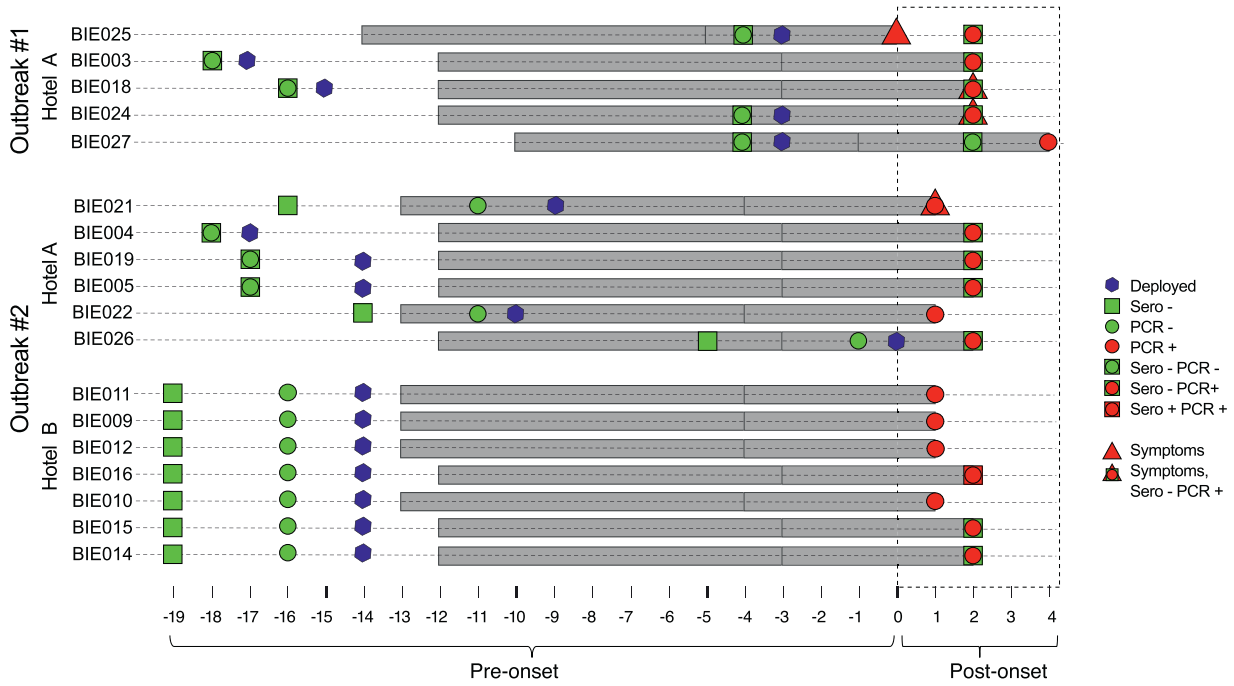

Fig 3. Employee timelines during initial suspected outbreak. Each dotted line indicates the timeline of one employee. Employee timelines are grouped by the infecting variant (outbreak) and the hotel at which they were quarantined. Symbols represent events as follows: Green boxes = seronegative test; red box = seropositive test; green circle = PCR negative test; red circle = PCR+ test; blue hexagon = deployment; triangles = reported symptoms. Superimposed symbols indicate multiple events on the same day. The timeline is set at Day 0 at the first reported onset of symptoms. The dotted box indicates the postonset period. Grey bars indicate the maximum viral incubation periods and horizontal lines within these boxes indicate the average incubation periods.

7 individuals shared the exact same timeline of a negative serological test, a PCR-negative test 3 days later, and deployment 2 days after the negative PCR test and confirmation of zero symptoms. Individuals at Hotel B always arrived at the hotel and were deployed at the same time, thus limiting exposure to additional employees through the 'revolving door' observed at Hotel A. On the platform, only 1 person in Outbreak #2 was mildly symptomatic with congestion (BIE021), which the individual believed was due to allergies. One of the asymptomatic individuals was seropositive (BIE016) at day 2.

While genetic data cannot identify the index case in either outbreak #1 or #2, it is likely that at least 2 employees that had quarantined at Hotel A were incubating the 2 viral strains (B.1.2 and B.1.234) prior to deployment. All 18 employees tested positive over 4 days, which, based on an average incubation period, would suggest that they were exposed at a similar time. Furthermore, 5 individuals at Hotel A (BIE025, BIE034, BIE027, BIE021, and BIE026) tested positive within the 14 day maximum incubation time and 3 of these cases boarded the platform within the median incubation period. All employees that quarantined at hotel B had deployed 15 days prior to testing positive, which is outside of a long incubation period. Furthermore, ten out of eleven employees that were serology tested during the outbreaks, were serology negative, suggesting a relatively recent infection. Because of the variable presentation of COVID-19, it is unclear exactly how many people were incubating the virus prior to deployment, but the data strongly suggest that many employees were infected offshore.

Outbreak #3 occurred 4 months later and consisted of a group of foreign contractors who entered Louisiana through the New Orleans International Airport. These individuals tested negative before traveling to the US and none reported symptoms. Two days after arriving to the US, they travelled to the Gulf Coast for quarantine, were re-tested, and all were confirmed as COVID-19-positive. Sequencing revealed that they were all infected with P.3, a relatively rare variant originally identified in the Philippines and designated a "variant of interest" at the time by the World Health Organization. This highlights the capability of WGS to objectively identify related infections and the vulnerability of this particular employee population to introductions of novel SARS-CoV-2 lineages, which could have varied transmission dynamics. These cases were immediately reported to the local department of public health for follow up contact tracing and are described in more detail in a separate publication[29]. Importantly, all of these individuals were identified prior to deployment.

In Cluster #2, sequences from 3 individuals grouped closely on the tree, although did not technically meet the definition of an outbreak as previously defined. The epidemiological history also suggested that this cluster (Pango lineage B.1.519) did not represent a direct work-related transmission (Table 1). In late February, BIE077 was symptomatic on a platform, tested positive, and was medevacked to shore to quarantine. BIE097 was first identified as positive eleven days later, and BIE108 was identified as positive in early May. To further investigate this cluster, we performed an additional phylogenetic analysis of all B.1.519 cases in Louisiana (Supplementary Fig 1; n = 188). This analysis clearly shows that BIE077 is unrelated to BIE097 and suggests a distant relationship of BIE077 and BIE108. It may be that an intermediate, asymptomatic case was not sampled, which could have come from the community.

Table 1
Epidemiological history of cluster #2

Sequence ID	Subject ID	Location	Sample day
BIE077	A	Platform A	1
BIE097a	B	Platform B	11
BIE097b	B	NA	28
BIE108	C	Predeployment	34

DISCUSSION

In this study, we implemented SARS-CoV-2 genomic surveillance as an evidence-based infection control approach in collaboration with an oil and gas company with offshore platforms in the Gulf of Mexico. Louisiana experienced one of the earliest and fastest accelerating COVID-19 outbreaks, coinciding with the Mardi Gras celebrations in February 2020.[15] The work detailed herein began in January 2021, as our infection rates were subsiding from their previous record peaks and the company IH in the Gulf of Mexico began to ease their quarantine protocols that had been in place for a year. Shortly thereafter, 2 distinct outbreaks were identified on platforms, suggesting that the reduction of quarantine to 24 hours after a negative test may have played a role. Subsequently, from February to April 2021, positive cases were primarily confined to pre-deployment testing.

We found that an initial suspected cluster of 18 PCR-positive individuals on 1 platform over a 5-day period was actually 2 distinct and separate outbreaks, evidenced by the 2 separate clades on the phylogenetic tree and 2 different Pango lineage designations. Several findings might indicate that workers who had a shorter quarantine period at Hotel A prior to deployment were index cases for both outbreaks. First, during offshore testing, 4 employees that had quarantined at Hotel A were symptomatic (BIE025, BIE31, BIE24, BIE021) suggesting they had been infected for at least 2 days; employees that quarantined at Hotel B only reported symptoms after removal from the platform, if at all. Second, several studies found that PCR tests are generally accurate at 3-5 days postinfection[30,31] and 4 of the positive individuals from Hotel A had deployed within this window; Hotel B employees/contractors were deployed for 14 days prior to testing positive.

The mobile nature of offshore platform work, including medics who bypass testing protocols, likely resulted in some unsampled individuals who may have been involved in transmissions. Thus, while it appears Hotel A was likely the source, phylogenetic analysis cannot definitively identify an index case. However, the genomic data objectively shows that both outbreaks were limited to 18 individuals because, while additional infections were identified, no new spread of these variants were identified on several platforms that were monitored over the next 4 months. Without whole genome sequencing, industrial hygienists would be unable to determine if they had stopped a transmission chain, or if it was continuing to disseminate in the worker population.

The pre-deployment outbreak validates that on-going testing can quickly stop the spread of viruses among employees. This outbreak, which was associated with international contractors, highlights the particular risk that offshore platforms face due to the high number of company employees and contractors who arrive from a variety of international locations. This risk tracks with employees as well as the local population since the introduction of new viral variants can result in more rapid viral spread and infection in those vaccinated.[32–34] At the time, only 6 other P.3 lineages in the United States had been reported (as evidenced through the available sequences in a public genomic database, www.gisaid.org). Using WGS in conjunction with the epidemiological data, we determined when this rare lineage was likely introduced into Louisiana via a specific contracting company. This finding provided valuable information to the local department of public health's contact-tracing staff so that they could follow up and ensure this rare variant did not continue to spread in the community. Currently, no further transmission of P.3 has been reported in Louisiana.

This study demonstrates the power of genomic surveillance to objectively identify viral transmission in high-risk environments, such as offshore platforms. The genomic data, in combination with employee timelines was used to assess the effectiveness of quarantines and testing protocols. It does appear that longer quarantines were more effective than the quarantines that lasted the length of time it took receive negative test results. While longer quarantines are expensive and frustrating, they may be needed during times of high infection rates. Furthermore, without genomic data, IH have no way of knowing if their plan-of-action stopped a transmission chain, especially with a virus like SARS-CoV-2 that has varied incubation times, varied transmission potential, and varied presentation of symptoms.

Most SARS-CoV-2 surveillance studies by IH or OPH have occurred in healthcare settings; however, this study reveals that genomic data has applications in the unique offshore oil and gas industry for ongoing evaluation concerning the effectiveness of safety protocols. When employees and contractors know that their employer has tools that identify linked cases, they may be more likely to follow mandated safety protocols, which will improve the health of the workers, the industry, and their surrounding communities. Furthermore, the data provides information that public health agencies can use to improve community health. As a whole, the study illustrates the imperative for genomic epidemiologists and industry to collaborate in developing infrastructure and protocols to understand and slow the spread of infectious disease.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2022.05.008.

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