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Outbreak of P.3 (Theta) SARS-CoV-2 emerging variant of concern among service workers in Louisiana

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ABSTRACT

During routine industrial quarantine/premobilization procedures, four individuals who recently traveled from the Philippines tested positive for SARS-CoV-2. Subsequent genomic analysis showed that all four were infected with a relatively rare Variant of Interest (P.3, Theta) derived from a single origin. This demonstrates the importance of on-going genomic surveillance of SARS-CoV-2.

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Many industries have implemented extensive safety measures to reduce workplace transmission risk during the COVID-19 pandemic [1–4]. These policies have been imperative in the offshore oil and gas industry because rig workers travel from many states and internationally. The western and central Gulf of Mexico is one of the major offshore petroleum-producing areas, incorporating >1800 rigs and thousands of employees. To prevent COVID-19 outbreaks on offshore platforms, the industry has implemented, to varying degrees, strict pre-mobilization testing and quarantine policies.

Here, we report the detection of a rare Pango lineage [5], which infected four individuals who tested SARS-CoV-2 positive in May 2021 while quarantined in Louisiana, prior to deployment to an oil platform. These individuals were employed by a contractor to the oil and gas company, and all had recently traveled from the Philippines. Reconstruction of their timeline showed they tested negative in the Philippines, immediately prior to boarding a USA flight. Upon arrival in the USA, the workers went into a 14-day quarantine. They were retested after 9 days, at which point they were all qPCR+ and serology negative. None reported COVID-19 symptoms. The expected time interval between exposure and test positivity (3–4 days post-exposure for qPCR, >1 week post-exposure for serology), as well as the workers' testing timeline (Fig. 1A), suggest a recent exposure after their flight to the United States.

To further understand the nature of the outbreak, the samples were sent to an independent laboratory (BioInfoExperts, LLC,

Thibodaux, LA) for viral load and genomic evaluation (Table 1). Viral loads were quantified using ddPCR (BioRad). Sequencing was performed using the ARTIC protocol [6] on the Illumina MiSeq platform, followed by Pango lineage assignment [5] and mutation analysis using the FoxSeq software platform (<https://bioinfox.com/foxseq/>). All four samples were assigned to the Pango P.3. (Theta) lineage (GenBank Accession #: MZ368550–MZ368553).

Of the 300 sequences in the GISAID database (www.gisaid.org) assigned to this comparatively rare lineage, the majority are from the Philippines (n = 209), where the P.3 lineage likely emerged from the Central Visayas region in January 2021 [7]. This lineage is also present in other parts of Asia (n = 30), North America (n = 18), Europe (n = 33), Oceania (n = 7), Africa (n = 2), and South America (n = 1). The earliest identified P.3 infection in the United States was at the end of March in California (n = 3), with additional cases detected in Washington (n = 3), Maryland (n = 2), New Jersey (n = 2), Virginia (n = 1), and West Virginia (n = 1).

To investigate this potential outbreak, we downloaded all sequences assigned to the P3 lineage, aligned them using MAFFT [8], and inferred a maximum likelihood phylogenetic tree using IQTREE v2 [9] (Fig. 1B; Fig. S1; Table S1). The four Louisiana sequences are part of a well-supported clade whose basal members are all from the Philippines and are separated from the most related P.3 sequences by a long branch, likely resulting from the time separating the Louisiana samples from the other samples in the clade (2+ months). Elsewhere on the tree, well-supported clades of P.3 sequences from Europe, USA, Oceania, and/or Asia indicate multiple introductions to those locations. Interestingly, the most basal sequence of the entire P.3 clade (closest to the root of the tree) is

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Table 1
Laboratory results for four Louisiana cases.

Sample	rtPCR cycles			Pre-mobilization		Viral load	
	S	N	ORF1ab	PCR	Serology	N1	N2
BIE-120	16.44	16.685	16.286	+	—	20,000,000	20,000,000
BIE-121	17.43	16.496	16.608	+	—	230,967	214,658
BIE-122	11.469	12.289	10.811	+	—	55,109	54,942
BIE-123	18.69	20.724	18.881	+	—	231,714	231,714

A. Timeline for four Louisiana cases



B. Maximum Likelihood Phylogeny of P.3

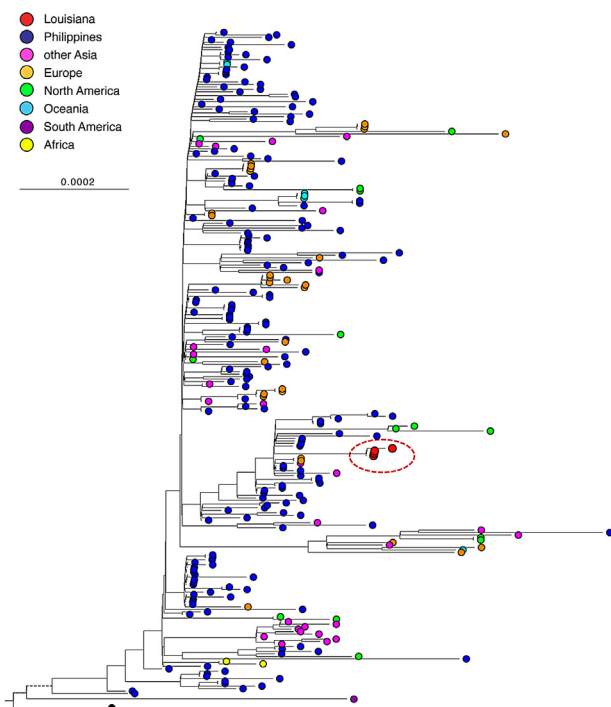


Fig. 1. (A) Timeline for four Louisiana cases. Circles represent days. (B) Maximum likelihood phylogenetic tree all sequences assigned to the P.3 lineage in GISAID as of 7/30/2021 ($n = 300$) were included, including the four Louisiana sequences described. The tree is rooted using the Wuhan reference (EPI_ISL_402124). The circles at each tip represents a sequence, colored according to the region of sampling per the legend. Branches are scaled in substitutions per site. Dotted branches indicate that a long branch was shortened for readability (see Appendix for complete tree).

from South America, although separated from the rest of the P.3 tree by a long branch.

P.3 is a considered a Variant Under Investigation (VUI) by Public Health England (PHE) [10] and was formerly considered a Variant of Interest by the World Health Organization. Along with other Variants of Concern (VOCs) classified by PHE, including B.1.1.7 (Alpha), P.1 (Gamma), and B.1.351 (Beta), all P.3 viruses contain multiple concerning mutations in the spike protein, including E484K, N501Y, and P681H [7], which have been linked to increased transmissibility and immune escape [11,12]. In addition, >95% of P.3 viruses exhibit spike protein mutations at D614G, H1101Y, E1092K, and V1176F. Two synonymous spike protein mutations also occur at positions 593G and 875S. The four new Louisiana sequences have a unique

spike mutation at Q1180H, which was not observed in any of the other P.3 sequences. A three amino acid deletion (LGV141_143del) in the spike protein reported as common in this lineage [7] was not found in the four Louisiana P.3 sequences.

The identification of a novel Louisiana P.3 cluster is of particular concern given the suite of spike mutations, the relatively low vaccination rate in Louisiana (<40%; [13]), and the close working conditions on the oil rigs. Our results show that industrial monitoring for COVID-19 remains crucial. Fortunately, due the testing policies in place, this particular variant was identified prior to deployment so that the workers continued quarantine until they were no longer considered a risk. No other employees are known to have contracted P.3.

The study confirms that SARS-CoV-2 surveillance, especially in populations prone to viral spread and the introduction of new variants of concerns such as Delta and its descendants, will remain important as the pandemic continues [14,15]. Finally, the study supports that collaborations between genomic epidemiologists and industrial hygienists can assist in understanding and controlling outbreaks in industries at high risk for infections.

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Competing interests

R.R., D.J.N., T.M.L. and S.L.L. are employed by BioInfoExperts LLC.

Ethical approval

A third party review from the WIRB-Copernicus Group (WCG IRB) concluded that this research was exempt from IRB review because it does not meet the definition of human subjects as defined in 45 CFR 46.102.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jiph.2021.11.011>.

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