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Monitoring International Travelers Arriving in Hong Kong for Genomic Surveillance of SARS-CoV-2

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We sequenced ≈50% of coronavirus disease cases imported to Hong Kong during March–July 2021 and identified 70 cases caused by Delta variants of severe acute respiratory syndrome coronavirus 2. The genomic diversity detected in Hong Kong was similar to global diversity, suggesting travel hubs can play a substantial role in surveillance.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineage B.1.617 (*1*) and 3 of its sublineages, B.1.617.1 (Kappa), B.1.617.2 (Delta), and B.1.617.3, were first detected in India. The Delta variant started circulating widely in different continents beginning in late March 2021 (*2*,*3*). It was initially classified as a variant of interest in April 2021 and then reclassified as a variant of concern in May 2021.

Hong Kong adopted an elimination strategy to control coronavirus disease (COVID-19). A previous study reported the use of stringent measures (e.g., mandatory COVID-19 testing, travel restrictions) to detect and prevent SARS-CoV-2 importation by COVID-19–positive travelers (*4*), thereby reducing the risk of new SARS-CoV-2 introductions, and also showed that regional and international airports could be useful sentinel surveillance sites to monitor SARS-CoV-2 circulation. In this study, we tested the feasibility of using surveillance strategies similar to those used in that study to monitor sequence diversity of Delta variant

SARS-CoV-2 among incoming travelers. Detection of B.1.617 variants at the end of March 2021 (*4*) prompted us to increase our sequencing efforts on imported COVID-19 cases. A total of 433 COVID-19 cases confirmed by reverse transcription PCR (RT-PCR) were imported during March 27–July 16, 2021; these cases accounted for 85.3% of all RT-PCR–confirmed COVID-19 cases in Hong Kong. We sequenced 49% (212) of those imported cases using next-generation sequencing technology (Appendix 1, https://wwwnc.cdc.gov/EID/article/28/1/21-1804-App1.pdf) and identified 42 Kappa and 70 Delta variant cases (Table). The same study reported that ≈80% of all imported COVID-19 cases in Hong Kong were asymptomatic at the time of RT-PCR testing (*4*). In this study, we observed a similar proportion (80.9%, N = 34) of asymptomatic Kappa variant cases but found that a significantly lower proportion, 52.8% (37/70), of Delta variant cases were asymptomatic ($p<0.001$ by χ^2 test). This observation aligns with previous findings that the Delta variant virus can induce more severe clinical symptoms (*5*).

All Kappa variant cases were imported from India, where the Kappa variant predominantly circulated (*6*). In contrast, the Delta variant cases were imported from 11 countries in Asia, Europe, and Africa (Table). Delta variant cases within a specific country often clustered together temporally (Figure), mainly because additional travel bans to countries with high COVID-19 circulation during the study period were implemented, preventing introduction of more cases from these countries to Hong Kong. For example, because of the COVID-19 upsurge in India in early April 2021, all passenger flights from India were prohibited from landing in Hong Kong after April 19. Similar travel restrictions were also imposed on flights from Brazil, Indonesia, Nepal, Pakistan, Philippines, South Africa, and the United Kingdom at different times during the study period. These travel restrictions

Table. Severe acute respiratory syndrome coronavirus 2 lineages identified from coronavirus disease cases imported to Hong Kong, March 27–July 16, 2021*

Date	Lineage	Total no. cases	Countries affected (no. cases)
Mar 27–31	B.1.1.7	6	Pakistan (2), Philippines (3), Turkey (1)
	P.3	5	Philippines (4), United States (1)
	B.1	1	Philippines (1)
	B.1.351	1	Philippines (1)
	B.1.466.2	1	Indonesia (1)
	B.1.526	1	United States (1)
	B.1.617.1	1	India (1)
	B.1.617.2	1	India (1)
Apr 1–30	B.1.617.1	41	India (41)
	B.1.1.7	38	Canada (1), France (2), India (9), Japan (1), Nepal (3), Pakistan (10), Philippines (10), Turkey (1), United States/Turkey (1)
	B.1.617.2	24	India (11), Nepal (13)
	B.1.351	16	Indonesia (1), Kenya (1), Philippines (14)
	B.1.466.2	7	Indonesia (7)
	B.1.470	2	Indonesia (2)
	C.36.3	2	Egypt (2)
	B.1	1	Philippines (1)
	B.1.1	1	Philippines (1)
	B.1.351.3	1	Bangladesh (1)
	B.1.36.18	1	Canada (1)
	B.1.441	1	Indonesia (1)
	B.1.456	1	Pakistan (1)
	P.3	1	Indonesia (1)
May 1–31	B.1.617.2	5	France (1), India (1), Nepal (3)
	B.1.441	2	Indonesia (2)
	B.1	1	Philippines (1)
	B.1.1.317	1	Russia (1)
	B.1.1.7	1	UAE (1)
	B.1.36	1	Pakistan (1)
	B.1.470	1	Indonesia (1)
Jun 1–30	B.1.617.2	35	Bangladesh (4), Indonesia (14), Namibia (1), Russia (1), United Arab Emirates (2), United Kingdom (13)
	B.1.1.7	2	Indonesia (2)
	B.1	1	France (1)
	B.1.351	1	Philippines (1)
	B.1.466.2	1	Indonesia (1)
	B.1.621	1	Colombia (1)
Jul 1–16	B.1.617.2	5	Cyprus (1), Ghana (1), Russia (3)

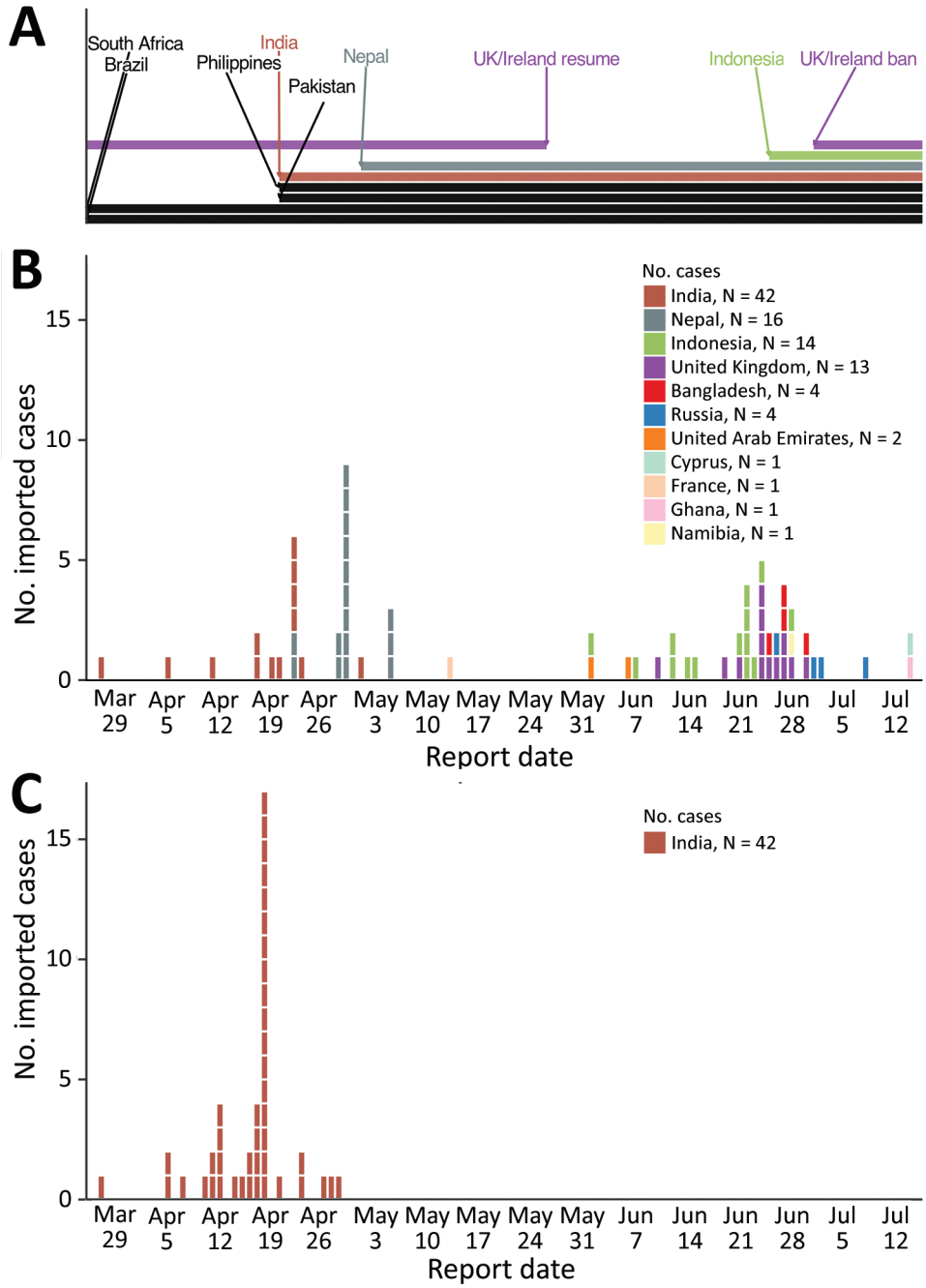

Figure. Imported Delta and Kappa variant severe acute respiratory syndrome coronavirus 2 infections, Hong Kong, March 27–July 16, 2021. A) Travel ban periods from each country to Hong Kong. B) Confirmed Delta variant cases, by country of origin. C) Confirmed Kappa variant cases; all were from India. All infections were confirmed by full-genome sequencing.

enabled us to capture viral sequence information from specific countries within limited identifiable time periods. Despite these restrictions, all 4 sublineages of B.1.617.2 (Delta I–IV) previously detected in other geographic locations (*7*) were detected in cases imported to Hong Kong (Appendix 1 Figure 1). Furthermore, we first detected these sublineages when they were in the early stages of global circulation (Appendix 1 Figure 2).

Although genomic sequencing has been used extensively to track SARS-CoV-2 transmission in specific geographic locations (*8*,*9*; H. Gu et al., unpub. data, https://doi.org/10.1101/2021.06.19.21259169), a global-level surveillance network using airports in different geographic locations might enable more feasible and cost-effective worldwide genomic surveillance. SARS-CoV-2 sequence information thus obtained, combined with relevant metadata, could strengthen current surveillance systems designed for other travel-related sources of illness and death (*10*). Specifically, we propose a multicenter surveillance network incorporating >1 travel hubs

from each subcontinent. Sample selections would ideally be proportional to the number of confirmed imported cases by countries of origin and by time. Additional efforts should be made to study cases imported from countries with limited public sequencing information. Data required for sharing would at a minimum include genomic sequences, sample locations, and sampling dates. To avoid data deanonymization, use of nonessential data (e.g., sex, age) could be restricted in or excluded from reports. The proposed surveillance network would take advantage of existing sequence-sharing platforms (e.g., GISAID, https://www.gisaid.org), but specific electronic tools and pipelines would need to be developed to enable timely, robust analyses.

During the study period, we found only sporadic local COVID-19 cases in Hong Kong (N = 10). There were 2 independent local Delta variant cases in which the infection was acquired at the Hong Kong airport (Appendix 1 Figure 1). For regions using elimination strategies to control COVID-19, these findings suggest that airports can be high-risk settings for transmitting SARS-CoV-2 and introducing new variants. The findings also support use of stringent control measures and guidelines for protecting staff who work in airports. Overall, our results suggest that key travel hubs can effectively be used as valuable surveillance sites to monitor SARS-CoV-2 sequence diversity.

Virus sequences reported in this study are available from GISAID (https://www.gisaid.org; Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/28/1/21-1804-App2.xlsx). The data and analyzing scripts used in the study can be accessed in a GitHub repository (https://github.com/Leo-Poon-Lab/HK-Delta-variants).

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Appendix

Supplementary Methods

Sequencing

To obtain high quality sequence results, we only selected clinical samples with a cycle threshold value <30 for the next-generation sequencing study. RNA samples of interest were sent to a World Health Organization reference laboratory at the University of Hong Kong for full genome analyses (IRB number: UW 20–168). We deduced near–full-length genomes from all available samples using an Illumina (https://www.illumina.com) sequencing protocol previously described by us (*1*,*2*). Briefly, the virus genome was reverse transcribed with multiple gene-specific primers targeting different regions of the viral genome. The synthesized cDNA was then subjected to multiple overlapping 2 kb PCRs for full-genome amplification. PCR amplicons obtained from the same specimen were pooled and sequenced using Nova sequencing platform (Illumina). The sequencing library was prepared by Nextera XT (Illumina). Generated sequencing reads were mapped to a reference virus genome by BWA-MEM2 (https://github.com/lh3/bwa) (*3*), and genome consensus was generated by iVar with the PCR primer trimming protocol (minimum sequence depth >100 for NovaSeq and >10 for iSeq samples (*4*). Deduced consensus sequences with genome coverage <70% or failed to pass quality check of PANGOLIN (https://cov-lineages.org/resources/pangolin.html) were excluded from the downstream analyses. A total of 258 imported cases were sequenced in this study; 46 yielded unsatisfactory results and were excluded in the downstream analyses.

Epidemiologic Data

The metadata of the imported cases including case report date, case arrival date, country of importation are freely available in the Hong Kong governmental Web sites (*5*). To avoid de-anonymization of the genetic sequences, all the sequences submitted to GISAID (https://platform.gisaid.org) were named by in-house sample identifier rather than official

case number. The sex and age of the cases are not provided by default. The sample collection/report dates were only revealed in year-month format.

Phylogenetic Analysis

Maximum likelihood phylogenies were estimated using IQ-TREE (v.2.1.3) (*6*), employing the JC nucleotide substitution model with Wuhan-Hu-1 (GenBank: MN908947.3) as the outgroup. Dating of the Hong Kong tree and reconstruction of the ancestral node sequences were performed using Treetime v 0.8.1 (https://github.com/neherlab/treetime) (*7*). Lineages were classified using PANGOLIN (v.3.1.7) (pangoLEARN version 2021–07–09) (*8*). The global sequences were subsampled evenly (N = 3, per country per week, total N = 1,380) from all the high-quality Delta-variant sequences from GISAID (assessed 2021–06–28).

Data Availability

Virus sequences reported in this study are available from GISAID (Appendix Table 1).The data and analyzing scripts used in the study can be accessed in a GitHub repository (https://github.com/Leo-Poon-Lab/HK-Delta-variants).

Local Cases Linked to the Airport

Case-patient A was a ground staff person who escorts aircrews in the airport. Epidemiologic investigations revealed that patient A had brief encounters with a COVID-19 cluster imported from Indonesia in the airport (*9*), and our genomic analyses of these cases confirmed the findings (Figure, case A, cluster 32). Case-patient B was a porter at the airport. Epidemiologic investigations revealed that patient B might have acquired the infection during his stay in a Russian cargo aircraft which briefly stayed in Hong Kong. Our sequence analyses also confirmed that the viral sequence from patient B was genetically most similar to our cases imported from Russia (Figure, clusters 27 and 28).

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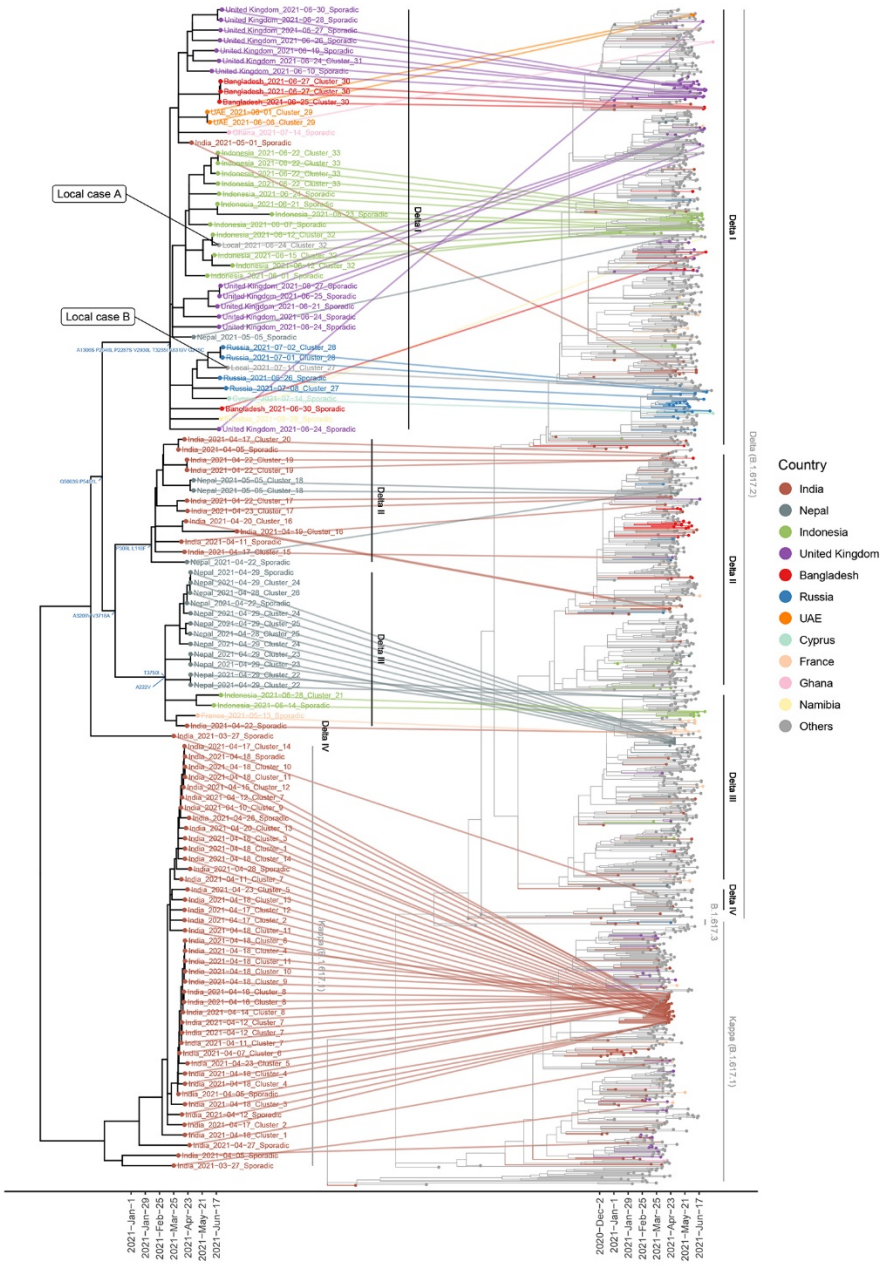
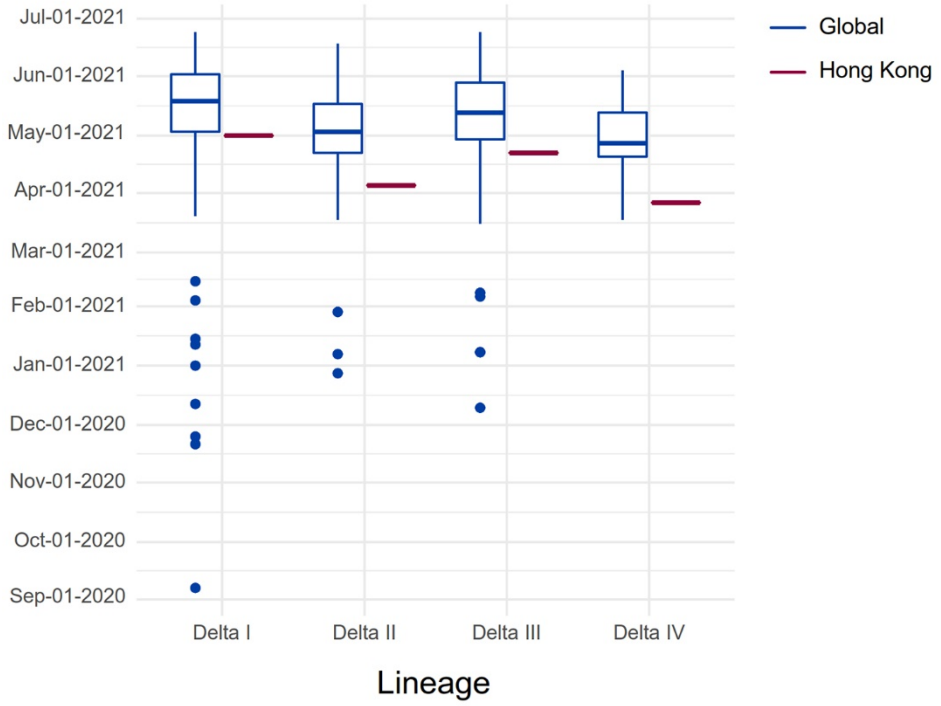


Appendix Figure 1. Phylogeny of SARS-CoV-2 B.1.617 variants identified in Hong Kong (left) and globally (right). The tips in the trees are colored according to the countries of origins. The global sequences were subsampled evenly (N = 3 per country per week, total N = 1,380) from all the high-quality Delta-variant sequences from GISAID (assessed 28 Jun 2021). Mutations defining the Delta sublineages were labeled at the ancestral nodes (*8*). Cases imported to Hong Kong that were epidemiologically linked are labeled with the same cluster number. Maximum likelihood phylogenies were estimated using IQ-TREE (v.2.1.3) (https://www.iqtree.org), employing the JC nucleotide substitution model with Wuhan-Hu-1 (GenBank accession no. MN908947.3) as the outgroup. Dating of the trees and reconstruction of the ancestral node sequences were performed using Treetime (v0.8.1) (https://treetime.readthedocs.io/en/latest). Lineages were classified using PANGOLIN (v3.1.7) (https://cov-lineages.org/resources/pangolin.html).



Appendix Figure 2. Detection dates of Delta variant sequences reported in the GISAID database (https://platform.gisaid.org). Distribution of dates of global subsampled data shown in box plots for different Delta sublineages. Dates represent outliers, minimum, 1st quartile, median, 3rd quartile, and maximum of each sublineage. Red line indicates detection date in Hong Kong for each sublineage.