Supplementary Appendix To:

The Clinical and Genomic Epidemiology of Seasonal Human Coronaviruses in Congregate Homeless Shelter Settings: A Repeated Cross-Sectional Study

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Supplemental Table 1 Homeless Shelter Study Sites

Shelter	Type	Grouping	Sleeping Quarters	Notes
Routine Surveillance and Surge Testing Sites				
A	Adult female (≥18 years)	Shelters: Adults ≥18 years old	Communal bunk beds	
B	Adult mix gender (≥18 years)	Shelters: Adults ≥18 years old	Communal bunk beds	
C	Adult mix gender (18-25 years)	Shelters: Adults 18-25 years old	Communal floor mats and bunk beds	
D	Family	Shelters: Adults and Children	Private rooms/shared rooms/communal floor mats	
E	Family	Shelters: Adults and Children	Private rooms/shared rooms/communal floor mats	
F	Adult male (≥18 years)	Shelters: Adults ≥18 years old	Communal bunk beds	
G	Adult mix gender (≥18 years)	Shelters: Adults ≥18 years old	Private rooms/shared rooms	Routine surveillance began on 4/12/2020 to replace L
H	Family	Shelters: Adults and Children	Private rooms/shared rooms	Routine surveillance began on 4/8/2020 to replace O
I	Adult male senior (≥50 years)	Shelters: Adults ≥ 50 years old	5 person dorms	Routine surveillance began on 9/26/2020 to replace M
J	Adult male (≥18 years)	Shelters: Adults ≥18 years old	Individual open cubicles	Routine surveillance began on 12/3/2020 to replace F
K	Adult mix gender (≥18 years)	Shelters: Adults ≥18 years old	Individual open cubicles	Routine surveillance began on 12/3/2020 to replace F
L	Adult mix gender (≥18 years)	Shelters: Adults ≥18 years old	Communal bunk beds	Closed on 4/9/2020 and moved residents to G
M	Adult male senior (≥50 years)	Shelters: Adults ≥ 50 years old	Communal floor mats	
N	Family	Shelters: Adults and Children	Private rooms/shared rooms	Open for 1 week before shutting down and then moving residents to H
O	Family	Shelters: Adults and Children	Private rooms/shared rooms	Closed on 4/6/2020 and moved residents to H
Surge Testing Site Only				
Other A	Adult male senior (≥50 years)	Shelters: Adults ≥ 50 years old	Communal floor mats	
Other B	Adult mix gender (≥18 years)	Shelters: Adults ≥18 years old	Private apartments	
Other C	Adult male senior (≥50 years)	Shelters: Adults ≥ 50 years old	Communal floor mats	
Other D	Adult mix gender (≥18 years)	Shelters: Adults ≥18 years old	Private apartments	
Other E	Adult male senior (≥50 years)	Shelters: Adults ≥ 50 years old	Communal floor mats	
Other F	Family	Shelters: Adults and Children	Private rooms/shared rooms/communal floor mats	
Other G	Adolescents (<18 years)	Shelters: Adults and Children	Communal bunk beds	
Other H	Adult mix gender (18-25 years)	Shelters: Adults 18-25 years old	Communal bunk beds	

Supplemental Table 2 Custom Arrayed Multi-Viral Pathogen RT-PCR Platform Over the Study Period

Dates During Study		Influenza			Parainfluenza	Enterovirus		Rhinovirus	Adenovirus	Seasonal Human Coronavirus				SARS-CoV-2	Respiratory Syncytial Virus		Metapneumovirus	Human Parechovirus	Human Bocavirus
Date Started	Date Ended	Influenza A	Influenza B	Influenza C	Parainfluenza (1-4)	Enterovirus	EVD68	Rhinovirus	Adenovirus	HCoV-HKU-1	HCoV-NL63	HCoV-229E	HCoV-OC43	SARS-CoV-2	RSVA	RSVB	Metapneumovirus	Human Parechovirus	Human Bocavirus
10/1/19	2/20/20	x	x	x	x	x	x	x	x	x		x			x	x	x	x	x
2/21/20	5/1/20	x	x	x	x	x	x	x	x	x		x			x	x	x	x	x
5/29/20	11/20/20	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	
11/23/20	5/31/21	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		

* From February 25, 2020, onward, samples were tested for SARS-CoV-2 in real-time by a RT-PCR assay. Specimens collected from January 1, 2020-February 25, 2020, were tested retrospectively for SARS-CoV-2. From May 29, 2020 onward, SARS-CoV-2 testing was integrated into the custom arrayed multi-viral pathogen RT-PCR platform.

Supplemental Table 3 GenBank Accession Numbers for Sequenced Seasonal Human Coronavirus Specimens

GenBank Accession Number	Date Collected	Shelter Site Location
ON136168	1/17/20	M
ON136169	4/8/21	F
ON136170	4/13/21	F
ON136171	5/22/21	F
ON136172	5/19/21	F
ON136173	4/15/21	F
ON461751	5/28/21	D
ON461752	5/28/21	C
ON461753	5/31/21	C
ON461754	11/2/19	E
ON461755	10/21/19	E
ON461756	10/29/19	E
ON461757	11/4/19	E
ON461758	11/7/19	E
ON461759	11/9/19	E
ON461760	11/11/19	E
ON461761	11/25/19	D
ON461762	12/2/19	C
ON461763	12/4/19	O
ON461764	12/11/19	L
ON461765	12/20/19	O
ON461766	12/20/19	M
ON461767	12/23/19	L
ON461768	2/10/20	L
ON461769	12/27/19	M
ON461770	12/27/19	M
ON461771	12/28/19	L
ON461772	12/30/19	L
ON461773	12/31/19	L
ON461774	1/3/20	L
ON461775	12/30/19	L
ON461776	12/31/19	O
ON461777	1/4/20	L
ON461778	1/6/20	L
ON461779	1/15/20	C
ON461780	1/16/20	L
ON461781	1/16/20	L

ON461782	12/19/19	B
ON461783	12/21/19	B
ON461784	12/31/19	L
ON461785	1/2/20	M
ON461786	1/7/20	M
ON461787	1/9/20	M
ON461788	1/16/20	M
ON461789	1/20/20	A
ON461790	1/28/20	O
ON461791	4/14/21	K
ON461792	2/3/20	O
ON461793	2/10/20	B
ON461794	10/26/19	M
ON461795	1/28/20	B
ON461796	4/8/20	D
ON461797	5/1/21	G
ON461798	5/20/21	G

Supplemental Table 4 Respiratory Viral Pathogen Detection Among Homeless Shelter Participants

	Number of Encounters by Pathogen Among Virus-positive Samples	Number of Encounters Where Pathogen Was the Only Virus Detected Among All Virus Positive Samples	Number of Seasonal Human Coronavirus Encounters with Co-detection of Viral Pathogen
Number of Encounters, N	**1,569**	**1,569**	**107**
Pathogen	**N(%)**	**n(%)**	**n(%)**
Adenovirus	133 (8·5)	99 (6·3)	0
Human bocavirus	14 (0·9)	6 (0·4)	0
Enterovirus	83 (5·3)	61 (3·9)	1 (0·9)
Influenza			
A	22 (1·4)	17 (1·1)	1 (0·9)
B	43 (2·7)	33 (2·1)	3 (2·8)
C	5 (0·3)	5 (0·3)	0
Metapneumovirus	14 (0·9)	10 (0·6)	1 (0·9)
Human parainfluenza virus (1-4)	32 (2·0)	26 (1·7)	0
Human parechovirus	3 (0·2)	1 (0·1)	0
Rhinovirus	1,066 (67·9)	986 (62·8)	13 (12·2)
Respiratory syncytial virus	20 (1·3)	15 (1·0)	2 (1·9)
Seasonal human coronavirus	107 (6·8)	88 (5·6)	-
SARS-CoV-2*	133 (10·2)**	123 (7·8)	0

*There were n = 22 inconclusive SARS-CoV-2 tests categorized as negative; there were no other pathogens detected in these samples and 17 were asymptomatic

**Among 1300 virus-positive samples in which SARS-CoV-2 testing was performed.

Supplemental Table 5 Seasonal Human Coronavirus Encounters by Age Group Among Samples Collected from Homeless Shelter Participants

Age group, years	All Encounters	All Seasonal Human Coronavirus Encounters	Percentage
	N	n	%
<5	654	6	0·9
5-11	885	4	0·5
12-17	506	1	0·2
18-49	7716	54	0·7
50-64	3795	35	0·9
≥65	908	7	0·8
Age group, years	**Viral Positive Encounters**	**All Seasonal Human Coronavirus Encounters**	**Percentage**
	N	**n**	**%**
<5	223	6	2·7
5-11	188	4	2·1
12-17	64	1	1·6
18-49	708	54	7·6
50-64	322	35	10·9
≥65	64	7	10·9

Supplemental Table 6 Seasonal Human Coronavirus Detection by Shelter Sites[1]

Type of Shelter	Number of Total Encounters	Number of Encounters with Viral Pathogen Detection	Seasonal Human Coronavirus Encounters Out of Total Encounters	Seasonal Human Coronavirus Encounters Out of Encounters with Viral Pathogen Detection
N	**14,464**	**1,569**	**107**	**107**
Surveillance Testing	**N**	**n (%)**	**n (%)**	**n (%)**
Shelters: Adults and Children	4761	756 (15·9)	29 (0·6)	29 (3·8)
Shelters: Adults ≥18 years old	6241	467 (7·5)	53 (0·9)	53 (11·4)
Shelters: Adults 18-25 years old	1179	120 (10·2)	6 (0·5)	6 (5·0)
Shelters: Adults ≥50 years old	849	103 (12·1)	18 (2·1)	18 (17·5)
Surge Testing				
Shelters: Adults and Children	318	30 (9·4)	0	0
Shelters: Adults ≥18 years old	704	39 (5·5)	0	0
Shelters: Adults 18-25 years old	143	11 (7·7)	0	0
Shelters: Adults ≥50 years old	269	43 (16·0)	1 (0·4)	1 (2·3)

[1]Percentages listed here are row percentages
[2]Among n = 18 seasonal human coronavirus positive encounters in shelters for older adults (≥50 years), there were 17 unique participants with samples collected from October 2019 through February 2020.

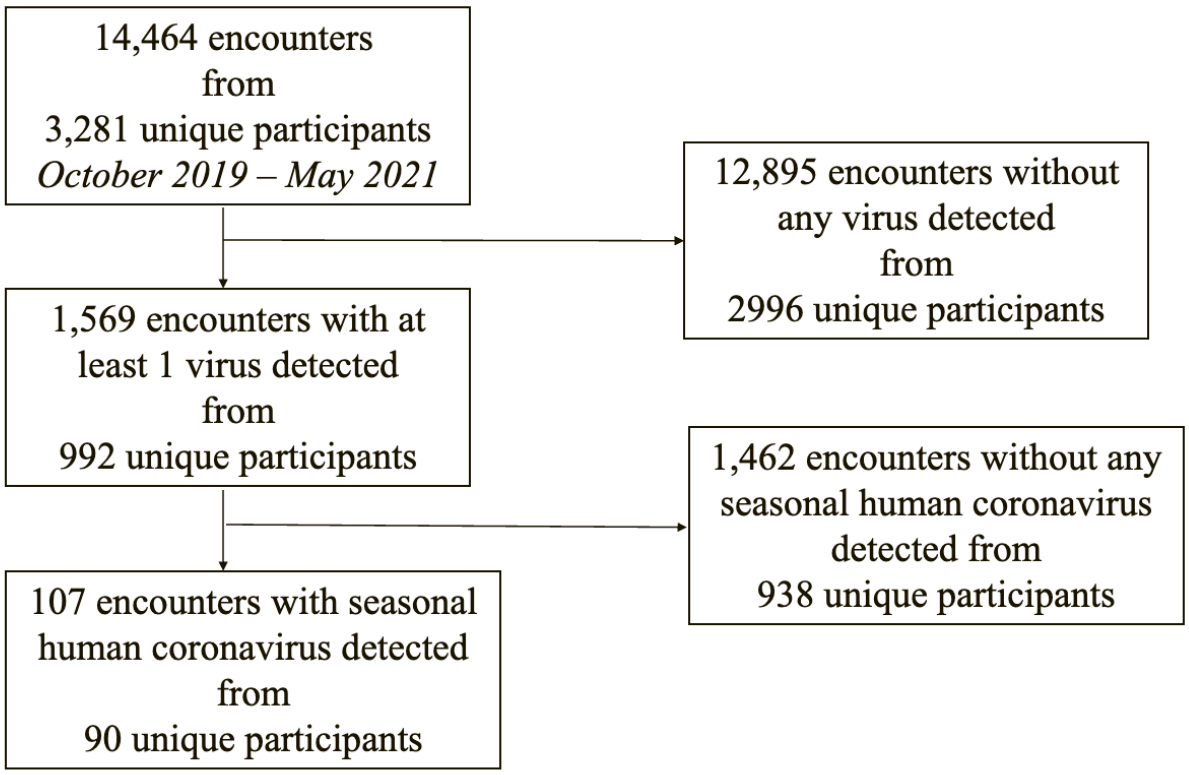
Supplemental Table 7 Shelter Participants with Multiple Seasonal Human Coronavirus Positive Samples

Participant	Infection Course	Total Days of Detection Within Same Infection Course*	Encounter Date	Seasonal Human Coronavirus Detected?	Seasonal Human Coronavirus Species	OpenArray Relative Cycle Threshold Value	Time Between Different Infections**
A	1	1 day	11/26/2019	No			42 days
			12/19/2019	Yes	HCoV-NL63	26·5	
			1/14/2020	No			
	2	8 days	1/28/2020	No			
			1/29/2020	Yes	HCoV-HKU1***	24·3	
			2/5/2020	Yes	HCoV-HKU1***	25·7	
			2/6/2020	No			
B	1	1 day	10/12/2019	No			142 days
			11/2/2019	Yes	HCoV-229E	17·8	
			12/19/2019	No			
	2	1 day	12/27/2019	No			
			3/23/2020	Yes	HCoV-NL63	26·4	
			4/10/2020	No			
C	1	20 days	1/20/2020	No			-
			1/29/2020	Yes	HCoV-NL63	19·8	
			2/17/2020	Yes	HCoV-NL63	23·5	
			2/22/2020	No			
D	1	6 days	4/6/2021	No			-
			4/15/2021	Yes	HCoV-HKU1***	14·3	
			4/20/2021	Yes	HCoV-HKU1***	27·6	
			4/22/2021	No			
E	1	6 days	4/3/2021	No			-
			4/8/2021	Yes	HCoV-HKU1A	10·4	
			4/13/2021	Yes	HCoV-HKU1A	20·3	
			4/24/2021	Yes	HCoV-HKU1***	27·2	
			4/29/2021	No			
F	1	1 day	4/8/2020	Yes	HCoV-OC43	18·7	415 days
			9/9/2020	No			
	2	1 day	12/3/2020	No			
			5/28/2021	Yes	HCoV-229E	12·6	
G	1	4 days	5/19/2021	No			-
			5/28/2021	Yes	HCoV-229E	10·6	
			5/31/2021	Yes	HCoV-229E	21·1	
H	1	6 days/1 day	12/16/2019	No			5 days
			12/23/2019	Yes	HCoV-HKU1B	21·5	

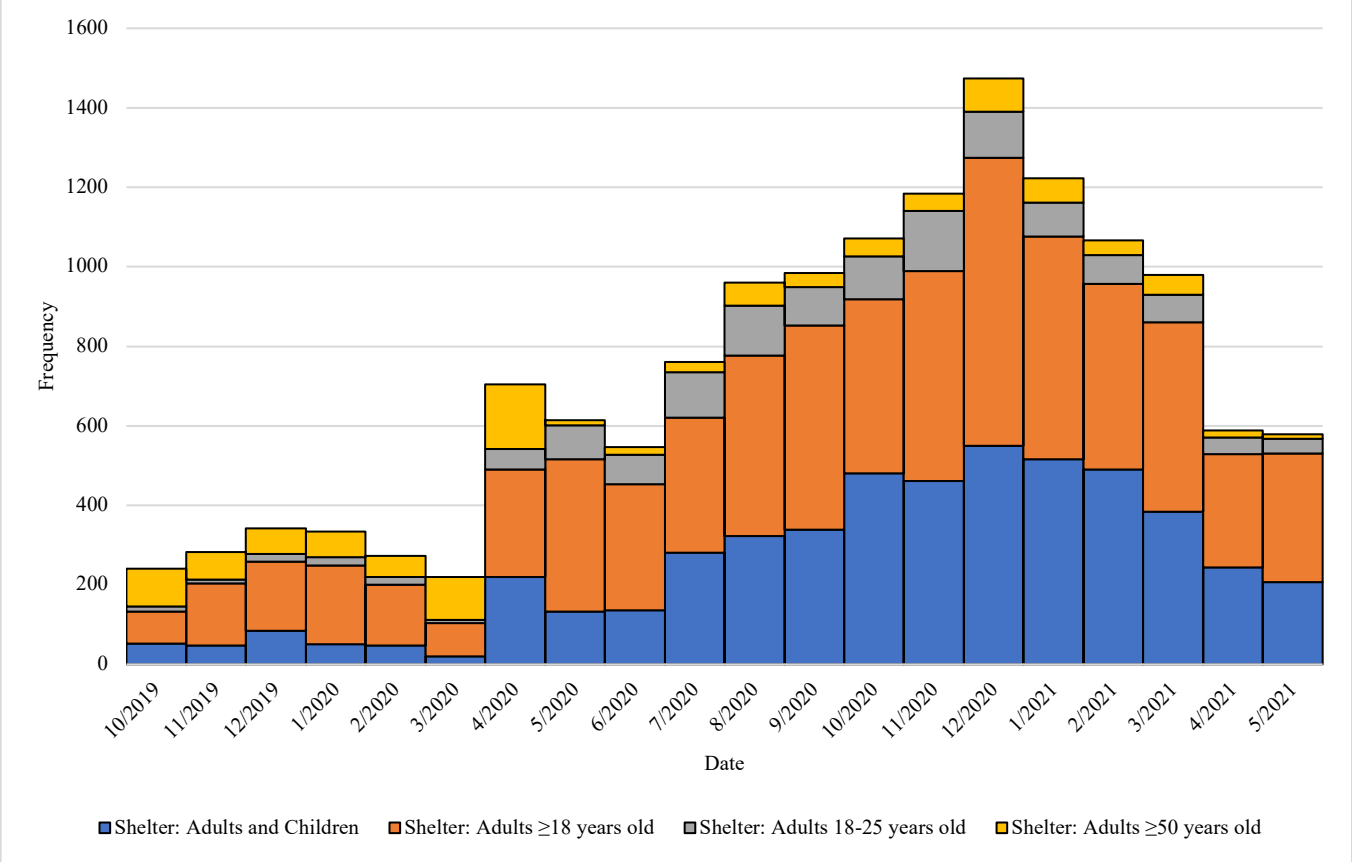
			12/28/2019	Yes	HCoV-HKU1***/HCoV-NL63	22·3	
			1/16/2020	No			
	2	1 day	1/27/2020	No			49 days
			2/10/2020	Yes	HCoV-HKU1B	16·5	
			4/16/2020	No			
I	1	4 days	12/12/2019	No			-
			12/20/2019	Yes	HCoV-HKU1***	15·4	
			12/23/2019	Yes	HCoV-HKU1***	25·4	
			1/29/2020	No			
J	1	3 days	12/17/2019	No			40 days
			12/19/2019	Yes	HCoV-NL63	17·8	
			12/21/2019	Yes	HCoV-NL63	15	
			12/30/2019	No			
	2	1 day	1/8/2020	No			
			1/28/2020	Yes	HCoV-OC43	11·9	
			2/7/2020	No			
K	1	7 days	12/26/2019	No			-
			12/28/2019	Yes	HCoV-HKU1B	11·9	
			12/30/2019	Yes	HCoV-HKU1B	15·7	
			12/31/2019	Yes	HCoV-HKU1B	17·7	
			1/3/2020	Yes	HCoV-HKU1B	19·3	
			1/4/2020	No			

*Total days of seasonal human coronavirus detection over same infection course calculated as the number of days of detection from the first day of detection to the last day of detection
**Time between different infections was calculated as the number of days between the date of first detection of the first seasonal human coronavirus infection and the date of first detection of the second seasonal coronavirus infection
***Unable to determine sub-type of HCoV-HKU1 infection

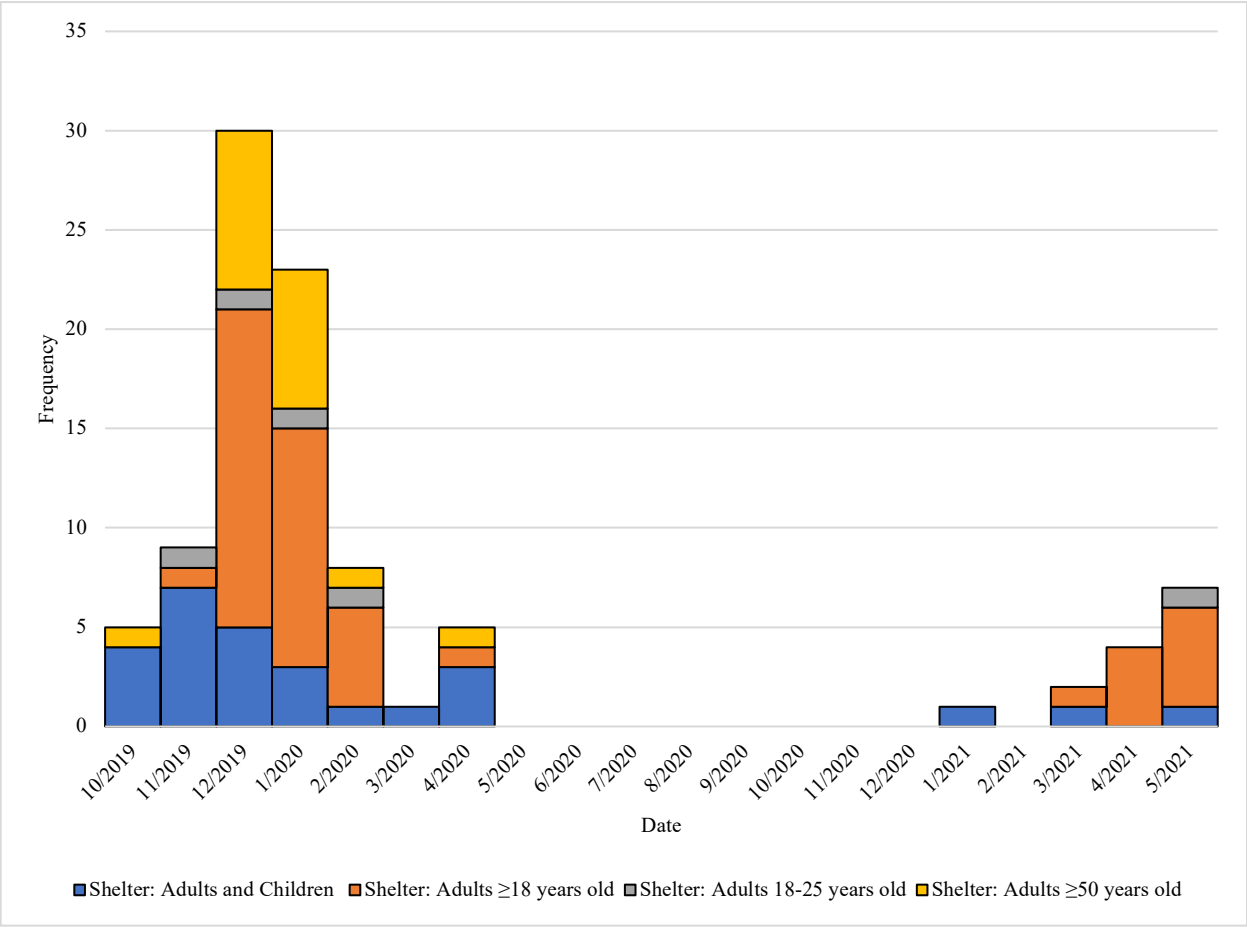
Supplemental Figure 1. Study Flow Diagram



Supplemental Figure 2 All Encounters by Shelter Type – October 2019 to May 2021



Supplemental Figure 3. Seasonal Human Coronavirus Cases Among Unique Participants by Shelter Type – October 2019 to May 2021 (n = 90)



Supplemental Figure 4: Phylogenetic Trees of Genomes from Shelter Samples and From GenBank by Seasonal Human Coronavirus Species

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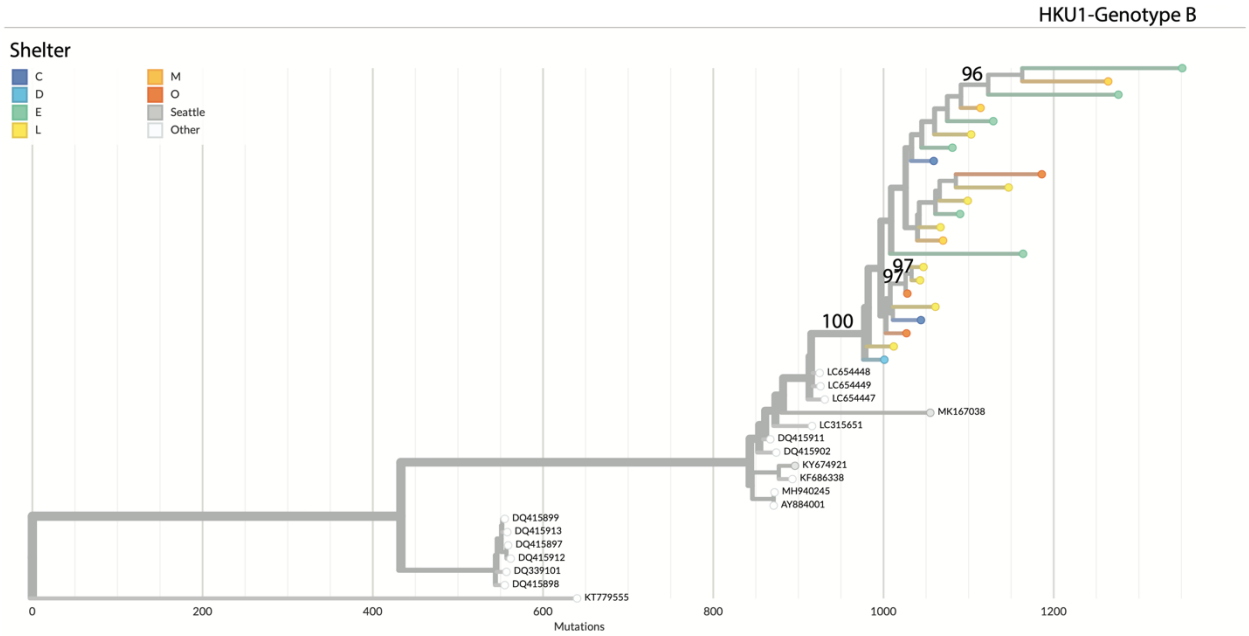

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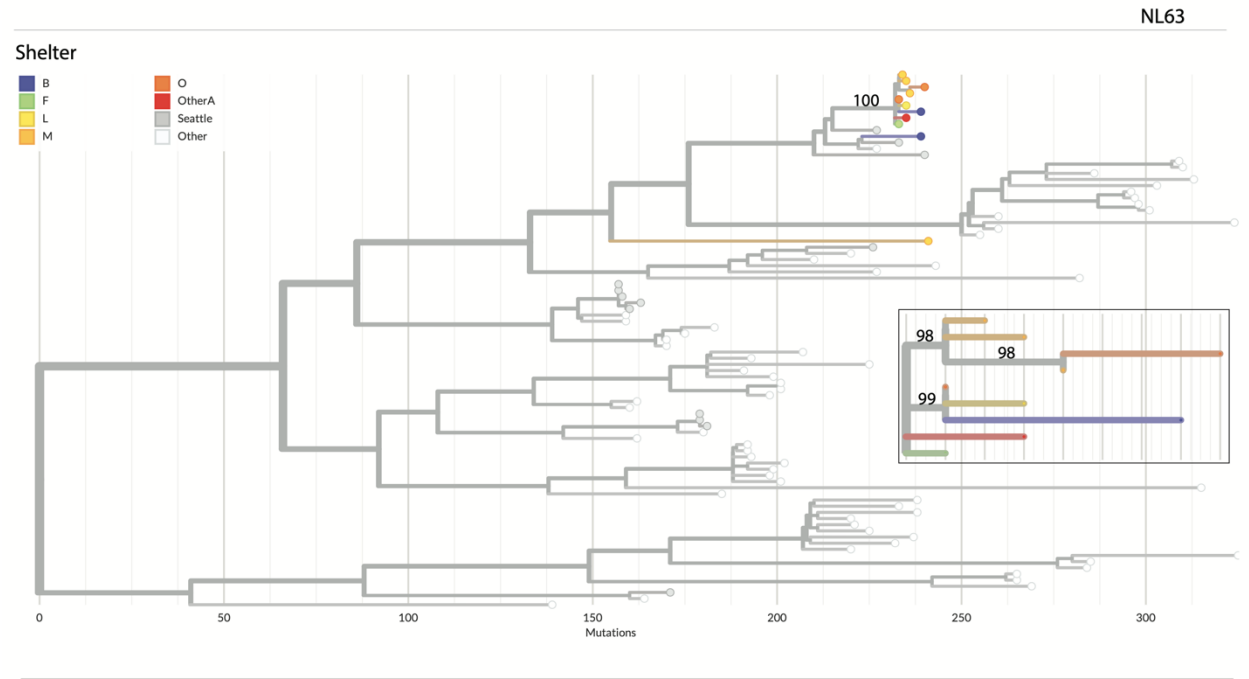

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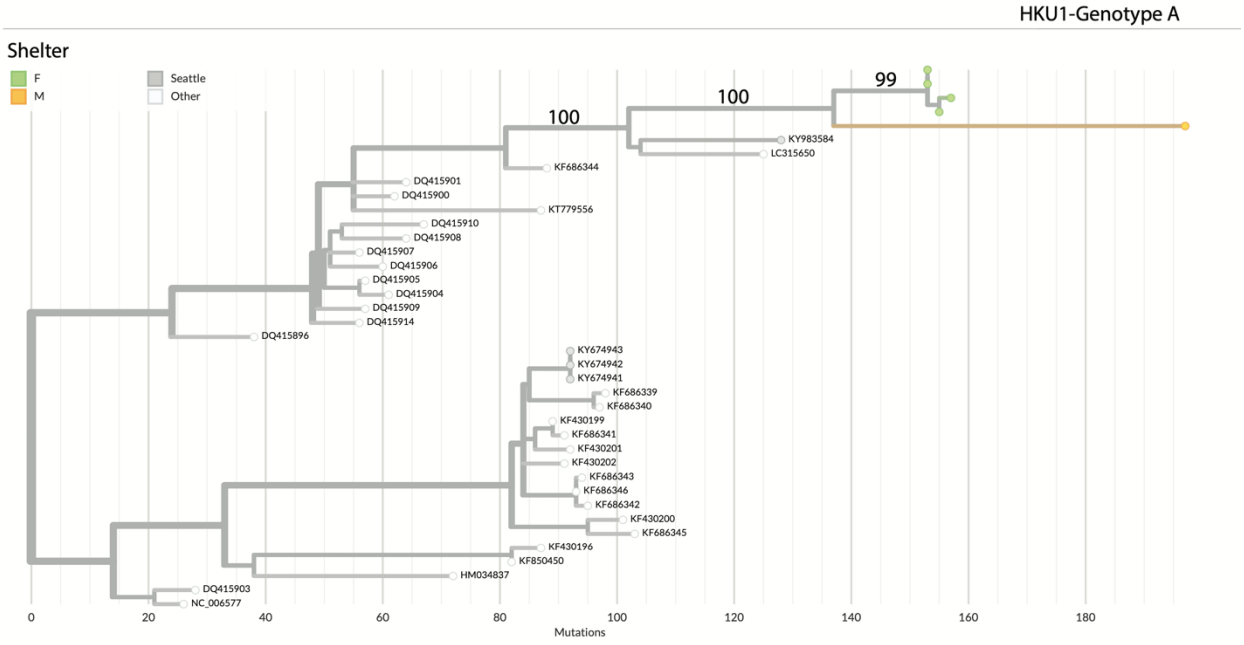
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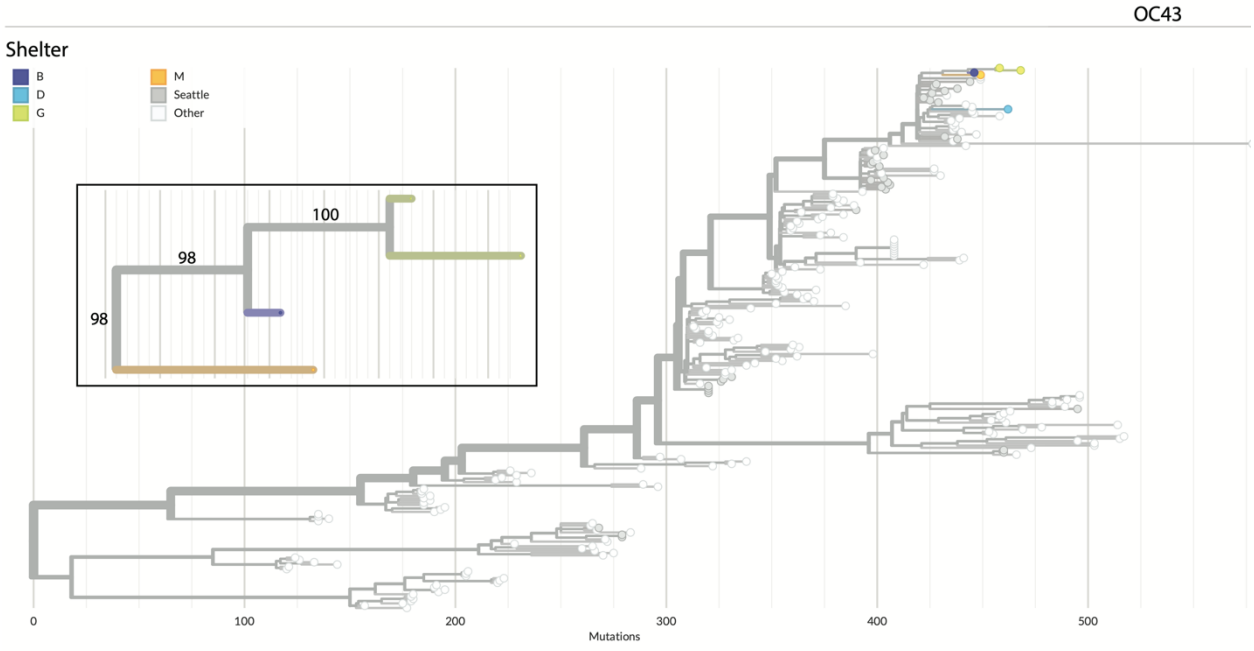
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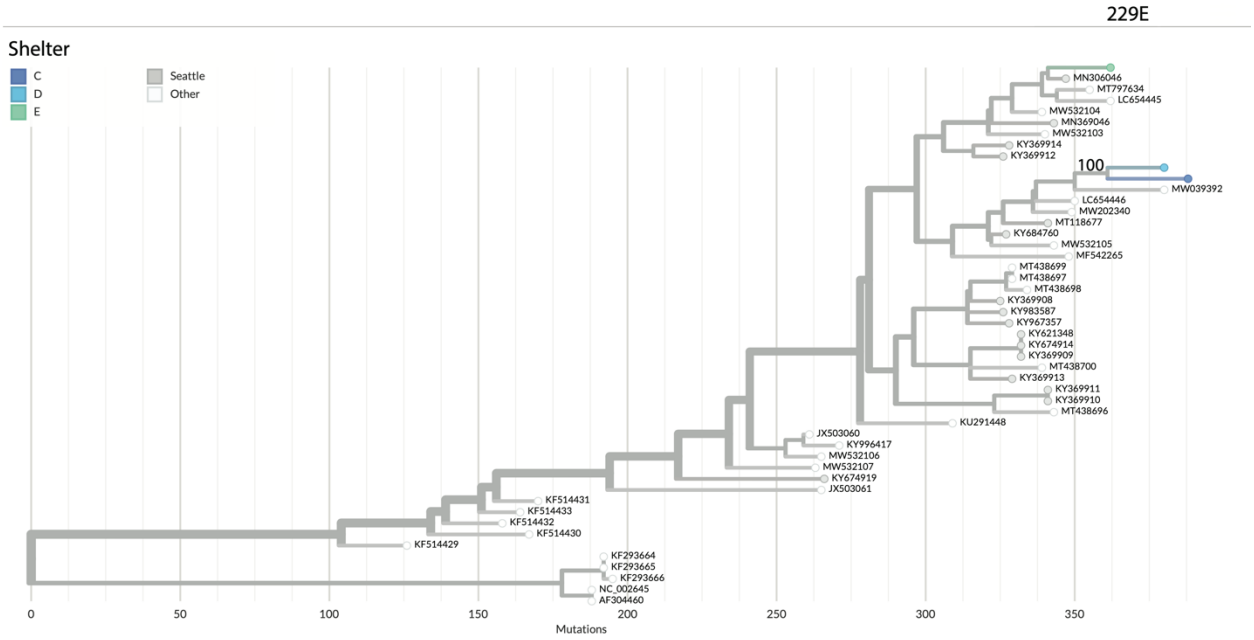
E)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Supplemental Methods

Study Design and Population

This study was a secondary analysis of cross-sectional data from a previously described cluster randomized control trial of influenza testing and treatment and a subsequent SARS-CoV-2 surveillance study in homeless shelter within the Seattle-King County, Washington area from October 2019-May 2021.[1,2] The influenza testing and treatment trial was a stepped-wedge cluster-randomized design clustered by homeless shelter (RCT) conducted between October 2019-March 2020 and October 2020-March 2021. Shelter residents aged ≥3 months with acute respiratory illness (ARI) symptoms onset within the last seven days were eligible for the study. ARI symptoms were defined as cough or at least two of the following symptoms: subjective fever, headache, sore throat, runny or stuffy nose, shortness of breath, and muscle or body aches; for participants aged <18 years, diarrhoea, rash and ear pain or discharge were included as well. Participants in the RCT study were screened and self-enrolled from staffed kiosks within each shelter site six days per week during these time periods. Individuals meeting these requirements, or their parent or guardian were electronically consented and filled out a questionnaire. A mid-turbinate nasal swab sample was obtained and sent for respiratory viral testing. A subsequent questionnaire and nasal swab were collected in the intervention arm at day 2 or 3 and at day 5, 6 or 7 after diagnosis and antiviral treatment. Once a month, shelter wide surveillance occurred when asymptomatic and minimally symptomatic individuals were enrolled.

Due to public health response to the SARS-CoV-2 spread in King County, the first year of the influenza trial intervention study ended early on April 1, 2020, and was replaced with an active surveillance study investigating SARS-CoV-2 epidemiology in homeless shelters. On-site recruitment was reduced to three days per week at each shelter when RCT study activities were not on-going (April 1, 2020-September 30, 2020, and April 1, 2021-May 31, 2021). Recruitment was opened to both shelter staff and residents aged ≥3 months regardless of symptoms from April 1, 2020, onwards. Individuals were limited to once weekly participation in the study unless new or worsening symptoms developed. Participants were not followed longitudinally but were permitted to enrol more than one time. In addition to routine surveillance where participants were recruited from staffed kiosks, one-day surge testing events were implemented with Public Health — Seattle & King County beginning March 30, 2020, as part of contact tracing efforts in shelter sites where cases of SARS-CoV-2 were detected. During surge testing events, testing was offered to all residents and shelter staff regardless of symptoms.

Prior to participation in the study, consent was required for individuals aged ≥18 years or from a guardian for those aged <18 years. Participants aged 13-17 years also provided written assent to enrol in the study. Multiple encounters from unique participants were linked together by name and birthdate. Data were de-identified prior to the preparation of this manuscript. Our study was approved by the University of Washington Institutional Review Board (Study 00007800) and was prepared using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Questionnaire and Shelter Site Data

Participants completed a sociodemographic and illness questionnaire at the time of nasal swab collection. Study staff and telephonic interpreters were available for assistance when needed. The questionnaire was administered on a tablet and data were stored on Research Electronic Data Capture (REDCap). Self-reported data collected included shelter site location, birthdate, sex, race and ethnicity, pregnancy status, and current tobacco and e-cigarette use status. Participants were asked if they had underlying medical conditions including neurological disease, cardiovascular disease, asthma, bronchitis, chronic obstructive pulmonary disease (COPD), hepatic disease, diabetes mellitus, immunosuppression, cancer or another condition not listed. The questionnaire also included self-reported symptoms associated with their illness course: runny nose or congestion, cough, sore throat, fatigue, myalgias, headaches, subjective fevers, shortness of breath, sweats, nausea or vomiting, chills, diarrhoea, rash, and ear pain or discharge. Loss of sense of taste or smell was added after April 1, 2020. On site study staff guided participants through symptom screening and the enrolment survey. Asymptomatic infection was defined as anyone not reporting any of the aforementioned symptoms up to the time of enrolment. Shelter facility specifics, including maximum resident capacity, available sleeping arrangements and targeted resident demographics that the shelter served were shared directly with the research team by shelter management.

Specimen Collection and Respiratory Viral Testing

Samples were initially obtained by mid-turbinate sterile nylon flocked swabs (Copan Diagnostics) by study staff from October 2019-July 22, 2020, and then subsequently from November 1, 2020-May 31, 2021. Anterior nares swabs were used from July 22, 2020-November 1, 2020, due to supply chain resource limitations. With the spread of SARS-CoV-2, sample collection was changed to a study staff supervised self-collected swabs. Comparability of a self-collected mid-turbinate swab to clinician-obtained nasopharyngeal sample has previously been described. Samples were stored at 4°C in universal transport media. Samples were purified for total nucleic acids using the Roche MagnaPure 96 DNA and viral NA small volume kit, Viral NA Universal SV 4·0 protocol (200µ input, 50µ elution) and tested by RT-PCR for multiple viral pathogens using a custom arrayed platform including: influenza (A, B and C), respiratory syncytial virus (A and B), human parainfluenza virus (1-4), HCoV, metapneumovirus, rhinovirus, enterovirus, human bocavirus, human parechovirus and adenovirus. Beginning November 23, 2020, the OpenArray platform identified HCoV by species including HCoV-HKU1, HCoV-NL63, HCoV-229E and HCoV-OC43. Beginning February 25, 2020, samples were tested for SARS-CoV-2 in real-time by a multiplexed RT-PCR assay targeting SARS-CoV-2 Orf1b and human RNase P genes in samples collected through March 18, 2020, and a multiplexed RT-PCR assay targeting SARS-CoV-2 Orf1b and S genes with FAM Fluor and the human RNase P gene with VIC or HEX fluor from March 19, 2020, onward. Specimens collected from January 1, 2020-February 24, 2020, were tested retrospectively using a single replicate Orf1b and RNase P multiplexed RT-PCR research assay to detect SARS-CoV-2 Orf1b. An OpenArray relative cycle threshold (Crt) value was calculated for virus-positive samples.

Genomic Sequencing and Analysis

We attempted genomic sequencing on HCoV-positive samples with Crt values <22. RNA was extracted from samples using the Roche MagnaPure 96 DNA and viral NA small volume kit, Viral NA Universal SV 4·0 protocol (200µ input, 50µ elution). Shotgun metagenomic sequencing libraries were prepared as previously described.[3,4] Briefly, RNA was DNase treated using the Turbo DNA-Free kit (Thermo Fisher). First-strand cDNA was synthesized using Superscript IV (Thermo Fisher) and random hexamers (Integrated DNA Technologies), and second-strand synthesis was performed with Sequenase version 2.0 DNA polymerase (Thermo Fisher). The resulting double-stranded cDNA was purified using AMPure XP beads (Beckman Coulter). Libraries were constructed using the Nextera DNA Flex pre-enrichment kit (Illumina) and cleaned using 0·8 volumes of AMPure XP beads. For samples where genomes could not be recovered using shotgun sequencing, we performed hybridization capture using biotinylated oligonucleotide probes (Integrated DNA Technologies). The resulting libraries in both cases were sequenced on an Illumina NextSeq 2000 using a 1x100 read format.

Raw reads were assembled into consensus genomes using the Seattle Flu Study assembly pipeline (https://github.com/seattleflu/assembly). Briefly, low quality bases and adapter sequences are trimmed from the ends of raw reads and short reads are removed. Processed reads are then mapped to reference genomes representing the four HCoV species (HCoV-OC43 GenBank KU131570.1, HCoV-NL63 NC_005831.2, HCoV-229E NC_002645.1, HCoV-HKU1 NC_006577.2). SNPs are called from these alignment files and are applied to the reference genomes to generate four consensus genomes for each sample. The consensus genome with the least missing data (lowest percent Ns) for each sample was selected for inclusion in further analyses. Sequences were uploaded to GenBank (Supplemental Table 3).

Among samples with completed genome sequencing, one genome per HCoV species from each unique participant were used to exclude multiple specimens from the same illness course. Maximum likelihood phylogenetic trees were constructed for each HCoV species (with separate trees for HCoV-HKU1A and HCoV-HKU1B) that included the shelter genomes and all full length (>25,000bp) genomes available in GenBank (Supplemental Figure 2). Shelter genomes for each HCoV species were then compared to assess clustering by shelter site (Supplemental Table 1) and time of sample collection. Genomic sequences collected before the pandemic (April 2020 and earlier) and during the pandemic (January 2021 and later) were compared.

Seasonal Human Coronavirus Species Identification

For samples that underwent genomic sequencing, the reference genome associated with the highest quality consensus genome was presumed to represent the sample's species. These prospective species assignments were confirmed by creating a maximum likelihood phylogenetic tree including all sample genomes and the four reference genomes. All other samples had species determination by multiplex RT-PCR or were sent for species-specific primer PCR amplification. HCoV species were confirmed using type-specific FAM fluor assays from ThermoFisher: Vi06439674_s1 (HKU1); Vi06439673_s1 (NL63); Vi06439671_s1 (HK229EU1); and

Vi06439646_s1 (OC43). PCR reactions used AgPath-ID One-Step RT-PCR kit, with 17.5µl buffer, 1.4µl enzyme, 1.5µl assay mix, and 5µl template RNA per 30µl reaction. All amplifications were carried out in ABI 7500 thermocyclers, and results were analysed with thresholds set to 0·1 and baselines set to 3-15.

References

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