Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Household Transmission during the Omicron Era in Massachusetts: A Prospective, Case-Ascertained Study using Genomic Epidemiology

Jaspreet Banga[1]*, Taylor Brock-Fisher[2,3]*, Brittany A. Petros[2,4]*, Eric Y. Dai[5], Ariana T. Leonelli[5], Sabrina T. Dobbins[2], Katelyn S. Messer[2], Audrey B. Nathanson[5], Amelia Capone[6], Nancy Littlehale[6], Viola Appiah-Danquah[5], Siang Dim[5], Gage K. Moreno[2], Maura Crowther[5], Kannon A. Lee[5], Katherine C. DeRuff[2], Bronwyn L. MacInnis[2], Michael Springer[7], Pardis C. Sabeti[2,3,8,9], Kathryn E. Stephenson[1,5,10,11,#]

*These authors contributed equally.

[1]Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston, MA, USA

[2]The Broad Institute of MIT and Harvard, Cambridge, MA, USA

[3]Department of Organismic and Evolutionary Biology, Faculty of Arts and Sciences, Harvard University, Cambridge, MA, USA

[4]Harvard/MIT MD-PhD Program, Boston, MA, USA

[5]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA, USA

[6]Beth Israel Lahey Health Primary Care, Chelsea, MA, USA

[7]Department of Systems Biology, Harvard Medical School, Boston, MA, USA

[8]Harvard T.H. Chan School of Public Health, Boston, MA, USA

[9]Howard Hughes Medical Institute, Chevy Chase, MD, USA

[10]Harvard Medical School, Boston, MA, USA

[11]Ragon Institute of Massachusetts General Hospital (MGH), Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA, USA

#Corresponding Author: Kathryn E. Stephenson, MD, MPH, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, E/CLS-1037, 300 Brookline Ave, Boston, MA 02215. Email: kstephen@bidmc.harvard.edu.

Key Points: When community transmission of SARS-CoV-2 is high, distinguishing household transmissions from independent introductions is difficult with either epidemiologic or genomic data alone. Here, we conducted daily nasal sampling with genomic sequencing to understand the dynamics of viral transmission within households.

Abstract:

Background:

Households are a major setting for SARS-CoV-2 infections, but there remains a lack of knowledge regarding the dynamics of viral transmission, particularly in the setting of widespread pre-existing SARS-CoV-2 immunity and evolving variants.

Methods:

We conducted a prospective, case-ascertained household transmission study in the greater Boston area in March-July 2022. Anterior nasal swabs, along with clinical and demographic data, were collected for 14 days. Nasal swabs were tested for SARS-CoV-2 by PCR. Whole genome sequencing was performed on high-titer samples.

Results:

We enrolled 33 households in a primary analysis set, with a median age of participants of 25 years old (range 2-66); 98% of whom had received at least 2 doses of a COVID-19 vaccine. 58% of households had a secondary case during follow up and the secondary attack rate (SAR) for contacts infected was 39%. We further examined a strict analysis set of 21 households that had only 1 PCR+ case at baseline, finding an SAR of 22.5%. Genomic epidemiology further determined that there were multiple sources of infection for household contacts, including the index case and outside introductions. When limiting estimates to only highly probable transmissions given epidemiologic and genomic data, the SAR was 18.4%.

Conclusions:

Household contacts of a person newly diagnosed with COVID-19 are at high risk for SARS-CoV-2 infection in the following 2 weeks. This is, however, not only due to infection from the household index case, but also because the presence of an infected household member implies increased SARS-CoV-2 community transmission. Further studies to understand and mitigate household transmission are needed.

INTRODUCTION

In March 2022, the SARS-CoV-2 Omicron variant BA.2 and its descendants caused multiple outbreaks across the greater Boston area of Massachusetts[1]. More than 95% of the population of Massachusetts at that time had received at least one dose of a COVID-19 vaccine, and a large number of individuals had been infected with the Omicron BA.1 variant only a few months prior[1]. Despite this presence of partial population immunity, there were over 200,000 new COVID-19 cases reported in Massachusetts between March and July 2022, coincident with the dropping of mask requirements and a reduction in social distancing policies.

Throughout the COVID-19 pandemic, households have been a major setting for SARS-CoV-2 transmission[2]. A meta-analysis of 135 studies published in 2022 found that the secondary attack rate (SAR) – the risk of transmission from index case to household contact – of exposed household contacts was 30-36% during the Alpha and Delta SARS-CoV-2 waves and 43% during the early (BA.1) Omicron period[2]. Factors associated with transmission risk were the vaccine status of the exposed contact, household density (i.e., the number of people sleeping in the same room), and immunological factors such as the magnitude of cross-reactive memory T cells to SARS-CoV-2 antigens[3-5]. In general, the SARs reported in households, and other residential settings[6], have been far higher than reported in other settings such as schools, where a SAR of 2.8% was reported for exposed students in 10 Massachusetts schools during the Delta wave[7].

Despite the large number and range of household studies performed to date, there remains a lack of precision and detail regarding the dynamics of SARS-CoV-2 household transmission. These studies are each individually small in size, and most lack careful characterization on the genomic level. This uncertainty is particularly important in the increasingly common scenario where there is (1) widespread pre-existing SARS-CoV-2 immunity, either from vaccination or infection, (2) emergence of new SARS-CoV-2 strains that are partially immune evasive, and (3) few infection control precautions in home and community environments. Knowing the risk of household transmission in this scenario, as well as identifying factors that can predict lower SARs, would enhance our ability to counsel patients appropriately and meaningfully reduce SARS-CoV-2 infections in households.

To address this research gap, we conducted a household SARS-CoV-2 transmission study in the greater Boston area in March-July 2022 in which we used clinical, demographic, viral kinetic, and genomic data for high-resolution analyses of SARS-CoV-2 transmission dynamics.

METHODS:

We conducted a prospective, observational, case-ascertained transmission study of household contacts of index SARS-CoV-2 cases identified in the greater Boston and Chelsea areas of Massachusetts in the United States. The primary recruitment pathway was via ambulatory COVID-19 testing sites associated with Beth Israel Deaconess Medical Center (BIDMC).

Ethics

The BIDMC Committee on Clinical Investigation, its Institutional Review Board (IRB), approved the study protocol (#2022P000021). Consent procedures were completed via phone/video conferencing or in-person. All enrolled participants provided verbal informed consent. Participants who met applicable IRB guidelines for provision of assent underwent an age-appropriate assent process; informed consent for study participation was obtained from each participant's parent or legal guardian before any study-specific procedures were performed. Sequencing of SARS-CoV-2-positive specimens was covered under protocol #1612793224, reviewed and approved by the Massachusetts Institute of Technology (MIT) IRB.

Study Population

An index case was eligible for inclusion if the participant (1) had a positive SARS-CoV-2 test either by rapid diagnostic assay or RT-PCR no more than 5 days prior to enrollment and had no more than 5 days of acute respiratory illness symptoms prior to testing, and (2) lived, and had plans to live in his/her household for the follow-up period of 14 days, and (3) was not hospitalized and had not been hospitalized since the date of illness onset, and (4) was at least 2 years old. An index case was excluded from the study if the participant did not live in a household (e.g., lived alone or in a congregate setting), or if they reported that any other person in the household had an acute respiratory illness or tested positive for SARS-CoV-2 in the 7 days before the test date or illness onset date of the index case.

A household contact was eligible for inclusion if the participant (1) routinely slept (at least half of the nights in the last month) in the same household as the index case, and (2) slept in the

household at least once between 1 day prior to the earliest of the index case's illness onset or positive test date and (3) had plans to live in the household for the follow-up period, and (4) was at least 2 years old.

Respiratory Specimen Collection

Each enrolled index case and household member self-collected an anterior nasal swab (Rhinostics dry swab in a sterile tube; https://rhinostics.com/) on each day of the 14-day follow-up period. The swab was self-collected regardless of whether the individual had acute respiratory illness signs or symptoms. Samples were dropped off by the participants at the BIDMC Clinical Research Center or the Chelsea clinic. A subset of participants who were treated with nirmatrelvir-ritonavir were asked to extend the follow-up period and continue self-swabbing for an additional 7 days in order to monitor for potential virologic rebound[8].

Questionnaires

At enrollment, each consenting household member and index case, or his/her parent/guardian, was asked to provide information on participant demographics, recent exposures to COVID-19, presence of high-risk conditions, prior and current symptoms of COVID-19, COVID-19 vaccination status, household characteristics, interactions with other enrolled and non-enrolled household members and people outside the home, and other social or medical history, including other vaccination history, as deemed appropriate. An optional follow-up questionnaire was administered to participants on days 7 and 14.

SARS-CoV-2 Viral Load Quantification

Viral loads were measured with the Quaeris SARS-CoV-2 Assay, a real-time reverse transcription polymerase chain reaction (rRT–PCR) test, using the Luna Probe One-Step RT-qPCR Kit (No ROX) [NEB E3007][9]. The SARS-CoV-2 primer and probe set detects RNA from the SARS-CoV-2 N1 and RdRP genes, and uses the human RNase P gene as a positive control. When received by the laboratory, samples were rehydrated with 300 µl phosphate buffered saline (PBS), inactivated at 65ºC, and subsequently used directly as input for the Quaeris assay without extraction. rRT-PCR was performed on an Applied Biosystem QuantStudio 7 instrument (software version 1.7). Liquid handling was automated using either the Tecan Fluent 1080, the Hamilton Star, or the Multidrop combi dispenser. N1 gene cycle threshold (Ct) values are reported. Viral rebound was defined as at least 2 negative (Ct≥35) PCR results followed by at least 2 positive (Ct<35) results.

SARS-CoV-2 Whole Genome Sequencing

Anterior nasal swabs that were positive for SARS-CoV-2 with Ct values of maximally 32 via the Quaris assay were sequenced as previously described[10]. Briefly, following inactivation with Buffer AVL, RNA was extracted from the samples using the MagMAX mirVana Total RNA Isolation Kit for the Kingfisher Flex (Thermo Fisher #A27828) according to the manufacturer's instructions. The ARTIC v4.1 primer set was used to amplify SARS-CoV-2 genetic material, from which Illumina DNA Prep sequencing libraries were prepared and sequenced on the NextSeq 550[11].

SARS-CoV-2 Genomic Data Analysis

Sequencing reads were demultiplexed, filtered to remove adapter and contaminant sequences, depleted of reads mapping to the human genome, and assembled by alignment to the reference sequence NC045512.2 via the viral-ngs v2.1.33 pipeline[12]. The following optional inputs were used with the assemble_refbased workflow to accommodate amplicon-based sequencing: major_cutoff = 0.5, min_coverage = 20, skip_mark_dupes = TRUE, and trim_coords_bed = "gs://pathogen-public-dbs/v1/amplicon_primers-ARTICv4.1_NC_045512.2.bed".

Lineages were assigned to viral genomes using Pango v4.1.3 pango-data v1.17[13]. Consensus single nucleotide variants (SNVs) were determined after removing ambiguous sites and sites prone to amplicon sequencing error[14]. Intrahost single nucleotide variants (iSNVs) were called using LoFreq and were filtered as follows: (i) masking of known problematic sites in SARS-CoV-2 genome, (ii) site read depth of minimally 100; (iii) allele frequency of minimally 3%; and (iv) no evidence of strand bias (via Fisher's exact test with $p > 0.05$)[11,15].

Transmission Analysis

To infer the relatedness of cases within a household, the most complete genome (i.e., the genome with the highest percentage of unambiguous sites) was used for each person with a minimum unambiguous genome length of 15,000 bp. The genetic (SNV) distance between pairs of cases was used alongside the serial interval, viral kinetics, iSNV information, and test status of other individuals at baseline to classify households into transmission categories. All pairs of cases within each household were assessed, excluding those inconsistent with the longitudinal testing data.

Transmissions were categorized as highly probable, possible, or unlikely. Pairs with a genetic distance of less than 2 SNVs and an iSNV in a putative donor that reached consensus in the putative recipient (i.e., transmission of a minor variant through a tight transmission bottleneck) were considered highly probable[16,17]. An index-contact pair in the strict analysis set (i.e., from a household with a single infected participant and documented PCR- contacts at baseline) with a genetic distance of less than 3 SNVs and a serial interval of less than 7 days were also considered highly probable transmissions. Pairs with a genetic distance of less than 3 SNVs and a serial interval of less than 7 days, but without iSNV support, were categorized as a possible transmission, though we cannot rule out a non-household recent common ancestor (i.e.., a shared exposure)[18,19]. Pairs with a genetic distance of 3 or more SNVs or a serial interval of minimally 7 days were considered unlikely. Highly probable and possible transmission links were plotted.[20,21]

Phylogenetic Analysis

Contextual genomes (10 BA.1 sequences each from Massachusetts and the United States, and 50 BA.2 sequences each from Massachusetts and the United States, isolated between 2022-02-01 and 2022-08-01) were downloaded from NCBI GenBank. These sequences, along with the most complete genome assembled per study participant (of minimally 15,000 bp), were aligned to the reference sequence (NC_045512.2) using Nextclade v.2.14.1[22]. A phylogenetic tree was estimated using IQ-TREE v.2.2.2.6[23] and was visualized and annotated using ggtree v.3.8.2[24] in R v.4.3.1.

RESULTS

Screening, Enrollment, and Follow-up

Enrollment began on March 3, 2022 and continued until July 9, 2022, after local SARS-CoV-2 case counts had declined[1]. We enrolled 38 households containing 38 index cases and 77 household contacts; Figure 1. One household was subsequently found to be ineligible and 4 households did not complete 14 days of follow up. The remaining 33 households (33 index cases and 66 household contacts) constituted our primary analysis set; 85% of these households (28/33) had all household members participate. Of the 33 households in the primary analysis set, 3 households had an index case that was PCR- at the time of first research swab, and 9 households had at least one household contact that were PCR+ at the time of first research swab. The remaining 21 households (21 index cases and 40 PCR- household contacts) constituted our strict analysis set. The median number of persons per household in the primary analysis set was 3.5 (range, 2-5; Supplementary Table 2). The median house size was 1400 square feet (range, 740-3300; Supplementary Table 2).

Study Population

Demographic and clinical characteristics of index cases and household contacts in the primary analysis set are shown in Table 1 and Supplemental Table 1. The median age was 25 years (range, 5-64) for index cases and 39 years (range, 2-66) for household contacts. 23% of participants were children in elementary or middle school. Participants were predominantly White and Non-Hispanic. 81% of participants reported having no medical conditions. The most common reported medical conditions were asthma (12%), autoimmune or immune system

disease (8%), and high blood pressure (4%). 10% of participants reported having COVID-19 at some point prior to the study (though symptom- and testing-based ascertainment bias is likely). 98% of participants received at least 2 vaccine doses. The median duration between most recent vaccination or most recent infection was 160 days (range 0-307).

SARS-CoV-2 Infections

Of the 33 households in the primary analysis set, 19 (58%) had a household contact who tested positive by PCR during follow up (Figure 2A and Table 2). Of the 66 household contacts in the primary analysis set, 26 tested positive, indicating a putative SAR of 39%; 11 of these cases were detected on day 1 of swabbing. If limiting analysis to the 21 households in the strict analysis set, 9 (43%) households had a new incident infection during follow up, corresponding to 9 infections out of 40 household contacts, or a putative SAR of 22.5%.

In the primary analysis set of 33 households, the median minimal Ct - corresponding to peak viral load - was 25.65 for index cases and 23 for infected household contacts (Supplemental Table 3). Median duration of viral shedding was 6.5 days and 8 days for index cases and contacts, respectively, and the median time to positive SARS-CoV-2 test from index diagnosis date was 3 days. The lower viral load and decreased duration of shedding for index cases is most likely due to the fact that cases were detected partway through infection. Eleven participants reported taking nirmatrelvir-ritonavir; 3 participants had viral rebound (Supplemental Figure 1A: MPO-02, BQE-01 and BQE-02)[8].

SARS-CoV-2 Sequencing

For the primary analysis set, approximately 1300 total anterior nasal swab samples were collected and analyzed via the rRT-PCR Quaris assay. 181 of these samples, from 48 unique individuals across 26 unique households, had a diagnostic Ct under 32 (mean = 30.1) and were thus subject to genomic sequencing. 178/181 (98.3%) samples had a nonzero unambiguous assembly length with a median length of 29,321bp. 143 samples, which came from 44 unique individuals among 24 unique households, produced partial genomes of at least 15,000 bp in length (Supplemental Table 4). 15 households yielded partial genomes from multiple individuals within the household. The vast majority of the sequences were BA.2 descendants, consistent with national trends (Figure 2B), though a BA.1 sequence was collected early in enrollment.

Focusing first on analyzable households in the strict analysis set (N=6), we found that four households had viral sequences that were either identical between index and secondary cases or differed by only 1-2 SNVs (Figure 3A, Supplemental Table 5). In these cases, transmission from index to secondary case was considered highly probable. In two households, we identified significant viral variation between sequences isolated from the index and secondary contacts, strongly suggesting that the secondary cases were independent introductions (Figure 3B). In household XOA, the index case was infected with a BA.2.13 variant, while the contact was infected with BA.2.12.1; there was a 13 SNV difference between sequences. In household WMD, the index case was infected with BA.2.12.1, and the contact was infected with BA.2; there was a 20 SNV difference between sequences. (Of the 3 remaining households with secondary cases in the strict analysis set (AXB, JDU and NKF), sequences were not available or couldn't be analyzed; viral kinetic curves for these households are in Figure 3C). When

296 excluding the 2 household contacts that were infected outside of the household, the SAR among
297 individual household contacts in the strict analysis set was 18.4% (Table 2).
298
299 Analyzing transmission networks among all the households in the primary analysis set revealed
300 several interesting patterns. In 5 households, there were clusters of infections (2-3 per household)
301 that were all genetically linked to the index case (Figure 4A (a representative example), and
302 Supplemental Figure 1A). In these households, there were 0 SNVs different between index and
303 contact sequences, but the close overlap in diagnosis dates suggests that either a shared common
304 exposure or intra-household transmission was possible. In 3 households, there were two distinct
305 clusters of infections detected (Figure 4B (a representative example), and Supplemental Figure
306 1B). In these households, the index case was infected with a virus that was 3-4 SNVs different
307 than the viruses that circulated in the rest of the household. In 1 household (Figure 4C), there
308 was evidence that the index case was co-infected with 2 contemporaneously circulating strains
309 (BA.2.1.12 and BA.2.10) while the contact was infected with solely BA.2.1.12; given the close
310 diagnosis dates, this could be explained by either a shared social network or transmission with a
311 bottleneck.
312
313 **Potential Predictors of Transmission**
314 To explore potential predictors of transmission between an index case and household contact, we
315 examined the 7 household contacts where transmission from the index was highly likely
316 compared to the 28 participating contacts that remained uninfected. Using Wilcoxon rank sum
317 and Fisher's exact tests (Table 3), we found that there was no association between infection
318 status and the contact's prior COVID-19 vaccination, number of prior vaccines received, history

of prior infection, days since vaccination and/or infection, age or sex assigned at birth. There was also no association between infection status and the associated index case's prior COVID-19 vaccine status, minimum Ct value, or duration of SARS-CoV-2 shedding, and no association between infection status and the number of persons in the household or and square footage. The small sample size and homogeneity of many of these variables (e.g., vaccine history) limited the power of our analysis to detect predictors of transmission risk.

DISCUSSION

Here we studied the transmission dynamics of SARS-CoV-2 within households using prospective daily PCR surveillance, clinical and demographic data, and genomic epidemiology. Our study is well-positioned to reflect the transmission dynamics of households consisting of relatively young, healthy individuals with pre-existing immunity to SARS-CoV-2. It was conducted during a wave of Omicron BA.2 and its sub-lineages. Our study population consisted primarily of families with 2-5 members; 23% of our participants were children in elementary or middle school. Participants were highly vaccinated, having received a median of 3 COVID-19 vaccines. Only 10% had a known prior COVID-19 diagnosis and the population was overall very healthy.

Our study found that the risk of SARS-CoV-2 in exposed households is very high, consistent with previous reports. In our primary analysis set, we found that 58% of enrolled households went on to have a second SARS-CoV-2 case in the household over the next 2 weeks. Nearly a third of households had a positive secondary case identified on the very first day of testing. At

the individual level, we found that 39% of household contacts tested positive for SARS-CoV-2 during follow up. Following the infection of a household contact, putatively negative household members must make decisions around travel and attending school or work. Here, we provide data that could inform this value-based decision.

We further aimed to more rigorously estimate the risk of SARS-CoV-2 transmission directly from an index case, performing a strict analysis including only households that had one confirmed PCR+ index case and only PCR- household contacts at the beginning of surveillance. In this analysis, we identified 9 cases among 40 household contacts, or a SAR of 22.5%. Using genomic epidemiology, we then excluded 2 cases as these were likely acquired outside of the household. We thus determined that SAR was 18.4%. Our study was conducted when multiple BA.2 descendants were circulating, and this genetic diversity improved our ability to rule out putative transmission events.

Using genomic epidemiology across all the households, we found that the infectious source of secondary cases was varied. The most common source of infection was the index case, but other sources included an imported case (outside the household), shared exposures with the index, or other infected contacts within the home. Our findings, particularly evidence of shared exposures and imported cases, highlight that infection within a household serves as a proxy for broader risk for community transmission, emphasizing the importance of maintaining vigilant public health measures both inside and outside of the home. More public health attention should be focused on mitigating this risk via vaccination and therapeutic advances, enhanced and early case detection and isolation, and non-pharmaceutical interventions such as masking and ventilation.

SAR estimates have varied considerably across multiple studies, due to varying study designs, populations, and circulating SARS-CoV-2 variants. During the Alpha and Delta waves, the SAR for exposed household contacts was reported between 30-36%, rising to 43% during the early (BA.1) Omicron period[2]. Here we report that during a BA.2 wave (and its descendants), the SAR was 39% using a larger primary set of households, 22.5% with a strict set of households, and 18.4% when further incorporating genomic data. This lower estimate using genomic data is likely a more accurate reflection of the true risk of household exposure.

Our small sample size substantially limited our power to assess predictors of transmission, especially because prior COVID-19 vaccination was near universal in our study cohort. Given the complexity of estimating the magnitude of pre-existing SARS-CoV-2 immunity by history alone, particularly in the context of both infection and prior vaccines, future household transmission studies would benefit from larger cohorts and baseline immunologic assessments of humoral, cellular and mucosal immune responses.

In summary, we determined that the risk of household transmission of SARS-CoV-2 is very high, even when using genomic epidemiology to exclude imported cases. Further interventions to block household transmission should be studied, especially in the now-common scenario when population immunity and vaccine coverage are high but viral variants are increasingly immune evasive.

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Conflicts of Interest

P.C.S. is a co-founder of, shareholder in, and scientific advisor to Sherlock Biosciences, Inc and Delve Bio; she is also a Board member of and shareholder in Danaher Corporation. P.C.S. has filed IP related to genome sequencing and analysis. The authors declare no other conflicts of interests.

411 **FIGURE LEGENDS**

412

413 **Figure 1**. **Trial profile.**

414

415 **Figure 2. Household diagram.** (A) Individual households are labeled with a 3-letter identifier
416 and represented with grey boxes. Households in both the primary and strict analysis sets are
417 shown. Each member of the household is shown within the grey box, with their outcome color-
418 coded as described in the legend. (B) Phylogenetic map of positive samples.

419

420 **Figure 3. Viral load curves and transmission plots by household in the strict analysis set.**
421 (A) Households where transmission was determined to be highly probable are shown. (B)
422 Households where transmission was determined to be unlikely are shown. (C) Households that
423 did not yield sufficient sequencing data are shown.

424

425 **Figure 4. Representative viral load curves and transmission plots from primary analysis**
426 **set.** (A) A representative household is shown where either a shared exposure (i.e., social
427 network) exists or within-household transmission has occurred, as all cases are genetically linked
428 to the index case. (B) A representative household is shown where two separate infection clusters
429 were identified. (C) A household is shown where a putative co-infection was identified in the
430 index case.

431

432 **Supplemental Figure 1. Additional viral load curves and transmission plots by household in**
433 **the primary analysis set.** Households are shown where either a shared exposure exists or

434 possible transmission has occurred, as all cases are genetically linked to the index case.

435 Households are shown where two separate infection clusters were identified.

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TABLES

Table 1. Baseline Characteristics of Study Population

Characteristic	All Participants (N=99)	Index Cases (N=33)	Contacts (N=66)
Age (Median, Min-Max)	27 (2-66)	25 (5-64)	39 (2-66)
Sex assigned at birth (N, %) –			
Male	44 (48.4)	14 (43.8)	30 (50.9)
Female	47 (51.7)	18 (56.3)	29 (49.2)
Missing	8	1	7
Race (N, %) –			
American Indian or Alaskan Native	10 (11.0)	3 (9.4)	7 (11.9)
Asian	2 (2.2)	1 (3.1)	1 (1.7)
Black or African American	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
White	79 (86.8)	28 (87.5)	51 (86.4)
Multiple	0	0	0
Prefer Not to Report	0	0	0
Other	0	0	0
Missing	8	1	7
Ethnicity (N, %) –			
Hispanic or Latino / Latinx	3 (3.3)	1 (3.1)	2 (3.4)

Not Hispanic or Latino / Latinx	88 (96.7)	31 (96.9)	57 (96.6)
Missing	8	1	7
Body Mass Index (Median, Min/Max)	22.9 (14.1-7.2)	23.0 (16.1-37.1)	22.7 (14.1-47.2)
Missing	19	9	10
Medical Conditions (N, %)			
None	69 (81.2)	21 (72.4)	48 (85.7)
Cancer	0	0	0
Diabetes	1 (1.2)	1 (3.5)	0
Asthma or wheezing	10 (11.8)	5 (17.2)	5 (8.9)
COPD/Emphysema	0	0	0
High blood pressure	3 (3.5)	1 (3.5)	2 (3.6)
Coronary artery disease	0	0	0
Congestive heart failure	0	0	0
Peripheral artery disease	0	0	0
Other*	2 (2.4)	1 (3.5)	1 (1.8)
Missing	14	4	10
Autoimmune or Immune System Diseases, Any** (N, %)	7 (8.2)	1 (3.7)	6 (10.3)
Missing	14	6	8
Prior Diagnosis of COVID-19 (N, %)	9 (9.5)	1 (3.2)	8 (12.5)
Missing	4	2	2

Days between most recent diagnosis and enrollment (Median, Min-Max)	59 (18-780)	780	57 (18-462)
Hospitalized for COVID-19 (N, %) – *missing 1*	0	0	0
Prior Receipt of a COVID-19 Vaccine (N, %) – ***missing 2***	95 (97.9)	32 (100)	63 (96.9)
Number of doses (Median, Min-Max)	3 (2-4)	3 (2-4)	3 (2-4)
Days between most recent COVID-19 vaccination and enrollment (Median, Min-Max) – *missing 4*	165 (1-308)	165 (48-308)	165 (1-307)
Days between most recent diagnosis OR most recent COVID-19 vaccination (whichever is more recent) and enrollment (Median, Min-Max)	160 (0-308)	164.5 (0-308)	160 (1-307)
Missing	2	1	1

*Reported by participant: “heart aneurysm” (1); history of stroke (2)
**Reported by participant: “irritable bowel syndrome, eczema and allergies” (1); “vitiligo” (1); “diverticulosis” (1); “Reynaud’s disease” (1)

Table 2. SARS-CoV-2 Secondary Attack Rates

	# Events	Total Households	Total Contacts	SAR (%)
Primary Analysis Set				
Households with 2º cases	19	33	-	57.6
Individual 2º cases	26	-	66	39.4
Strict Analysis Set				
Households with 2º cases	9	21	-	42.9
Households with 2º cases, excluding households with outside infection	7	19	-	36.8
Individual 2º cases	9	-	40	22.5
Individual 2º cases, excluding individuals with outside infection	7	-	38	18.4

Table 3. Household Contact Characteristics by Infection Status (Strict Analysis Set), Excluding 2^0 Cases with Outside Infections

	Infected Contact (N=7)	**Not Infected Contact (N=28)**	**p-value**
Characteristic of Household Contact			
Age (Median, Min-Max)	46 (13-50)	28.5 (10-64)	0.76[a]
Male sex assigned at birth (N, %) Missing	2 (33.3) 1	13 (59.1%) 6	0.37[b]
Number of COVID-19 vaccines received (Median, Min-Max) Missing	3 (2-3) 0	3 (2-4) 1	1.00[b]
Days from last infection/vaccination (Median, Min-Max) Missing	146 (119-193) 0	138 (-1-197) 6	0.45[a]
Characteristic of Associated Index Case			
Lowest Ct value (Median, Min-Max)	27.8 (18.2-35)	25 (15.9-33.9)	0.19[a]
Duration of shedding in days (Median, Min-Max) Missing	7 (3-9) 0	8 (1-15) 0	0.41[a]
Characteristic of Associated Household			
Number of persons in household (Median, Min-Max) Missing	4 (2-4) 0	4 (2-4) 1	0.10[b]
Square footage (Median, Min-Max) Missing	1140 (740-3300) 2	1000 (740-3300) 15	0.59[a]

a. Wilcoxon rank sum test
b. Fisher's Exact

SUPPLEMENTAL TABLES

Supplemental Table 1. Additional Baseline Data

Characteristic	**All Participants (N=99)**	**Index Cases (N=33)**	**Contacts (N=66)**
Currently Working / Employment (N, %) – ***missing 13***			
Yes	57 (66.3)	18 (69.2)	39 (65.0)
No	29 (33.7)	8 (30.8)	21 (35.0)
Missing	13	7	6
Student (N, %) – ***missing 9***			
Yes	32 (35.6)	13 (43.3)	19 (31.7)
No	58 (64.4)	17 (56.7)	41 (68.3)
Missing	9	3	6
If yes (N=32), level (N, %)			
Elementary	11 (34.4)	5 (38.5)	6 (31.6)
Middle School	12 (37.5)	5 (38.5)	7 (36.8)
High School	5 (15.6)	2 (15.4)	3 (15.8)
Community College	0	0	0
College or University	4 (12.5)	1 (7.7)	3 (15.8)
Currently Uses eCigarettes/Vape (N, %) – ***missing 9***	1 (1.1)	0	1 (1.6)
Currently Smokes Cigarettes (N, %) – ***missing 9***	1 (1.1)	0	1 (1.6)

History of Smoking Cigarettes (N, %) – ***missing 10***	11 (12.4)	3 (10.3)	8 (13.3)
If yes, packs per day (N, %)			
0.5	7 (63.6)	1 (33.3)	6 (75.0)
1.0	4 (36.4)	2 (66.7)	2 (25.0)
1.5	0	0	0
2.0	0	0	0
2.5	0	0	0
3 or more	0	0	0
If yes, how many years (N, %)			
1-5	8 (72.7)	2 (66.7)	6 (75.0)
5-10	0	0	0
10-15	1 (9.1)	0	1 (12.5)
15-20	0	0	0
20 or more	2 (18.2)	1 (33.3)	1 (12.5)
Concurrent Use of Steroids or Immune Suppressing Medications (N, %) – ***missing 12***	6 (6.9)	1 (3.6)	5 (8.5)
Concurrent use of NSAIDs – *missing 13*	38 (44.2)	10 (37.0)	28 (47.5)

Supplemental Table 2. Baseline Characteristics of Study Households in Primary Analysis Set

Characteristic	**Total Households (N=33)**
Number of persons in household (Median, Min-Max)	3.5 (2-5)
House size (in square feet) as reported by index (Median, Min-Max)	1400 (740-3300)
Missing or unknown data from 11 households	

Supplemental Table 3. SARS-CoV-2 Viral Kinetics in Primary Analysis Set, Excluding 2 Cases of Outside Introduction

Outcome	**Index Cases (N=30)[a]**	**Infected Contacts (N=26)**
Lowest Ct (median, min-max)	25.65 (15.9-35)	23 (8.1-36.2)
Duration of viral shedding[b] (median, min-max)	6.5 days (1-19)	8 days (1-20)
Time to first positive SARS-CoV-2 from index diagnosis date	N/A	3 days (0-10)

a. Excluding 3 index cases who were PCR negative at baseline.
b. From first positive SARS-CoV-2 test.

Supplemental Table 4. Sequencing Performance

Household	Participant	Samples	Samples Producing Genome with Minimum Unambiguous Genome Length of 15 kbp
AXB	1	1	0
	2	6	5
JRP	1	5	5
	2	6	6
ODK	1	1	1
EGI	1	6	6
	4	5	4
CWA	1	4	3
	2	4	3
VBL	1	1	1
TSE	1	1	1
	2	6	5
KQX	1	1	1
DUC	1	2	1
	2	8	7
MPO	2	5	4
	3	9	7
	4	4	3
HXJ	1	4	1
	3	5	5
BQE	1	7	5
	2	5	5
SVW	1	1	1

IRG	1	2	2
	2	6	6
	3	4	3
	4	8	5
LCT	1	7	5
	4	11	9
	5	9	9
PFK	1	1	1
	4	2	1
JDU	1	1	1
XOA	1	6	1
	2	4	4
REZ	1	1	0
WMD	1	2	1
	3	4	3
HSA	1	1	1
	2	5	3
YVB	1	1	0
NKF	1	1	1
	2	1	0
QEC	1	1	1
OXS	1	2	2
	2	1	1
	4	2	2
LME	1	1	1

Supplemental Table 5. Household Transmission Analysis

Household	Transmission Direction	SNV Distance	Difference in Dates of Diagnosis (Days)	iSNV Information	Transmission Pair Categorization	Household Transmission Categorization
JRP	1>2	1	4		Highly probable	Highly probable
EGI	1>4	1	4		Highly probable	Highly probable
CWA	1>2	0	2	C21721T; T22917A; C23673T present at mixed sites in 1 (possible BA.2.12.1 & BA.2.10 co-infection) and fixed in 2	Possible	Possible
	2>1	0	-2		Possible	
TSE	1>2	3	5		Unlikely	Unlikely
	2>1	3	-5		Unlikely	
DUC	1>2	0	2		Possible	Possible
	2>1	0	-2		Possible	
MPO	4>2	0	3		Possible	Possible
	4>3	0	6		Possible	
	2>4	0	-3		Possible	
	2>3	0	3		Possible	
HXJ	1>3	0	1	share C23393T at < 10% frequency	Possible	Possible
	3>1	0	-1		Possible	
BQE	2>1	0	2		Possible	Possible
	1>2	0	-2		Possible	
IRG	1>4	3	2		Unlikely	Mixed household
	4>1	3	-2		Unlikely	
	4>2	0	3		Possible	
	4>3	0	5		Possible	
	2>3	0	2		Possible	
LCT	4>1	4	1		Unlikely	Mixed household

	1>4	4	-1		Unlikely	
	4>5	0	5		Possible	
PFK	1>4	0	6		Highly probable	Possible
XOA	1>2	13	9	share C3832T at < 10% frequency	Unlikely	Unlikely
WMD	1>3	20	12		Unlikely	Unlikely
HSA	1>2	2	5		Highly probable	Possible
OXS	1>2	0	2		Possible	Possible
	1>4	0	2		Possible	
	2>1	0	-2		Possible	
	2>4	0	0		Possible	
	4>1	0	-2		Possible	
	4>2	0	0		Possible	

FIGURE 1

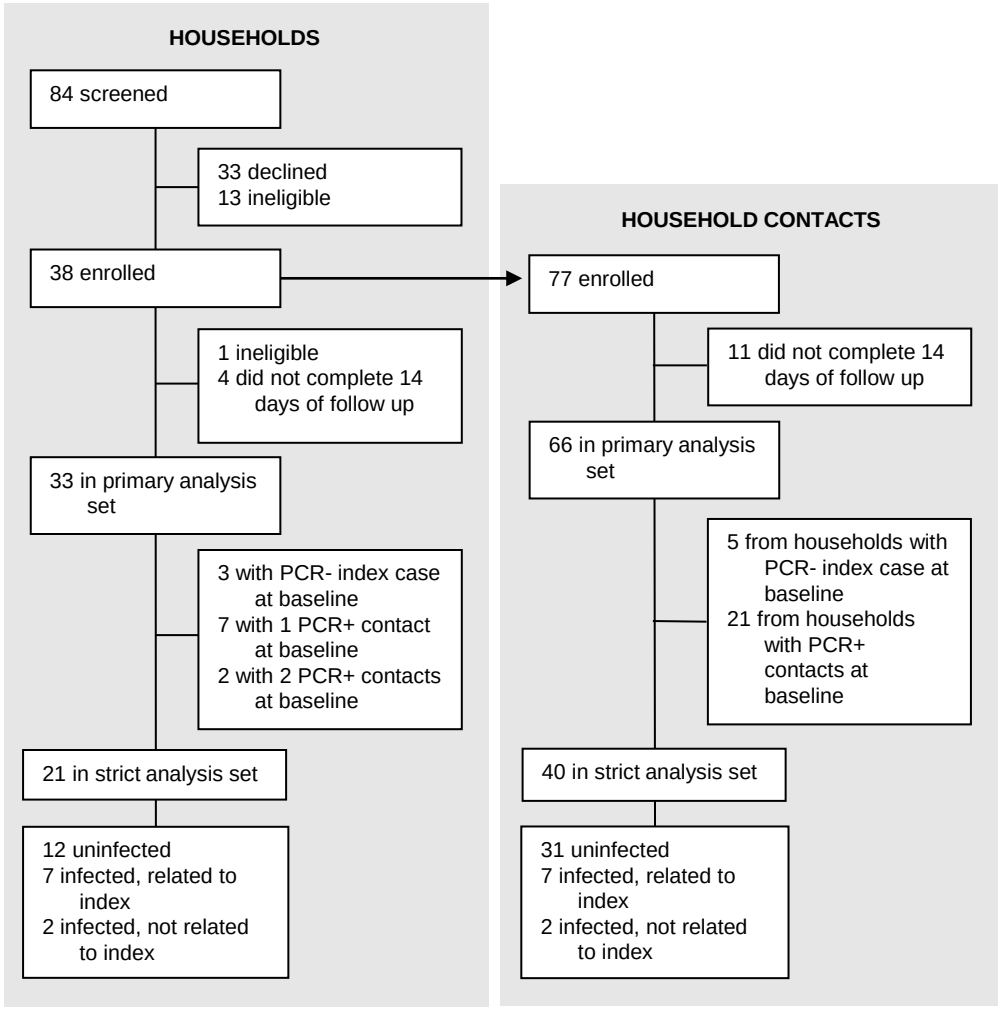
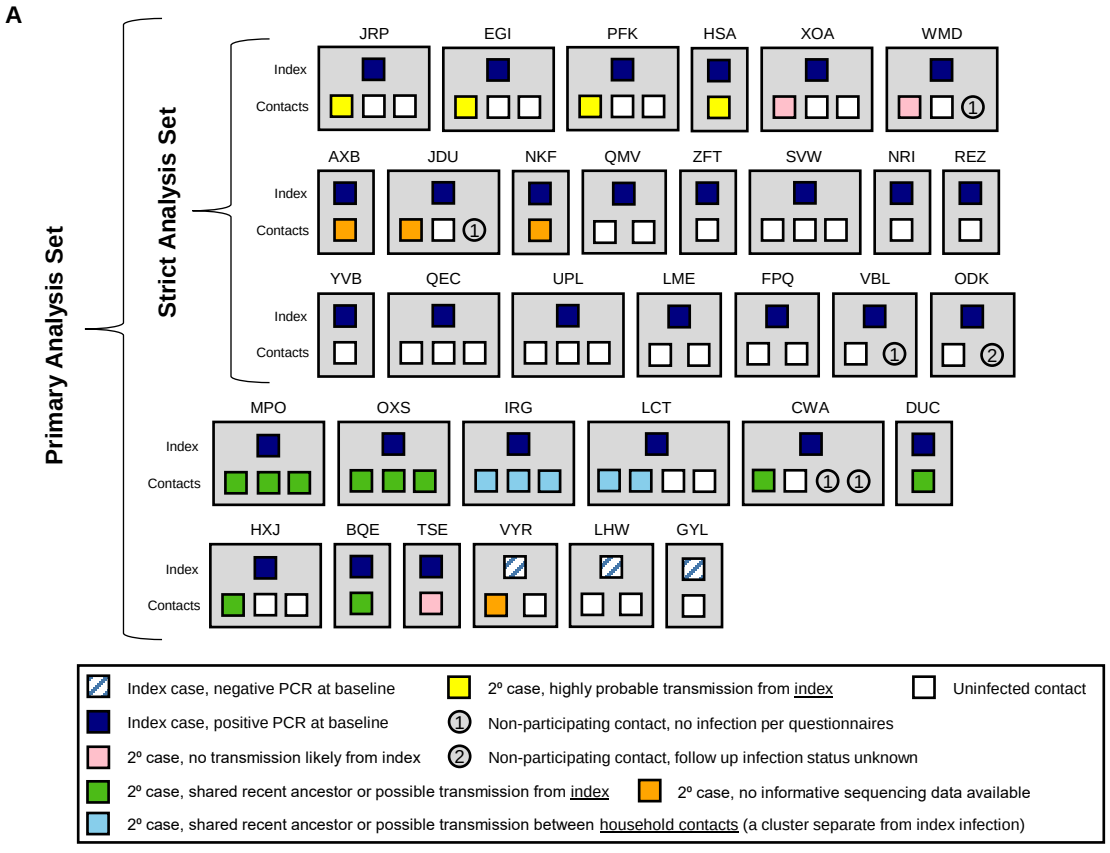

HOUSEHOLDS
84 screened
33 declined
13 ineligible
38 enrolled
1 ineligible
4 did not complete 14 days of follow up
33 in primary analysis set
3 with PCR- index case at baseline
7 with 1 PCR+ contact at baseline
2 with 2 PCR+ contacts at baseline
21 in strict analysis set
12 uninfected
7 infected, related to index
2 infected, not related to index
HOUSEHOLD CONTACTS
77 enrolled
11 did not complete 14 days of follow up
66 in primary analysis set
5 from households with PCR- index case at baseline
21 from households with PCR+ contacts at baseline
40 in strict analysis set
31 uninfected
7 infected, related to index
2 infected, not related to index

FIGURE 2


A
Primary Analysis Set
Strict Analysis Set
JRP
EGI
PFK
HSA
XOA
WMD
AXB
JDU
NKF
QMV
ZFT
SVW
NRI
REZ
YVB
QEC
UPL
LME
FPQ
VBL
ODK
MPO
OXS
IRG
LCT
CWA
DUC
HXJ
BQE
TSE
VYR
LHW
GYL
Index
Contacts
Index case, negative PCR at baseline
Index case, positive PCR at baseline
2º case, no transmission likely from index
2º case, shared recent ancestor or possible transmission from index
2º case, shared recent ancestor or possible transmission between household contacts (a cluster separate from index infection)
2º case, highly probable transmission from index
Non-participating contact, no infection per questionnaires
Non-participating contact, follow up infection status unknown
2º case, no informative sequencing data available
Uninfected contact

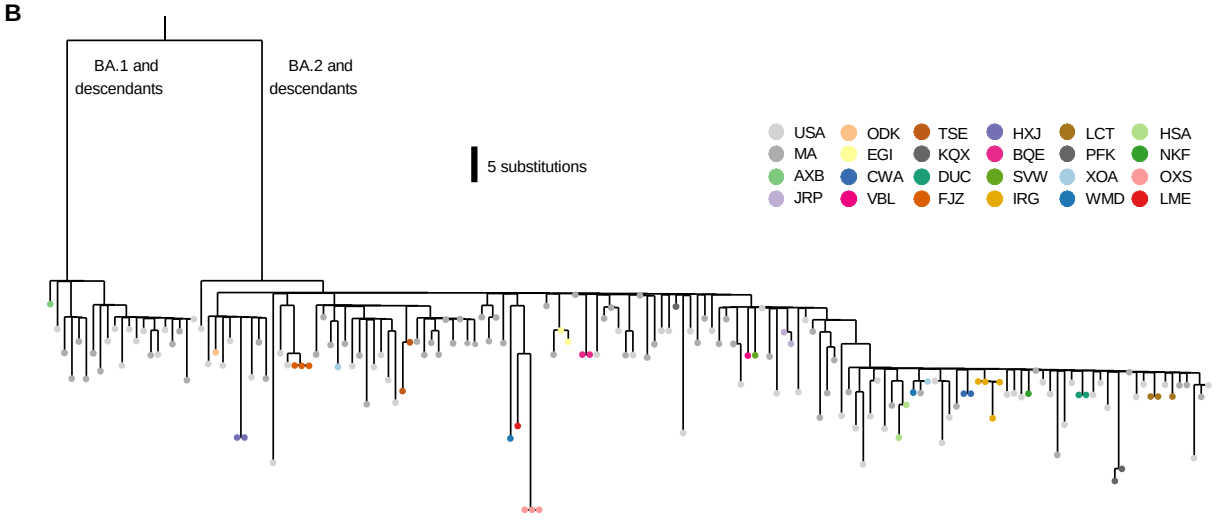

B
BA.1 and descendants
BA.2 and descendants
5 substitutions
USA
MA
AXB
JRP
ODK
EGI
CWA
VBL
TSE
KQX
DUC
FJZ
HXJ
BQE
SVW
IRG
LCT
PFK
XOA
WMD
HSA
NKF
OXS
LME

FIGURE 3

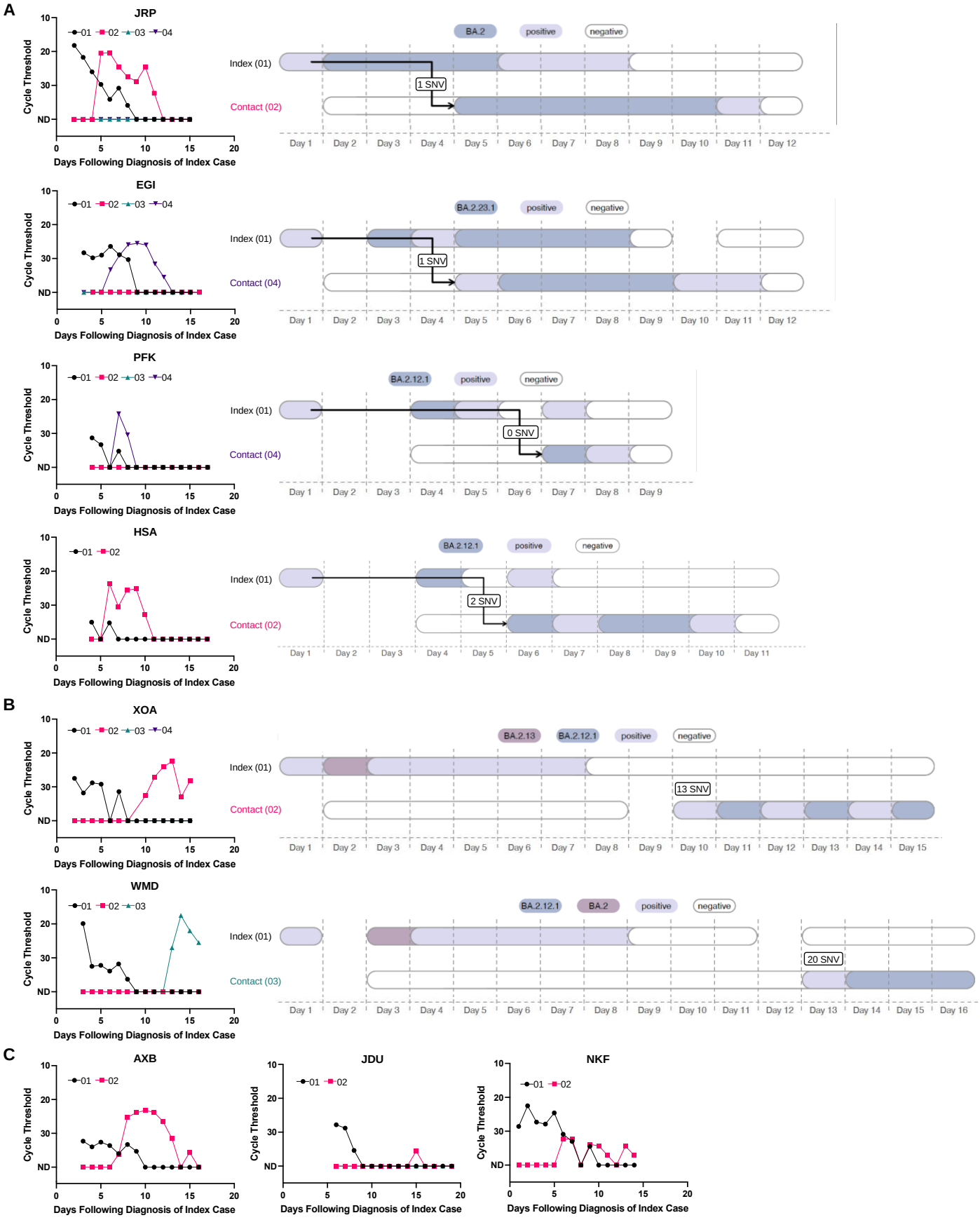
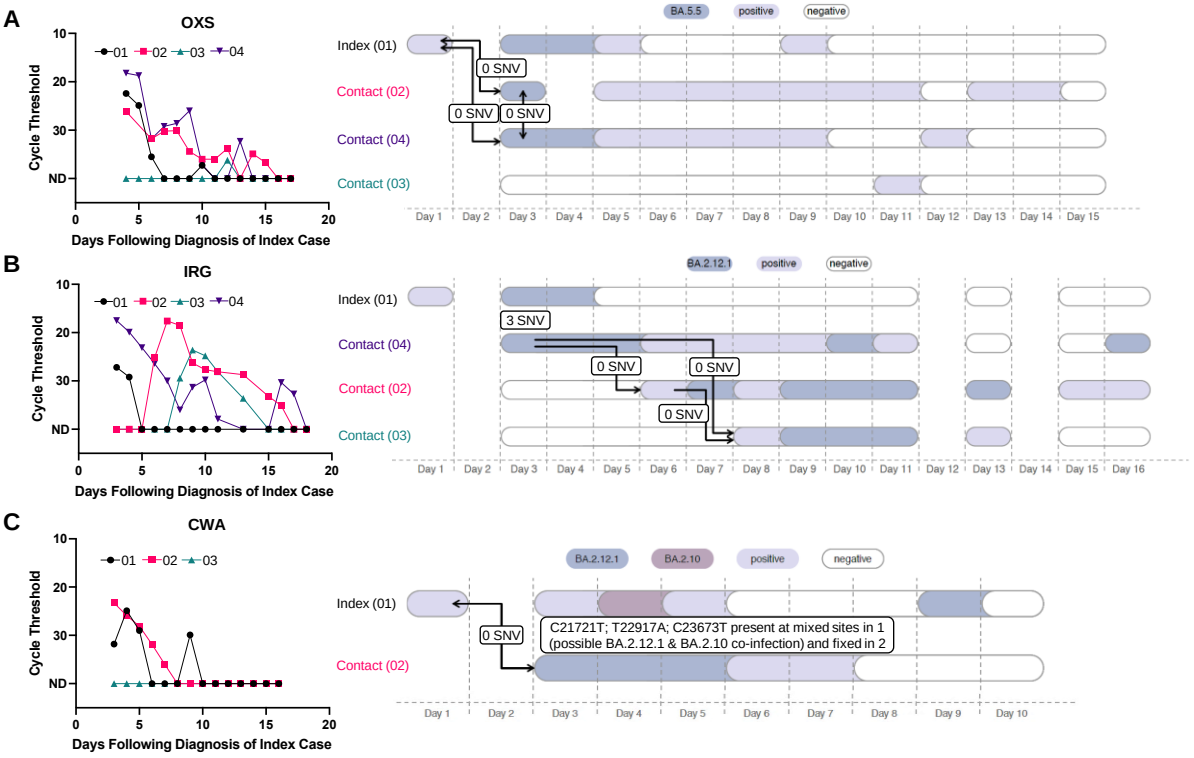

A
JRP
Cycle Threshold
Days Following Diagnosis of Index Case
BA.2
positive
negative
Index (01)
Contact (02)
1 SNV
EGI
BA.2.23.1
Contact (04)
PFK
BA.2.12.1
0 SNV
HSA
2 SNV
B
XOA
BA.2.13
13 SNV
WMD
Contact (03)
20 SNV
C
AXB
JDU
NKF

FIGURE 4


A
OXS
Cycle Threshold
10
20
30
ND
01
02
03
04
0
5
10
15
20
Days Following Diagnosis of Index Case
BA.5.5
positive
negative
Index (01)
Contact (02)
Contact (04)
Contact (03)
0 SNV
0 SNV
0 SNV
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
B
IRG
Cycle Threshold
10
20
30
ND
01
02
03
04
0
5
10
15
20
Days Following Diagnosis of Index Case
BA.2.12.1
positive
negative
Index (01)
Contact (04)
Contact (02)
Contact (03)
3 SNV
0 SNV
0 SNV
0 SNV
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
Day 16
C
CWA
Cycle Threshold
10
20
30
ND
01
02
03
0
5
10
15
20
Days Following Diagnosis of Index Case
BA.2.12.1
BA.2.10
positive
negative
Index (01)
Contact (02)
0 SNV
C21721T; T22917A; C23673T present at mixed sites in 1
(possible BA.2.12.1 & BA.2.10 co-infection) and fixed in 2
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10

SUPPLEMENTAL FIGURE 1