High SARS-CoV-2 household transmission rates detected by dense saliva sampling

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Summary

By repeated saliva self-sampling combined with NPS, OPS, and serology in 85 households, we report the highest SARS-CoV-2 household transmission rates to date. Households are pivotal in SARS-CoV-2 transmission and salivary sampling may assist in infection control in this setting.

Abstract

Background: Understanding the dynamics of SARS-CoV-2 household transmission is important for adequate infection control measures in this ongoing pandemic.

Methods: Households were enrolled upon a PCR-confirmed index case between October and December 2020, prior to the COVID-19 vaccination program. Saliva samples were obtained by self-sampling at day 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42 from study inclusion. Nasopharyngeal swabs (NPS) and oropharyngeal swabs (OPS) were collected by the research team at day 7 and capillary blood samples at day 42. Household secondary attack rate (SAR) and per-person SAR were calculated based on at least one positive saliva, NPS, OPS, or serum sample. Whole genome sequencing was performed to investigate the possibility of multiple independent SARS-CoV-2 introductions within a household.

Results: Eighty-five households were included consisting of 326 (unvaccinated) individuals. Comparable numbers of secondary cases were identified by saliva (133/241; 55.2%) and serum (127/213; 59.6%). The household SAR was 88.2%. The per-person SAR was 64.3%. The majority of the secondary cases tested positive in saliva at day 1 (103/150; 68.7%). Transmission from index case to household member was not affected by age or the nature of their relationship. Phylogenetic analyses suggested a single introduction for the investigated households.

Conclusion: Households have a pivotal role in SARS-CoV-2 transmission. By repeated saliva self-sampling combined with NPS, OPS, and serology, we found the highest SARS-CoV-2 household transmission rates reported to date. Salivary (self-)sampling of adults and children is suitable and attractive for near real-time monitoring of SARS-CoV-2 transmission in this setting.

Key words: SARS-CoV-2; COVID-19; household transmission; saliva

Introduction

Since the first identification in December 2019, coronavirus disease 2019 (COVID-19) numbers continue to increase and have passed 245 million cases globally.[1] Insight into transmission dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is of major importance for infection control measures.

While the current standard testing method for SARS-CoV-2 detection is mainly via reverse transcription polymerase chain reaction (RT-PCR) on nasopharyngeal swabs (NPS),[2] other specimens like saliva may also offer a good source for detection by RT-PCR.[3, 4] Multiple studies have demonstrated a comparable or higher sensitivity of detecting SARS-CoV-2 using saliva compared to NPS, both in adults and children.[5-9] Furthermore, RT-PCR on saliva effectively identifies SARS-CoV-2 even before the period of infectiousness[10] and particularly high sensitivity rates of RT-PCR on saliva have been found among studies involving asymptomatic individuals.[11, 12] Compared to NPS, salivary testing is less invasive and samples can be easily collected by individuals themselves (including children with assistance of their parents) without need of qualified personnel, allowing frequent sampling. Moreover, salivary self-sampling has been shown to be more sensitive than self-administered nasal swabs.[5] Salivary self-sampling could therefore contribute to improved monitoring and lower costs.[13]

As not only symptomatic, but also asymptomatic and pre-symptomatic individuals are considered to be potential sources of new infections,[14, 15] high-density salivary sampling (i.e. frequent sampling in a short time period) for RT-PCR testing of SARS-CoV-2 can be applied to investigate SARS-CoV-2 transmission in households. Households are considered to be one of the most frequent settings of SARS-CoV-2 transmission with close contact between members over long time within confined spaces without use of personal protective equipment (PPE).[16]

The reported secondary attack rates (SARs) of previous household studies show a wide range of transmission from 6% to 50%, depending on the study setting, study period, frequency of testing, testing method, and specimen types analysed.[17-24] Since the majority of these studies have been

performed in adults, uncertainty still exists about the SARs in children and their role as index case in household transmission.

Upcoming variants associated with higher transmissibility[25] contribute to the persistence of the SARS-CoV-2 pandemic. A better understanding of SARS-CoV-2 transmission dynamics, including the role of children, will support the rationale behind public health policies, like school closure and other infection control measures. The aim of this study is to assess household transmission dynamics of SARS-CoV-2 by frequent saliva sampling in combination with NPS, OPS and serology, to determine factors associated with transmission, and to investigate the suitability of salivary (self-) sampling to monitor SARS-CoV-2 household transmission.

Methods

Study design

In this prospective cohort study "SARSLIVA" (SARS-CoV-2 in saLIVA), households were eligible in case of an index case under the age of 65 years SARS-CoV-2 infection with RT-PCR confirmed SARS-CoV-2 on a combined NPS and oropharyngeal swab (OPS) during the previous 72 hours (index case) and with at least two additional household members willing to participate in the study. Between October and December 2020, prior to the COVID-19 vaccination program, participants were recruited by the Public Health Services Kennemerland, The Netherlands, or, in case of employees of the Spaarne Gasthuis hospital Haarlem/Hoofddorp, The Netherlands, by the hospital's Infection Control Department. Index cases could be either symptomatic or asymptomatic.

This study was reviewed and approved by the Medical Ethical Committee of the Vrije Universiteit university Medical Centre (VUmc), The Netherlands (reference number 2020.436).

Sample collection

Saliva samples were obtained by participants themselves at home at day 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42 (with day of inclusion as day 0). At day 7, NPS and OPS were collected from all participants by

the research team during a home visit. At day 42, capillary blood samples were collected by the research team during a second home visit for serological analyses. See Supplementary Materials for details.

Questionnaires

An online baseline questionnaire was obtained at day 0. This questionnaire contained questions on household composition, household characteristics, smoking, medical history, self-reported ethnicity, and educational level. Each night before the pre-defined time points for saliva collection, online COVID-19 symptomatology questionnaires were sent to the participants, consisting of self-perceived severity scores ("no", "mild", "moderate", "severe") per symptom. Medical records were obtained if participants were admitted to a hospital or visited a general practitioner during the study period.

Case definitions

The first member of each household with a RT-PCR confirmed SARS-CoV-2 infection included in the study was defined as the index case. As this index case was not necessarily the primary case (the first SARS-CoV-2 infection) in a household, we performed a sensitivity analysis with a more stringent index case definition (see Statistical methods). SARS-CoV-2 infection during follow-up was defined as either at least one positive SARS-CoV-2 RT-PCR result on one of the saliva samples, on NPS or OPS at day 7, or detection of serum antibodies at day 42, regardless of the presence of symptoms. COVID-19 disease severity was classified with a four-degree scale per time point, derived from national and international guidelines[26, 27]: (1) no coronavirus-related symptoms, (2) mild symptoms (rhinitis, pharyngitis, mild dyspnoea, mild or moderate coughing, olfactory dysfunction or gustatory dysfunction), (3) moderate symptoms (moderate or severe dyspnoea, severe coughing, temperature > 38°C or a pneumonia diagnosed by a physician), and (4) hospital admission due to coronavirus-related symptoms. A maximum severity score over all time points was calculated per participant.

Molecular diagnostics and serology

Initial combined NPS/OPS of the index cases as well as NPS and OPS obtained at day 7 were analysed for the presence of SRAS-CoV-2 by the Regional Public Health Laboratory Kennemerland, Haarlem, The Netherlands as described previously. SARS-CoV-2 viral loads in saliva samples were analysed by the laboratory of the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands as described before.[28, 29] See Supplementary Materials for details.

Amplicon-based SARS-CoV-2 sequencing for was performed on the positive saliva sample with the highest viral load for each individual using the Nanopore protocol "PCR tiling of COVID-19 virus (Version: PTC_9096_v109_revE_06FEB2020)" which is based on the ARTIC v3 amplicon sequencing protocol.[30, 31] See Supplementary Materials for details.

Sera were tested for the presence of IgG antibodies reactive with the SARS-CoV-2 spike trimer, S1, and N antigens in a protein microarray, in duplicate 2-fold serial dilutions starting at 1:20, essentially as described previously.[32] For each antigen, a 4-parameter log logistic calibration curve was generated and EC50 antibody titres were calculated. Raw data were processed with the R 4.04 statistical software.[33]

Statistical methods

The household SAR was calculated by dividing households with secondary transmission by the total number of households. Per-person SAR was calculated by dividing the number of secondary cases by the number of participating household members. Logistic regression models were used to compare characteristics of index cases and household members and to assess the relation between household SAR and per-person SAR and the characteristics of households, index cases, and household members. Statistical analyses were performed with R version 3.6.2 (R Core Team, Vienna, Austria). P-values <0.05 were considered significant.

To account for the influence of our index case definition on the (per-person) SARs, a sensitivity analysis was performed in which household and per-person SARs were calculated after excluding households in which it was uncertain whether the index case was the primary case. Households were excluded when household member had (1) a RT-PCR confirmed SARS-CoV-2 infection 1-14 days prior to the index case or (2) reported symptoms 1-14 days prior to the index case and tested positive in either saliva, NPS, OPS, or serum during follow-up.

Results

Baseline characteristics

In total 390 index cases were approached of whom 91 were included. Six dropped out resulting in a total of 85 households consisting of 326 (unvaccinated) participants (85 index cases and 241 household members). For all participants (n=326), protocol adherence for collection of the specimens was 92.4% (94.8% saliva, 92.9% NPS, 93.6% OPS, and 88.4% serum).

The median age of the index cases was 40.0 (IQR 22.0-48.0) years and 17/85 (20.0%) index cases were younger than 18 years old (Table 1). The median age of household members was 20.0 (IQR 12.0-45.0) years and 106/241 (44.0%) household members were younger than 18 years old. The majority of the index cases were female (56; 65.9%). Fifty-five (64.7%) index cases had mild symptoms, 22 (25.9%) moderate symptoms and seven (8.2%) were asymptomatic during the study period. One (1.2%) index case had severe symptoms leading to hospital admission. Roughly half of the participating household members were asymptomatic (121/241; 50.2%), regardless of their test results. Ninety-four (39.0%) had mild symptoms, 25 (10.4%) had moderate symptoms, and one (0.4%) had severe symptoms leading to hospital admission. The median time between index symptom onset and a positive test result (in symptomatic cases) was one day (IQR 1.0-2.0) and the median study enrolment (day 0) was four days (IQR 3.0-4.0) after symptom onset.

The median size of the households was four (IQR 3.0-4.0) participating household members (Table 2).

SARS-CoV-2 detection in different specimens and phylogenetic results

Of the household members, 64.3% tested positive in at least one saliva, NPS, OPS, or serum specimen (155/241 secondary cases; Figure 1). Household members tested positive for SARS-CoV-2 in saliva in 55.2% (133/241) and for SARS-CoV-2 antibodies in serum at day 42 in 59.6% (127/213). Only 32.6% (71/218) and 25.9% (57/220) were positive in NPS and OPS at day 7 respectively. Sixteen household members tested positive for SARS-CoV-2 in saliva only, resulting in a decline to 57.7% (139/241) in the proportion of secondary cases if saliva would not have been obtained. Of these household members, nine tested negative for SARS-CoV-2 antibodies in serum and in seven household members serum was not collected. Twenty household members tested positive for SARS-CoV-2 antibodies in serum only. Of the secondary cases, 58 (37.4%) were asymptomatic (Figure 2). The highest proportion of asymptomatic secondary cases tested positive in serum (45/109; 41.3%), followed by saliva (44/121; 36.4%).

To investigate the possibility of multiple independent SARS-CoV-2 introductions within a household, SARS-CoV-2 genomes were analysed by whole genome sequencing. For 103 individuals originating from 60 households successful sequence analyses was possible. Each household shows a distinct cluster in phylogenetic analyses with minimal sequence differences (Figure 3), indicative of a single introduction within each household. However, in certain cases very similar sequences were observed between different households, representing infections in those households with closely related variants. For certain households only a single genome could be determined, for which no conclusions could be drawn.

Secondary attack rates

Secondary infection (based on saliva, NPS, and OPS RT-PCR results and serological analyses) was detected in 75/85 households, leading to a household SAR of 88.2% (Table 2). The median household size did not significantly differ between households with and without secondary transmission (4.0 (IQR 3.0-4.0) vs. 3.5 (IQR 3.0-4.0); $p = 0.274$).

Secondary transmission was detected in households of 16/17 index cases under the age of 18 (Table 3). No significant differences in secondary transmission were observed between the age groups of the index cases. Median Cp-values of the index case initial NPS/OPS were not significantly different between households with and without secondary transmission (25.0 (IQR 22.1-29.6) vs. 23.9 (IQR 22.8-26.4), $p = 0.837$).

At the household member level, secondary infection was detected in 155/241 individuals, leading to a per-person SAR of 64.3% (Table 4). The majority of the secondary cases already tested positive in saliva at sampling day 1 (103/150; 68.7%). The median age did not differ between secondary and non-secondary cases (19.0 (IQR 12.0-44.0) vs. 21.0 (IQR 10.3-47.8), $p = 0.678$) and no significant differences in secondary transmission were observed between the age groups of the household members. The relationship between index case and household member had no remarkable influence on per-person SAR. The proportion of per-person secondary transmission from index case parent to child and from index case to partner were similar (78/117 children; 66.7% and 39/58 partners; 67.2% respectively) (Table 4).

Sensitivity analysis

The sensitivity analysis excluding 27 households (27 index cases and their 75 household members) with a possible other primary case than our defined index case resulted in a household SAR of 82.8% (48/58 households) and a per-person SAR of 54.2% (90/166 household members). Characteristics of index cases, households, or household members associated with secondary transmission were not remarkably different between the primary and sensitivity analysis (Supplementary Tables 1-3).

Discussion

In this study we have investigated household transmission dynamics of SARS-CoV-2 by frequent and dense saliva sampling in combination with serology, NPS, and OPS in 85 households with a RT-PCR confirmed index case. We found a household SAR of 88.2%, the highest rate of SARS-CoV-2 household transmission reported up to date. The majority of the secondary cases were identified by saliva only. As approximately two thirds of the secondary cases were already detected at the first sampling event, our study underlines that household transmission occurs rapidly. Secondary transmission was detected from and to different age groups and relationships within households, indicating that children, as well as adults, are at risk of infection and spreading of SARS-CoV-2 among their household members. Additional phylogenetic analyses suggest a single introduction for the investigated households.

A possible explanation for the high SARs in our study could be that other studies performed repeat sampling only in case of symptomatology, thereby ignoring possible asymptomatic cases within the household.[18, 20, 24, 34, 35] In addition, the high frequency and density of salivary sampling may have contributed to the higher SARs in our study compared to earlier reports.[19, 22, 35] We found the highest sensitivity of SARS-CoV-2 detection by salivary testing combined with serological analyses. However, the performance of salivary testing alone was comparable and its timely results could serve infection control purposes while serological analyses could not. Two other prospective household studies used saliva as specimen to detect SARS-CoV-2 transmission.[19, 23] One of these studies[23] reported a lower per-person SAR of 43%, possibly because salivary sampling was performed less frequently than in our study and additional sampling was performed only in case of symptoms. In our study, we found that 58 out of the 155 secondary cases were asymptomatic and the per-person SAR would have declined from almost 65% to 40% if only symptomatic individuals would have been included. Saliva identified over 75% (44/58) of the asymptomatic secondary infections. This confirms saliva (self-)sampling being highly effective in detecting asymptomatic SARS-CoV-2 infections in adults and older children.[11, 12] In addition, the non-invasive character of

salivary (self-)sampling facilitates frequent use for near-real time monitoring in symptomatic as well as asymptomatic individuals. Salivary (self-) sampling of household members in the first week after symptom onset or positive test of a household index case could therefore improve infection control in this setting. Although in the context of high transmission rates, household isolation may be more practical and cost-effective.

Other factors that may have affected SARs are the study period including vaccination status of the participants (the COVID-19 vaccination program had not started yet), quarantine policies, and dominating variants (Nextclade 20A, 20B, 20E (EU1)) associated with different transmission rates.[36] All participants were recruited in the second wave of COVID-19 infections in the Netherlands (approximately from July 2020 to January 2021), in which working at home was encouraged or enforced and school classes generally took place at home, hereby possibly increasing the risk of household transmission.[16]

In this study, we aimed to identify factors associated with secondary transmission. We found no apparent association between index case, household, or household member characteristics and secondary transmission. These findings are not fully in line with previous studies. In our study, transmission from index case to household member was not affected by age or the nature of their relationship. In contrast, several studies indicate that children (<18 years) play a minor role in household transmission of SARS-CoV-2, but caution must be applied here as the number of index children in these studies was low.[17, 19, 21, 24] In these studies, as well as in our study, the number of younger index children (<12 years) was too small to draw reliable conclusions regarding their role in transmission. We can however conclude that introduction and spreading of SARS-CoV-2 in households appears difficult to prevent as it occurs fast and involves household members of different age groups.

A limitation of our study is that index cases were defined as the first included household member. However, not all index cases might have been the primary cases within the household and co-primary cases were not considered which could have caused an overestimation of the per-person

SAR. Additional serological analyses at study enrolment could have confirmed seroconversion of individuals for whom no positive saliva or OPS/NPS specimens were available, although due to limited circulation of SARS-CoV-2 prior to our study period, chances of previous infection are considered low. In addition, our sequencing results and phylogenetic results show no evidence of multiple introductions within one household. However, not for every infected individual sequencing analyses were successful. Our reported SARs therefore represent the maximum contribution from household transmission, as we cannot fully exclude introductions from outside the households. Lastly, as no transmission occurred in solely 10 out of 85 households in our study, comparison of the households with and without transmission was hampered.

In summary, this study reveals that households have a pivotal role in SARS-CoV-2 transmission, as we found the highest household SARS-CoV-2 transmission rates reported so far. Household transmission occurs fast, hampering quick identification of primary cases and underlining the importance of prompt isolation and rapid testing of all household members, regardless of their age and presence of symptoms. Salivary (self-)sampling of adults and children is suitable and attractive for near real-time monitoring of SARS-CoV-2 transmission in this setting.

NOTES

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MAvH, KT, EAMS, AM, EMdK, and WRM contributed to the conception and design of the study; LMK, SFvL, MEH, JGCS, EMdK, JCDK, and MAvH participated in acquisition of data; RM, SE, and DE coordinated the laboratory analyses; LMK, SFvL, JGCS, ANS, ECC, SE, DE, and MAvH were responsible for data analyses and interpretation; LMK, JGCS, SE, and DE verified the underlying data; LMK, SFvL, JGCS, SE, DE, and MAvH wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflicts of interests

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Figure legends

Figure 1 - Secondary transmission in household members (n=241) defined with different sample type results

In this 'upset plot' each column is a pattern of co-occurrences of positivity (filled and connected dots indicate a positive test, grey dots indicate a negative or missing test result). The rows indicate the different tests, with the bar chart to the right the number of occurrences of positivity of each test. Below each column is a bar chart indicating the number of occurrences of the pattern.

Data on saliva, NPS, OPS, and serology were available for 241, 218, 220, and 213 household members respectively; 155 household members were positive in either saliva, NPS, OPS, or serology (secondary cases); saliva positivity was defined as ≥1 RT-PCR positive saliva sample at day 1-42; serology positivity was defined as IgG antibody positivity for ≥1 antigen (SARS-CoV-2 spike trimer, S1, or N).

NPS = nasopharyngeal swab; OPS = oropharyngeal swab

Figure 2 - Secondary transmission in household members (n=241) defined with different sample type results and symptom status

In this 'upset plot' each column is a pattern of co-occurrences of positivity (filled and connected dots indicate a positive test, grey dots indicate a negative or missing test result). The rows indicate the different tests, with the bar chart to the right the number of occurrences of positivity of each test. Below each column is a bar chart indicating

the number of occurrences of the pattern. 120 household members were symptomatic and 121 household members were asymptomatic; data on saliva, NPS, OPS, and serology were available for 120, 109, 108, and 104 symptomatic household members respectively; data on

saliva, NPS, OPS and serology were available for 121, 109, 112, and 109 asymptomatic household members respectively; 97 symptomatic and 58 asymptomatic household members were positive in

either saliva, NPS, OPS, orserology (secondary cases); saliva positivity was defined as ≥1 RT-PCR positive saliva sample at day 1-42; serology

positivity was defined as IgG antibody positivity for ≥1 antigen (SARS-CoV-2 spike trimer, S1, or N); asymptomatic household members were defined as not reporting symptoms on any of the examinations (day 1-42).

NPS = nasopharyngeal swab; OPS = oropharyngeal swab

Figure 3 - Phylogenetic analysis of SARS-CoV-2 sequences within households (60 households, 103 individuals)

Sequences were obtained from saliva samples with the highest viral load and are labelled per household. Households with three or more available sequences are indicated in colour.

Tables

Table 1 – Baseline characteristics (n=326)

	No. (%)[a]				
	Total number of participants	Index cases	Household members		
Total participants	326	85	241		
Characteristics				**p**	**OR [95%CI]**
Child (<18 years) **(%)**	123 (37.7%)	17 (20.0%)	106 (44.0%)	<0.001	0.32 [0.18-0.57]
Adult (%)	203 (62.3%)	68 (80.0%)	135 (56.0%)	<0.001	3.14 [1.74-5.66]
Age (median (IQR)	28.5 (13.0-46.0)	40.0 (22.0-48.0)	20.0 (12.0-45.0)	<0.001	1.03 [1.02-1.05]
Age group, years (%)					
< 12 years	59 (18.1%)	1 (1.2%)	58 (24.1%)	-	1 (ref)
12-17 years	64 (19.6%)	16 (18.8%)	48 (19.9%)	0.005	19.33 [2.47-151.11]
18-39 years	74 (22.7%)	25 (29.4%)	49 (20.3%)	<0.001	29.59 [3.89-226.36]
40-49 years	78 (23.9%)	25 (29.4%))	53 (22.0%)	<0.001	27.36 [3.58-208.97]
50-65 years	51 (15.6%)	18 (21.2%)	33 (13.7%)	<0.001	31.64 [4.04-247.85]
Sex (female) (%)	157 (48.2%)	56 (65.9%)	101 (41.9%)	<0.001	2.68 [1.60-4.49]
BMI class[b] (%)					
Normal weight	195 (59.8%)	45 (52.9%)	150 (66.1%)	-	1 (ref)
Obesity	23 (7.1%)	10 (11.8%)	13 (5.7%)	0.038	2.56 [1.05-6.24]
Overweight	87 (26.7%)	28 (32.9%)	59 (26.0%)	0.108	1.58 [0.90-2.77]
Underweight	7 (2.1%)	2 (2.4%)	5 (2.2%)	0.736	1.33 [0.25-7.11]
Underlying medical condition (%)	37 (11.3%)	10 (11.8%)	27 (11.2%)	0.888	1.06 [0.49-2.29]
Cardio vascular disease	10 (3.1%)	2 (2.4%)	8 (3.3%)		
Lung disease	1 (0.3%)	1 (1.2%)	0 (0.0%)		

Immune disorder	1 (0.3%)	1 (1.2%)	0 (0.0%)		
Diabetes	3 (0.9%)	1 (1.2%)	2 (0.8%)		
Rheumatic disorder	2 (0.6%)	1 (1.2%)	1 (0.4%)		
Other	24 (7.4%)	5 (5.9%)	19 (7.9%)		
Smoking = Yes (%)	11 (3.4%)	4 (4.7%)	7 (2.9%)	0.433	1.65 [0.47-5.79]
Nationality (other)[c] (%)	4 (1.2%)	2 (2.4%)	2 (0.8%)	0.298	2.86 [0.40-20.60]
Positive saliva day 1[d]	176 (54.0%)	73 (85.9%)	103 (42.7%)	<0.001	13.37 [5.90-30.26]
Symptom status[e]					
Severe symptoms[f]	2 (0.6%)	1 (1.2%)	1 (0.4%)	0.052	17.29 [0.98-306.28]
Moderate symptoms	47 (14.4%	22 (25.9%)	25 (10.4%)	<0.001	15.21 [5.86-39.46]
Mild symptoms	149 (45.7%)	55 (64.7%)	94 (39.0%)	<0.001	10.11 [4.40-23.23]
Asymptomatic	128 (39.2%)	7 (8.2%)	121 (50.2%)	-	1 (ref)

[a] Some numbers might not add up to 326 due to missing values.

[b] BMI categories for index cases and household members 2-18 year were defined as BMI z-score (<-2 = underweight, BMI z-score -2-1 = normal weight, BMI z-score 1-2=overweight, BMI z-score >2= obesity); BMI categories for index cases and household members ≥18 years defined were as BMI <18.5 = underweight, BMI 18.5-25 = normal weight, BMI 25-30 = overweight, BMI >30 = obesity.[37]

[c] Other than Dutch.

[d] Saliva at day 1 available for 315 participants.

[e] Maximum over 10 time points.

[f] Hospital admission due to coronavirus-related symptoms.

OR = odds ratio; CI = confidence interval

Table 2 - Household characteristics (n=85)

	No. (%)[a]				
	Total house holds	Households with secondary transmission	Households without secondary transmission		**Secondary attack rate (%)**
Total households	85	75	10		88.2
Characteristics				**p**	**OR [95% CI]**
Median household size (IQR)	4.0 (3.0-4.0)	4.0 (3.0-4.0)	3.5 (3.0-4.0)	0.274	1.76 [0.64-4.87]
Median household size, no of person					
3	29 (34.1%)	24 (32.0%)	5 (50.0%)	-	1 (ref)
4	42 (49.4%)	38 (50.7%)	4 (40.0%)	0.343	1.98 [0.48-8.11]
5	13 (15.3%)	12 (16.0%)	1 (10.0%)	0.426	2.50 [0.26-23.86]
6	1 (1.2%)	1 (1.3%)	0 (0.0%)	-	-
Educational level[b]					
High	57 (70.4%)	51 (70.8%)	6 (66.7%)	-	1 (ref)
Middle/low	24 (29.6%)	21 (29.2%)	3 (33.3%)	0.797	0.82 [0.19-3.60]
Median number of bedrooms per household (IQR)	4.0 (3.0-5.0)	4.0 (3.0-5.0)	3.5 (2.3-4.8)	0.627	1.17 [0.62-2.19]
Number of bedrooms					
2	5 (5.9%)	2 (2.7%)	3 (30.0%)	-	1 (ref)
3	34 (40.0%)	32 (42.7%)	2 (20.0%)	0.007	24.00 [2.43-236.89]
4	21 (24.7%)	19 (25.3%)	2 (20.0%)	0.024	14.25 [1.42-143.19]
5	20 (23.5%)	18 (24.0%)	2 (20.0%)	0.027	13.5 [1.34-135.98]

≥6	5 (5.9%)	4 (5.3%)	1 (10.0%)	0.214	6.00 [0.35-101.57]
Median number of toilets per household (IQR)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	0.799	0.88 [0.32-2.40]
Number of toilets					
1	6 (7.1%)	5 (6.7%)	1 (10.0%)	-	1 (ref)
2	64 (75.3%)	57 (76.0%)	7 (70.0%)	0.676	1.63 [0.17-16.02]
3	12 (14.1%)	11 (14.7%)	1 (10.0%)	0.602	2.20 [0.11-42.74]
≥4	3 (3.5%)	2 (2.6%)	1 (10.0%)	0.577	0.40 [0.02-10.02]
Pets	48 (56.5%)	41 (54.7%)	7 (70.0%)	0.365	0.52 [0.12-2.15]

[a] Some numbers might not add up to 85 due to missing values.

[b] Educational level was categorised as high if at least one household member aged ≥21 years had completed at least vocational or university education and middle/low for all others.

OR = odds ratio; CI = confidence interval

	No. (%)[a]				
	Total index cases	Households with secondary transmission	Households without secondary transmission	**p**	**Secondary attack rate (%)**
Total index cases	85	75	10		88.2
Characteristics					**OR [95% CI]**
Child (<18 years) (%)	17 (20.0%)	16 (21.3%)	1 (10.0%)	0.413	2.44 [0.29-20.72]
Adult (%)	68 (80.0%)	59 (78.7%)	9 (90.0%)	0.413	0.41 [0.05-3.48]
Age (median (IQR))	40.0 (22.0-48.0)	41.0 (24.0-48.0)	33.0 (21.5-42.8)	0.388	1.02 [0.98-1.07]
Age group, years (%)					
< 12 years	1 (1.2%)	1 (1.3%)	0 (0.0%)	-	-
12-17 years	16 (18.8%)	15 (20.0%)	1 (10.0%)	-	1 (ref)
18-39 years	25 (29.4%)	19 (25.3%)	6 (60.0%)	0.170	0.21 [0.02-1.95]
40-49 years	25 (29.4%)	23 (30.7%)	2 (20.0%)	0.834	0.77 [0.06-9.22]
50-65 years	18 (21.2%)	17 (22.7%)	1 (10.0%)	0.932	1.13 [0.07-19.74]
Sex (female) **(%)**	56 (65.9%)	50 (66.7%)	6 (60.0%)	0.677	1.33 [0.35-5.16]
BMI class[b] (%)					
Normal weight	45 (52.9%)	39 (52.0%)	6 (60.0%)	-	1 (ref)
Obesity	10 (11.8%)	10 (13.3%)	0 (0.0%)	-	-
Overweight	28 (32.9%)	25 (33.3%)	3 (30.0%)	0.741	1.28 [0.29-5.60]
Underweight	2 (2.4%)	1 (1.3%)	1 (10.0%)	0.206	0.15 [0.01-2.80]
Underlying medical	10	8 (10.7%)	2 (20.0%)	0.4	0.48

condition[c] (%)	(11.8%)			78	[0.09-2.65]
Smoking = Yes (%)	4 (4.7%)	4 (5.3%)	0 (0.0%)	-	-
Nationality (other)[d] (%)	2 (2.4%)	2 (2.7%)	0 (0.0%)	-	-
Symptom status[e]				0.639	
Severe symptoms[f]	1 (1.2%)	1 (1.3%)	0 (0.0%)	-	-
Moderate symptoms	22 (25.9%)	21 (28.0%)	1 (10.0%)	0.400	3.50 [0.19-64.67]
Mild symptoms	55 (64.7%)	47 (62.7%)	8 (80.0%)	0.979	0.98 [0.10-9.25]
Asymptomatic	7 (8.2%)	6 (8.0%)	1 (10.0%)	-	1 (ref)
Cp-value initial combined NPS/OPS (median(IQR))	24.9 (22.2-29.3)	25.0 (22.1-29.6)	23.9 (22.8-26.4)	0.837	1.01 [0.89-1.16]
Days of symptoms before test (median(IQR))	1.0 (1.0 - 2.0)	1.0 (1.0-2.0)	1. 1.0-2.7)	0.482	0.92 [0.71-1.17]

Table 3 – Index case characteristics (n=85)

[a] Some numbers might not add up to 85 due to missing values.

[b] BMI categories for index cases and household members 2-18 year were defined as BMI z-score (<-2 = underweight, BMI z-score -2-1 = normal weight, BMI z-score 1-2=overweight, BMI z-score >2= obesity); BMI categories for index cases and household members ≥18 years defined were as BMI <18.5 = underweight, BMI 18.5-25 = normal weight, BMI 25-30 = overweight, BMI >30 = obesity.[37]

[c] Cardiovascular disease, lung disease, immune disorder, diabetes, rheumatic disorder, and other disorders.

[d] Other than Dutch.

[e] Maximum over 10 time points.

[f] Hospital admission due to coronavirus-related symptoms.

OR = odds ratio; CI = confidence interval

Table 4 – Household member characteristics (n=241)

	No.(%)[a]				
	Total number of household members at risk	Secondary case	No secondary case	p	**Secondary attack rate (%)**
Total household members	241	155	86		64.3
Characteristics					**OR [95%CI]**
Child (<18 years) (%)	106 (44.0%)	68 (43.9%)	38 (44.2%)	0.962	0.99 [0.58-1.68]
Adult (%)	135 (56.0%)	87 (56.1%)	48 (55.8%)	0.962	1.01 [0.60-1.72]
Age (median (IQR))	20.0 (12.0-45.0)	19.0 (12.0-44.0)	21.0 (10.3-47.8)	0.678	1.00 [0.98-1.01]
Age group, years (%)					
< 12 years	58 (24.1%)	34 (21.9%)	24 (27.9%)	-	1 (ref)
12-17 years	48 (20.0%)	34 (21.9%)	14 (16.3%)	0.194	1.71 [0.76-3.86]
18-39 years	49 (20.4%)	35 (21.6%)	14 (16.3%)	0.170	1.77 [0.79-3.97]
40-49 years	53 (22.1%)	32 (22.6%)	21 (24.4%)	0.851	1.08 [0.50-2.30]
50-65 years	33 (13.8%)	20 (12.9%)	13 (15.1%)	0.853	1.09 [0.45-2.60]
Sex (female) **(%)**	101 (41.9%)	62 (40.0%)	39 (45.3%)	0.420	0.80 [0.47-1.37]
BMI class[b] (%)					
Normal weight	150 (66.1%)	95 (65.5%)	55 (67.1%)	-	1 (ref)
Obesity	13 (5.7%)	9 (6.2%)	4 (4.9%)	0.672	1.30 [0.38-4.43]
Overweight	59 (26.0%)	37 (25.5%)	22 (26.8%)	0.933	0.97 [0.52-1.82]
Underweight	5 (2.2%)	4 (2.8%)	1 (1.2%)	0.458	2.32 [0.25-21.24]
Underlying medical condition[c] (%)	27 (11.2%)	14 (9.0%)	13 (15.1%)	0.155	0.56 [0.25-1.25]
Smoking = Yes (%)	7 (2.9%)	4 (2.6%)	3 (3.5%)	0.689	0.733 [0.16-3.35]
Nationality (other)[d] (%)	2 (0.8%)	0 (0.0%)	2 (2.4%)	-	-
Relationship to index case					

Child[e]	117 (48.5%)	78 (50.3%)	39 (45.3%)	-	1 (ref)
Spouse	58 (24.1%)	39 (25.2%)	19 (22.1%)	0.939	1.03 [0.53-2.01]
Other adult	3 (1.2%)	3 (1.9%)	0 (0.0%)	-	-
Parent	44 (18.3%)	25 (16.1%)	19 (22.1%)	0.248	0.66 [0.32-1.34]
Sibling	19 (7.9%)	10 (6.5%)	9 (10.5%)	0.239	0.56 [0.21-1.48]

[a] Some numbers might not add up to 241 due to missing values.

[b] BMI categories for index cases and household members 2-18 year were defined as BMI z-score (<-2 = underweight, BMI z-score -2-1 = normal weight, BMI z-score 1-2=overweight, BMI z-score >2= obesity); BMI categories for index cases and household members ≥18 years defined were as BMI <18.5 = underweight, BMI 18.5-25 = normal weight, BMI 25-30 = overweight, BMI >30 = obesity.[37]

[c] Cardiovascular disease, lung disease, immune disorder, diabetes, rheumatic disorder, and other disorders.

[d] Other than Dutch.

[e] Children could be either < 18 years old or >18 years old if their role was a child within a household.

OR = odds ratio; CI = confidence interval

Figure 1

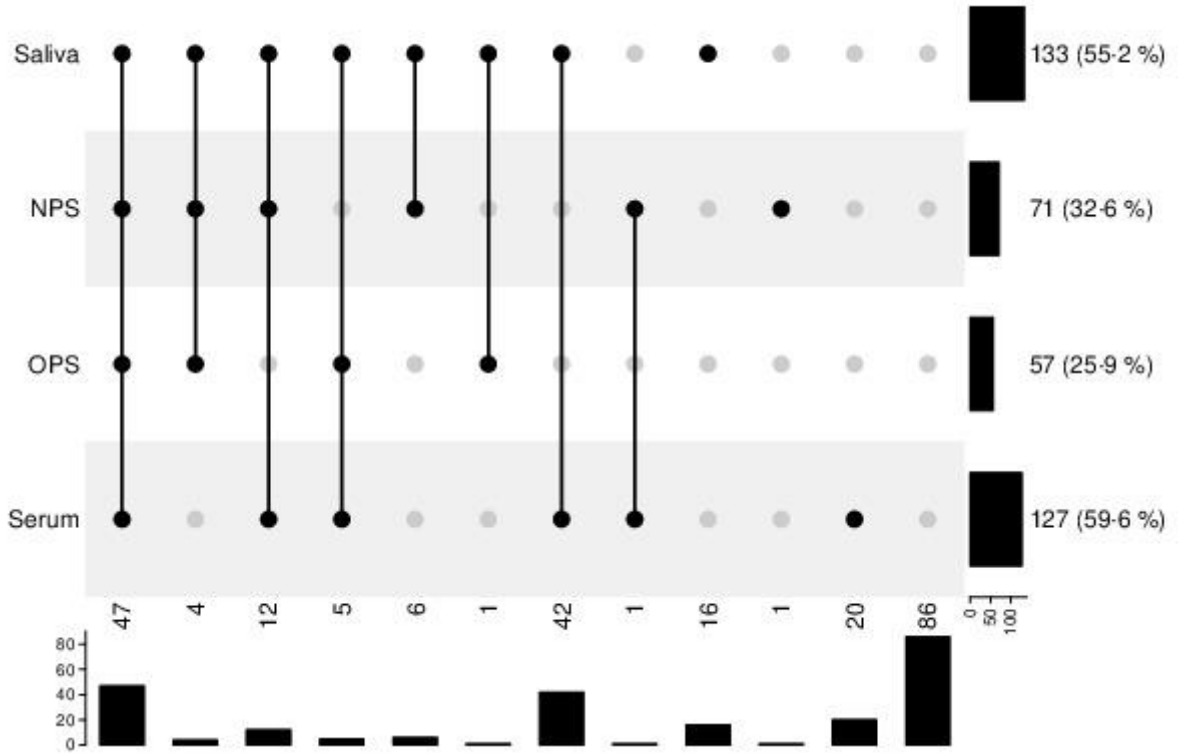

Figure 2

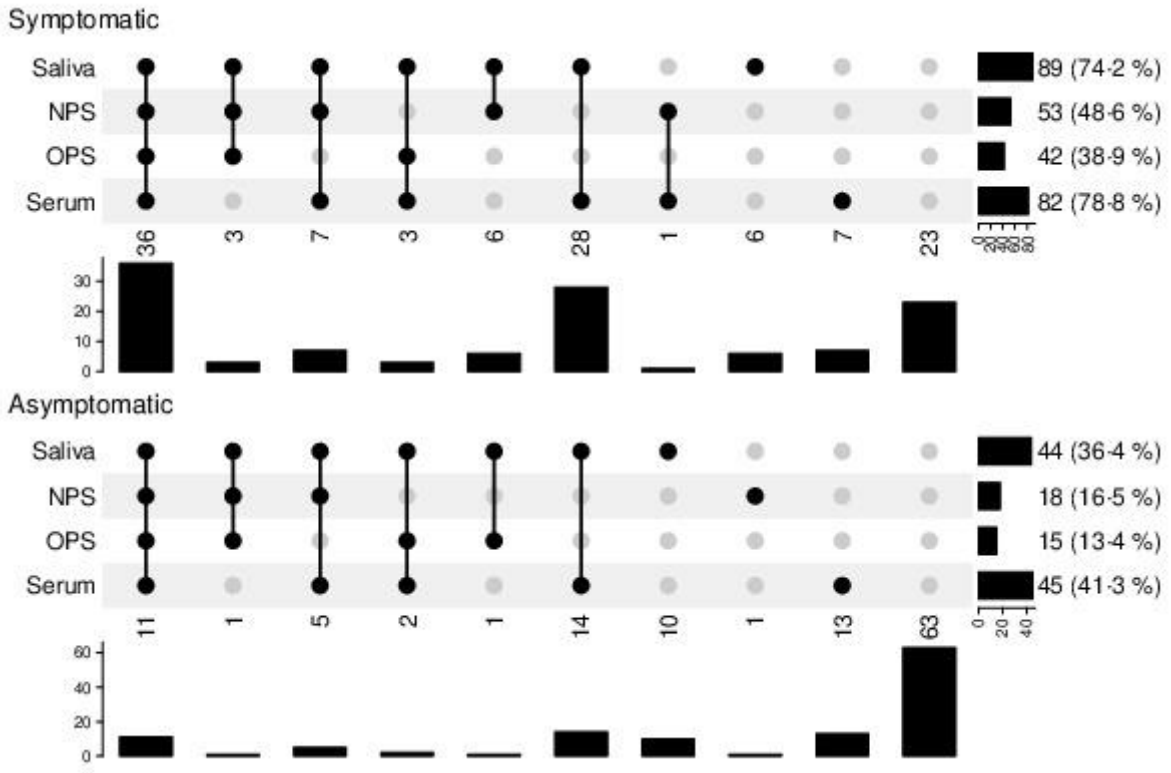

Figure 3