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MAJOR ARTICLE

SARS-CoV-2 in Exhaled Aerosol Particles from COVID-19 Cases and Its Association to Household Transmission

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Background. Coronavirus disease 2019 (COVID-19) transmission via exhaled aerosol particles has been considered an important route for the spread of infection, especially during super-spreading events involving loud talking or singing. However, no study has previously linked measurements of viral aerosol emissions to transmission rates.

Methods. During February–March 2021, COVID-19 cases that were close to symptom onset were visited with a mobile laboratory for collection of exhaled aerosol particles during breathing, talking, and singing, respectively, and of nasopharyngeal and saliva samples. Aerosol samples were collected using a BioSpot-VIVAS and a NIOSH bc-251 2-stage cyclone, and all samples were analyzed by RT-qPCR for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA detection. We compared transmission rates between households with aerosol-positive and aerosol-negative index cases.

Results. SARS-CoV-2 RNA was detected in at least 1 aerosol sample from 19 of 38 (50%) included cases. The odds ratio (OR) of finding positive aerosol samples decreased with each day from symptom onset (OR 0.55, 95 confidence interval [CI] .30–1.0, $P = .049$). The highest number of positive aerosol samples were from singing, 16 (42%), followed by talking, 11 (30%), and the least from breathing, 3 (8%). Index cases were identified for 13 households with 31 exposed contacts. Higher transmission rates were observed in households with aerosol-positive index cases, 10/16 infected (63%), compared to households with aerosol-negative index cases, 4/15 infected (27%) (χ^2 test, $P = .045$).

Conclusions. COVID-19 cases were more likely to exhale SARS-CoV-2-containing aerosol particles close to symptom onset and during singing or talking as compared to breathing. This study supports that individuals with SARS-CoV-2 in exhaled aerosols are more likely to transmit COVID-19.

Keywords. exhaled aerosol; singing; aerosol sampling; airborne SARS-CoV-2.

Singing and talking have been linked to several super-spreading events during the coronavirus disease 2019 (COVID-19) pandemic [1–6]. Additional common denominators at these events have been many people in the same room, often poor ventilation, a long (>30 minute) period of exposure and a usually asymptomatic index person. Many of these case reports have concluded that transmission primarily occurred through the inhalation of aerosols. Nevertheless, no study has yet been able to directly link measurements of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in aerosols with a higher transmission rate.

Because more than 50% of COVID-19 transmission originate from presymptomatic or asymptomatic individuals [7], and these are unlikely to cough or sneeze extensively, other nonsymptomatic respiratory activities have been assumed to induce disease transmission. The potential for COVID-19 transmission through exhaled aerosols (aerosols defined as solid or liquid particles <100 μm suspended in a gas) during singing and talking has been investigated by us and other groups [8–10]. In line with a pre-COVID-19 study [11], the results show that aerosol emissions increase during talking, as compared to breathing, and even more during singing. The louder the vocalization, the higher the aerosol emissions.

Recently, two studies examined the amount of SARS-CoV-2 RNA in exhaled aerosols during breathing, talking and singing [12, 13]. Both studies found positive aerosol samples, and Adeniaye et al also demonstrated cell-culture infectivity in two SARS-CoV-2 positive aerosol samples. However, cases in these studies were on average sampled on days 4–5 from symptom onset. Considering that transmission is most likely to occur close to symptom onset [14], more data are needed from the initial phase of the infection. In addition, no direct connection between exhaled SARS-CoV-2 aerosols and the risk of transmission has previously been shown.

This study aimed to detect, quantify and characterize SARS-CoV-2 RNA in the exhaled aerosols of COVID-19 cases during

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the early viral phase of the infection and nonsymptomatic respiratory activities: breathing, talking, and singing. Moreover, we investigated household transmission dynamics and differences in households with aerosol-positive versus aerosol-negative index cases.

METHOD

Study Design and Setting

COVID-19 cases living in the vicinity of Malmö, Sweden, that were close to symptom onset were visited at their homes for sampling of SARS-CoV-2 in upper airways and in exhaled aerosols. All specimens were analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) for detection of SARS-CoV-2 RNA. In addition, questionnaires regarding symptoms and subsequent household transmission were filled out at the time of enrollment and during a follow-up phone call, respectively. Sample collection was performed during February and March 2021, a period when the Alpha-variant increasingly constituted from 16% to 83% of cases and Beta- and Gamma-variants <1% [15].

Case Inclusion

Cases with COVID-19 were identified through the contact tracing services at Skåne University Hospital. Cases with <6 days of symptoms and preferably one or several household contacts were asked to participate in the study (Figure 1). For those that volunteered, a research team drove a mobile laboratory installed in a small truck to the home address of the case and quarantined household contacts. Regardless of symptoms, volunteering household contacts over the age of 12 were tested with a nasopharyngeal antigen test (Panbio COVID-19 antigen rapid test, Abbott). Household contacts that were either antigen test-positive or had COVID-19-like symptoms were asked to join the study. If at least 1 of their nasopharyngeal or saliva sample was positive in the RT-qPCR analysis, they were included.

In total, 41 cases were enrolled in the study. Two cases enrolled due to COVID-19 like symptoms were subsequently excluded as they did not have COVID-19, and 1 was excluded due to technical problems during sampling. The remaining 38 cases had a median age of 34 years (range 12–59), of which 21 (55%) were female.

Test Procedure

Included cases filled out a questionnaire on experienced symptoms and COVID-19 vaccination status. A nasopharyngeal swab sample and a posterior oropharyngeal saliva sample were collected in 1.5 mL NaCl solution and in a 50 mL sputum collector, respectively, and stored at −80 °C. The posterior oropharyngeal saliva (hereafter referred to as saliva) sample was

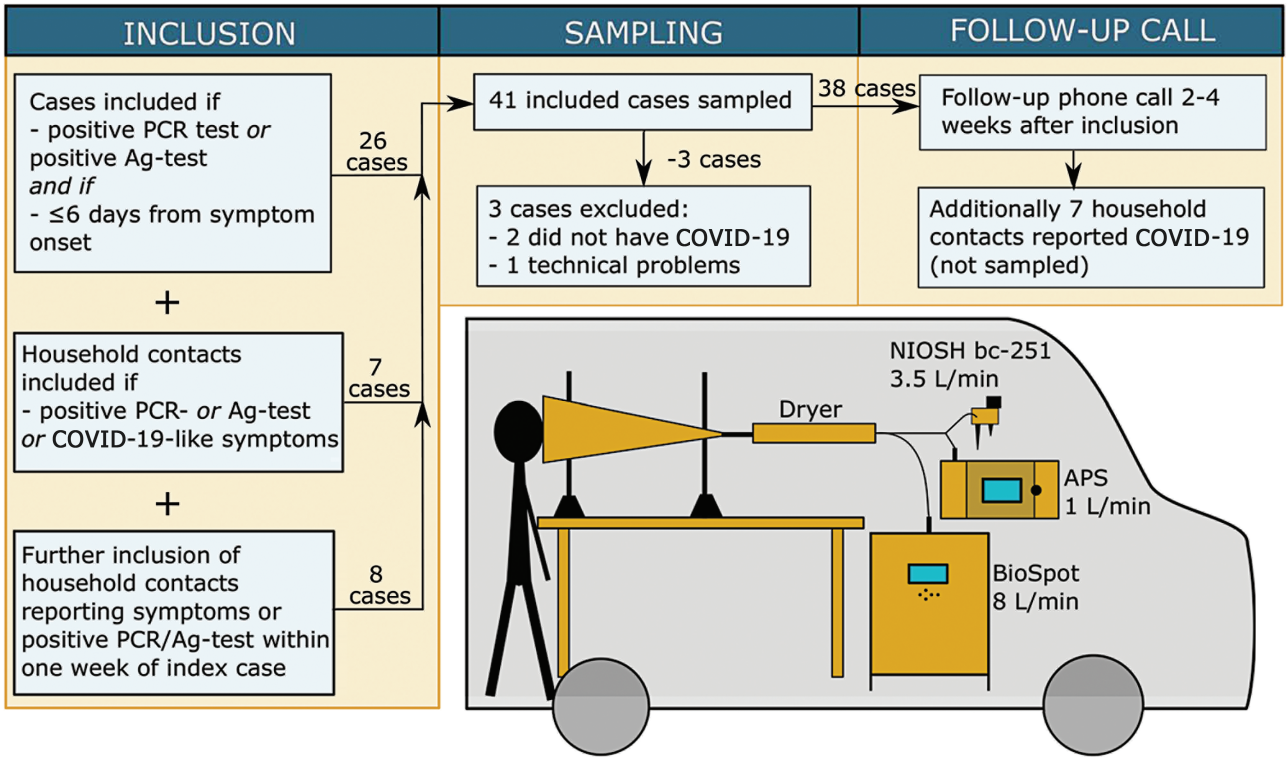

Figure 1. Study design from (1) inclusion of cases and household contacts, (2) sampling SARS-CoV-2 in exhaled aerosols and upper respiratory tract and (3) follow-up call where household transmission was evaluated. Abbreviations: APS, aerodynamic particle sizer; COVID-19, coronavirus disease 2019; NIOSH, National Institute for Occupational Safety and Health; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

an effort to represent the hypopharyngeal region (closer to the vocal cords).

Cases were positioned in the mobile laboratory with their face in the opening of a metal funnel and asked to breathe normally, although exhaling through the mouth, for 10 minutes. After the breathing session, cases were asked to talk freely for 10 minutes, and thereafter sing any song(s) for 10 minutes. Participants were allowed to sing or talk in their preferred language. The majority chose Swedish or English (other languages: Dari, Finnish, French, Greek, Polish, and Turkish).

Household Transmission

One case from each household was contacted 2–4 weeks after sampling to compile additional information about household cases and follow-up on symptom progression. Household transmission rates were investigated in association to aerosol-positive or aerosol-negative index cases. Included cases were classified as index cases, household cases, or none (including those where more than 1 from the same household were infected simultaneously or where there were no susceptible household contacts). We defined index cases as those who were infected with COVID-19 outside the home and who solely exposed household contacts during home quarantine. Households where index cases were included after day 3 from symptom onset were excluded from the transmission analysis, to avoid misclassification of aerosol-positivity. A household case was defined as a susceptible contact (not previously reported COVID-19, nor fully vaccinated) who had symptom onset ≥ 3 days after the index case, and who was PCR-positive (or had COVID-19-like symptoms for children under the age of 6, whom according to the local COVID-19 strategy were not tested by PCR) within 14 days. All household cases were not sampled.

Aerosol Collection Setup

Aerosol particles were collected from the end of the funnel with a condensational growth tube collector (BioSpot-VIVAS, Aerosol Devices Inc.) operating at 8 L min^{-1}, and using a 2 stage cyclone sampler (NIOSH bc-251) or a filter cassette operating at 3.5 L min^{-1} or 4 L min^{-1}, respectively (Figure 1). In addition, the aerosol particle size and concentration were monitored using an aerodynamic particle sizer (APS, Model 3321, TSI Inc.). A heating blanket or a diffusion dryer was used to avoid water vapor condensation in the tubes. The BioSpot sample was exchanged between breathing, talking, and singing, respectively, whereas the NIOSH sampler or total filter was kept running for all exercises (30 minutes) of the same person. All samples were stored at −80 °C until analysis. Details on the methodology are found in Supplementary Materials.

Theoretical calculations on aerosol particle losses (sedimentation and impaction) in the collection setup resulted in an aerodynamic particle cutoff size, d_{50}, around 12 µm for the BioSpot.

RNA Extraction and RT-qPCR Analysis

RNA was extracted using the QIAamp Viral RNA Mini kit (Qiagen) according to the protocol of the manufacturer. For BioSpot samples, the double amount of sample (280 µL) was used to improve sensitivity. Viral RNA was extracted from 2 quarters of each filter using the Nuclisens MiniMag (Biomérieux) kit. We compared 3 methods for filter extractions (details in Supplementary Materials).

RT-qPCR was performed using the qPCRBIO Probe 1-step virus detect kit (Pcr biosystems) targeting the N1 and N2 genes [16]. Primers and probes were used at a concentration of 0.4 µM and 0.16 µM, respectively. We used 10 µL of sample for each reaction in order to improve the sensitivity. A dilution series of a known concentration of DNA (Integrated DNA Technologies) was used for quantification. Samples were defined as positive if at least 1 of the duplicates had a cycle threshold (Ct)-value ≤ 40. In addition, the nasopharyngeal sample for 1 case from each household was analyzed by PCR targeting the N501Y mutation associated with the Alpha variant.

Statistics

Differences in continuous variables between aerosol-positive and aerosol-negative cases were assessed with the Mann-Whitney *U* test, and for counts and percentages the χ^2 test or Fisher's exact test (where appropriate) were used. Associations between symptom characteristics and positive aerosol samples were investigated with univariate logistic regression. One case was asymptomatic at the time of enrollment and was therefore excluded from the statistical analyses involving days from symptom onset and reported symptoms. To account for heterogeneity in transmission dynamics within different households, a random-effects logistic regression was performed as complement to the χ^2 test. Statistical analyses were performed using STATA version 13 (StataCorp LLC).

Ethics

All participants received oral and written information and signed a written consent. This study was approved by the Swedish Ethical Review Authority (2020-07103).

RESULTS

We detected SARS-CoV-2 RNA in exhaled aerosol particles from 19 of 38 (50%) cases. A higher fraction of cases had positive aerosol samples closer to symptom onset (Figure 2A). The odds ratio (OR) for positive aerosol samples decreased significantly with each day from symptom onset (OR 0.55, 95 confidence interval [CI] .30–1.0, $P = .049$). Twenty-two cases were

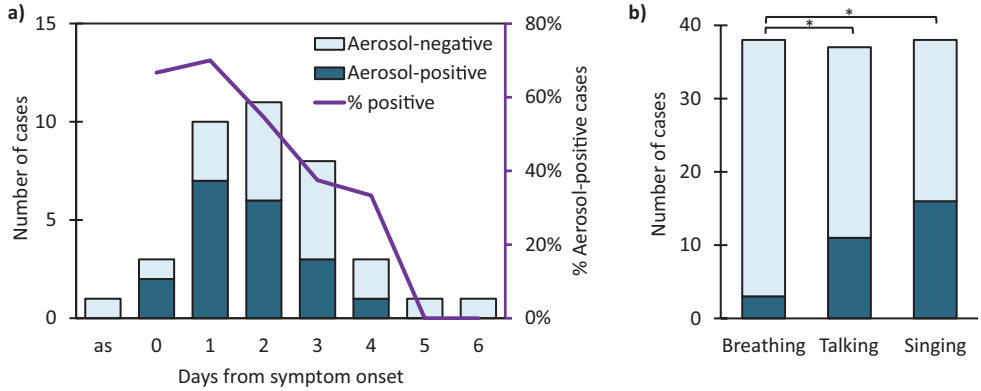

Figure 2. *A*, Number of cases with at least 1 positive aerosol sample (*dark blue*) or all negative (*light blue*) on days 0–6 from symptom onset. The purple line shows the percentage of cases with at least 1 positive aerosol sample (right *y*-axis). *B*, Cases with positive (*dark blue*) and negative (*light blue*) aerosol samples from breathing, talking, and singing. Abbreviation: as = asymptomatic at the time of enrollment. *Indicate significance between fractions using Fisher's exact test.

infected with the Alpha variant, 15 with pre-Alpha variants, and 1 sample failed in the analysis.

Household Transmission

Cases included in the study belonged to 25 different households, and in 16 an index case could be identified (in 9 households cases were infected simultaneously or had no susceptible household contact). Of the 16 index cases, 7 were aerosol-positive, 6 were aerosol-negative, and 3 were excluded due to inclusion in the study on day 4 or more (Figure 3). The secondary attack rate in households with aerosol-positive index cases was 63% (10 of 16 exposed contacts), higher (P = .045) than the secondary attack rate in households with aerosol-negative index cases 27% (4/15 exposed contacts) (details in Supplementary Table 1). The association persisted in a sensitivity analysis where symptomatic children not confirmed with PCR test were excluded as household cases (P = .04). Only a trend was found using a random-effects logistic regression (OR 5.7, 95 CI .52–65, P = .16), a test that takes into account possible heterogeneity between different households. No evidence for subsequent transmission within the households after the initial exposure was found. There were no differences in home size or mitigation strategies among households with aerosol-positive index cases and households with aerosol-negative cases (Supplementary Table 2).

Breathing, Talking Versus Singing RNA Emission Rates

There was a higher fraction of positive aerosol samples from singing, 42% (16/38), and from talking, 30% (11/37, 1 sample missing) than from breathing, 8% (3/38) (P = .001 and P = .019, respectively) (Figure 2B). About half of the aerosol positive cases, 52% (10/19), were positive in more than 1 type of aerosol sample; however, only 2 cases were positive in all 3 breathing, talking, and singing samples.

We determined the SARS-CoV-2 RNA emission rates for the cases with positive BioSpot aerosol samples based on the RNA concentration in the samples and the collection airflow rates. The median (range, n) emission rates were 70 (20–220,

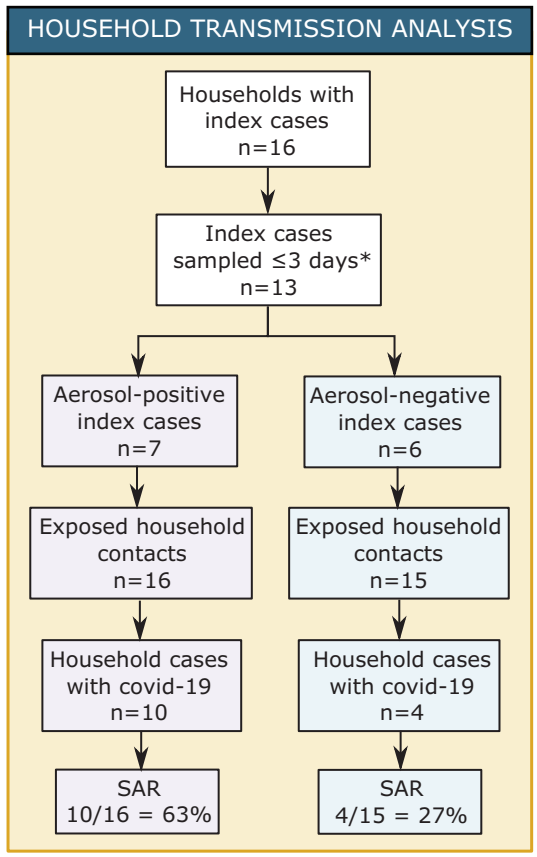

Figure 3. Box diagram of index cases and household contacts included in the household transmission analysis. Days* = days from symptom onset. Abbreviations: COVID-19, coronavirus disease 2019; SAR, secondary attack rate.

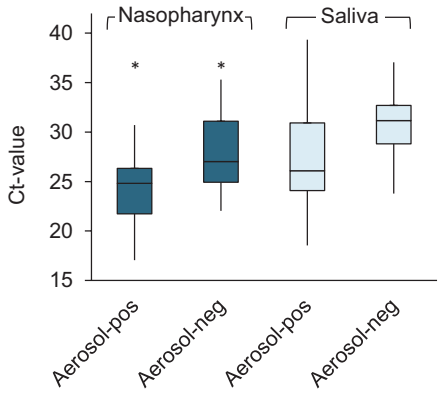

Figure 4. Ct-values of nasopharyngeal and saliva samples for the group of aerosol-positive and aerosol-negative cases. *Indicate significant differences between groups, $P < .05$. Abbreviation: Ct, cycle threshold.

3) RNA copies/minute for breathing, 110 (30–3100, 11) RNA copies/minute for talking, and 80 (20–7800, 16) RNA copies/minute for singing (Supplementary Figure 2). The highest emission rates for talking and singing were collected from a person on the day of symptom onset (day 0).

Aerosol Particle Size Fractions Containing SARS-CoV-2

Five of 20 cases had at least 1 positive aerosol particle size-fraction in the NIOSH sampler. Two samples from the particle size fraction >4 µm were positive, 4 from 1–4 µm, and 3 from <1 µm. For the other 18 cases where a filter collecting all particle sizes was used instead of the NIOSH sampler, 6 cases had positive total filter samples (Supplementary Table 3).

Ct-Values in Upper Respiratory Tract

Aerosol-positive cases had lower Ct-values (higher viral load) in nasopharyngeal samples than aerosol-negative cases ($P = .02$) (Figure 4), but no difference was seen for saliva samples ($P = .11$) (Supplementary Figure 1). Among index cases (n = 13), however, there was no difference in nasopharyngeal Ct-values between aerosol-positive and aerosol-negative cases ($P = .89$) (Supplementary Table 1).

Symptoms Associated With Aerosol-Positive Cases

Cases who were aerosol-positive had shorter time from symptom onset ($P = .045$) to sampling and more often reported cough ($P = .008$) (Table 1). Cases reporting cough had an OR of 13 (95 CI 1.4–120, $P = .02$) to have positive aerosol samples.

DISCUSSION

This study shows an association between SARS-CoV-2 RNA in exhaled aerosols from index cases during breathing, talking, and singing and a higher secondary attack rate in households. Moreover, we found 4 factors that increase the risk for SARS-CoV-2-positive aerosol samples: voicing (singing and talking as compared to breathing), short time from symptom onset, a low Ct-value from a nasopharyngeal sample, and reporting cough as a symptom. These findings support that exhaled aerosols from cases in the early phase of infection are of special concern for transmission of COVID-19.

This study is the first to link SARS-CoV-2 aerosol emissions to COVID-19 transmission patterns, indicating a twice as high secondary attack rate (63% compared to 27%) when the index case exhaled detectable viral RNA levels in the early phase of the infection. This finding further supports the hypothesis that exhaled SARS-CoV-2 aerosols may determine individual transmissibility and secondary attack rate. The aerosol emission rates can vary both between cases and within one case over time. This variation and possibly other factors may have influenced the results. Index cases were generally sampled later than infected household contacts due to the study design (median days from symptom onset was 3 for index cases and 1 for nonindex cases). Because Coleman et al saw an association between days from symptom onset and aerosol-positivity, index cases on day 4 or more were excluded from the analysis as they may be misclassified as aerosol-negative due to late sampling [12]. The low number of households (n = 13) eligible for the analysis limited statistical power. Nevertheless, this finding is noteworthy and needs to be further investigated and confirmed.

The characteristics of positive aerosol samples in our study are in general agreement with previous results: about half of

Table 1. Time From Symptom Onset and Self-Reported COVID-19 Symptoms

Clinical Presentation	Positive Aerosol (n = 19)	Negative Aerosol (n = 19)	*P*
Time from symptom onset, median (IQR)	2 (1–2)	3 (2–3)	.045
Cough, n (%)	18 (95%)	11 (58%)	.008
Headache, n (%)	15 (79%)	13 (68%)	.46
Sore throat, n (%)	13 (68%)	9 (47%)	.19
Rhinorrhea, n (%)	12 (63%)	10 (53%)	.51
Fever, n (%)	7 (37%)	7 (37%)	1
Loss of smell or taste, n (%)	3 (16%)	2 (11%)	.63
Dyspnea, n (%)	3 (16%)	2 (11%)	.63
Vomiting or diarrhea, n (%)	1 (5%)	0%	.31

Abbreviations: COVID-19, coronavirus disease 2019l IQR, interquartile range.

the cases exhaled detectable amounts of SARS-CoV-2 RNA, and the highest RNA levels were exhaled during singing [12, 13]. The majority of our aerosol samples contained <1000 RNA copies (corresponding to <200 RNA copies/min), which is similar to the sample concentrations found by Coleman et al [12]. However, 3 cases in our study emitted more than 1000 RNA copies per minute, the highest reaching 7800 copies per min. The corresponding sample concentrations of these samples are similar to the highest concentration found in the study by Adenaiye et al that was confirmed infectious in cell culture [13].

We found detectable SARS-CoV-2 RNA levels in the aerosol particle size fractions >4 μm, 1–4 μm, and <1 μm, and more positive samples in the size fraction 1–4 μm. This finding is in line with our previous study on (noninfectious) aerosol emission rates from singing, where a major part of the aerosol mass was in the size range 1–4 μm [8]. Santarpia et al, who used a similar aerosol sampler, found most SARS-CoV-2 from the rooms of hospitalized COVID-19 patients in the <1 μm fraction and were only able to replicate viruses from this size fraction in cell cultures [17].

Aerosol droplets from the respiratory system can be generated by several mechanisms: (i) from thin film ruptures in the small airways during breathing, primarily generating aerosol particles in the size range 0.2–1.0 μm [18, 19], (ii) from the vibrations of the vocal chords during vocalization, primarily generating aerosol particles in the size range 1–5 μm [8, 9], and (iii) from mouth and lips movements during articulation, primarily generating aerosol droplets in the size range >30 μm [18, 20]. In addition, high-velocity airflows in the upper respiratory tubes has been suggested to induce surface instabilities leading to droplet budding from the lining fluid [21]. The aerosol emission rates from breathing, talking, and singing in previous studies [8, 9] agree with the virus-containing aerosol samples and size fractions found here. Thus, our results suggest that we collected aerosol particles from thin film rupture during breathing, and from thin film rupture and vocal cords vibrations during speaking and singing. Some part of the mouth- and lips-generated droplets may have been collected as well; however, particles >20 μm likely deposited in the collection setup prior to sampling instruments.

Although aerosol-positive cases had significantly lower Ct-values (higher viral load) in nasopharyngeal samples than aerosol-negative cases, no difference was seen between aerosol-positive index cases and aerosol-negative index cases. Moreover, as shown in Figure 4, some aerosol-positive cases had high Ct-values; thus, we recognize that upper respiratory tract samples do not fully explain which cases exhale SARS-CoV-2 aerosols.

Only 5 cases in this study had received vaccination for COVID-19, and only 1 was fully vaccinated (2 doses). Two of these 5 cases had at least 1 positive aerosol sample, yet cases were too few to draw any conclusions.

A major challenge in this study was to find cases in the early phase of the infection, and it has been shown that timing is crucial for collection of airborne infectious agents [22]. Although we designed this study to screen household contacts using an on-site antigen test, only 1 asymptomatic case was enrolled. The reason is likely because the antigen test used for screening has been shown to have a lower sensitivity for cases with Ct-values >25 [23, 24] and for presymptomatic cases [25].

As a consequence, this study has a bias toward symptomatic cases close to symptom onset. It is not unlikely that some cases emit SARS-CoV-2 in aerosols also later on during the disease, as found by Coleman et al (days 5–8), as well as before symptom onset [12]. However, as COVID-19 cases are most infectious close to symptom onset [26], it is of high relevance to focus on the early phase of the infection. For future studies, it would be important to include more asymptomatic and presymptomatic cases and in addition, to study the effect of vaccination on viral aerosol emissions.

CONCLUSION

In this study we show that 50% of the included cases emitted detectable levels of SARS-CoV-2 RNA in exhaled aerosols during non-symptomatic respiratory activities. Voicing (singing and talking) generated more positive samples and higher sample concentrations than breathing. We also present an association between SARS-CoV-2 RNA in exhaled aerosol particles and a higher household transmission rate, further demonstrating the importance of aerosol transmission in the early phase of COVID-19 infections.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. C.-J. F. reports being contributing author to Swedish guidelines for clinical managements of COVID-19 and

member of the board of the Swedish Medical Association of Infection Control (SHLF), both unpaid. J. L. reports receiving payment for lectures by Camfil paid to institution and payment for expert advice on protection for airborne diseases at University Hospital in 2020 paid as personal salary. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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