

## Transmission of SARS-CoV-2 on a Patient Transport Van

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## **ABSTRACT**

We report 2 episodes of potential SARS-CoV-2 transmission from infected van drivers to passengers despite masking and physical distancing. Whole genome sequencing confirmed relatedness of driver and passenger SARS-CoV-2. With the heater operating, fluorescent microspheres were transported by airflow >3 meters from the front to the back of the van.

**Keywords:** SARS-CoV-2, motor vehicle, transmission, aerosol, ventilation

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Motor vehicle travel with multiple occupants poses a risk for transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1-3]. In an outbreak in China in January 2020, an infected source passenger in a bus was linked to multiple infections in fellow passengers, including individuals seated more than 2 meters away [2]. However, in that outbreak, transmission risk may have been relatively high because the passengers were not wearing masks and the ventilation system was set on a recirculating mode [2]. In situations where shared travel cannot be avoided, the Centers for Disease Control and Prevention (CDC) recommends that drivers and passengers wear facemasks, optimize physical distancing, and take measures to improve ventilation when possible by opening windows or setting the ventilation system on non-recirculation mode [1]. It is not known if these measures are effective in minimizing the risk for transmission. Here, we report an investigation of 2 episodes in which patient transport van drivers with coronavirus disease 2019 (Covid-19) were suspected of transmitting the virus to passengers.

## **METHODS**

### **Contact Tracing Investigation**

The study protocol was approved by the Cleveland VA Medical Center's Institutional Review Board. A patient van driver was diagnosed with COVID-19 on December 3, 2020 and a second driver was diagnosed on January 23, 2021. The Infection Control Department conducted contact tracing for both infections beginning 2 days prior to the onset of symptoms [4]. The drivers and passengers were interviewed regarding their interactions and masking and hygiene practices. All passengers were asked to submit nasopharyngeal swab specimens for SARS-CoV-2 reverse transcriptase polymerase-chain reaction (RT-PCR) testing.

## **Whole Genome Sequencing**

Whole genome sequencing of driver and passenger SARS-CoV-2 was performed on an Illumina MiSeq sequencing platform (see Supplement for detailed methods); 3 samples from unrelated COVID-19 patients were included for comparison. Sequences were aligned to a reference COVID-19 sequence (NCBI accession: NC\_045512.2). Variant positions were defined as any genomic position in which an alternate allele was the predominant (>50%) allele present in a sequenced sample. Sequences were assigned to a transmission cluster if they had  $\leq 2$  base differences based on their variant positions. Consensus sequences were classified into 3 different commonly referenced clade classification systems, including Nextstrain (nextstrain.org), Global Initiative on Sharing Avian Influenza Data (GISAID), and Pangolin (github.com/cov-lineages/pangolin).

## **Evaluation of Airflow and Microsphere Dispersal in the Van**

During both trips the same van was used, the windows were closed, ventilation was not on recirculation mode, and heaters were operated with medium fan speed. We used a smoke-emitting pen (S220 Smoke Pen, Regin) to assess airflow under these conditions. Smoke was released in the passenger seats and the direction of air movement was assessed. Fluorescent microspheres (Cospheric) were used to assess the potential for particles to travel in air currents to the back of the van and to settle on surfaces. Microspheres with 1-5  $\mu\text{m}$  and 212-250  $\mu\text{m}$  diameter were chosen to be consistent in size with both small respiratory aerosols (1-10  $\mu\text{m}$ ) and larger droplets (100-1000  $\mu\text{m}$ ) [5]. Dry powder containing 15,941 of the larger microspheres (100 mg) was poured over 10 seconds adjacent to the front vents. A Preval sprayer (Nakoma Products, LLC) aerosol-based spray system was used to release 6 mL of droplets containing  $\sim 3.2 \times 10^7$  of the smaller microspheres (0.18 mg) over 5 minutes from the

driver's seat directed toward the front vents; based on particle counter readings the device disperses predominantly 0.3 to 5  $\mu\text{m}$  liquid droplets.

After 5 minutes, a 395 nm ultraviolet blacklight flashlight (TaoTronics) was used to detect the larger microspheres on passenger seats. To assess for dispersal of the smaller microspheres, glass slides were placed on each row of seats and an air sampler (TE-BC251 NIOSH Bioaerosol Cyclone (TISCH Environmental) was used to obtain air from the back seat. The air sampler was run for 5 minutes (25 L total volume) starting at the time of microsphere release. The slides were covered with clear tape after 5 minutes. Microspheres were visualized with a microscope under 395 nm ultraviolet light fluorescence at 40x magnification. The number of microspheres recovered from air samples and detected per  $\text{cm}^2$  on the slides was calculated. Testing was performed in triplicate.

## **RESULTS**

### **Contact Tracing Investigation**

The first driver transported 4 patients on 2-hour trips to and from the hospital on December 2, 2020, one day prior to onset of symptoms (Figure 1.A). The quantification cycle of the driver's nasopharyngeal specimen was 15.9, indicating a high viral burden. The driver and all passengers wore cloth facemasks and only interacted during boarding and transportation. Of 4 passengers tested for SARS-CoV-2, 3 had positive results from nasopharyngeal specimens collected 6 to 8 days after exposure and 1 subsequently developed COVID-19 symptoms.

The second driver transported 3 patients on the same 2-hour trip in the same van on January 23, 2021, the day his symptoms began (Figure 1.B). The quantification cycle of the driver's nasopharyngeal swab specimen was 24, indicating a high viral burden. One passenger was diagnosed with COVID-19 4 days later, whereas the other passengers

remained asymptomatic and tested negative. The van driver did not wear a mask, but the passengers wore cloth facemasks.

### **Whole Genome Sequencing**

All samples had >99% of the genome covered with a depth greater than 100X. SARS-CoV-2 sequences for each van driver were distinct, whereas passenger sequences for each episode were related to the corresponding driver sequences ( $\leq 2$  base differences based on their variant positions) (Figure 1.C).

### **Air Flow and Microsphere Dispersal in the Van**

When the heater was on, smoke released from the driver and passenger seats at headrest height rose and flowed toward the back of the van (Supplementary Figure 1). No released microspheres were recovered from the seats or air when the heater and fans were off. Table 1 shows the number of microspheres detected when the heater was running for 1 of the 3 experiments. In all passenger rows, the larger microspheres were visualized under black light on seats and the smaller microspheres were detected on glass slides placed on the seats. The smaller microspheres were also detected in air (>100 microspheres) collected from the back row.

### **DISCUSSION**

Our investigation strongly suggests transmission of SARS-CoV-2 by 2 patient van drivers to several passengers. The drivers posed a high risk for transmission because they were in the acute stage of infection with a high viral burden. The driver who transmitted SARS-CoV-2 to 3 passengers was pre-symptomatic, consistent with evidence that pre-symptomatic individuals may contribute substantially to transmission [6-7]. The other driver did not wear

a facemask, resulting in increased risk for transmission due to lack of source control. On both trips, individuals sitting more than 3 meters away in the back seat acquired SARS-CoV-2. Thus, transmission occurred on 1 of the trips despite following CDC recommendations that all riders wear facemasks and optimize physical distancing [1].

The evaluation of air flow within the van suggests that air currents produced by the heating and ventilation system may facilitate SARS-CoV-2 transmission. With the heater on, air flowed toward the back of the van and fluorescent microspheres consistent in size with small respiratory aerosols and larger droplets were dispersed from the front to the back of the van. Transmission of SARS-CoV-2 in restaurants over distances greater than 2 meters has been similarly linked to respiratory droplet dispersal by air conditioners [8-9]. Although opening windows may improve ventilation, the trips were taken on cold days and windows in the passenger rows of the van do not open.

Our findings suggest that improved strategies are needed to reduce the risk for transmission of SARS-CoV-2 in motor vehicles. One strategy may be to ensure that drivers and passengers wear facemasks that provide adequate fit and filtration [10-11]. The van riders involved in the current investigation wore single-layer cloth masks. Measures such as filtration to remove viral particles and upper room ultraviolet light disinfection have been used in buildings [12] but may not be feasible in vehicles. Our facility installed plexiglass dividers between the van drivers and passengers in response to the transmission clusters. However, limited information is available on the effectiveness of such barriers.

Our study has some limitations. Compliance with facemasks during the trips is uncertain. Fomite-mediated transmission is unlikely but cannot be excluded as passengers signed in with a shared pen and clipboard and dispersed respiratory droplets settling on surfaces could have been acquired on hands. We cannot exclude the possibility that the passengers acquired SARS-CoV-2 variants commonly circulating in the community.



However, only 3 of 76 (3.9%) additional SARS-CoV-2 samples sequenced at our facility between May 2020 and January 2021 were the lineages infecting the van riders (data not shown). Finally, it is not known if the simulations with fluorescent microspheres correlate well with transmission of viruses in respiratory secretions.

In conclusion, there is substantial risk for SARS-CoV-2 transmission on patient transport vans, even when riders wear masks and maintain physical distancing. Heater fans may create air currents that enhance dispersal of respiratory droplets. Additional measures are needed to reduce the risk for SARS-CoV-2 transmission on vehicles.

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## Notes

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**Table 1. Number of Fluorescent Microspheres Detected on Van Seats after Release from the Front of the Van**

Distance from driver	Size and formulation of microspheres	
	1-5 $\mu$ m aerosolized	210-250 $\mu$ m dry powder
1 meter (1 <sup>st</sup> row)	34	8
2.1 meters (2 <sup>nd</sup> row)	25	9
2.7 meters (3 <sup>rd</sup> row)	42	5
3.3 meters (back row)	17	1

The number of microspheres detected during 1 of 3 simulations is shown. The 210-250 $\mu$ m microspheres were directly visualized on seats with a black light; the 1-5 $\mu$ m microspheres that settled on glass slides placed on the seats were counted under 395 nm ultraviolet light fluorescence at 40x magnification and calculated per cm<sup>2</sup>.

## Figure legend

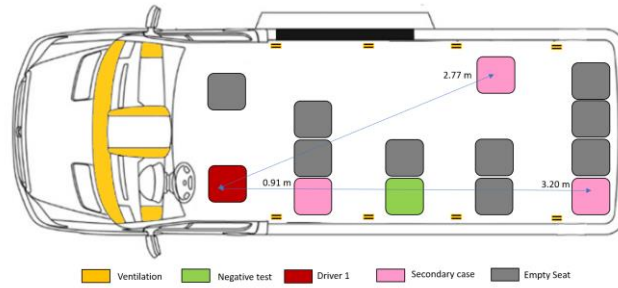
### **Figure 1. Passenger Seating Arrangement on the Van and Genetic Variant Profiles of the Sequenced SARS-CoV-2.**

A and B show a schematic of the van and passenger seating arrangement during the first and second van rides. C shows the genetic variant profiles of the sequenced SARS-CoV-2 relative to the Wuhan-Hu-1 reference genome for 6 SARS-CoV-2-infected individuals from the van rides and for 3 unrelated COVID-19 patients. Clade information was based on Nextstrain, Global Initiative on Sharing Avian Influenza Data (GISAID), and Pangolin lineage. Gradient intensity of the bar indicates the reference allele frequency at each variant nucleotide position.

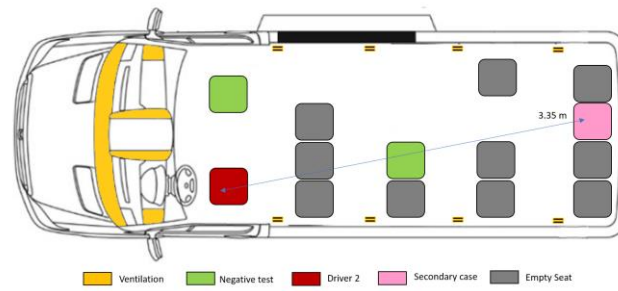
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Figure 1

A.



B.



C.

