Sewage as a Possible Transmission Vehicle During a Coronavirus Disease 2019 Outbreak in a Densely Populated Community: Guangzhou, China, April 2020

Jun Yuan,1,2,a Zongqiu Chen,1,2,a Chenghua Gong,1,2 Hui Liu,1,2 Baisheng Li,1,2 Kuibiao Li,3 Xi Chen,1 Conghui Xu,1 Qinlong Jing,1 Guocong Liu,3 Pengzhe Qin,1 Yufei Liu,1 Yi Zhong,1 Lijuan Huang,3 Bao-Ping Zhu,5,a and Zhicong Yang1

1Guangzhou Center for Disease Control and Prevention, Guangzhou, China, 2School of Public Health, Southern Medical University, Guangzhou, China, 3Guangzhou Yuexiu District Center for Disease Control and Prevention, Guangzhou, China, 4Guangdong Center for Disease Control and Prevention, Guangzhou, China, and 5Independent Scholar, Atlanta, Georgia, USA

Background. Sewage transmission of SARS-CoV-2 has never been demonstrated. During a COVID-19 outbreak in Guangzhou, China in April 2020, we investigated the mode of transmission.

Methods. We collected clinical and environmental samples from quarantined residents and their environment for RT-PCR testing and genome sequencing. A case was a resident with a positive RT-PCR test regardless of symptoms. We conducted a retrospective cohort study of all residents of cases’ buildings to identify risk factors.

Results. We found 8 cases (onset: 5–21 April). During incubation period, cases 1 and 2 frequented market T where a COVID-19 outbreak was ongoing; cases 3–8 never visited market T, lived in separate buildings and never interacted with cases 1 and 2. Working as a janitor or wastepicker (RR = 13; 95% CIexact, 2.3–180), not changing to clean shoes (RR = 7.4; 95% CIexact, 1.8–34) and handling dirty shoes by hand (RR = 6.3; 95% CIexact, 1.4–30) after returning home were significant risk factors. RT-PCR detected SARS-CoV-2 in 19% of 63 samples from sewage puddles or pipes, and 24% of 50 environmental samples from cases’ apartments. Viruses from the squat toilet and shoe-bottom dirt inside the apartment of cases 1 and 2 were homologous with those from cases 3–8 and the sewage. Sewage from the apartment of cases 1 and 2 leaked out of a cracked pipe onto streets. Rainfall after the onset of cases 1 and 2 flooded the streets.

Conclusions. SARS-CoV-2 might spread by sewage, highlighting the importance of sewage management during outbreaks.

Keywords. COVID-19; SARS-CoV-2; disease outbreak; infectious disease transmission; sewage management.

Since coronavirus disease 2019 (COVID-19) was first detected in Wuhan, Hubei Province, China [1], it has affected almost every country, causing more than 37 million cases and 1 million deaths globally as of 11 October 2020 [2]. In Guangzhou City (population, approximately 15 million), in southern China, the first wave of the COVID-19 outbreak, with 365 cases, occurred from 19 January 2020 through 5 March 2020 and was related to travelers from Hubei Province. The second wave, with 384 cases, occurred from 10 March through 25 May and was mainly related to travelers from overseas.

On 5 April, a woman who resided in a crowded, urban, low-income, migrant community in Guangzhou developed a cough and headache. Her husband developed similar symptoms on 10 April. After their symptoms failed to resolve, they were admitted to a hospital designated for COVID-19 treatment on 13 April. Their throat swabs, taken on 13 April, tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). Subsequently, they were isolated for treatment. Their rectal swabs obtained on 14 April also tested positive.

During the next 2 weeks, several additional cases occurred. We investigated this outbreak to identify the sources of infection and mode of transmission and to recommend prevention and control measures.

METHODS

Epidemiologic Investigation

After the 2 initial cases occurred, the Guangzhou Center for Disease Control and Prevention (GZCDC), following the national COVID-19 quarantine guidelines, placed all 212 residents of the same or immediately adjacent buildings of the first 2 cases (Figure 1A) in a hotel for centralized quarantine. The hotel was distant from residential areas and had an independent sewage treatment system and a separate medical waste disposal process. Each person under quarantine stayed in a single-occupancy room and could not leave. Medical staff took their temperature twice daily, checked for...
symptoms, and collected throat swabs every 2–3 days and a rectal swab at the start of the quarantine. If symptoms suggestive of COVID-19 were to appear, the individuals would be immediately transported to a designated hospital. The quarantine would be lifted after 14 days if all samples had tested negative.

An additional 112 residents of buildings within 20 meters of the 2 initial cases (Figure 1B) were placed under involuntary home quarantine. The other 2552 residents in the residential area were given voluntarily stay-at-home orders. For each person under involuntary or voluntary quarantine, a throat swab sample was collected for COVID-19 screening. Community health workers checked for suspected COVID-19 symptoms (eg, fever, cough, decreased sense of smell, sore throat, runny nose, conjunctival inflammation, and diarrhea) by telephone. If any of these symptoms were to appear, medical staff would visit the person’s home for confirmation. If confirmed, he/she would be immediately transported to a designated hospital for isolation, where 2 throat swabs would be taken 3 days apart to detect SARS-CoV-2 RNA.

We defined a confirmed case as having a throat or rectal sample tested positive for SARS-CoV-2 RNA by RT-PCR, regardless of symptoms.

Environmental Sampling
We used sterile premoistened swabs with universal transport medium containing Hanks’ balanced salt solution, amino acids, and glycerin to collect environmental samples. In the apartments where the cases lived, we collected samples from frequently touched surfaces, including doorknobs, armrests, buttons, remote controls, water glasses, cutlery, and desktops. In the bathrooms, we collected samples from the squat toilets, floor drains, and sinks. We also collected dirt samples from shoe bottoms and bicycle tires. On the streets, we used sterile straws to collect the sewage from puddles on the ground and stored it in universal transport medium.

RNA Extraction and RT-PCR Testing
Clinical and environmental samples were placed in containers and transported to GZCDC’s Virology Laboratory within 2 hours of sample collection, following the standard biosafety protocol [3]. Viral RNA was extracted from a 200-µL respiratory sample with the Viral Nucleic Acid Isolation Kit (Magnetic Beads) in the SSNP-2000A automatic nucleic acid extraction system (bioPerfectus Technologies, Taizhou, China). RT-PCR was conducted using the Novel Coronavirus 2019 Nucleic Acid Test Kit (bioPerfectus Technologies, Taizhou, China) in the Applied Biosystems ViiA7 instruments (Applied Biosystems, Hong Kong, China) following the manufacturers’ instructions. The TaqMan probe–based kit was designed to detect the ORF1ab and N genes of SARS-CoV-2 in 1 reaction. Thermal cycling was performed for 10 minutes at 50°C for reverse transcription, followed with 1 minute at 97°C, and then 45 cycles for 5 seconds at 97°C and for 30 seconds at 58°C.

SARS-CoV-2 Genome Sequencing
Genome sequencing was conducted on 10 clinical samples and 4 environmental samples using the general multiplex PCR method [4, 5]. Briefly, the multiplex PCR method was performed with 2 pooled primer mixtures; the cDNA reverse-transcribed with random primers was used as a template. After 35 rounds of amplification, the PCR products were collected and quantified, followed with end-repairing and barcoding ligase. Around 50 fmol of final library DNA was loaded onto the MinION device (Oxford Nanopore Technologies, Oxford, United Kingdom). The nanopore sequencing platform takes less than 24 hours to obtain 10Gb of sequencing data, achieving between 0.3 and 0.6 million reads per sample. The ARTIC bioinformatics pipeline for COVID-19 was used to generate consensus sequences and call single nucleotide changes relative to the reference sequence [6].

Phylogenetic Analysis
We downloaded 53 genomic sequences of the SARS-CoV-2 virus from the Global Initiative on Sharing All Influenza Data (GISAID) databases. Multiple sequence alignment of all 67 coronavirus genomes was performed with the Multiple Alignment using Fast Fourier Transform. All 67 coronavirus genomes were used for phylogenetic tree analysis using the MEGA X software based on the maximum likelihood method (bootstrap = 1000).

Retrospective Cohort Study
Using a structured questionnaire, we interviewed all residents of buildings B and C where the 6 secondary cases occurred. We collected data on sociodemographics (eg, age, sex, occupation, education, income) and potential exposure risk factors (eg, handwashing in various situations and frequency; frequency of leaving the house and by what mode [walking or bicycling]; facemask use; air conditioner use; frequency of opening windows for ventilation; frequency of handling dirty shoes by hand; and frequency of cleaning the floor). For young children, we interviewed their parents or grandparents. We used StatXact 9 (Cytel, Inc, Cambridge, MA) to compute Fisher exact 95% confidence intervals (CIexact) for the relatives risks.

The GZCDC Ethics Committees approved this study.

RESULTS
Cases 1 and 2 made their living by transporting goods between wholesale markets throughout Guangzhou. They visited market T (located in a distinctly different community approximately 2 km away from their home) together on 27 March and 3 April and stayed there for about 5 hours during each trip. During their trips, they took off their facemasks multiple times while
Figure 1. Spacial distribution of coronavirus disease 2019 cases and results of environmental testing during an outbreak in Guangzhou, China, April 2020. A, The 8 cases were found in 3 buildings, A–C. In all case patients’ homes, samples collected from shoe bottoms and squat toilets tested positive for SARS-CoV-2. A sample collected from a bicycle tire in building C also tested positive. Eleven sewage samples collected near the 3 buildings tested positive. B, Residents in the outbreak community were quarantined (solid red: centralized quarantine; red polygon: involuntary home quarantine; yellow polygon: voluntary stay-at-home order). A total of 108 environmental samples were collected from inside or around the cases’ homes (including 10 squat toilet swabs, 29 shoe bottom dirt samples, 3 dirt samples from bicycle tires, 30 other samples, 36 sewage samples collected in buildings besides A–C). None of the other samples tested positive, except 1 sewage sample collected near building D, shown in Figure 1A. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
talking to other workers and their foreign employers, drinking water, and having other interactions with numerous people. Meanwhile, a COVID-19 outbreak was occurring (between 20 March and 14 April) among persons working at or related to market T. During 4–21 April, GZCDC conducted a RT-PCR screening for all 5622 people who had visited market T in the previous month and identified 35 symptomatic or asymptomatic infections. On 4 April, market T was closed, and the couple never returned to the market afterward. In addition to market T, the couple did not visit other places with active outbreaks during the 2 weeks before their symptom onset.

The intensive case finding identified 8 cases in this community of 2888 residents (attack rate = 2.8/1000). These included the 2 initial cases, 4 subsequent symptomatic cases with onset during 17–21 April who tested positive for SARS-CoV-2 by throat swabs, and 2 asymptomatic persons whose throat swabs, collected on 16 and 18 April, respectively, tested positive for SARS-CoV-2.

The 8 cases were 4 couples who resided in 4 apartments in 3 adjacent buildings. The median age was 58 years (range, 48–73). They worked as transporters of goods, cleaners (ie, persons doing janitorial or other general cleaning jobs), or wastepickers (ie, persons salvaging reusable materials from dumped trash for personal use or sale). The 6 symptomatic cases presented with cough (83%), fever (67%), expectoration (67%), headache (50%), tachypnea (33%), chest tightness (33%), abdominal pain (33%), vomiting (33%), and sore throat (17%). The onset dates of cases 1 and 2 were 5 days apart, whereas the onset (or specimen collection) dates of cases 3–8 clustered in 6 days between 16 April and 21 April. Heavy rainfall occurred on 5 April (33.5 mm) and 6 April (38.3 mm), the first 2 days of case 1's symptom onset, whereas minor rainfall occurred on 11 April (1.8 mm), 1 day after case 2's symptom onset (Figure 2).

Cases 1 and 2 lived on the second floor of 2-story building A. Cases 3, 4, 7, and 8 resided in adjacent 6-story building B. Cases 3 and 4 resided on the second floor while cases 7 and 8 resided on the first floor, and they used separate exits. Cases 5 and 6 lived on the second floor of 6-story building C, adjacent to building B. Building A only had 1 exit facing south, away from buildings B and C. The alleys on the west and east sides of building A were separate from those of buildings B and C (Figure 1A). Review of the closed-circuit television (CCTV) footage confirmed that residents of building A did not interact with residents of buildings B and C.

In-depth interviews revealed that cases 3–8 never visited market T or other places with outbreaks and had no contact with any COVID-19 patients. They did not know and had not interacted with cases 1 and 2 and had separate daily walking routes. Cases 7 and 8, a couple, had a brief conversation with case 3 on 13 April in a well-ventilated, open space. At that time, case 3 was asymptomatic and wore a facemask, and her throat swab collected on 14 April tested negative. On 16 April, she had another throat swab collected, which tested positive. Otherwise cases 3–8 had no interactions with one another.

Inspection showed that the sewage pipe for the toilet of cases 1 and 2, which ran along the wall outside building A, had a hole about 100 cm² in size, located a few centimeters above ground. In an experiment, we poured water into the toilet of cases 1 and 2. The water gushed out of the hole into the alley, flowed into the 5 drains around buildings A–D (Figure 1A), and soaked the entrances of buildings B and C.

Based on the cases' onset dates, spatial distribution, and contact history, we hypothesize that cases 3–8 were infected by cases 1 and 2 through the leaked sewage.

In the retrospective cohort study, the risk of SARS-CoV-2 infection among the 33 residents of buildings B and C was significantly associated with working as cleaners or wastepickers (risk ratio [RR] = 13; 95% CIexact, 2.3–180), not changing to clean shoes upon returning home (RR = 7.4; 95% CI exact, 1.8–34), and handling dirty shoes by hand after returning home (RR = 6.3; 95% CI exact, 1.4–30). Leaving the house more than once a day had a borderline significant association (RR = 4.0; 95% CI exact, .95–19). Age ≥50 years and monthly household income of less than $360 also had a significant association with COVID-19 infection. No significant associations were found for all other risk factors examined (Table 1).

On 22 April, GZCDC conducted an extensive environmental sampling in the community. Before the samples were taken, cases 1 and 2 had left their apartment for isolation for 9 days; cases 3 and 4, for 8 days; and cases 5–8 for 5 days. Of the 199 environmental samples collected, 25 (13%) tested positive. The sewage samples; the sewage pipe swabs near buildings A, B, and C; and swabs inside the apartments where cases 1–8 resided had high positivity rates. Of note, 4 of the 6 dirt samples collected from shoe bottoms in the apartments of cases 1–8 and from a bicycle tire in an apartment with no cases in building C tested positive. Conversely, none of the 72 samples collected from the apartments in other buildings except for buildings A–C tested positive (Table 2).

The genome sequencing showed that the viruses from the squat toilet and the shoe bottom dirt in the apartment of cases 1 and 2, from cases 3–8, and from the sewage had a 99.996% similarity. The viruses from 4 patients during the market T outbreak, from cases 3 and 4, and from the other samples differed by only 1 nucleotide (Figure 3).

We attempted to culture the viruses from the clinical specimens of cases 3–8 and all positive environmental samples. No samples yielded viable viruses. Clinical specimens of cases 1 and 2 had been disposed of and were unavailable for culture.

On 20 April, the GZCDC and the city government fixed the broken pipe, disinfected the sewage system, and thoroughly cleaned and disinfected the apartments of all cases and the alleys around buildings A, B, and C. No new cases have occurred since that time.
DISCUSSION

Considering that COVID-19 has an incubation period of 1–14 days [7], the primary case in this outbreak (onset April 5) likely was infected on either 27 March or 3 April when she visited market T. Her husband, who last visited market T on 3 April and had onset on 10 April, might have been infected in market T or from his wife. Cases 3–8 were unlikely to have been infected from market T because they did not visit market T during their incubation periods. They were also unlikely to have been directly infected by cases 1 and 2 based on their exposure history and onset dates.

On the other hand, our retrospective cohort study showed that working as cleaners or wastepickers, not changing to clean shoes, and handling dirty shoes by hand after returning home were significant risk factors. The environmental investigation identified SARS-CoV-2 in sewage around buildings A, B, and C; in the dirt on shoe bottoms and a bicycle tire; and inside the apartments where cases resided. The genome sequencing showed that the viruses from cases 3–8 were homologous with the virus identified inside the apartment of cases 1 and 2; and the spatial distribution of cases 3–8 was consistent with the pattern of sewage contamination. Also, after the presumptive source of infection (ie, the contaminated sewage that flooded the street) was eliminated, no new cases have occurred. Together, these findings suggest a possible scenario that the viruses shed by cases 1 and 2 entered the sewage system; the sewage leaked out of the broken pipe; the rainfall on 5, 6, and 11 April flooded the streets around buildings A, B, and C; and cases 3–8 carried the contaminated

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Table 1. Risk Factors Significantly Associated With Severe Acute Respiratory Syndrome Coronavirus 2 Infection During a Coronavirus Disease 2019 Outbreak: Guangzhou, China, April 2020

| Risk Factor With Significant Associations | n | Number of Cases | Attack Rate (%) | Risk Ratio (95% Fisher Exact Confidence Interval) |
|------------------------------------------|---|----------------|----------------|-----------------------------------------------|
| Age (years)                              |   |                |                |                                               |
| ≥50                                      | 11| 5              | 46             | 10 (1.6–130)                                  |
| <50                                      | 22| 1              | 4.6            | Referent                                      |
| Monthly household income                 |   |                |                |                                               |
| <$360 approximately                      | 18| 6              | 33             | ≈ (1.5–∞)                                    |
| ≥$360 approximately                      | 15| 0              | 0              | Referent                                      |
| Occupation                               |   |                |                |                                               |
| Cleaner/wastepicker                      | 9 | 5              | 56             | 13 (2.3–180)                                 |
| Other                                    | 24| 1              | 4.2            | Referent                                      |
| Changing to clean shoes upon returning home | |                |                |                                               |
| No                                       | 7 | 4              | 57             | 7.4 (1.8–34)                                 |
| Yes                                      | 26| 2              | 7.7            | Referent                                      |
| Handling shoes by hand after returning home | |                |                |                                               |
| Yes                                      | 8 | 4              | 50             | 6.3 (1.4–30)                                 |
| No                                       | 25| 2              | 8.0            | Referent                                      |
| Frequency of leaving the house           |   |                |                |                                               |
| ≥2 times/day                             | 11| 4              | 36             | 4.0 (1.95–19)                                |
| 0–1 time/day                             | 22| 2              | 9.1            | Referent                                      |

Results of a retrospective cohort study conducted among 33 residents of buildings B and C in a low-income community.

*Other potential risk factors explored included sex; education; handwashing in various situations and frequency; usual mode of leaving the house (walking or bicycling); facemask use; air conditioner use; frequency of opening windows for ventilation; frequency of cleaning floors; and frequency of handling dirty shoes by hand. These factors were not significantly associated with coronavirus disease 2019.
sewage home on their shoe bottoms and bicycle tires, leading to this outbreak.

Multiple studies have shown that SARS-CoV-2 RNA can be detected in patients’ fecal matter and rectal swabs [8–11]. Two other related coronaviruses, that is, the severe acute respiratory syndrome virus and the Middle East respiratory syndrome coronavirus, have also been detected in fecal and sewage samples [12]. Additionally, SARS-CoV-2 has been isolated in human feces after at least 4 days [13–16]. Further, the receptor for SARS-CoV-2, the angiotensin-converting enzyme 2, is highly expressed on differentiated enterocytes. Recent studies have shown active replication of SARS-CoV-2 in human intestinal organoids [16, 17], suggesting that the intestinal tract could facilitate the transmission of SARS-CoV-2. Also, a recent study conducted in a hospital designated for COVID-19 treatment showed that 50% of the samples from the shoe bottoms of the intensive care unit medical staff tested positive for SARS-CoV-2 and 3 positive samples were detected from the floor of the dressing room of medical staff, indicating that shoe bottoms can potentially serve as a vehicle for SARS-CoV-2 transmission [18]. To our knowledge, however, no direct evidence currently exists on sewage transmission of SARS-CoV-2 [19]. Our investigation, for the first time, shows epidemiologically that sewage contaminated by COVID-19 patients’ fecal matter could cause outbreaks.

Research has shown that virus shedding may be the heaviest at the beginning of symptom onset [20]. During this outbreak, rainfall occurred on the first 2 days of case 1’s symptom onset and the day after case 2’s symptom onset. Thus, the first 2 cases might have heavily shed virus into the sewage system at the start of their symptoms. Subsequently, the heavily contaminated sewage flooded the streets by the rain, which was carried into the homes of the residents of surrounding buildings.

Older age and low household income were significant risk factors. These associations might be due to the fact that older and low-income persons were more likely to be cleaners or wastepickers, both occupations that have a higher risk of sewage exposure. Due to the small sample size, this hypothesis could not be fully elucidated.

The outbreak occurred in a crowded, urban, migrant community in China. These communities often have a high population density, poor sanitation, inadequate infrastructure, and crowded living spaces. These conditions create an ideal environment for pathogen transmission [18], leading to frequent outbreaks.

Our study had several limitations. First, we did not have direct evidence of sewage transmission, as viral culture of clinical and environmental samples yielded no viable viruses. Cases 1 and 2 had been isolated for treatment on 13 April; cases 3 and 4 on 14 April; and cases 5–8 on 18 April, whereas environmental samples were collected on 22 April, that is, 4–9 days after the cases had left their apartments. By that time, the viruses might have degraded in the environment, which might explain the unsuccessful viral culture. Second, the viruses from the clinical samples of cases 1 and 2 had been disposed of and could not be sequenced. As a result, we were unable to directly show their homogeneity with those in subsequent cases, although we did demonstrate homogeneity of the virus found in the apartment of cases 1 and 2 with the other viruses. Third, the small number of cases led to imprecise relative risk estimates and rendered it impossible to control for confounding in this retrospective, cohort study. Fourth, cases 7 and 8 had a brief interaction with case 3 on 13 April. While the possibility that cases 7 and 8 were infected by case 3 could not be completely ruled out, this scenario is unlikely because the conversation occurred in an open space and case 3 wore a facemask and likely had very low viral load at the time.

**CONCLUSIONS**

Our investigation, for the first time, provides epidemiologic and laboratory evidence that contaminated sewage might cause
COVID-19 outbreaks. This findings highlights the importance of sewage management for preventing and controlling COVID-19, especially in densely populated, low-income urban communities with poor sanitation and hygiene conditions. We recommend regular inspection and maintenance of sewage systems to ensure their integrity and functionality.

Notes

Author contributions. Authorship was determined by the authors' contributions as follows: J. Y. provided oversight for the design and implementation of the field epidemiologic investigation and approved the final version of the manuscript; Z. C. prepared the first draft of the manuscript; B. L. and K. L. led the laboratory investigation; H. L. designed the retrospective cohort study; C. G., H. L., X. C., C. X., Q. J., G. L., P. Q., Y. L., Y. Z., and L. H. conducted the field epidemiologic investigation and sample collection; B. P. Z. provided guidance on the statistical analysis and critically revised the manuscript; and Z. Y. was the guarantor of the integrity of the entire study.

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