

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

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Summary: During a COVID-19 outbreak in a densely populated community in Guangzhou, China, SARS-CoV-2 might have been transmitted by sewage. This finding highlighted the importance of sewage management for controlling COVID-19 outbreaks.

Abstract

Background. SARS-CoV-2 has been identified in the fecal matter of COVID-19 patients. However, sewage transmission has never been shown. In April 2020, a COVID-19 outbreak occurred in a densely populated community in Guangzhou, China. We investigated this outbreak to identify the mode of transmission.

Method. A home quarantined order was issued in the community. We collected throat swab samples from the residents and environmental samples from the surfaces inside and around the houses, and conducted RT-PCR testing and genome sequencing. We defined a case as a resident in this community with a positive RT-PCR test, with or without symptoms. We conducted a retrospective cohort study of all residents living in the same buildings as the cases to identify exposure risk factors.

Result. We found eight cases (four couples) in this community of 2888 residents (attack rate=2.8/1000), with onset during April 5–21, 2020. During their incubation periods, Cases 1-2 frequented market T with an ongoing outbreak. Cases 3-8 never visited market T during incubation period, lived in separate buildings from, and never interacted with, Cases 1-2. Retrospective cohort study showed that working as cleaners or waste picker (RR=13, 95% CI_{exact}: 2.3-180), not changing to clean shoes after returning home (RR=7.4, 95% CI_{exact}: 1.8-34), collating and cleaning dirty shoes after returning home (RR=6.3, 95% CI_{exact}: 1.4-30) were significant exposure risk factors. Of 63 samples collected from street-sewage puddles and sewage-pipe surfaces, 19% tested positive for SARS-CoV-2. Of 50 environmental samples taken from cases' apartments, 24% tested positive. Viral genome sequencing showed that the viruses identified from the squat toilet and shoe-bottom dirt inside the apartment of Cases 1-2 were homologous with those from Cases 3-8 and those identified from sewage samples. The sewage pipe leading from the apartment of Cases 1-2 to the drainage had a large hole above ground. Rainfalls after the onset of Cases 1-2 flooded the streets.

Conclusion. Our investigation has for the first time pointed to the possibility that SARS-CoV-2 might spread by sewage. This finding highlighted the importance of sewage management, especially in densely-populated places with poor hygiene and sanitation measures, such as urban slums and other low-income communities in developing countries.

Keywords: COVID-19; SARS-CoV-2; disease outbreak; infectious disease transmission; epidemiology; risk factor; sewage management

Introduction

Since the Coronavirus Disease 2019 (COVID-19) was first detected in Wuhan, Hubei Province, China¹, it has affected almost all countries, causing more than 31 million infections and 961,700 deaths globally as of September 20, 2020².

In Guangzhou City (population: approximately 15 million), southern China, the first wave of COVID-19 outbreak, with 365 cases, occurred during January 19–March 5, 2020 and was related to travelers from Hubei Province. The second wave, with 384 cases, occurred during March 10–May 25 and was mainly related to travelers from overseas.

On April 5, 2020, 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 April 10. After their symptoms failed to resolve, they were admitted to a hospital designated for COVID-19 treatment on April 13. Their throat swabs taken on April 13 tested positive for the Severe Acute Respiratory Syndrome virus-2 (SARS-CoV-2) by real-time reverse transcriptase–polymerase chain reaction (RT-PCR). Subsequently, they were isolated for treatment. Their rectal swabs obtained on April 14 also tested positive.

During the next two 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.

Method

Epidemiologic Investigation

After the two 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 two cases (Figure 1a) for centralized quarantine in a hotel, which 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 may 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 appeared, they 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 20m of the two initial cases (Figure 1b) were placed under involuntary home quarantine. The other 2552 residents in the residential area were given voluntarily stay-at-home order. 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 (e.g., fever, cough, decreased sense of smell, sore throat, runny nose, conjunctival inflammation and diarrhea) by telephone. If any

of these symptoms appeared, 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 two throat swabs would be taken three days apart to detect the 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 pre-moistened swabs with universal transport medium containing Hanks' balanced salt solution, amino acids, and glycerin to collect environmental samples. In the apartments of the buildings where the cases lived, we collected samples from frequently-touched surfaces, including doorknobs, armrests, buttons, remote controls, water glasses, cutlery, and desktops. In the toilets, we collected samples from the close stools, 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 two hours of sample collection, following the standard biosafety protocol³. Viral RNA was extracted from a 200 μ L 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 gene of SARS-CoV-2 in one reaction. Thermal cycling was performed at 50°C for 10m for reverse transcription, followed by 97°C for 1m and then 45 cycles of 97°C for 5s, 58°C for 30s.

SARS CoV-2 Genome Sequencing

Genome sequencing was conducted on ten clinical samples and four environmental samples, using the general multiplex PCR method⁴⁻⁵. Briefly, the multiplex PCR was performed with two pooled primer mixture, and the cDNA reverse transcribed with random primers were used as a template. After 35 rounds of amplification, the PCR products were collected and quantified, followed with end-repairing and barcoding ligation. Around 50fmol of final library DNA was loaded onto the MinION device (Oxford Nanopore Technologies, Oxford, UK). The nanopore sequencing platform takes less than 24h to obtain 10Gb of sequencing data, achieving between 0.3–0.6 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 sequence⁶.

Phylogenetic Analysis

We downloaded 53 genomic sequences of the SARS-CoV-2 virus from the GISAID databases. Multiple sequence alignment of all 67 coronavirus genomes was performed using 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 six secondary cases occurred. We collected the data on sociodemographics (e.g., age, sex, occupation, education, income) and potential exposure risk factors, e.g., handwashing in various situations and frequency; frequency of leaving house and by what mode (walking or bicycling); facemask use; air conditioner use; and frequencies of opening windows for ventilation, changing, collating, or washing shoes, and cleaning the floor. For young children, we interviewed their parents or grandparents. We used StatXact® 9 (Cytel, Inc., Cambridge, MA) to compute Fisher's exact 95% confidence intervals (95% CI_{exact}) for the relative risks.

This study was approved by the ethics committees of GZCDC (approval number GZCDC-ECHR-2020A0004).

Results

Cases 1-2 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 March 27 and April 3, and stayed there for about five hours during each trip. During their trips, they took off their facemasks multiple times while 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 March 20 and April 14 among persons working at, or related to, market T. During April 4–21, 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 April 4, market T was closed, and the couple never return to the market afterwards. Besides market T, the couple did not visit other places with active outbreaks during the two weeks before their symptom onset.

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

The eight cases were four couples residing in four apartments inside three adjacent buildings. The median age was 58 (range: 48–73) years. They worked as transporters of goods, cleaners or waste pickers. The six 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 1-2 were five days apart, whereas the onset (or specimen collection) dates of Cases 3-8 clustered in six days between April 16 and 21. Heavy rainfall occurred on April 5 (33.5mm) and 6 (38.3mm), the first two days of Case 1's symptom onset, whereas a minor rainfall occurred on April 11 (1.8mm), a day after Case 2's symptom onset (Figure 2).

Cases 1-2 lived on the second floor of a two-story building, A. Cases 3-4 and 7-8 resided in the adjacent, six-story building, B, with Cases 3-4 on the second floor and Cases 7-8 on the first floor. Cases 5-6 lived on the second floor of a six-story building, C, adjacent to building B. Building A only had one 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 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 1-2, and had separate daily walking routes. Cases 7-8, a couple, had a brief conversation with Case 3 on April 13 in a well-ventilated open space. At that time Case 3 was asymptomatic and wore a facemask, and her throat swab collected on April 14 tested negative. On April 16, 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 1-2, which ran along the wall outside building A, had a hole about 100cm² in size, located a few centimeters above ground. In an experiment, we poured water into the toilet of Cases 1-2. The water gushed out of the hole onto the alleys, flowed into the five 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 hypothesized that Cases 3-8 were infected by Cases 1-2 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 waste pickers (RR=13, 95% CI_{exact}: 2.3-180), not changing to clean shoes upon returning home (RR=7.4, 95% CI_{exact}: 1.8-34), and collating and cleaning dirty shoes 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}: 0.95-19). Age \geq 50 years and monthly household income <2500 yuan (about USD\$360) also had a significant association with COVID-19 infection. No significant associations were found for all other risk factors examined (Table 1).

On April 22, GZCDC conducted an extensive environmental sampling in the community. Before the samples were taken, Cases 1-2 had left their apartment for isolation for nine days; Cases 3-4, eight days; and Cases 5-8, five 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, four of the six dirt samples collected from shoe-bottoms in the apartments of Case 1-8 and from a bicycle tire in an apartment with no cases in the building C, tested positive. Conversely, none of the 72 samples collected from the apartments in other buildings besides 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 1-2, from Cases 3-8, and from the sewage had a 99.996% identity. The viruses from four patients during the market T outbreak, from Cases 3-4, and from the other either samples differed by one nucleotide only (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 1-2 had been disposed of and were unavailable for culture.

On April 20, 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 afterwards.

Discussion

Considering COVID-19 has an incubation period of 1-14 days⁷, the primary case in this outbreak (onset: April 5) likely was infected on either March 27 or April 3 when she visited market T. Her husband, who last visited market T on April 3 and had onset on April 10, 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 1-2 based on their exposure history and onset dates.

On the other hand, the retrospective cohort study showed that working as cleaners or waste pickers, not changing to clean shoes, and collating or cleaning dirty shoes 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 virus from Cases 2-8 were homologous with the virus identified inside the apartment of Cases 1-2; and the spatial distribution of Cases 3-8 is consistent with the direction of the sewage flow. Also, after the presumptive source of infection (i.e., the contaminated sewage that flooded the street) was eliminated, no new cases have occurred. Together, these findings suggested a possible scenario that, the viruses shed by Cases 1-2 entered the sewage system; the sewage leaked out of the broken pipe; the rainfalls on April 5, 6, and 11 flooded the streets around

buildings A, B, and C; and Cases 3-8 carried the contaminated sewage home on their shoe bottoms and bicycle tires, leading to this outbreak.

Multiple studies have shown that the SARS-CoV-2 RNA can be detected in patients' fecal matter and rectal swabs⁸⁻¹¹. Two other related coronaviruses, i.e., the severe acute respiratory syndrome virus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), have also been detected in fecal and sewage samples¹². Additionally, the SARS-CoV-2 has been isolated in human feces after at least 4 days¹³⁻¹⁶. Further, the receptor for SARS-CoV-2, the angiotensin-converting enzyme 2 (ACE2), is highly expressed on differentiated enterocytes. Recent studies have shown active replication of SARS-CoV-2 in human intestinal organoids¹⁶⁻¹⁷, 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 ICU medical staff tested positive for SARS-CoV-2, and three 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 transmission¹⁸. To our knowledge, however, no direct evidence currently exists on sewage transmission of SARS-CoV-2¹⁹. Our investigation for the first time has shown 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 onset²⁰. During this outbreak, a rainfalls occurred on the first two days of Case 1's symptom onset and the day after Case 2's symptom onset. Thus, the first two 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 of waste pickers, who had 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 community in China. Due to poor planning and management, these communities often have a high population density, poor sanitation, inadequate infrastructure, and crowded living spaces. These conditions create an idea environment for pathogen transmission¹⁸, 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 1-2 had been isolated for treatment on April 13; Cases 3-4, April 14; and Cases 5-8, April 18, whereas environmental samples were collected on April 22, i.e., 4-9 days after the cases had left their apartments. By then the viruses might have degraded in the environment, which might explain the unsuccessful viral culture. Second, the viruses from the clinical samples of Cases 1-2 had been disposed of and could not be sequenced; hence we were

unable to directly show their homogeneity with those in subsequent cases, although we did demonstrated homogeneity of the virus found in the apartment of Cases 1-2 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 the retrospective cohort study. Fourth, Cases 7-8 had a brief interaction with Case 3 on April 13. While the possibility that Cases 7-8 was 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.

Conclusion

Our investigation for the first time provided epidemiologic and laboratory evidence that contaminated sewage might cause COVID-19 outbreaks. These 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.

Author Contribution

Authorship was determined by authors' contributions, as follows. Jun Yuan provided oversight for the design and implementation of the field epidemiologic investigation, and approved the final version of the manuscript. Zongqiu Chen prepared the first draft of the manuscript. Baisheng Li and Kuibiao Li led the laboratory investigation. Hui Liu designed the retrospective cohort study. Chenghua Gong, Hui Liu, Xi Chen, Conghui Xu, Qinglong Jing, Guocong Liu, Pengzhe Qin, Yufei Liu, Yi Zhong, Lijuan Huang conducted the field epidemiologic investigation and sample collection. Bao-Ping Zhu provided guidance on the statistical analysis and critically revised the manuscript. Zhicong Yang was the guarantor of the integrity of the entire study. All authors declared no conflict of interest.

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Conflict of interest: All the authors have no conflict to declare.

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Figure legends:

Figure 1. Spatial distribution of COVID-19 cases and results of environmental testing during an outbreak: Guangzhou, China, April 2020

a. The eight cases were found in three 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. 11 sewage samples collected near the three 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). 108 environmental samples were collected inside or around 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 other samples tested positive, except one sewage sample collected near buildings D, shown in Figure 1a.

Figure 2. Symptom onset dates of cases (or, for Cases 3 and 5, specimen collection dates) during a COVID-19 outbreak: Guangzhou, China, April 2020

Figure 3. Phylogenetic tree of full genome sequences of SARS-CoV-2 strains from COVID-19 patients(▲)and environment(Δ) during an outbreak:Guangzhou City, Guangdong Province, China, April 2020

a. Case A-D represent the 4 viruses isolated from the market T outbreak.

Table 1. Risk factors significantly associated with SARS-CoV-2 infection during a COVID-19 outbreak: Guangzhou, China, April 2020*

Risk factors with significant associations†	n	Num. cases	Attack rate (%)	RR (95% CI _{exact})‡
Age (years)				
≥50	11	5	46	10 (1.6-130)
<50	22	1	4.6	
Household income (yuan/month)				
<2500§	18	6	33	∞ (1.5-∞)
≥2500	15	0		
Occupation				
Cleaner/Waste picker	9	5	56	13 (2.3-180)
Other	24	1	4.2	
Changing shoes upon returning home				
Not changing to clean shoes	7	4	57	7.4 (1.8-34)
Changing to clean shoes	26	2	7.7	
Collating/cleaning shoes after returning home				
Yes	8	4	50	6.3 (1.4-30)
No	25	2	8.0	
Frequency of leaving the house				
≥2 times/day	11	4	36	4.0 (0.95-19)
0-1 time/day	22	2	9.1	

* 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 changing, collating, or washing shoes. These factors were not significantly associated with COVID-19.

‡ RR = risk ratio; CI_{exact} = Fisher's exact confidence interval.

§ 2500 yuan ≈ US\$360

Table 2. SARS-CoV-2 test results of environmental samples during a COVID-19 outbreak: Guangzhou, China, April 2020

Location and objects where samples were taken	Num. samples collected	Num. SARS-CoV-2 (+) samples	% Positive
All samples	199	25	13
Sewage-related samples	63	12	19
Swabs of sewage pipes around building A-C	7	3	43
Sewage collected on the street near building A-C	20	8	40
Sewage collected in buildings besides A-C	36	1	2.8
Apartments in Buildings A-C where Cases 1-8 lived	50	12	24
Squat toilet	8	4	50
Shoe bottom dirt	6	4	67
Other	36	4	11
Apartments in Buildings A-C without cases	14	1	7.1
Squat toilet swabs	1	0	0
Shoe-bottom dirt	7	0	0
Bicycle-tire dirt	1	1	100
Other	5	0	0
Apartments in other buildings besides A-C	72	0	0
Squat-toilet swabs	10	0	0
Shoe-bottom dirt	29	0	0
Bicycle-tire dirt	3	0	0
Other	30	0	0.0

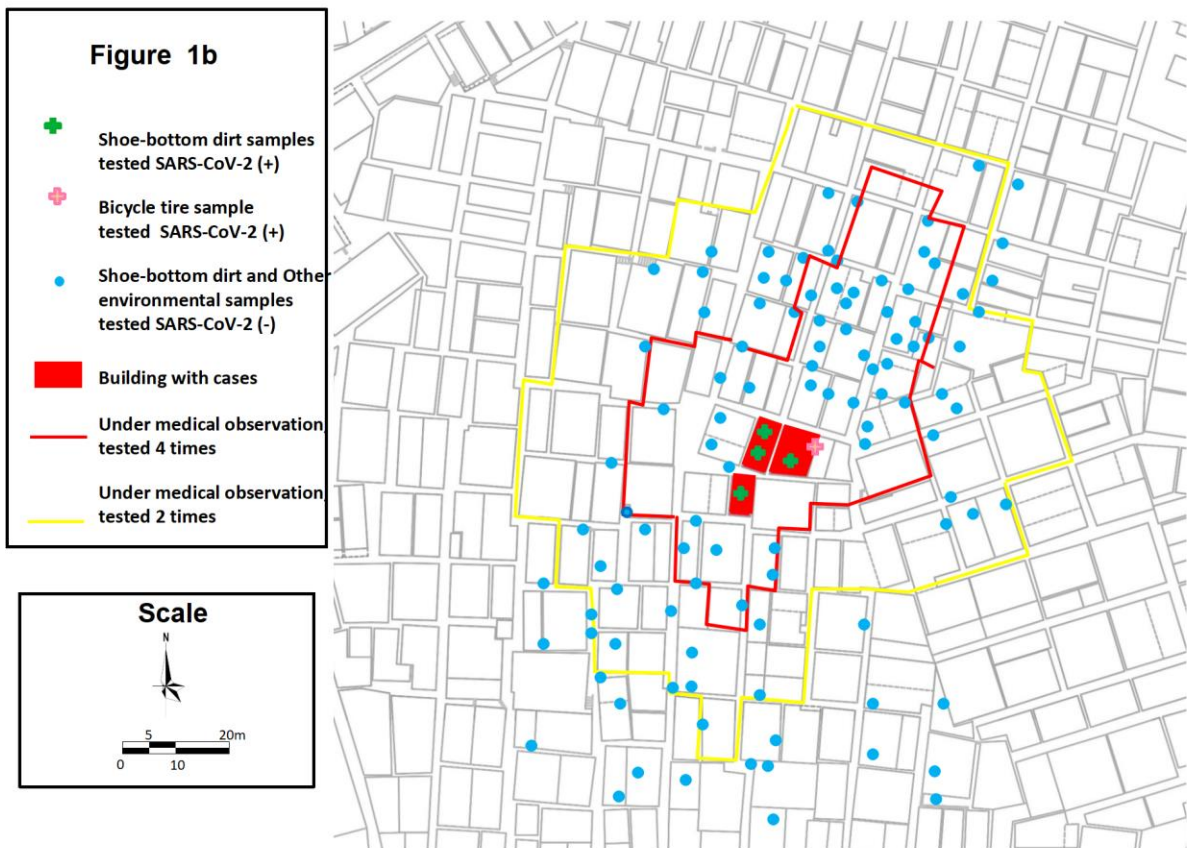
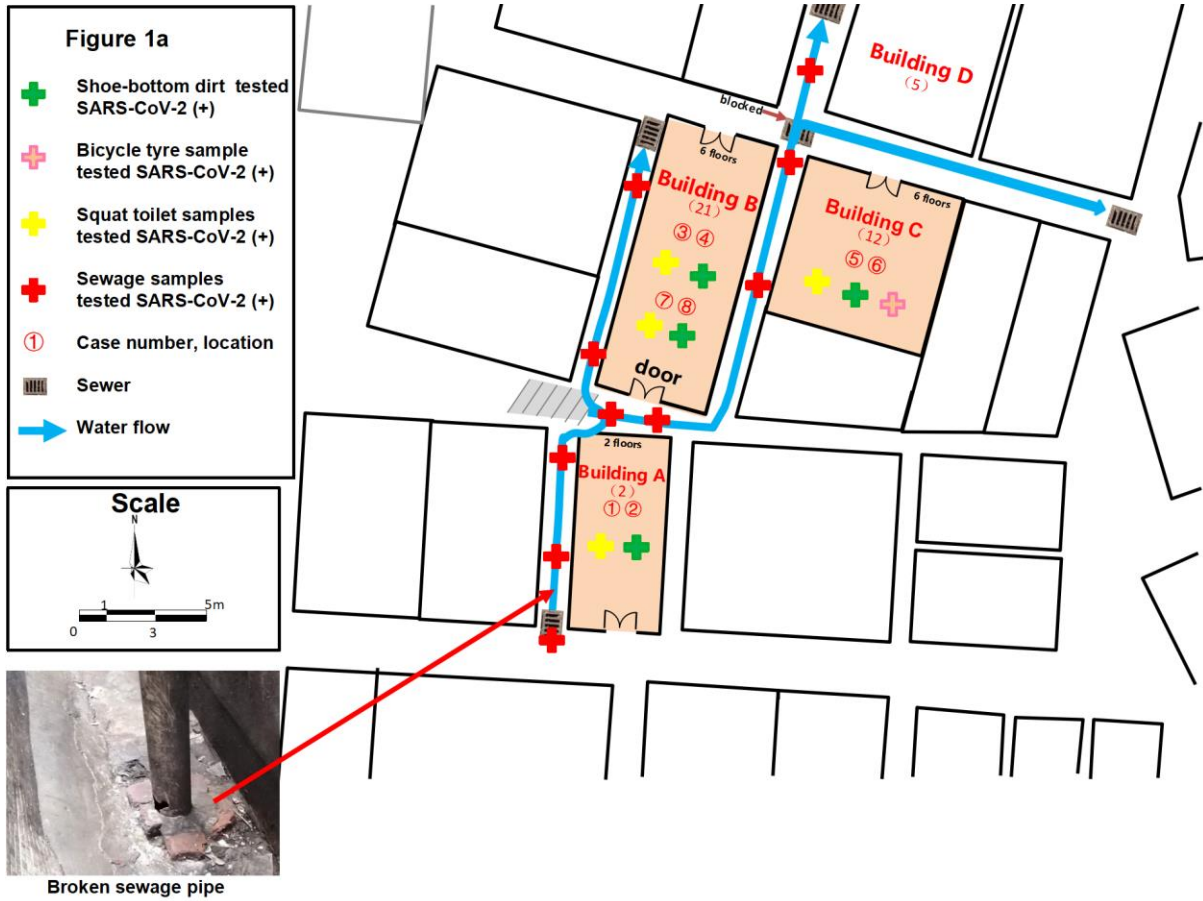


Figure 2

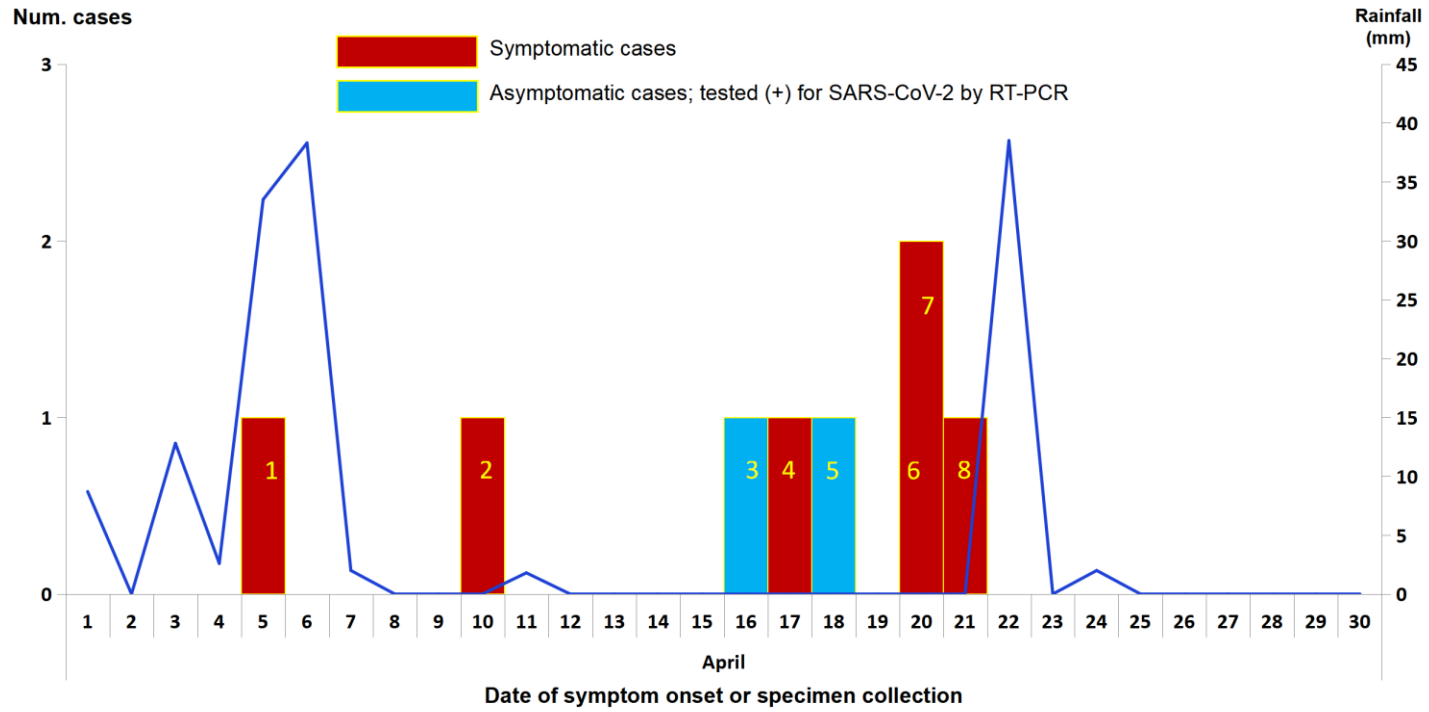


Figure 3

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