

Transmission potential of asymptomatic and paucisymptomatic SARS-CoV-2 infections: a three-family cluster study in China

Xiao-Lin Jiang^{1,a}, Xiao-Li Zhang^{2,a}, Xiang-Na Zhao^{3,a}, Cun-Bao Li⁴, Jie Lei¹, Zeng-Qiang Kou¹, Wen-Kui Sun¹, Yang Hang¹, Feng Gao⁵, Sheng-Xiang Ji², Can-Fang Lin⁴, Bo Pang¹, Ming-Xiao Yao¹, Benjamin D. Anderson⁶, Guo-Lin Wang⁷, Lin Yao⁷, Li-Jun Duan⁷, Dian-Ming Kang^{1,b}, Mai-Juan Ma^{7,b}

1. Shandong Provincial Center for Disease Control and Prevention, Jinan, China
2. Linyi Center for Disease Control and Prevention, Linyi, China
3. Institute of Disease Control and Prevention, Chinese People's Liberation Army, Beijing, China
4. Lanshan District Center for Disease Control and Prevention, Linyi, China
5. Linyi People's Hospital, Linyi, China.
6. Global Health Research Center, Duke Kunshan University, Kunshan, Jiangsu, China
7. State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China.

^aX.-L. J., X.-L. Z., and X.-N. Z. are joint first authors.

^bD.-M. K. and M.-J. M are senior authors and contributed equally to this article

Corresponding author: Mai-Juan Ma, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China (mjma@163.com)

Alternate corresponding author: Dian-Min Kang, Dian-Ming Kang, Department of viral Infectious disease control and prevention, Shandong Provincial Center for Disease Control and Prevention, Jinan 250014, China (dmkang66@163.com)

Brief summary

We report a unique three-family cluster of infection with SARS-CoV-2. The transmission of SARS-CoV-2 by individuals with asymptomatic or paucisymptomatic infections is likely occurred. SARS-CoV-2 was detected in contaminated environments of one household.

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Abstract

Data concerning the transmission of SARS-CoV-2 in asymptomatic and paucisymptomatic patients are lacking. We report a three-family cluster of infections involving asymptomatic and paucisymptomatic transmission. Eight (53%) of 15 members from three families were confirmed with SARS-CoV-2 infection. Of eight patients, three were asymptomatic and one was paucisymptomatic. An asymptomatic mother transmitted the virus to her son, and a paucisymptomatic father transmitted the virus to his three-month-old daughter. SARS-CoV-2 was detected in the environment of one household. The complete genomes of SARS-CoV-2 from the patients were >99.9% identical and were clustered with other SARS-CoV-2 sequences reported from China and other countries.

Keywords. SARS-CoV-2; COVID-2019; asymptomatic; paucisymptomatic; transmission

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that causes coronavirus disease 2019 (COVID-19), emerged in December 2019 in Wuhan, China [1]. It has since been declared a global pandemic with over 1,000,000 cases reported as of April 3, 2020 [2]. Person-to-person transmission has been established [3-8], and asymptomatic transmission of SARS-CoV-2 has been reported [9]. However, studies on the potential transmission of SARS-CoV-2 by asymptomatic persons and those with mild illness have been limited [10]. Herein, we report a 3-family cluster study of eight patients associated with asymptomatic and pauciasymptomatic (one mild symptom only) SARS-CoV-2 transmission in Shandong Province, China.

METHODS

Epidemiological Investigation

The first positive SARS-CoV-2 patients in this cluster were identified on January 21, 2020 triggering an epidemiological investigation by the local center for disease control and prevention. To identify the possible infective source, the epidemiological investigation focused on exposure history before the onset of illness, such as travel history to Wuhan or Hubei Province, visiting live animal markets, and contact history with febrile persons. Medical records were also closely reviewed to verify the timelines of events and clarify clinical progressions.

To examine possible environmental contamination of SARS-CoV-2 in households, select surfaces that may be frequently touched by family members were sampled in the bedroom (door handle, bedside light switch, and sliding of wardrobe door), kitchen (door handle, faucet switch, light switch, rice cooker plug), and bathroom (door handle, handrail, the surface of the toilet bowl, sink). One swab per site (room) with multiple surfaces was collected.

All close contacts of SARS-CoV-2 positive patients were traced, including family members who lived with the patients and individuals who had contact with the patients within 1 meter without wearing proper personal protection. Close contacts were quarantined at home and monitored for fever ($\geq 38^{\circ}\text{C}$) and symptoms. In addition, nasopharyngeal swabs of close contacts were collected every 24

hours from day 1 to 14 to detect SARS-CoV-2 by molecular assay. If any close contact had positive detection of SARS-CoV-2, they were sent to a hospital for isolation and treatment.

SARS-CoV-2 Molecular Detection, Sequencing, and Phylogenetic Analysis

All collected environmental and patient samples were stored at -80°C before being transported using cold chain to a biosafety level 2 enhanced laboratory to perform molecular detection of SARS-CoV-2. A real-time reverse transcriptase PCR (rRT-PCR) Test Kit (GZ-D2RM, Shanghai GeneDx Biotech Co., Ltd) targeting the *ORF1ab* and *N* genes of SARS-CoV-2 was used. A cycle threshold (Ct) value less than 37 was interpreted as positive for SARS-CoV-2 RNA and a Ct value of 40 or more was defined as a negative test. A medium load (weakly positive), defined as a Ct value of 37 to less than 40, required confirmation by retesting. Positive samples were sequenced directly from the original specimens as previously described [11]. The maximum likelihood phylogenetic tree of the complete genomes was conducted by using RAxML software (version 8.2.9) [12] with 1000 bootstrap replicates, employing the general time-reversible nucleotide substitution model.

RESULTS

Description of SARS-CoV-2 Positive Patients

Patients 1 (62-year-old woman) and 2 (65-year-old man) were a couple who lived with their son (Patient 3), daughter-in-law (Patient 4), and two grandchildren. Patient 1 presented with cough, rhinorrhea, and sputum on January 12, 2020 (Figure 1). On January 15, she visited a health clinic and was diagnosed with a common cold. She was prescribed intravenous infusions of ampicillin and sulbactam, ribavirin, and traditional medicine for five days. On January 16, she developed a fever (38°C). On January 17, Patient 2 reported symptoms of fever (37.8°C), cough, sputum, earache, and upset stomach. He was also diagnosed with a common cold at the health clinic and received the same prescription as Patient 1 for three days. However, their symptoms did not resolve at the conclusion of the treatment regimen leading both to seek care at a local hospital on January 21. Nasopharyngeal swabs were collected from both patients at the hospital and confirmed positive for

SARS-CoV-2 by rRT-PCR. Thereafter, they were admitted to the isolation ward of the hospital for treatment. Major symptoms during hospitalization for both patients included fever, cough, and fatigue (Figure 1).

Patients 3 (37-year-old male) and 4 (35-year-old female), a young couple, were close contacts of Patients 1 and 2. They were self-quarantined at home for 14 days starting on the day of the hospital admission of Patients 1 and 2. Their nasopharyngeal swabs were collected on January 23 for SARS-CoV-2 testing. Patient 3 was confirmed positive that same day for SARS-CoV-2 by rRT-PCR, but had no symptoms. Patient 4 tested negative for SARS-CoV-2 but was later confirmed to be positive for SARS-CoV-2 on January 25 when a repeat pharyngeal swab was collected and tested. Upon hospital admission, Patient 3 developed a slightly dry and itchy throat. The cough was a major symptom during hospitalization, and two days fever and four days fatigue of the total hospital stay was also recorded. Patient 4 had no identified clinical symptoms (Figure 1).

Patient 5, a 53-year-old female, lived with her son (Patient 6) and parents, and was a close contact of Patients 3 and 4, being the mother-in-law of Patient 3 and the mother of Patient 4. She was self-quarantined at home beginning the day of Patient 3's confirmation (January 23). On January 24, her nasopharyngeal swab was collected to test for SARS-CoV-2 infection, which was later confirmed positive by rRT-PCR, despite her lack of symptoms. She also did not show any clinical symptoms of infection during hospitalization (Figure 1). Patient 6, a 28-year-old male, was identified as the close contact of his mother (Patient 5). He was self-quarantined at home beginning the day of his mother's (Patient 5) confirmed infection (January 24). His nasopharyngeal swab was collected on January 25 and confirmed SARS-CoV-2 positive by rRT-PCR. On admission in the afternoon on January 25, he developed a fever (37.5°C). Major symptoms during hospitalization included fever and cough, and the symptoms persisted more than two weeks.

Patient 7, a 35-year-old man, lived with his wife and three children, and was identified as the close contact of Patient 3. He was self-quarantined at home beginning the day of Patient 3's confirmed infection (January 23). On January 25, his nasopharyngeal swab was collected and tested positive for SARS-CoV-2 by rRT-PCR. During hospitalization, he was paucisymptomatic, with only an occasional cough. Patient 8, a 3-month-old female infant, was the close contact of Patient 7, her father. On January 27, her nasopharyngeal swab was collected and was a weak positive for SARS-

CoV-2. A repeat pharyngeal swab was collected on January 29 and was positive for SARS-CoV-2. The infant had no clinical symptoms before, during, or after hospitalization.

The chest CT images on admission or hospitalization showed that Patients 1-6 had ground-glass opacities. However, no significant abnormalities were observed for Patients 7 and 8 (Supplemental Figure 1). As of February 17, 2020, all patients recovered and were discharged to home isolation for 14 days.

Exposure Histories

Patients 1 and 2 traveled to their hometown in Xiaogan December 29, 2019 to January 15, 2020 (Figure 1). Xiaogan is a city adjacent to the epidemic center of Wuhan where the first case was identified on January 1, 2020. Moreover, they had changed trains at the Hankou railway station in Wuhan. Patients 3 and 4 had not traveled to Wuhan. They and their parents live together, eat together, and have frequent face-to-face interactions. Facemasks or other personal protective equipment (PPE) were not used at home. Patient 5 had contact with Patients 3 and 4 several times at a factory that they jointly operated. Patient 5 also visited the home of Patients 1-4 on the evening of January 21 and stayed one night. On the morning of January 22, Patient 5 was driven home by Patient 3. During these contacts, no facemasks or other PPE were used. Patient 6 did not report close contact with known COVID-19 cases except for his mother. Patient 7 reported that he had a frequency of 2 to 3 times daily contact with Patient 3 at the factory from January 15 to 18 and dined with Patient 3 and other colleagues on January 18. He did not report close contact with any known COVID-19 cases except for Patient 3. Except for contact with her father, patient 8 had no known contact with COVID-19 patients.

SARS-CoV-2 in Samples from Patients and Environments and Phylogenetic Analysis

SARS-CoV-2 was detected in nasopharyngeal swabs of all patients during hospitalization, including asymptomatic and paucisymptomatic patients (Figure 1). A total of 15 (5 per household) surface swab samples were collected from the bedrooms (n=9, three per household), kitchens (n=3, one per household), and bathrooms (n=3, one per household) of patient homes. Two (13.3%) of 15 swabs (one from the bedroom of Patient 3 and another one from his family kitchen) were positive for SARS-CoV-2 by rRT-PCR.

The full-genome sequences for eight patients were obtained and have been deposited in GISAID (accession numbers EPI_ISL_414934–414941). The full-genome for the two environmental swabs positive for SARS-CoV-2 were not obtained due to low-coverage genomes. The full-genomes of eight patients were >99.9% identical across the whole genome. Phylogenetic analysis revealed that the viruses from patients were clustered in the same clade and were genetically similar to other SARS-CoV-2 sequences reported from China and other countries (Figure 2). No significant mutation site was identified in the eight SARS-CoV-2 viral sequences compared with previous strains in China and other countries.

Close contacts

Fifteen contacts of either Patient 1 or Patient 2 were identified, and two contacts (Patients 3 and 4) were confirmed with COVID-19. A total of 88 contacts of Patient 3 were identified, and two contacts (Patients 5 and 7) tested positive for SARS-CoV-2. Seventy-three close contacts of Patient 5 were identified, and one contact (Patient 6) tested positive for SARS-CoV-2. Two contacts were identified for Patient 6 and all tested negative. Twenty-one contacts of Patient 7 were identified and one contact (Patient 8) tested positive. Of 101 close contacts identified for Patient 8, all tested negative. No other close contacts of the patients were identified during the 14-day follow-up. Thus, a crude estimation of the attack rate is 3.8% (4/105) for symptomatic and 1% (2/195) for asymptomatic and paucisymptomatic.

DISCUSSION

We report a unique three-family cluster of infection with SARS-CoV-2, in which eight of 15 members were confirmed with SARS-CoV-2 infection. Particularly interesting is that of 6 secondary patients, two were asymptomatic, one was paucisymptomatic, and three were symptomatic. Our findings show that the transmission of SARS-CoV-2 by individuals with asymptomatic or paucisymptomatic infections is possible. Patients 1 and 2 were likely first exposed to SARS-CoV-2 after visiting their hometown in Xiaogan Hubei Province, China. Their son (Patient 3) and daughter-in-law (Patient 4, asymptomatic), whom they live with, were later found to be infected with SARS-CoV-2. Patient 5 (asymptomatic) was identified to be infected with SARS-CoV-2 after frequent contact with Patients 3 and 4 during work and home visits. She transmitted the virus to her son (symptomatic) whom she lives with. Patient 7 (paucisymptomatic) was found to be infected with SARS-CoV-2 after frequent contact with Patient 3 during work. He likely transmitted the virus to his daughter (Patient 8, asymptomatic). In addition, consistent with previous studies [5-8], the transmission of SARS-CoV-2 during the incubation period of Patient 3 likely occurred. Patients 5 and 7 were infected after their exposures to a presymptomatic Patient 3 during working or home visits. These findings may help explain the rapid spread of SARS-CoV-2 between person-to-person.

The currently available evidence shows that SARS-CoV-2 is transmitted between people through droplets and close contact [13]. A recent study showed extensive environmental contamination by a SARS-CoV-2 patient [14], suggesting the contaminated environment as a potential medium of transmission. In this study, we detected SARS-CoV-2 in two environmental swabs from the household of Patient 3. Such detection of SARS-CoV-2 in contaminated environments of the household may provide an additional contribution to virus transmission among family members as the virus can remain viable and infectious on the surface up to seven days [15]. However, the direct research-based evidence describing exactly how SARS-CoV-2 is transmitted is limited, and further studies are required.

We cannot rule out the possibility of unknown COVID-19 patients (e.g., asymptomatic carriers) transmitting the virus. However, according to screening protocols implemented by the provincial, municipal, and county-level Center for Disease Control and Prevention, all close contacts were

traced, and all patients with positive rRT-PCR results in this study were confirmed by whole-genome sequencing, including those who were asymptomatic or paucisymptomatic (Patients 4, 5, 7, and 8).

CONCLUSION

The transmission potential by individuals with asymptomatic and paucisymptomatic infection and the detection of SARS-CoV-2 in contaminated environments create challenges in control and prevention for the disease. Further studies are needed to investigate the contribution of persons with asymptomatic or paucisymptomatic SARS-CoV-2 infection and the relationship with transmission of the virus in the household, occupational, and community settings.

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Potential conflicts of interest

All authors: No reported 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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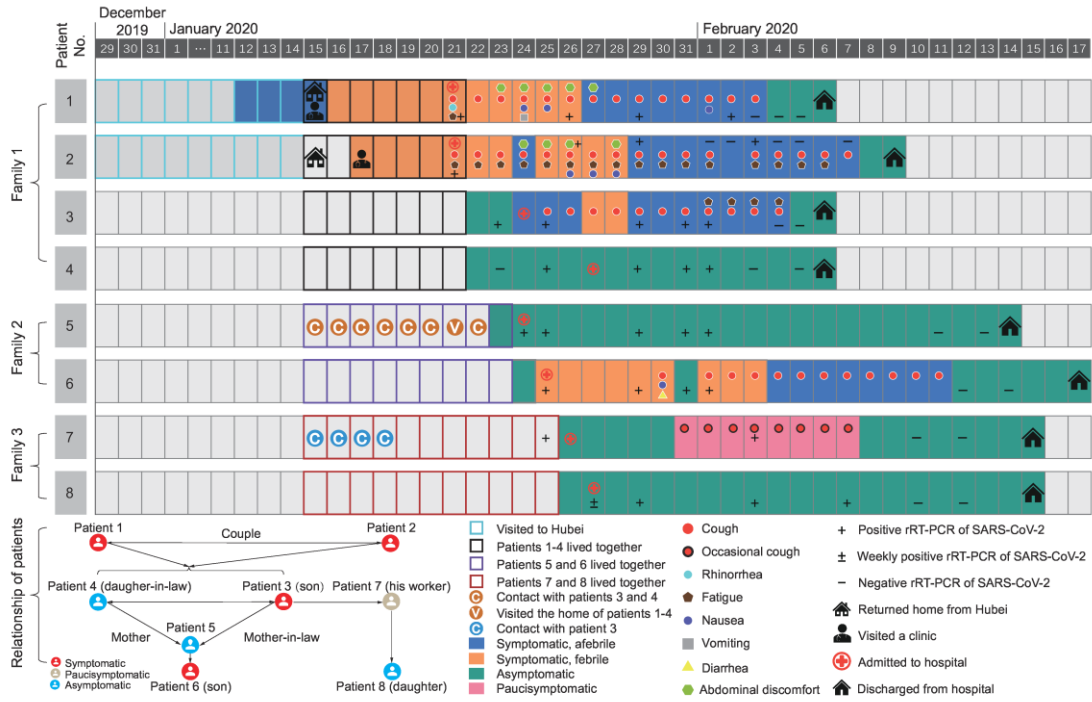
Figure legends

Figure 1. Timeline of relevant exposures and clinical symptoms of eight patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. rRT-PCR: real-time reverse transcriptase-polymerase chain reaction.

Figure 2. Phylogenetic analysis of full-length genomes of SARS-CoV-2 in eight patients. Red text indicates SARS-CoV-2 detected in the patients in the present study.

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Figure 1



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Figure 2

