Clinical and Epidemiological Features of a Family Cluster of Symptomatic and Asymptomatic Severe Acute Respiratory Syndrome Coronavirus 2 Infection

Gerhard K. Wolf, Thomas Glueck, Johannes Huebner, Maximilian Muenchhoff, Dieter Hoffmann, Lars E. French, Oliver T. Keppler, and Ulrike Protzer

1Children’s Hospital Traunstein, LMU Munich, Traunstein, Germany; 2Division of Infectious Diseases, Kliniken Südostbayern, Trostberg, Germany; 3Division of Pediatric Infectious Disease, Dr. von Hauner Children’s Hospital, LMU Munich, Munich, Germany; 4German Center for Infection Research, Munich Partner Site, Germany; 5Max von Pettenkofer Institute, LMU Munich, Munich, Munich, Germany; 6Institute of Virology, School of Medicine, Technical University of Munich/Helmholtz Zentrum München, Munich, Germany; and 7Department of Dermatology and Allergology, LMU University Hospital, Munich, Germany

In a family experiencing coronavirus disease 2019, the parents and 2 children aged 2 and 5 years became infected but the youngest child was not infected. Both children initially shed infectious virus, but cleared the virus after 5 to 6 days in the nasopharynx. However, viral RNA was continuously detected in the children’s stool for more than 4 weeks.

Keywords. children; COVID-19; gastrointestinal; RNA persistence; SARS-CoV-2.

The novel coronavirus disease 2019 (COVID-19) was introduced into Germany initially by a Chinese business delegate around 19–21 January 2020 near the city of Munich. Fellow coworkers who attended business meetings with that person were identified as contacts; several of them subsequently fell ill and were hospitalized [1]. The father of the children reported in this study had no direct contact with the Chinese visitor but met with a German contact person who got infected (Figure 1). Secondary and tertiary transmission is possible in this cluster.

METHODS

The family was hospitalized and patients were seen by an infectious diseases specialist and a pediatrician on a daily basis. Clinical and laboratory results were documented. Nasopharyngeal swabs, stools samples, and blood were collected; immediately stored at 4°C; and transported to the diagnostic laboratory for analysis within 24 hours. Nasopharyngeal swabs were used for virus culture in a biosafety level 3 laboratory on Vero cells in medium that contained antibiotics and antifungals. After 24 and 48 hours, cells were observed for cytopathic effect, and cell culture supernatant was passaged onto fresh cells. Infection was confirmed using immunofluorescence staining with the father’s serum. Nucleic acids were extracted using the Abbott mSample Preparation Systems from 500 µL resuspended nasopharyngeal swabs, blood, or stool suspensions and analyzed for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA using real-time polymerase chain reaction (PCR). Digital droplet PCR (ddPCR) was used to quantify SARS-CoV-2 RNA from stool samples. Samples were quantified in duplicate using the One-Step reverse transcriptase-ddPCR Advanced Kit for Probes (BioRad) on the BioRad QX200 platform. Primer and probe sequences were used to detect the SARS-CoV-2 nucleoprotein gene (N) as published by the Centers for Disease Control and Prevention (CDC) [2]. Results are shown for the CDC N1 reaction. Digital droplet PCR methods were validated using dilution series of a commercially available plasmid containing the complete N-gene with known copy numbers (2019-nCoV CDC RUO Plasmid Controls, IDT). Representative raw data are shown in Supplementary Figure 1. Ethical approval was obtained by the institutional ethics review board of the University Hospital of Technical University of Munich, Germany.

RESULTS

The father fell ill with flu-like symptoms and muscle pain on 24 January and tested positive for SARS-CoV-2 on 29 January. By order of the local health authorities, the family was taken into isolation at a local hospital. Upon the family’s request and as the father had been symptomatic for 5 days prior to admission, they stayed together in a large room with separate bathroom, separate dining room, and play area for the children. All healthcare workers including those involved in the family’s care always wore full protective equipment including N95 mask, gloves,
gown, and face shield. The parents were asked to wear masks; wearing masks was not practical for the children.

At the time of admission, the father had flu-like symptoms, mild respiratory distress, a dry cough, a white cell count of 2.100/µL, with an absolute neutrophil count (ANC) of 900/µL. During the course of his stay, he developed moderately severe disease with partial respiratory insufficiency, which was successfully managed with high-flow oxygen. Respiratory specimens became negative for SARS-CoV-2 from 6 February onward (Figure 1).

The mother still tested SARS-CoV-2 negative on 29 January but developed a low-grade fever and malaise on 30 January. However, she showed only minimal symptoms and mild leukopenia of 3.600/µL. Her PCR test for SARS-CoV-2 in respiratory material was negative on 30 January and 1 February, but showed high viral titers on 3 February in a pharyngeal swab and in a stool sample. As with his sister, infectious virus was easily grown from the nasopharyngeal swab material on 3 and 4 February. Her laboratory test results revealed marked leukopenia, with a white cell count of 1.100/µL, and C-reactive protein was 12.6 mg/L. He cleared the virus from the upper respiratory tract by 10 February, but stool samples remained PCR-positive for 4 weeks.

Child B, a 2-year-old male, developed vomiting on 31 January and low-grade fever on 2 February, but only for a few hours, and then remained asymptomatic. He tested negative for SARS-CoV-2 on 30 January and 1 February in respiratory material but showed high viral titers on 3 February in a pharyngeal swab and in a stool sample. As with his sister, infectious virus was easily grown from the nasopharyngeal swab material on 3 and 4 February. His laboratory test results revealed marked leukopenia, with a white cell count of 2.500/µL, an ANC of 1.100/µL, and a platelet count of 151 000/µL, and C-reactive protein was 12.6 mg/L. He cleared the virus from the upper respiratory tract by 10 February, but stool samples remained PCR-positive for 4 weeks.

The viral load in stool samples from children A and B was quantified for all samples available using digital droplet PCR, as summarized in Figure 2. Interestingly, both child A and child B developed Beau lines of their fingernails 3 weeks after symptom onset.
Child C, a 7-month-old female who was breastfed, was asymptomatic throughout the observation period and never developed fevers or any other symptoms, despite continuous exposure to her parents and siblings. She remained SARS-CoV-2 PCR-negative in repeat testing of pharyngeal swab and stool specimens over the entire observation period. Her hemoglobin was 13.5 g/dL, white cell count was 6,400/µL, with an ANC of 2,100/µL, an ALC of 4109/µL, a platelet count of 284,000/µL, and C-reactive protein of 0.0 mg/L.

Additional infections by influenza A or B, parainfluenza, human metapneumovirus, respiratory syncytial virus, and adenovirus were excluded in all family members. The order for hospital quarantine of the family was waived by the local health authorities after 14 days, when all family members tested SARS-CoV-2 PCR-negative in 2 consecutive nasopharyngeal swabs.

The leading symptoms in our pediatric patients were mild and predominantly gastrointestinal, such as vomiting and transient diarrhea accompanied by low-grade fever. This is coincident with detection of the highest viral genome copy numbers in stool specimens from both children. However, respiratory symptoms were absent. In contrast, respiratory, but not gastrointestinal, symptoms were reported in 10 children in China outside Wuhan [8]. While viral RNA was detected in nasopharyngeal swabs for up to only 7 days in the 2 infected children we report on here, stool samples remained PCR-positive for more than 4 weeks. This is in accordance with reports that describe viral shedding in stools of infected children [6, 8]. The relevance of viral shedding in stool for virus transmission is unclear to date. Recovery of infectious virus from stool has been reported for an adult patient [9], indicating the possibility of transmission via the fecal–oral route.

Gastrointestinal involvement is known for beta-coronaviruses in animals and has been described for Middle East respiratory syndrome (MERS) [10] and SARS [11]. MERS coronaviruses have been shown to readily replicate in human intestinal epithelium [12]. Currently, the viral receptor for SARS-CoV-2 is thought to be the same as for the original SARS-CoV, ACE-2 [13, 14]. This cell surface protein is highly expressed in oral and intestinal mucosa [15], favoring that this emerging virus has an intestinal tropism in addition to targeting the respiratory system.

The occurrence of nail damage in both children could be attributed to the infection itself, as previously suggested for other systemic infections such as mumps and syphilis [16], or to high levels of stress caused by the circumstances of this quarantine isolation.

Experience with the related SARS-CoV-1 showed that case fatality rates were only 1.7% in children aged <19 years compared with 25.5% in adults aged 60–79 years [17]. Although the children described in the current study developed high viral titers in nasopharyngeal mucosa and stool, they did not develop any severe symptoms. Furthermore, the 7-month-old child did not become infected despite intense and continued exposure to her parents and siblings and despite being breastfed by her mother who was symptomatic and shed virus, albeit at low levels.

Our experience with this family cluster shows that it will be very important to define how long patients, especially children,
with SARS-CoV-2 infection shed the virus and be infectious, for how long strict hygiene measures need to be taken, and when children can be safely reintegrated into child care.

**Supplementary Data**

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

**Note**

*Potential conflicts of interest.* All authors: No reported conflicts of interest. 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.

**References**

1. Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med 2020; 382:970–1.
2. National Center for Immunization and Respiratory Diseases, D.o.V.D. Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus. 2020. February 27, 2020 [cited 2020 3/6/2020]; Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-detection-instructions.html. Accessed 6 March 2020.
3. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study [published online ahead of print April 27, 2020]. Lancet Infect Dis. doi:10.1016/S1473-3099(20)30287-5.
4. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020; 382:1199–207.
5. Chang, Lin M, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA 2020; 323:1092–3.
6. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat Med 2020; 26:502–5.
7. Wei M, Yuan J, Lu Y, Pu T, Yu X, Zhang ZJ. Novel coronavirus infection in hospitalized infants under 1 year of age in China. JAMA 2020;323:1313–4.
8. Cai J, Xu J, Lin D, et al. A case series of children with 2019 novel coronavirus infection: clinical and epidemiological features [published online ahead of print February 28, 2020]. Clin Infect Dis. doi:10.1093/cid/ciaa198.
9. Zhang Y, Chen C, Zhu S, et al. Isolation of 2019-nCoV from a stool specimen of a laboratory-confirmed case of the coronavirus disease 2019 (COVID-19). China CDC Weekly 2020; 2:123–4.
10. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis 2013; 13:752–61.
11. Leung WK, To KF, Chan PK, et al. Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. Gastroenterology 2003; 125:1011–7.
12. Zhou J, Li C, Zhao G, et al. Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus. Sci Adv 2017; 3:eaa04966.
13. Kuhn JH, Li W, Choe H, Farzan M. Angiotensin-converting enzyme 2: a functional receptor for SARS coronavirus. Cell Mol Life Sci 2004; 61:2738–43.
14. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579:270–3.
15. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020; 12:8.
16. Braswell MA, Daniel CR 3rd, Brodell RT. Beau lines, onychomadesis, and retronychia: a unifying hypothesis. J Am Acad Dermatol 2015; 73:849–55.
17. Jia N, Feng D, Fang LQ, et al. Case fatality of SARS in mainland China and associated risk factors. Trop Med Int Health 2009; 14 Suppl 1:21–7.