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BRIEF REPORT

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

Gerhard K. Wolf,[1] Thomas Glueck,[2] Johannes Huebner,[3] Maximilian Muenchhoff,[4,5] Dieter Hoffmann,[4,6] Lars E. French,[7] Oliver T. Keppler,[4,5] and Ulrike Protzer[4,6]

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

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Correspondence: Gerhard K. Wolf, Kinderklinik Traunstein, Cuno-Niggl-Str 3, 83278 Traunstein, Germany (gerhard.wolf@lmu.de).

Here, we describe the clinical and virological characteristics of 3 children in a family cluster who experienced infection with severe acute respiratory syndrome coronavirus 2

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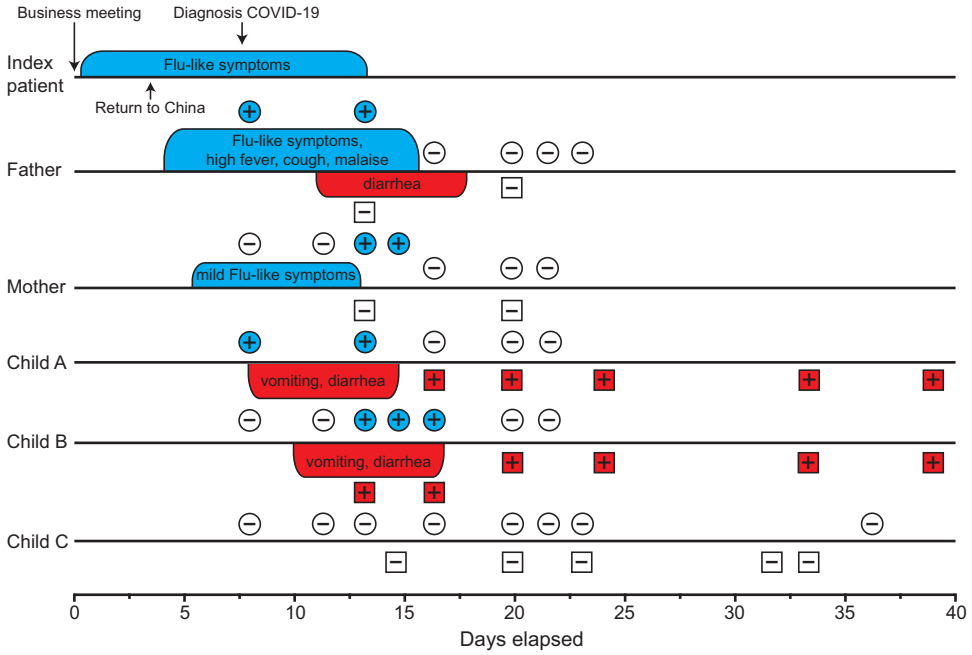

Figure 1. Timeline of symptoms and severe acute respiratory syndrome coronavirus 2 PCR results. Clinical symptoms are summarized for each case over time beginning on 21 January 2020 (day 0), with respiratory and systemic symptoms shown in blue and gastrointestinal symptoms shown in red. PCR results are indicated for respiratory material shown as circles (positive results in blue) and stool samples shown as squares (positive results in red). Abbreviations: COVID-19, coronavirus 2019; PCR, polymerase chain reaction.

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 a pharyngeal swab was still negative on 1 February, turned positive on 3 and 4 February, but was reported negative again after 6 February. Both adults never had viral RNA detected in stool specimens.

The couple has 3 children who were cohospitalized. Child A, a 5-year old female, developed symptoms of gastroenteritis with soft stools, fever, and vomiting on 29 January and tested positive for SARS-CoV-2 in a pharyngeal swab the same day. On admission, she had a systolic ejection murmur with no signs of cardiac injury and was afebrile and asymptomatic. Her hemoglobin was 18.9 g/dL, white cell count was 6.000/μL, with an ANC of 2.100/μL and an absolute lymphocyte count (ALC) of 3420/μL. The platelet count was 169 000/μL, and C-reactive protein was 1.4 mg/L. She did not develop any respiratory symptoms but tested PCR-positive again in nasal and pharyngeal swabs on 3 February when infectious virus could be grown from swab material. She turned PCR-negative for SARS-CoV-2 again by 6 February. However, multiple stool specimens continued to be PCR-positive for up to 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 an ALC of 1250/μL. His hemoglobin was 16.9 g/dL, platelet count was 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.

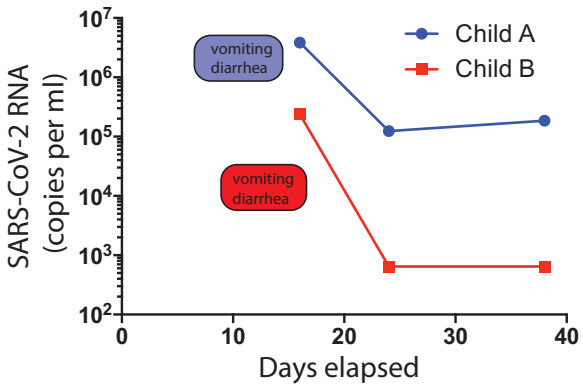

Figure 2. Viral load results for child A (red) and child B (blue) for available stool samples. Total copy numbers are indicated, adjusted to 1-mL stool sample using digital droplet polymerase chain reaction with Centers for Disease Control and Prevention N1 primer-probe sequences. The timeline is the same as in Figure 1 starting on 21 January 2020 (day 0). Durations of gastrointestinal symptoms are indicated for each child. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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. However, the children were not allowed to return to daycare and nursery school as long as viral RNA shedding in the feces continued.

DISCUSSION

In this family cluster, the secondary attack rate was 75% compared with the 15% reported in a case series by Bi et al [3] Analysis of the transmission chain indicates that the incubation period may be as short as 72 hours. There is evidence that children often have clinically asymptomatic SARS-CoV-2 infections; there is an overall low prevalence in young children in particular [4–6]. Of 9 infants aged <1 year hospitalized in China between December 2019 and February 2020, most had mild respiratory symptoms or fever [7]. Our observations confirm that toddlers or older children can be infected by this novel coronavirus and shed infectious virus for several days. However, although continuously exposed through all 4 other family members, the 7-month-old child remained SARS-CoV-2–negative, indicating that infants may either lack a receptor or factor essential for the virus or may have a more effective innate immune response to protect them from infection.

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 betacoronaviruses 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.

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