Clinical and Epidemiological Features of a Family Cluster of Symptomatic and Asymptomatic SARS-CoV-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, Germany

[2]Division of Infectious Diseases, Kliniken Südostbayern, 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

[5]Max von Pettenkofer Institute, LMU Munich, Germany

[6]Institute of Virology, School of Medicine, Technical University of Munich / Helmholtz Zentrum München, Germany

[7]Department of Dermatology and Allergology, LMU University Hospital Munich, Germany

Abstract

This report describes the clinical and virological characteristics of three children in a family cluster experiencing infection with SARS-CoV2. While the youngest child was not infected, both parents and the two 2- and 5 years-old children became infected. The children were only briefly symptomatic with predominant gastrointestinal symptoms. They initially shed infectious virus from the upper respiratory tract, but cleared the virus after five to six days in the nasopharynx. However, SARS-CoV2 RNA was continuously detected in the stools of the children for more than 4 weeks indicating a predominant replication within the gastrointestinal tract.

Key words:
SARS-CoV2, COVID-19, children, SARS, gastrointestinal, RNA-persistence

Introduction

The novel coronavirus (SARS-CoV-2) was introduced into Germany initially by a Chinese business delegate around January 19-21, 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 disease specialist and a pediatrician on a daily basis. Clinical as well as laboratory results were documented. Nasopharyngeal swabs, stools samples and blood were collected, immediately stored at 4°Celsius and transported to the diagnostic laboratory for analysis within 24 hours. Nasopharyngeal swabs were used for virus culture in a BSL3 lab on Vero cells in medium containing 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 by immunofluorenscence staining using 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 SARS-CoV2 RNA by real-time PCR. Digital droplet PCR (ddPCR) was used to quantify SARS-CoV2 RNA from stool samples. Samples were quantified in duplicate using the One-Step RT-ddPCR Advanced Kit for Probes (BioRad) on the BioRad QX200 platform. Primer and probe sequences were used for detection of the SARS-CoV2 nucleoprotein gene (N) as published by the 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 is 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 January 24 and was tested positive for SARS-CoV-2 on January 29. 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 already been symptomatic for five days prior to admission, they stayed together in a large room with separate bathroom, separate dining room and play area for the children. All health care workers including involved in the care of the family always wore full protective equipment including N95 mask, gloves, gown and face shield. The parents were asked to wear masks, but for the children this turned out to be impractical.

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

The mother still tested SARS-CoV-2 negative on January 29, but developed low-grade fever and malaise on January 30. She, however, 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 February 1, turned positive on February 3 and 4, but was reported negative again after February 6.

Both adults never had viral RNA detected in stool specimens.

The couple has three children that were co-hospitalized.
Child A, a 5-year old girl, developed symptoms of gastroenteritis with soft stools, fever and vomiting on January 29 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 ALC of 3420 / µl. Platelet count was 169,000 / µl, and C-reactive protein was 1.4 mg/L. She did not develop respiratory symptoms at all, but tested PCR-positive again in nasal and pharyngeal swabs on February 3 when infectious virus could be grown from swab material. She turned PCR-

negative for SARS-CoV-2 again by February 6. However, multiple stool specimens continued to be PCR-positive for up to four weeks.

Child B, a 2-year old boy, developed vomiting on January 31 and low-grade fever on February 2, but only for a few hours and remained asymptomatic since. He tested negative for SARS-CoV-2 on January 30 and February 1 in respiratory material, but showed high viral titers on February 3 in a pharyngeal swab and in a stool sample. As with his sister, infectious virus was easily grown from the nasopharyngeal swab material on February 3 and 4. His lab tests revealed marked leukopenia with a white-cell count of 2.500 / µl and an ANC of 1.100 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 February 10, but stool samples also remained PCR positive for four 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 B developed Beau lines of their finger nails 3 weeks after symptom onset.

Child C, a 7-month old girl who was breast-fed, 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 per µl, with an ANC of 2.100 per µl and an ALC of 4109 / µl, platelet count was 284,000 per µl, and C-reactive protein was 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 had tested SARS-COV2-PCR negative in two consecutive nasopharyngeal swabs. However, the children were not allowed to return to day care and nursery school as long as viral RNA shedding continued in the feces.

Discussion:

In this family cluster the secondary attack rate was 75%, compared to the 15% reported in a case series by Bi et al. (Preprint server https://www.medrxiv.org/content/10.1101/2020.03.03.20028423v1). 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 and an overall low prevalence in particular in young children [3-5]. Of nine infants less than one year of age hospitalized in China between December 2019 and February 2020, most had mild respiratory symptoms or fever [6]. 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 four other family members, the 7-months old 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 protecting 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 highest viral genome copy numbers in stool specimens from both children. Respiratory symptoms, however, were absent. In contrast, respiratory, but no gastrointestinal symptoms, were reported in 10 children in China outside Wuhan [7]. While viral RNA was detected in nasopharyngeal swabs only for up to 7 days in the two infected children we report, stool samples remained PCR-positive for more than 4 weeks. This is accordance with reports describing viral shedding in stools of infected children [5, 7]. The relevance of viral shedding in stool for virus transmission to date is unclear. Recovery of infectious virus from stool has been reported for an adult patient [8], indicating the possibility of transmission via the fecal-oral route.

Gastrointestinal involvement is known for beta-Coronaviruses in animals and has been described for MERS [9] and SARS [10] before. MERS-coronaviruses have been shown to readily replicate in human intestinal epithelium [11]. Currently, the viral receptor for SARS-CoV2 is thought to be the same as for the original SARS-CoV, ACE-2 [12, 13]. This cell surface protein is highly expressed in oral and intestinal mucosa [14], 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[15], or also 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 <19 years, compared to 25.5% in adults 60-79 years [16]. Although the children described in the current study developed high viral titers in nasopharyngeal mucosa and stool, they didn't 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, and especially children with SARS-CoV-2 infection, may 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.

Literature:

1. Rothe, C., et al., *Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany.* N Engl J Med, 2020.
2. National Center for Immunization and Respiratory Diseases (NCIRD), 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.
3. Li, Q., et al., *Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia.* N Engl J Med, 2020.
4. Chang, et al., *Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China.* JAMA, 2020.

5. Xu, Y., et al., *Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. .* Nature Medicine, 2020.
6. Wei, M., et al., *Novel Coronavirus Infection in Hospitalized Infants Under 1 Year of Age in China.* JAMA, 2020.
7. Cai, J., et al., *A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features.* Clin Infect Dis, 2020.
8. Zhang, Y., 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**(8).
9. Assiri, A., 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**(9): p. 752-61.
10. Leung, W.K., et al., *Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection.* Gastroenterology, 2003. **125**(4): p. 1011-7.
11. Zhou, J., et al., *Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus.* Sci Adv, 2017. **3**(11): p. eaao4966.
12. Kuhn, J.H., et al., *Angiotensin-converting enzyme 2: a functional receptor for SARS coronavirus.* Cell Mol Life Sci, 2004. **61**(21): p. 2738-43.
13. Zhou, P., et al., *A pneumonia outbreak associated with a new coronavirus of probable bat origin.* Nature, 2020.
14. Xu, H., et al., *High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa.* Int J Oral Sci, 2020. **12**(1): p. 8.
15. Braswell, M.A., C.R. Daniel, 3rd, and R.T. Brodell, *Beau lines, onychomadesis, and retronychia: A unifying hypothesis.* J Am Acad Dermatol, 2015. **73**(5): p. 849-55.
16. Jia, N., et al., *Case fatality of SARS in mainland China and associated risk factors.* Trop Med Int Health, 2009. **14 Suppl 1**: p. 21-7.

Fig. 1: Timeline of symptoms and SARS-CoV2 PCR-results. Clinical symptoms are summarized for each case over time beginning on January 21, 2020 (day 0) with respiratory and systemic symptoms shown in cyan and gastrointestinal symptoms shown in red. PCR-results are indicated for respiratory material shown as circles (positive results in cyan) and stool samples shown as squares (positive results in red).

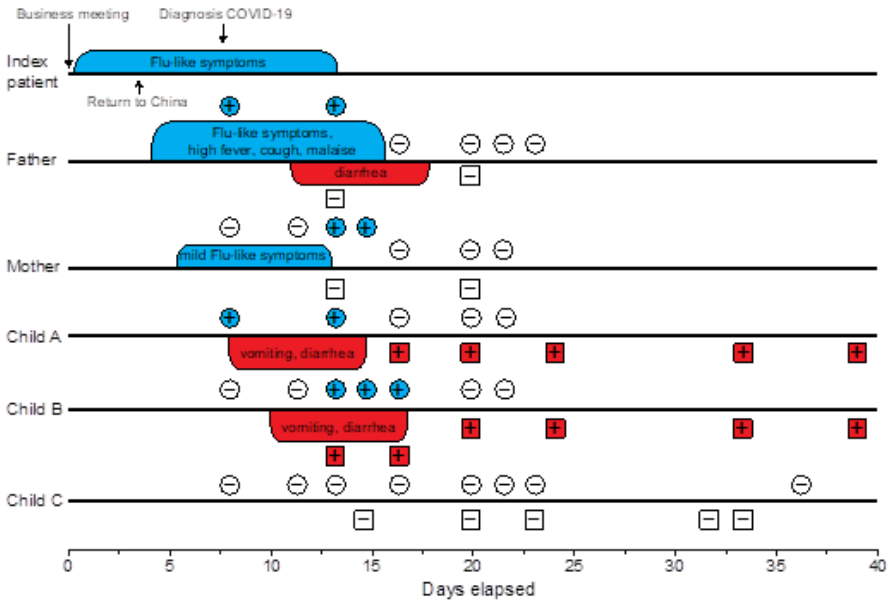

Fig. 2: Viral load results are shown for child A (red) and child B (blue) for available stool samples. Total copy numbers are indicated adjusted to 1ml stool sample using ddPCR with CDC N1 primer-probe sequences. Timeline is the same as in Figure 1 starting on January 21, 2020 (day 0). Durations of gastrointestinal symptoms are indicated for each child.