MEDDOCS Open Access Publisher

Annals of Community Medicine and Primary Health Care

Open Access | Short Communication

Shedding of Infectious Sars-Cov-2 in Two Asymptomatic Children

Noelia S Reyes[1]; Pamela E Rodriguez[1]; Victoria Diaz Balocchi[1]; Carmen Ricarte Tech[1]; Natalia Echegoyen[2]; Mariana Viegas[3]; Augusto Varese[4]; Ana Ceballos[4]; Martin E Stryjewski[5]; Marcela Echavarria[1,2]

[1]Virology Unit (CEMIC-CONICET), Centro de Educación Médica e Investigaciones Clínicas (CEMIC), University Hospital. Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

[2]Virology Laboratory, CEMIC. Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

[3]Virology Laboratory Ricardo Gutierrez Children´s Hospital, (CONICET). Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

[4]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires (CONICET), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

[5]Department of Medicine and Division of Infectious Diseases, (CEMIC), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

***Corresponding Author(s): Echavarría Marcela**

Virology Unit (CEMIC-CONICET), Centro de Educación Médica e Investigaciones Clínicas (CEMIC), University Hospital, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

Email: mechavarria@cemic.edu.ar

Received: Aug 30, 2022

Accepted: Sep 30, 2022

Published Online: Oct 03, 2022

Journal: Annals of Community Medicine and Primary Health Care

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Introduction

Asymptomatic infections with SARS-CoV-2 are associated with viral transmission and have a key role in the propagation of the pandemic [1]. Understanding viral shedding during asymptomatic infections is critical. Unfortunately, data on asymptomatic SARS-CoV-2 infection in children is extremely limited [2].

In adult patients with SARS-CoV-2 infection RT-PCR in respiratory samples can be positive 3 days up to several weeks from symptoms onset. Furthermore, some patients can become positive again after a period of negative testing [3]. In children, the mean time of positive RT-PCR is 11.1 days in symptomatic and 9.4 days in asymptomatic subjects [4]. However, a positive RT-RCR test does not necessarily reflect shedding of viable virus.

Infectiousness of SARS-CoV-2 can begin 2-3 days prior to symptoms onset and declines 7 days from symptoms onset Viable SARS-CoV-2 detected in cell culture virus is isolated in as-

Cite this article: Echavarria M, Reyes NS, Rodriguez PE, Balocchi VD, Ricarte C, et al. Shedding of Infectious SARS-CoV-2 in Two Asymptomatic Children. Ann Community Med Prim Health Care. 2022; 1(1): 1008.

ymptomatic adults mostly within 7 days after the initial positive RT-PCR [5]. In symptomatic children, viable virus was detected in cell culture up to 5 days after onset of symptoms [6]. Longer viral shedding has been described in very few pediatric cases either from critical patients or from children with oncohematologic diseases. Specifically, viable SARS-CoV-2 was isolated up to day 54 in a critical pediatric patient and up to 139 days in an immunocompromised child with severe COVID-19 [2]. To our knowledge, there is a lack of data on isolation of SARS-CoV-2 in asymptomatic and otherwise healthy children.

The objective of this study was to determine the presence of viable virus by cell culture in saliva samples from two asymptomatic and otherwise healthy children infected with SARS-CoV-2.

Materials and methods

A prospective study in a family group infected with SARS-CoV-2 was conducted. Family members were prospectively followed for up to 28 days (during April of 2021). Demographic and clinical data were collected. This study was approved by the Ethics Committee of CEMIC (Protocol: 1298/20).

Sequential saliva and fecal samples were obtained every 3 days. Nucleic acid was extracted from 100µl and eluted in 15µl using manual columns (Quick-RNA TM Viral Kit, Zymo Research CORP.) following manufacturer's recommendation.

Detection of SARS-CoV-2 was performed with an in-house one-step real time RT-PCR multiplex assay targeting the E gene of SARS-CoV-2 and the human RNAsa P gene as an internal control, in a CFX 96 Deep Well™ Real Time System (BioRad). A positive result was considered when the human RNAse gene or the internal amplification control were positive and the cycle threshold (Ct) value was less than 40 [7].

To analyze the signature amino acid mutations on the Spike protein of the variants of SARS-CoV-2, Sanger sequencing of segment 29 of the CDC amplification protocol that includes amino acids 428 to 750 was performed [8].

SARS-CoV-2 isolation was performed in a BSL3 facility at Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires. Vero cells monolayers were inoculated with 300 ul of pre-filtered infectious saliva sample diluted in 300ul of DMEM (Sigma) supplemented with Fetal Bovine Serum (4%), streptomycin (50 ug/ml), penicillin (50 U/ml) and amphotericin B (125 ng/ml). Cells were monitored for virus-associated Cytopathic Effect (CPE) for 96 hours. Positive supernatants were confirmed by RT-qPCR.

SARS-CoV-2 serology was evaluated in both children using COVIDAR IgG assay, which uses a combination of a trimer stabilized spike protein and the Receptor Binding Domain (RBD) in a single Enzyme-Linked Immunosorbent Assay (ELISA) plate (Fundación Instituto Leloir- CONICET-Laboratorio Lemos, Argentina) [9].

Results

A breakthrough male case with COVID-19 was identified on April 6th, 2021 (Patient#1). His family included his wife (43 years old, Patient#2), a 9 years old boy (Asymptomatic#1) and a 12 years old girl (Asymptomatic#2). Both parents were healthcare workers who had completed the Sputnik V vaccine (2 doses) in February, 2021. All subjects were previously healthy. The family returned from a short holiday trip on April 4th, 2021. On the same day, Asymptomatic#1 developed pharyngitis. A rapid pharyngeal test obtained the following day was positive for *Streptococcus pyogenes,* and he received antibiotics. His nasopharyngeal swab (NPS) for SARS-CoV-2 was negative.

On April 6th (day 1), Patient#1 (the index case) developed fever and myalgia and his NPS was SARS-CoV-2 positive. On the same day, Patient#2, who was asymptomatic, also tested positive for SARS-CoV-2. The following day, both asymptomatic children were RT-PCR negative for SARS-CoV-2 in saliva and NPS samples. On day 7, Patient#2 developed COVID-19 symptoms including fever, myalgia, arthralgia and headache. On day 8, both asymptomatic children became SARS-CoV-2 positive. Both adults developed mild COVID-19 and remained RT-PCR positive for 21 and 25 days. Children remained asymptomatic throughout the study period and they had RT-PCR positive in saliva for 25 and 28 days. Viable viruses were detected in children by cell culture on days 8 and 17 **(Figure 1)**. Positive cell culture samples correlated with RT-PCR Ct values ranging from 22.3 to 33.4. In addition, stool samples were SARS-CoV-2 positive in both children for up to 21 and 28 days.

Viral sequencing in Patient#2 and both children showed 4 mutations corresponding to E484K, N501Y, D614G and H655Y, consistent with B.1.1.28.1 lineage (Variant Gamma or Variant P.1). Both asymptomatic children seroconverted and showed detectable SARS-CoV-2 anti-Spike IgG levels (65 and 227 UI/ml).

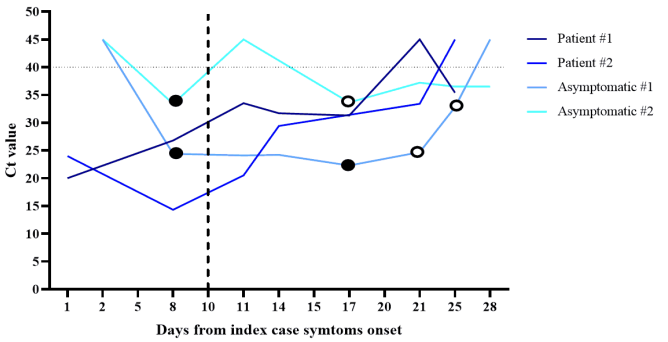

Figure 1: SARS-CoV-2 isolation and detection by RT-PCR in saliva and NPS samples.

Sequential samples of Patient #1, #2, Asymptomatic #1 and #2. Filled circle: positive culture. Empty circle: negative culture. Dotted vertical line: Theoretical de-isolation date. Ct value ≥ 40 are RT-PCR negative results.

Discussion

SARS-CoV-2 pandemic affects mostly adult patients and shedding time of viable SARS-CoV-2 has been well established. However, data on viable shedding in asymptomatic healthy children is lacking. In this study, we describe the presence of viable SARS-CoV-2 from saliva samples in two asymptomatic healthy children.

Given the low rate of infections in pediatrics, asymptomatic children, even those with close contacts to positive cases, are usually not screened for SARS-CoV-2. In this study, two asymptomatic children living with their infected parents who were prospectively followed, showed RT-PCR positivity and viable virus. This observation underscores the potential role of asymptomatic children in the spread of the virus, especially considering that most children remain asymptomatic [10].

Interestingly, the asymptomatic child with viable viral shedding for at least 17 days from index case's symptoms onset,

would have been potentially contagious beyond the isolation period that was suggested, in this moment, by the Ministry of Health in the region. The isolation period for asymptomatic close contacts of a positive case had been determined for 10 days from the case's symptoms onset.

Successful cell culture isolation was associated with Ct values lower than 23 [11]. In our study, isolation was successful even on samples with higher Ct values. This finding suggests that at least in children Ct value >23 cannot rule out the presence of viable virus. Murata et al. found similar results, from nasopharyngeal swab samples from an older adult who became infected with SARS-CoV-2 on a cruise ship [5]. In this study, sequence analysis demonstrated the presence of Gamma variant (lineage P1), which was circulating in Argentina in 2021, but was later displaced by Omicron variant [12]. Whether this variant remains contagious for longer periods or has a different kinetic in children is still to be determined.

RT-PCR in saliva samples was shown to be convenient and successful in detecting SARS-CoV-2 in symptomatic adult patients [7]. In our study, saliva samples were also useful in detecting SARS-CoV-2 in asymptomatic children. Furthermore, these samples were also useful for successful viral isolation in cell culture. As nasopharyngeal swabs can be painful and bothersome, particularly in children, saliva samples represent a more convenient, non-invasive and painless option [13,14]. Confirming the presence of true infections, both children were found to have anti-S IgG for SARS-CoV-2 in subsequent serum samples.

The main limitation of this study is that only two children were evaluated. Despite this limitation, our observation showed that the presence of viable virus in saliva samples from asymptomatic children can last for at least 10 days from the initial PCR positivity and can represent a source for spreading.

In summary, our observation underscores the importance of testing asymptomatic children since they can also shed viable virus for several days. Given the difficulties for obtaining nasal swabs in children, saliva samples can provide a reasonable alternative for detection of SARS-CoV-2.

Acknowledgment

We thank the study volunteers for providing the analyzed samples. This study was funded through grants Fundación "Norberto Quirno" N° 01/20; Fondo Nacional de Ciencia Tecnología e Innovación, Grant/Award Number: IP COVID19:938.

Conflicts of interest

MS is a consultant to Basilea, a speaker for Pfizer and the principal investigator in Argentina for NIH grant UM1AI104681. The rest of the authors have no conflict of interest.

References

1. Guo Z, Xiao D. Epidemiological analysis of asymptomatic SARS-CoV-2 transmission in the community: an individual-based model. Sci Rep. 2021; 11: 6251.

2. Sahbudak Bal Z, Ozkul A, Bilen M, Kurugol Z, Ozkinay F. The Longest Infectious Virus Shedding in a Child Infected With the G614 Strain of SARS-CoV-2. Pediatr Infect Dis J. 2021; 40: e263-65.

3. Fu Y, Han P, Zhu R, Bai T, Yi J, et al. Risk factors for viral RNA shedding in COVID-19 patients. Eur Respir J. 2020; 56.

4. Xu CLH, Raval M, Schnall JA, Kwong JC, Holmes NE. Duration of Respiratory and Gastrointestinal Viral Shedding in Children with SARS-CoV-2: A Systematic Review and Synthesis of Data. Pediatr Infect Dis J. 2020: E249-56.

5. Murata T, Sakurai A, Suzuki M, Komoto S, Ide T, et al. Shedding of Viable Virus in Asymptomatic SARS-CoV-2 Carriers. MSphere. 2021; 6: 1-7.

6. L'Huillier AG, Torriani G, Pigny F, Kaiser L, Eckerle I. Culture-Competent SARS-CoV-2 in Nasopharynx of Symptomatic Neonates, Children, and Adolescents. Emerg Infect Dis. 2020; 26: 2494-2497.

7. Echavarria M, Reyes NS, Rodriguez PE, Ypas M, Ricarte C, Rodriguez MP, et al.: Self-collected saliva for SARS-CoV-2 detection: a prospective study in the emergency room. J Med Virol. 2021:0-14.

8. Clinton R. Paden, Ying Tao, Krista Queen, Jing Zhang, Yan Li, Anna Uehara, et al. Rapid, Sensitive, Full-Genome Sequencing of Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis. 2020; 26: 2401-2405.

9. Ojeda DS, Lopez Ledesma MMG, Pallarés HM, Costa Navarro GS, Sanchez L, Perazzi B, et al.: Emergency response for evaluating SARS-CoV-2 immune status, seroprevalence and convalescent plasma in Argentina. PLoS Pathog. 2021; 17: 1-18.

10. Felsenstein S, Hedrich CM. SARS-CoV-2 infections in children and young people. Clin Immunol. 2020; 220.

11. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis. 2020; 71: 2663-2666.

12. Ministerio de Salud de la Nación Argentina: SITUACIÓN DE NUEVAS VARIANTES SARS-CoV-2 EN ARGENTINA. 2022.

13. Tutuncu EE, Ozgur D, Karamese M. Saliva samples for detection of SARS-CoV-2 in mildly symptomatic and asymptomatic patients. J Med Virol. 2021; 93: 2932-2937.

14. Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, et al.: Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study. Clin Microbiol Infect. 2020.