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Case Report

SARS-CoV-2 infection diagnosed only by cell culture isolation before the local outbreak in an Italian seven-week-old suckling baby

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ABSTRACT

SARS-CoV-2 emerged in China in December 2019 and has now been declared a pandemic by the World Health Organization. This paper described the case of a 7-week-old suckling baby from Italy who was SARS-CoV-2-positive only by the cell culture method, with no clinical suspicion of and/or risk factors for SARS-CoV-2 infection. The baby was referred to hospital, with signs and symptoms of upper respiratory tract infection, before the virus had spread to the province. Nasal and pharyngeal swabs and a nasopharyngeal aspirate were used for conventional and molecular diagnostic assays not including the SARS-CoV-2 virus. Bacteria referred to the resident population were revealed in nasal and pharyngeal swabs. No viruses were detected using both immunofluorescence assay and nucleic acid amplification assays in the nasopharyngeal aspirate. The baby was discharged in good condition after 3 days of hospitalisation. Later, a cytopathic effect on the cell monolayers currently used for respiratory viruses was observed and the viral particles were identified as *Coronaviridae* by transmission electron microscopy. SARS-CoV-2 was identified by RT-PCR performed both on cell culture and on the stored aliquot of the original sample. The virus isolate was named SARS-Cov-2/human/Parma/1/2020. Cell culture still remains the only reference diagnostic method also for emerging viruses, allowing it to reveal cytopathogenic viruses and demonstrate their infectivity.

Introduction

A novel coronavirus was initially detected in Wuhan, China (starting in December 2019) in patients with severe pneumonia of unknown origin (Chen and Yu, 2020). Genome sequencing allowed the virus to be classified into the subgenus *Sar-becovirus* of the genus *Betacoronavirus*; it was termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the illness it causes as coronavirus disease 2019 (COVID-19). On 11 March 2020 the World Health Organization declared SARS-CoV-2 a pandemic, considering that >118,000 cases of the coronavirus illness had been diagnosed in over 110 countries around the world (World Health Organization, 2020). The first case of SARS-CoV-2 infection in Northern Italy was declared on 7 February 2020 in a Chinese subject recently returned from Wuhan. The infection became epidemic in Northern Italy since 21 February 2020 when autochthonous cases were identified (Spiteri et al., 2020). At the date of manuscript submission, Italy was the second country with the highest number of SARS-Cov-2-infected subjects after the USA (Ministero della Salute, 2020).

In the area of Parma, where the current laboratory is located, SARS-CoV-2 became epidemic on 3 March 2020 and had reached 2083 cases on 3 April 2020. Older age and comorbidities were associated with more severe clinical pictures, while the clinical course was generally milder in younger people. Available data regarding the clinical symptoms of the infection were mostly obtained from the elderly or those aged >18 years (Salehi et al., 2020, Huang et al., 2020). It is believed that there is no literature evidence of SARS-CoV-2 virus infection, including virus

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isolation, in suckling babies and very little evidence for newborns (Lu and Shi, 2020, Wang et al., 2020). In the reported cases, laboratory diagnosis was only performed by molecular methods.

This paper describes the case of a 7-week-old suckling baby from Italy who was infected by SARS-CoV-2 virus presenting fever and polypnea.

Case report

A 7-week-old baby was referred to the Neonatology ward of the University Hospital of Parma (Italy) on the night of 25 February 2020 with a fever of 38 °C and vomiting at home. On hospital admission he showed mild leukopenia, fever of 37.5 °C, pharyngeal hyperaemia, inflammation of the upper respiratory tract, no signs and symptoms of pneumonia, and with normal serum inflammatory markers. Based on this clinical picture, an upper respiratory tract infection was postulated, making X-rays unnecessary. No suspicion and no specific risk factors of SARS-CoV-2 infection were formulated, as the virus had not yet spread to Parma province.

In order to assess the presence of respiratory bacteria and/or viruses, a nasopharyngeal aspirate, nasal swab and pharyngeal swab were sent to the Microbiology and Virology Units of the same hospital. The nasal and pharyngeal swabs were positive for *Staphylococcus aureus*. The nasopharyngeal aspirate was tested by: (i) immunofluorescence assay (IFA) to detect respiratory syncytial virus (RSV) antigens directly in sample exfoliated cells; (ii) the same technique after a 24-h incubation of inoculated cell monolayers grown on a chamber slide to detect influenza (A and B), parainfluenza (1–3), RSV-specific antigens (De Conto et al., 2019); and (iii) real-time PCR and real-time RT-PCR to detect nucleic acids of a large panel of respiratory viruses (adenovirus, influenza A virus H1 and H3, influenza B virus, parainfluenza virus 1–4, RSV A and B, metapneumovirus, bocavirus 1–4, rhinovirus A, B and C, enterovirus, coronavirus 229E, NL63 and OC43) by using the Allplex™ Respiratory Panels assay (Seegene INC., Seoul, Korea). All of these tests gave negative results. In parallel, the same sample was inoculated in cell monolayers to detect cytopathogenic respiratory viral agents (De Conto et al., 2019). On daily microscopic observation, a cytopathic effect was detected in VERO and LLC-MK2 cells at day 10 post-infection (Figure 1A–D). As a first identification level, electron microscopy of the inoculated cell supernatants after 1% formaldehyde inactivation, ultracentrifugation and negative staining, according to standard procedures (Calderaro et al., 2014), enabled the detection of viral particles belonging to the *Coronaviridae* family (Figure 1E).

Subsequently, an RT-PCR assay for detecting SARS-CoV-2 nucleic acid was performed according to the protocol of the Centers for Disease Control and Prevention of Atlanta, USA, which confirmed the presence of SARS-CoV-2 RNA at a low cycle threshold (N gene, 15.43 for VERO cell extract; N gene, 15.51 for LLC-MK2 cell extract). The concomitant presence of other respiratory viruses was excluded by applying the same molecular assay on the inoculated VERO and LLC-MK2 cell extracts. According to the naming convention proposed by the Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020), the cell culture isolate was named SARS-Cov-2/human/Parma/1/2020. The presence of SARS-CoV-2 RNA was assessed by the real-time RT-PCR assay retrospectively performed on the aliquot of the same patient's nasopharyngeal aspirate stored at −80 °C.

Only the culture isolation of this cytopathogenic agent allowed its final identification as SARS-CoV-2. The clinical characteristics of the case and the time period in which the baby came to the hospital did not allow suspicion of this specific infection, making it unnecessary to submit the baby to chest X-ray and CT scan. The baby was discharged from the hospital in a good condition on 28 February 2020, and remained healthy in the following weeks. Pharyngeal swabs over 2 consecutive days were performed by the Public Service of Health, 2 weeks after his discharge from the hospital, and both were negative for SARS-CoV-2 RNA.

The origin of infection of the baby remains unclear. In the period before admission to hospital the parents were healthy, although the mother had a slight malaise that did not stop her from breastfeeding. They had never been tested for SARS-CoV-2 because they were healthy until 17 March 2020. However, after the baby was discharged, they remained at home in self-isolation together with the baby.

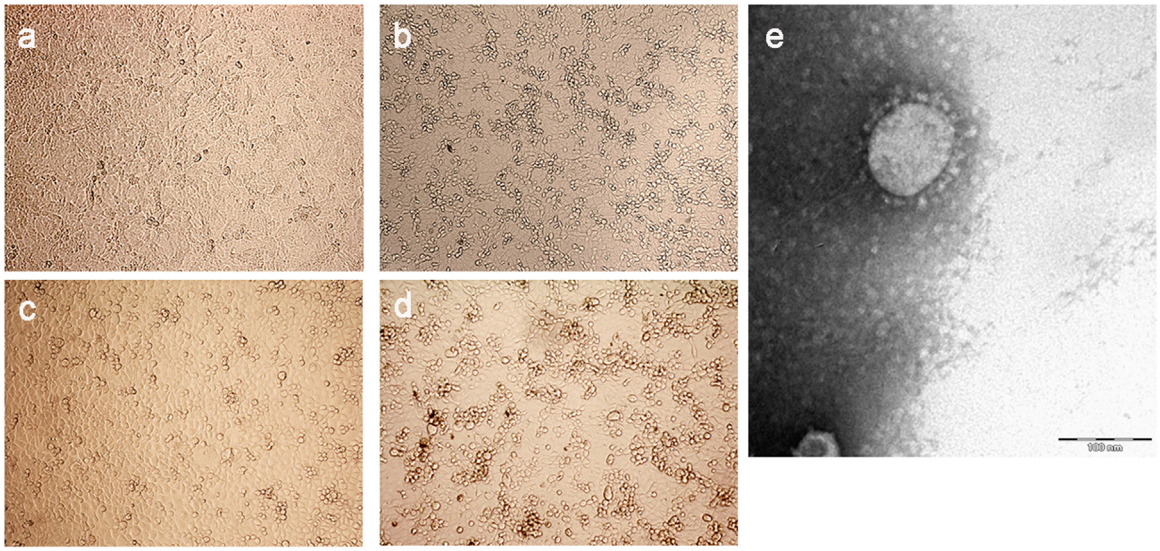

Figure 1. (A–D) VERO and LLC-MK2 cell monolayers were inoculated with the nasopharyngeal aspirate and observed daily to detect cytopathic effects. The images show control uninfected cells (a: VERO; c: LLC-MK2) compared with infected cells at day 10 post-infection (b: VERO; d: LLC-MK2). Magnification: 2000 ×. (e) Virion particle belonging to the *Coronaviridae* family observed by electron microscopy. The cell supernatants collected from inoculated VERO and LLC-MK2 cell monolayers were first fixed with 2% formaldehyde in phosphate buffered saline for 1 h before ultracentrifugation (1 h, 25,000 rpm) and preparation of the grid by negative staining. Bar: 100 nm.

Discussion

It is believed, in the international literature at the time of this manuscript submission, that there have been no other reports of infants of this age describing the laboratory diagnosis of SARS-CoV-2 infection, including virus isolation together with RNA detection.

The present results highlight the importance of viral culture to be used in parallel with molecular techniques, as it is the only reference laboratory method able to reveal the presence of cytopathogenic viral agents and demonstrate their infectivity in cases of emerging viruses. This is of relevance in those cases, such as the one described here, in which the symptoms are mild and the laboratory becomes an essential support to promptly report the presence of this pandemic virus for preventing its spread. This result shows that diagnosis should not be limited to molecular tools in laboratories that have the facility to perform *in vitro* virus cultivation to obtain different viral strains for further genomic comparative analysis, as this helps in clarifying the mechanisms involved in the limited virulence of SARS-CoV-2 in young people and babies. Moreover, this result could help to improve diagnosis using innovative tools such as the Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry assay for respiratory viruses (Calderaro et al., 2016). The cultivability of this agent also provides an important tool with which to obtain large amounts of virus for further studies, such as experimental trials for the development of new antiviral drugs and comparative genomic analyses with the SARS-CoV-2 viral strains isolated from adult and paediatric populations in different places. This may help to understand differences, if any, and to find out why young people are mildly affected.

Authors' contributions

AC designed the study. AC, MCA, FDC, MB, PM, SM, FF, FP performed laboratory experiments. AC, CC performed structural studies. AC, MCA, FDC, MB, CC collected data and samples. AC, FDC, CC, analysed data. AC, MCA, FDC, MB prepared the manuscript.

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Competing interests

The authors declare no competing interests.

Informed consent

The samples analysed in this study were sent to the University Hospital of Parma for routine diagnostic purposes, and the laboratory diagnosis results were reported in the medical records of the patients as answer to a clinical suspicion; ethical approval at the University Hospital of Parma is required only in cases in which the clinical samples are to be used for applications other than diagnosis.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.05.035.

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