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Case report

Sars-CoV-2 isolation from a 10-day-old newborn in Italy: A case report

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ABSTRACT

This report describes the evolution of COVID-19 in a 10 day-old-baby. The mother developed the disease immediately after childbirth and therefore a vertical transmission can be excluded. The isolation of the virus in cell culture with a cytopathic effect already visible after 48 h, indicates that the viral load of the newborn was quite high, but not serious course of the disease was observed. This paper wants to highlight the possible role of newborns and children in the spread of the disease.

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Introduction

It is not yet clear why children, and especially newborns, are less susceptible to COVID-19 than adults [1], despite viral infections being much more frequent from childhood onwards. One hypothesis is related to vaccinations that are carried out in the first weeks of life and which would favor an increase of a non-specific immunity related to an increase in interleukin-2 (IL-2) or other factors not yet known [2]. Probably some interactions between the newborn and the most common respiratory viruses of childhood would stimulate an innate resistance to RNA viruses [3]. Another hypothesis concerns angiotensin-converting enzyme 2 (ACE2), a membrane-bound aminopeptidase which is highly expressed in pulmonary alveolar epithelial cells and small

intestine enterocytes. Although ACE2 has been identified as a receptor for Sars-CoV-2, it is plausible that ACE2 tissue distribution differs between adults and children or that ACE2 binding capacity in children is lower than in adults [4].

One reason why infant do not develop severe forms of the disease could be due to the fact that the immune system of infants is not mature at all and therefore probably is not yet able to start the cytokine storm that occurs in COVID-19 infection of the adults [5]. In fact, from a clinical point of view, COVID-19 generally occurs in children with moderate or low fever accompanied by mild and non-specific symptoms. In some cases, gastrointestinal symptoms such as diarrhea, abdominal distension and aversion to food may occur in newborns [6].

Hypoxia and increased respiratory rate were recorded only in a small subgroup of children, which in severe cases does not respond to standard oxygen therapy and requires nasal cannula or mask. Based on the reported cases, most of these children have a benign course which resolves within 1–2 weeks after the onset of the disease. Infants with COVID-19 need more careful and cautious observation because they are more likely to present with non-specific symptoms such as lethargy and dehydration [4,7,8].

Case report

On 09 August 2020, a 34-year-old pregnant woman was admitted to the "Mater Dei" hospital (Bari, Apulia region, Italy) and

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46 following the standard procedures before the entrance was
47 subjected to the nasopharyngeal swab for Sars-CoV-2 detection,
48 resulting negative despite manifesting cold symptoms, but
49 without fever. Also an internal reaction control was used in order
50 to exclude a false negativity. On the same day the woman gave
51 birth to a child weighing 3.100 Kg. Three days later mother and son
52 came back home and after 10 days from the birth, the newborn
53 developed low-grade fever and mild respiratory symptoms. For
54 this reason he was transferred to the pediatric hospital "Giovanni
55 XXIII" (Bari, Apulia region, Italy), but he couldn't be hospitalized
56 because he was less than 30 days old. On 20 August 2020 he was
57 transferred to the neonatology department of "Miulli" hospital
58 (Acquaviva delle Fonti, Apulia region, Italy) for suspected sepsis
59 since he manifested fever with a temperature of 37.8 °C.
60 Immediately he was subjected to the following tests: complete
61 blood count, C reactive protein, film array for the detection of
62 Adenovirus, Coronavirus 229E-HKQ1-NL63-OC43, Metapneumo-
63 virus human, Rhinovirus, Enterovirus, Influenza A-B, Parainfluenza
64 1-2-3-4, RSV, Pertussis, Chlamydia and Mycoplasma pneumonia.
65 No lombar puncture was performed at the beginning, because the
66 newborn showed mild respiratory symptoms and therefore a
67 respiratory infection was suspected at first instance.

68 The values of the complete blood count were normal e C
69 reactive protein resulted negative. Moreover, was not revealed the
70 presence of respiratory pathogens. Finally, it was decided to
71 perform also the canonical nasopharyngeal swab to verify an
72 eventual Sars-CoV-2 infection.

73 The real time PCR specific for Sars-CoV-2 was performed by
74 Allplex 2019-nCoV Assay Kit (Seegene, Seoul, Korea) on the CFX96
75 Real Time System C1000 Thermal Cycler (Bio-Rad, Hercules,
76 California-USA). The test resulted positive showing the amplifi-
77 cation of the E gene with CT 9.7; RdRp gene with CT 13.3; and N
78 gene with CT 14.5. After the results, the newborn was transferred
79 to the infectious disease department of the pediatric hospital
80 "Giovanni XXIII" (Bari). The day after, on 21 August, all his four
81 family members (mother, father and two young brothers) were
82 also tested for Sars-CoV-2, because the father complained of
83 headache and sore throat and one of the children had 375 °C fever.
84 Mother, father and the older brother resulted positive to the test,
85 while the youngest one resulted negative, probable because in
86 those days he had not been in strict contact with his family, but he
87 was living temporarily with his maternal grandparents. It's
88 important to underline that the paternal grandmother during the
89 days following the birth of the child complained a strong
90 headache and cold flu symptoms and she went to visit the family
91 at their home. The old woman was subjected to Sars-CoV-2 swab
92 in the same day of the other relatives and she resulted to be
93 positive. Probably she was the person who infected the others.
94 Since that time all the family members were subjected to
95 mandatory quarantine under the direct responsibility of the local
96 health authorities.

97 The nasopharyngeal swab of the newborn was sent to the BSL-3
98 laboratory of the Istituto Zooprofilattico Sperimentale della Puglia
99 e della Basilicata (Foggia, Apulia region, Italy) for the isolation test.
100 Briefly, 500 µL of the swab medium was incubated with 250 µL of
101 an antibiotic solution for 1 h at room temperature. The suspension
102 was then inoculated in a T25 Cell Culture Flask containing a
103 monolayer of VeroE6 cells. The flask was incubated in a thermostat
104 at 37 °C for 1 h. After incubation, 5.5 mL of EMEM with 6 % fetal
105 bovine serum were added and incubated again at 37 °C. After 48 h
106 of incubation a quite good cytopathic effect was appreciable. After
107 further 24 h, an impressive cytopathic effect was highlighted
108 consisting of rounding and detachment of most of the cells (Fig. 1).
109 The replication of the virus was confirmed by the specific
110 biomolecular test for Sars-CoV-2 carried out on 200 µL of the
111 culture medium.

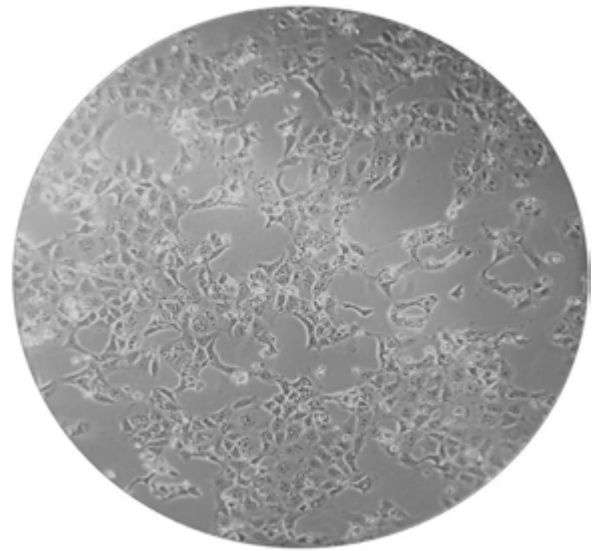


Fig. 1. Cytopathic effects consisting of rounding and detachment of cells in Vero E 6 cell cultures, 72 h after the inoculation of the virus from the swab medium.

112 The newborn manifested fever for 3 days following the
113 admission and then the health conditions resulted stable.

114 Discussion

115 The newborn almost certainly contracted Sars-CoV-2 from
116 one member of the family, presumably the paternal grandmoth-
117 er who, most likely, was infective in the days following the birth,
118 when she went to visit the newborn at his home. We do not
119 know if the newborn or another family member infected the
120 others, but it's very interesting that the viral load of the
121 newborn was very high as demonstrated either by PCR or by
122 isolation of the virus. The cytopathic effect on the Vero E6
123 monolayer was already evident after 48 h post inoculation and
124 was much clearer after 72 h (Fig. 1). Despite the high viral load,
125 the newborn never showed serious symptoms except mild
126 respiratory symptoms and a not high fever for 3 days following
127 the admission to the pediatric hospital. This case confirms what
128 has already been reported in other similar works, even in
129 presence of high viral loads, newborns usually show relatively
130 mild symptoms [9].

131 This case requires a reflection on the potentiality of Sars-
132 CoV-2 transmission from newborns and children that, maybe,
133 often is underestimated. Moreover, some traditional behaviors
134 of the Italian population and in particular of Southern Italy,
135 where it is customary to visit newborn children in the days
136 immediately following the return home, can amplify the
137 transmission of Sars-CoV-2 from unknown infected children.
138 In fact, newborns living inside home environment can spread-
139 large quantities of virus and lead to high levels of contamina-
140 tion, so as to be dangerous for anyone who comes into contact
141 with them. It's unlikely that that infant would generate effective
142 cough aerosols for infection, but however they can have
143 infectious secretions such as stool or saliva as reported in
144 literature [10,11].

145 Therefore, the aim of this paper is to report that also from Sars-
146 CoV-2 positive newborns is possible to isolate alive virus even in
147 presence of moderate symptoms, as showed by the isolation on cell
148 culture of this study, indicating that they could be dangerous for
149 the dissemination of the virus.

Ethical considerations

Written informed consent was obtained from the patient for the publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request. All authors have made substantial contributions to the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

CRediT authorship contribution statement

Giuseppe Lenoci: Conceptualization, Project administration, Supervision, Visualization, Writing - original draft. **Domenico Galante:** Conceptualization, Supervision, Visualization, Writing - original draft. **Edmondo Ceci:** Conceptualization, Supervision, Visualization, Writing - original draft. **Viviana Manzulli:** Data curation, Formal analysis, Investigation. **Angela Maria Moramarco:** Investigation, Formal analysis, Investigation, Methodology, Software, Validation. **Anna Chiaramonte:** Formal analysis, Investigation, Methodology, Software, Validation. **Giuseppina Labarile:** Formal analysis, Investigation, Methodology, Software, Validation. **Simone Lattarulo:** Formal analysis, Investigation, Methodology, Software, Validation. **Annalisa Resta:** Formal analysis, Investigation, Methodology, Software, Validation. **Lorenzo Pace:** Data curation, Formal analysis, Investigation, Methodology. **Valeria Rondinone:** Data curation, Formal analysis, Investigation, Methodology. **Antonio Parisi:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization. **Dora Cipolletta:** Data curation, Formal analysis, Investigation, Methodology. **Leonardo Marino:** Formal analysis, Investigation, Methodology. **Iolanda Padalino:** Formal analysis, Investigation, Methodology. **Luigina Serrecchia:** Formal analysis, Investigation, Methodology. **Angela Aceti:** Formal analysis, Formal analysis, Investigation, Methodology. **Michela Iatarola:** Formal analysis, Investigation, Methodology. **Francesco Tolve:** Formal analysis, Investigation, Methodology. **Antonio Fasanella:** Project administration, Supervision, Visualization, Writing - original draft.

Declaration of Competing Interest

The authors report no declaration of interests.

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