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# Higher viral RNA detection in placenta biopsies than maternal serum in a term infant born to a mother with confirmed SARS-CoV-2 infection

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**Abstract**
The SARS-CoV-2 outbreak was declared a pandemic by the WHO in March 2020. The neonatal population is thought to have a low incidence and severity of the disease, but maternal-to-fetus transmission has not been well studied. We present a term infant born to a mother with mild symptoms of laboratory confirmed SARS-CoV-2 infection seven days prior to cesarean delivery. Four placental biopsies tested by RT-PCR for SARS-CoV-2 were positive, while a nasopharyngeal swab and rectal swab were negative. The infant was not symptomatic. No anti-SARS-CoV-2 maternal antibodies were detected in the infant's sera through the 28th postnatal day.

**Keywords:** Neonate, maternal, placenta, SAR-CoV-2

## Introduction
The World Health Organization declared the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection to be a pandemic in March 2020. The elderly and patients with pre-existing comorbidities are at greatest risk for severe disease and death [1], while clinical symptoms in healthy children are comparatively mild [2, 3], and infections in infants are thought to be uncommon and present with non-specific symptoms. As with other viral infections, there is a possibility of maternal-fetal transmission in utero. Reported cases of newborn infants with SARS-CoV-2 infection raised concerns of vertical transmission, but postnatal contamination of samples with maternal secretions or feces at birth could not be ruled out.
Here we report the presence of the SARS-CoV-2 RNA on placental tissue samples from a term infant who was born to a mother with recent SARS-CoV-2 infection. Written informed consent was obtained from the mother for publication of this case report and any accompanying images.

## Case report
A 37-week gestation AGA Thai infant was born by elective cesarean section due to fetal growth restriction and maternal SARS-CoV-2 infection. The mother was a 20-year-old, previously healthy, primigravida from Samut Sakon province, the site of a large epidemic of SARS-CoV-2 infection in December 2020. Her antenatal care was initially uneventful and serology was unremarkable (VDRL titer non-reactive, anti-HIV antibody negative). She was diagnosed with fetal growth restriction at 25-weeks' gestation from sonographic parameters. Serologic investigation for TORCH infections was negative. A subsequent follow-up visit for fetal growth assessment suggested improvement.
At 35-weeks GA, she had close contact with a familial member who had mild upper respiratory tract symptoms and a positive real-time polymerase chain reaction (RT-PCR) to the SARS-CoV-2 RNA. Her first nasopharyngeal (NP) swab RT-PCR for SARS-CoV-2 was negative. However, a repeated NP RT-PCR 5 days later returned positive. She was admitted to Banphaeo General Hospital, a provincial tertiary-care center, for quarantine and delivery. The mother perceived regular active fetal movement and there was no sign of labour. She reported anosmia during home quarantine, but was afebrile and had no abnormal respiratory or gastrointestinal symptoms.

Her blood chemistry and chest x-ray were unremarkable.

On the 4th day after admission during the 36th week of gestation, the mother had irregular uterine contractions. A non-stress test revealed a normal fetal heart rate and a variable pattern. The amniotic membrane was intact. Following a multidisciplinary team consultation, an elective cesarean section was planned at 37-weeks GA. She was prescribed Favipiravir 1600 mg orally q 12h one day before undergoing cesarean section, and this was continued at 600 mg orally q 12h for another four days.

Cesarean section was performed uneventfully in a dedicated operating room. All health care providers donned full personal protective equipment to protect from aerosolized transmission. The amniotic fluid was clear. Following umbilical cord cutting, the infant was immediately separated to the next room for resuscitation. Umbilical cord blood was collected for SARS-CoV-2 antibody analysis. The 495 gram placenta appeared normal and was immediately placed inside a sterile, polyethylene bag in a cold storage container and sent for RT-PCR testing.

The female infant weighed 2265 grams at birth, was 46 cm in length, and her head circumference was 30 cm. The Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. She was placed in a closed incubator in a negative pressure room under full airborne isolation. The mother was not allowed contact with her infant. The infant's vital signs were T 36.6°C, pulse rate 150 bpm, respiratory rate 60 breaths/min, and the oxygen saturation was 97% in room air. She initially had borderline hypoglycemia (blood glucose was 44 mg/dL) which was resolved by the following day. She appeared well and her respiratory and gastrointestinal status were unremarkable. A chest X-ray that was performed on the 2nd day of life was also unremarkable.

The infant remained asymptomatic and was bottle-fed maternal breast milk. The mother was encouraged to wear a surgical mask and perform thorough hand washing before expressing her milk. A sample of the breast milk was sent for RT-PCR analysis. According to the national policy at time, this infant was separated from the mother who remained positive for SARS-CoV-2 infection. NP swab RT-PCR were performed on day-of-life (DOL) 2 and 3 and serum was obtained for RT-PCR for SARS-CoV-2 testing on DOL 3. She was discharged home on DOL 5. Universal metabolic, pulse oximetry, and hearing screening were normal. The mother was discharged after 5 days postpartum and was placed on home-quarantine. At the 28-day follow-up visit, both mother and infant were asymptomatic and the infant was gaining weight appropriately (weight 3,300 g, length 50 cm, and head circumference 33 cm). A final serum and nasopharyngeal swab work-up for SARS-CoV-2 infection was obtained during this visit.

**Quantitative analysis of SARS-CoV-2**

Placenta, blood, and other biological specimens were analyzed at the Thai Red Cross Emerging Infectious Diseases Health Science Centre, at the King Chulalongkorn Memorial Hospital, in Bangkok, Thailand. After thoroughly cleaning contaminated maternal blood from each surface with an absorbent sterile gauze, manual biopsies of 10x10x10 cu.mm were obtained from separate positions. Figure 1 (A and B) demonstrate the placental characteristics and sampling positions. One placental segment and a one cm-length of umbilical cord were sampled from the fetal side and three segments of placenta were removed from the maternal side. One fifth of each tissue was cut separately and put into a lysis buffer containing guanidine thiocyanate and immediately grinded using the FastPrep-24™ 5G tissue homogenizer (MP Biomedical, USA). The viral RNA was extracted using NUCLISENS® MINIMAG® kit (bioMérieux, France).

Viral RNA was extracted from the nasopharyngeal swab in viral transport media (VTM) using magLEAD® nucleic acid extraction kit following the manufacturer's instructions (Precision System Science, Japan). The presence of SARS-CoV-2 RNA was detected by real-time RT-PCR amplification of SARS-CoV-2 E, N and RdRp genes using RT-PCR kits (Allplex™ 2019-nCoV Assay, Seegene Inc., Korea). The cutoff as per the manufacturer's protocol is at Ct >40. Droplet digital PCR (Bio-Rad, USA) targeting the N gene was used to quantify the viral load of SARS-CoV-2 from different parts of the placenta (Table 1 to 3). The whole genome sequence of SARS-CoV-2 was conducted from the positive NP swab collected seven days pre-delivery, and from placenta tissue collected at the fetal side (tissue no. 2, Table 3) using the Respiratory Virus Oligo Panel library preparation and MiSeq sequencer (Illuminar, USA). The sequence was submitted to GISAID (accession number EPI_ISL_1117527and EPI_ISL_1117528 for placenta and NP swab, respectively.

Antibody responses to SARS-CoV-2 from all samples were analyzed for neutralization (NT), IgM, and IgG antibodies by the ELISA method using a commercial kit (Genescript, USA). Circulating NT antibodies against SARS-CoV-2 that block the interaction of the receptor binding domain (RBD) of the viral spike glycoprotein with the ACE2 cell surface receptor was determined and calculated as % inhibition. According to the manufacturer's protocol, the NT antibody level cutoff was 20% and the titer level for convalescent plasma is higher than 68% inhibition [4]. (Table 4).

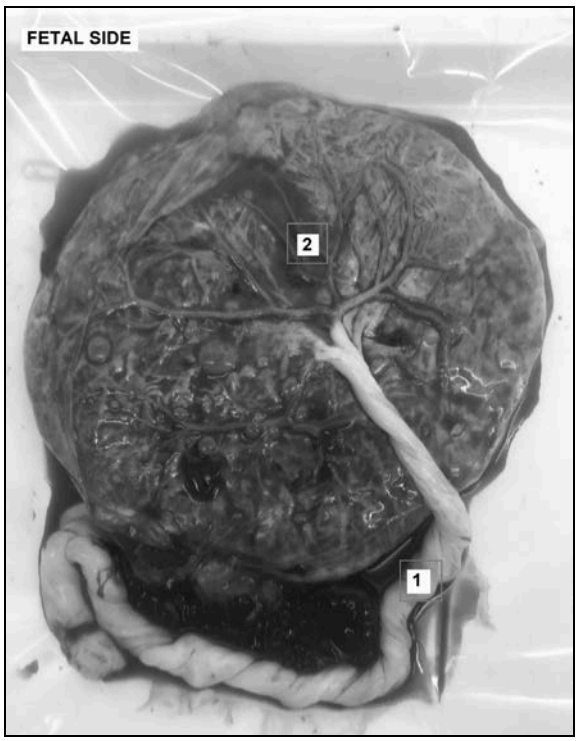


**Fig 1:** Timeline of maternal SARS-CoV-2 infection and investigations of the mother and infant

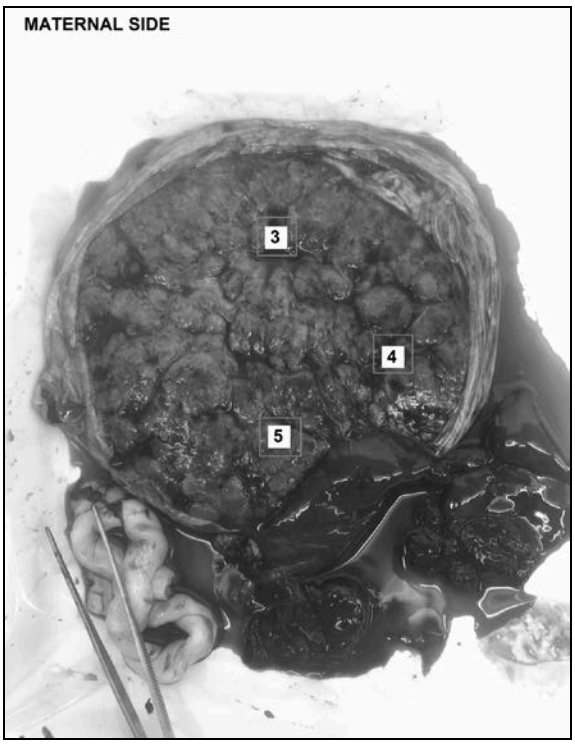


**Fig 2:** Placental biopsy sites (a) fetal side, and (b) maternal side

**Table 1:** Maternal samplings RT-PCR analysis for SARS-CoV-2

| Target Genes | Pre-delivery | | | Postpartum | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 days | 7 days | 1 day | 2 days | 5 days | | 28 days | |
| | NP swab | NP swab | Serum | NP swab | Feces | Breast milk | Serum | NP swab |
| E (Ct) | Neg | 18.24 | 29.92 | 20.62 | 34.42 | Neg | Neg | Neg |
| RdRP (Ct) | Neg | 20.67 | 33.46 | 24.64 | 35.30 | Neg | Neg | Neg |
| N (Ct) | Neg | 20.88 | 31.41 | 23.87 | 34.10 | Neg | Neg | Neg |
| Interpretation | Not detected | Detected | Detected | Detected | Detected | Not detected | Not detected | Not detected |

Viral RNA was extracted from the nasopharyngeal swab in viral transport media (VTM) using magLEAD® nucleic acid extraction kit following the manufacturer's instructions (Precision System Science, Japan). Ct cutoff <40 was considered positive

(Abbreviations: Ct: Cycle threshold; E, *envelope*; RdRP, *RNA dependent RNA polymerase*; N, *nucleocapsid protein*; Neg, negative; NP, nasopharyngeal)

**Table 2:** The infant's samplings RT-PCR analysis for SARS-CoV-2

| Target Genes | Umbilical cord blood | DOL 2 | | DOL 3 | | DOL 28 |
|---|---|---|---|---|---|---|
| | | NP swab | Serum | NP swab | Rectal swab | NP swab |
| E (Ct) | Neg | Neg | Neg | Neg | Neg | Neg |
| RdRP (Ct) | Neg | Neg | Neg | Neg | Neg | Neg |
| N (Ct) | Neg | Neg | Neg | Neg | Neg | Neg |
| Interpretation | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |

Ct cutoff <40 was considered positive

(Abbreviations: Ct: Cycle threshold; E, *envelope*; RdRP, *RNA dependent RNA polymerase*; N, *nucleocapsid protein*; DOL, day-of-life; Neg, negative; NP, nasopharyngeal;

**Table 3:** Placental samplings RT-PCR analysis for SARS-CoV-2

| Target Genes | Fetal side** | | Maternal side** | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| E (Ct) | 30.53 | 18.06 | 29.58 | 30.10 | - |
| RdRP (Ct) | 35.60 | 23.03 | 32.67 | 36.78 | - |
| N (Ct) | 31.30 | 19.94 | 29.06 | 30.88 | - |
| N*/Conc. (copies/ul) | 53.3 | 609,855 | 1,922 | 483 | - |
| Interpretation | Detected | Detected | Detected | Detected | Not detected |

Ct cutoff <40 was considered positive

*Viral load was detected by ddPCR targeted N gene, ** biopsy positions as shown in Fig 1 (A and B).

(Abbreviations: Ct: Cycle threshold; E, *envelope*; RdRP, *RNA dependent RNA polymerase*; N, *nucleocapsid protein*; DOL, day-of-life; NP, nasopharyngeal;

**Table 4:** Serum antibody analysis for SARS-CoV-2

| | Maternal serum | | Umbilical cord blood | Infant's serum | |
|---|---|---|---|---|---|
| | 1 day PTD | 28 days PP | | DOL 3 | DOL 28 |
| IgM Ab | Pos | Pos | Neg | Neg | Neg |
| IgG Ab | Pos | Pos | Neg | Neg | Neg |
| Neutralizing Ab* | Pos (33.06%) | Pos (91.26%) | Neg | Neg | Neg |

*Neutralizing antibody cutoff > 20% was considered positive

(Abbreviations: Ab, antibody; DOL, day-of-life; Ig, immunoglobulin; Neg, negative; Pos, positive; PP; postpartum; PTD, prior-to-delivery)

## Discussion

The mechanism and outcomes of infants exposed to maternal SARS-CoV-2 infection in-utero is not fully understood in terms of the route of transmission and the likelihood of congenital infection. Neonatal infants are thought to have a low incidence of infection and mild disease from SARS-CoV-2 infection [2]. One possibility is that the transferred maternal antibody may protect the fetus from serious illness [5], but maternal antibody takes several days to reach protective levels during which time the fetus would be at risk for congenital infection. In addition to postnatal infection from caregivers [6], case reports suggest the possibility of vertical transmission in newborn infants, most of whom were diagnosed using pharyngeal swabs [7-11]. It is unclear whether those infections resulted from maternal-fetal transmission in-utero or contamination with the mother's secretions at birth [12].

Besides pharyngeal swabs, a number of other sample sites from infected mothers have been analyzed to detect evidence of in-utero transmission. Chen *et al.* investigated amniotic fluid, breastmilk, and umbilical cord blood, but were unable to detect the SARS-CoV-2 virus or antibody [13]. Wang *et al.* investigated placenta, amniotic fluid, umbilical cord blood, gastric aspirate, and infant urine and feces which were also negative [14]. Interestingly, Penfield *et al.* demonstrated SARS-CoV-2 nucleic acid in placental swabs [15, 16], but acknowledged the possibility of contamination of maternal blood or secretion on the swab surface.

We found evidence of infection with SARS-CoV-2 RNA in placental and umbilical cord biopsies seven days after maternal onset of the disease. Although the RNA could have originated from maternal blood, we took steps to prevent such contamination. For example, samples were taken from multiple sites following surface blood removal and the interior of each biopsy segment was selected for analysis. Moreover, RNA was detected in 4 of 5 placental biopsy segments and the number of copies, particularly on the fetal side (Table 3), was higher than maternal serum (Table 1). Therefore, it is unlikely that contaminated maternal blood was the source of the RNA. Instead, the high levels of SARS-CoV-2 RNA we detected in the placenta were likely the result of the increased expression of angiotensin-converting enzyme 2 receptor at the maternal-fetal cell interface [16, 17].

The whole genome sequence of SARS-CoV-2 from the maternal NP swab specimen collected seven days before delivery and from the fetal side of the placenta are almost identical (2 of 29,847 nucleotides synonymous substitutions were found). These two substitutions were likely due to intra-host variation and the evolution dynamics of SARS-CoV-2 [18]. Despite a higher viral load on the fetal side and the absence of maternal transferred antibodies, our infant had no clinical signs of infection. This finding is similar to another case report of a maternal SARS-CoV-2 infection within seven days of delivery [13].

We followed the infant for 28 days and found no evidence of neonatal infection. This suggests that in-utero vertical transmission from the infected mother to the fetus is possible/probable/likely. Given the high viral load in the placenta, the organ represents a barrier to viral invasion of the fetus but the precise physiological mechanism of this protection remains unknown [19].

However, Vivanti *et al.* reported clear evidence of transplacental SARS-CoV-2 transmission in neonates [20]. Maternal and fetal factors associated with congenital infection merit further investigation.

Flennery *et al.* demonstrated maternal antibodies specific to SARS-CoV-2 in cord blood that could protect against vertical infection, but such antibody required time between the maternal onset of the disease and delivery of the neonate [5]. In our study, maternal antibody was positive on the first

day PTD but at non-protective levels (33.06% inhibition), rising to 99.26% at 28 days postpartum (Table 4). That our infant was seronegative from cord blood serum could be explained by the short time between the onset of maternal infection and delivery, and by the low levels of maternal antibody at delivery. Therefore, it is possible that an infant born to a mother with a SARS-CoV-2 infection shortly before delivery would be at low risk for vertical transmission.

In summary, SARS-CoV-2 can infect the placenta and potentially result in maternal-fetal transmission. While we did not detect maternal transfer antibodies, the infant was not infected. Factors associated with vertical infection of SARS-CoV-2 warrant further investigation.

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**Conflict of Interest**

The authors have no conflict of interest.

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