

Necrotising enterocolitis in infants with *in-utero* SARS-CoV-2 exposure in Tshwane, South Africa

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Background. Literature suggests an association between *in-utero* SARS-CoV-2 exposure and necrotising enterocolitis (NEC).

Objective. To determine whether admitted infants exposed to SARS-CoV-2 *in utero* had a higher NEC incidence than unexposed infants; and to describe the overall incidence of NEC during the COVID-19 pandemic to explore potential associations.

Methods. The evaluation period included 1-year pre-COVID-19 (April 2019 - March 2020) and a 2-year COVID-19 period (April 2020 - March 2022). An electronic database was used to identify infants and to collect routine data. Additional databases were used to identify mothers with COVID-19 and SARS-CoV-2-exposed neonates.

Results. All infants exposed to SARS-CoV-2 *in utero* tested negative for COVID-19 before admission, indicating no vertical transmissions. Among 32 SARS-CoV-2-exposed infants, one developed NEC (incidence 3.1%), compared with 2.5% in unexposed infants ($p=0.834$). NEC incidence was similar across periods (1.8% v. 2.5%; $p=0.147$). The median day of life of NEC diagnosis was 12 days for both periods. The sex distribution of infants with NEC was similar during both periods.

Conclusion. While this study did not demonstrate a statistically significant increase in NEC incidence during the COVID-19 pandemic, a potential association cannot be excluded, owing to the potential pathophysiological mechanisms that support this hypothesis.

Keywords. COVID-19; NEC; necrotising enterocolitis.

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Necrotising enterocolitis (NEC) is a gastrointestinal emergency primarily affecting premature infants.^[1,2] Risk factors include prematurity, low birthweight, intrauterine growth restriction and feeding with non-human milk.^[2] Incidence is inversely proportional to gestational age (GA) and birthweight,^[2] with an incidence of 7 - 11% in very-low-birthweight (VLBW) infants.^[1] The age of onset is inversely proportional to GA, with lower GA linked to later onset of NEC.^[1] Mortality ranges between 20% and 40%, and is higher in severe disease.^[1,2]

In utero exposure to, and/or postnatal infection with, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been associated with the development of NEC as reported in multiple studies.^[3-12] The SARS-CoV-2 virus is responsible for the clinical condition named coronavirus disease of 2019 (COVID-19). Eight studies reported on NEC in SARS-CoV-2-exposed infants,^[3-5,7-9,11,12] while four studies reported on NEC in SARS-CoV-2-positive infants.^[6-8,10] Of 16 infants, 62.5% ($n=10$) were premature.^[3,4,7-9,11,12] NEC onset ranged from 26 hours of life to seven weeks of age,^[3,10] with seven infants developing NEC in the first week of life.^[3-5,8,9] Pneumatosis intestinalis was the most common diagnostic sign,^[3,4,6,9,10] while two studies reported pneumoperitoneum only.^[5,8] A similar association was proposed in the 1980s, when coronavirus-like particles were found in NEC-affected gastrointestinal tissue samples.^[13]

The exact mechanism linking SARS-CoV-2 exposure and/or infection to NEC in infants is unclear, but it is hypothesised to be immune-mediated,^[3] as SARS-CoV-2 triggers a disproportionate inflammatory response, impaired adaptive immunity, and production of auto-antibodies.^[11] Jin *et al.*^[11] propose a persistent

GIT viral reservoir and inflammation post *in-utero* exposure, despite negative nasal reverse transcription-polymerase chain reaction (RT-PCR) tests after delivery. Liu *et al.*^[12] also suggest ongoing inflammation in SARS-CoV-2-exposed infants as IL-6 values were elevated. Additionally, angiotensin-converting enzyme 2 (ACE 2), the SARS-CoV-2 receptor, is abundant in the small intestine and the colon, possibly contributing to this association.^[6]

Further studies are required to investigate associations between maternal SARS-CoV-2 infection during pregnancy and NEC in infants. The present study aimed, firstly, to identify *in-utero* SARS-CoV-2-exposed infants in a tertiary neonatal unit to determine whether NEC incidence was higher than in SARS-CoV-2-unexposed infants. Secondly, the study compared the overall NEC incidence prior to and during the COVID-19 pandemic, possibly implying a link between *in-utero* SARS-CoV-2 exposure and NEC. This study also examined changes in sex distribution and age of NEC onset between these periods.

Methods

This research formed part of the Tshwane Maternal-Child COVID-19 study in the Tshwane District, Gauteng Province, South Africa. Ethics approval was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (reference: 822/2020). Approval was also granted by the National Health Research Database (reference: GP_202011_076) with all necessary institutional permissions. No identifying data were published, and in accordance with the ethics approval, patient consent was not required.

The period of evaluation was April 2019 to March 2022, including 1 year pre-COVID-19 (April 2019 - March 2020) and 2 years during the COVID-19 pandemic (April 2020 - March 2022). This latter period included four COVID-19 waves, namely 07 June - 22 August 2020, 15 November 2020 - 06 February 2021, 09 May - 18 September 2021, and 28 November 2021 - 05 February 2022, respectively. During this time, pregnant women with suspected or confirmed SARS-CoV-2 infection delivered at a neighbouring district hospital, designated for COVID-19. Hospitalised neonates born to these mothers were transferred to the tertiary-level neonatal unit after testing negative via rapid antigen or RT-PCR tests; rapid tests were confirmed by RT-PCR. All infants admitted to the neonatal unit were eligible for inclusion and infants remaining with their mothers at the neighbouring district hospital were excluded. Admissions and routinely collected data were obtained from the unit's electronic database. Infant feeding data were not available during the pre-COVID-19 and first pandemic year and were therefore not analysed.

During the COVID-19 pandemic, this tertiary hospital established a Google-based electronic paediatric and maternal SARS-CoV-2 testing database for all new admissions and lodging mothers. This database, the existing electronic neonatal unit's database, and the local infection control communication group were used to identify COVID-19-positive mothers and SARS-CoV-2-exposed neonates. Additionally, hospital records of infants transferred from the district hospital were retrieved to confirm or exclude *in-utero* SARS-CoV-2 exposure. The submitted National Surveillance facility-specific 'COVID-19 monitoring list' and the 'In-hospital COVID-exposed neonates data sheet' were also used to identify SARS-CoV-2-exposed neonates. In cases where maternal hospital numbers were available, the National Health Laboratory System was also used to check SARS-CoV-2 results to determine neonatal SARS-CoV-2-exposure. *In-utero* exposure was defined as a positive maternal SARS-CoV-2 rapid antigen or RT-PCR test during pregnancy or on the day of delivery. Infants exposed postnatally were excluded from *in-utero* exposure analysis. NEC analysis only included infants born during the study period. The number of admissions and birthweight data from both periods were collected from the neonatal unit's electronic admission records. Abdominal radiographs used to diagnose radiographic NEC (grade 2 and 3 using the Modified Bell's criteria) were reviewed on the hospital's imaging system by three resident neonatologists; uncertain cases were confirmed using hospital records. Additionally, from January 2021, the neonatal electronic database was used to identify cases of NEC. During the COVID-19 period, infants with additional NEC risk factors – cyanotic congenital heart disease (CCHD) ($n=2$), moderate to severe hypoxic-ischaemic encephalopathy (HIE) ($n=4$), or those referred with NEC from another hospital ($n=2$) – were excluded. The same criteria were applied to the pre-COVID-19 period, with eight NEC referrals excluded. An additional two NEC cases were excluded as they were born before the study period (March 2019).

For statistical analysis, continuous variables were checked for normality using the Shapiro-Wilk test and presented as means and standard deviations (SD) for normally distributed data or medians and interquartile ranges (IQRs) for skewed data. Data for the 2-year COVID-19 period were compared with the pre-COVID-19 period using a 2-sided z -test. A p -value <0.05 was considered statistically significant.

Results

There were 1 197 neonatal admissions during the one-year pre-COVID-19 period and 1 924 admissions during the two-year COVID-19 period. Fig. 1 outlines the included and excluded neonates during these two periods.

***In-utero* SARS-CoV-2 exposure during the COVID-19 period and the incidence of necrotising enterocolitis in SARS-CoV-2-exposed infants**

During the COVID-19 pandemic, 32 infants with *in-utero* SARS-CoV-2 exposure were identified using the methods described above. All infants with *in-utero* SARS-CoV-2 exposure were tested for COVID-19 using a SARS-CoV-2 RT-PCR, with no cases of *in-utero* SARS-CoV-2 transmission.

Among the 32 infants with documented *in-utero* SARS-CoV-2 exposure, one developed NEC, with an incidence of 3.1%. Of the 1 892 unexposed infants, 48 developed NEC, with an incidence of 2.5%. Although the NEC incidence was higher in the exposed group, the difference was not statistically significant ($p=0.834$). NEC incidence increased with decreasing birthweight, but no significant differences were found between exposed and unexposed infants across different weight categories (see Table 1).

Overall incidence of necrotising enterocolitis: Comparing pre-COVID-19 and COVID-19 periods

During the pre-COVID-19 period, 21 infants developed NEC, with an overall incidence of 1.8%. A total of 49 infants were diagnosed with NEC during the 2-year COVID-19 period, with an overall incidence of 2.5%. This difference was not statistically significant ($p=0.147$). The incidence of NEC during the four COVID-19 waves and prior to each of the corresponding waves was analysed. Although the incidence was higher during the first three waves compared with the pre-wave periods, this was not statistically significant (3.2% v. 1.5%, $p=0.298$; 3.5% v. 2.9%, $p=0.726$; 2.8% v. 1.1%, $p=0.134$, respectively). However, during the fourth wave, there was a higher incidence in the pre-wave period compared with the fourth wave, although this was not statistically significant (4.1% v. 2.7%, $p=0.435$).

Necrotising enterocolitis cases by epidemiological week of birth and epidemiological week of diagnosis

Fig. 2a shows the number of NEC cases per epidemiological week of birth during the pre-COVID-19 and COVID-19 periods (corresponding with the period of potential *in-utero* SARS-CoV-2 exposure). Overall, the number of cases per week was not significantly different for the pre-COVID-19 and COVID-19 periods (0.4 v. 0.5; $p=0.490$). The COVID-19 period was also analysed to determine whether there was a difference in cases per week in the pre-wave periods compared with the corresponding four waves. Although the cases per week were higher in the first, second and third waves compared with the corresponding pre-wave period, this was not statistically significant (0.5 v. 0.3 ($p=0.465$); 0.6 v. 0.4 ($p=0.412$); and 0.5 v. 0.2 ($p=0.095$), respectively). Conversely, the number of cases per week was significantly higher in the pre-wave four period compared with the fourth wave (0.9 v. 0.5; $p=0.031$). Fig. 2b shows the number of NEC cases per epidemiological week of diagnosis of NEC. However, no trends could be identified.

Birthweights of infants with necrotising enterocolitis

The median (IQR) birthweights of infants with NEC during the pre-COVID-19 and COVID-19 periods were not significantly different (1 118 g (920 - 1 662) v. 1 324 g (1 059 - 1 795); $p=0.242$). Birthweight categories were also compared between the pre-COVID-19 and COVID-19 periods. In summary, NEC was more common in infants weighing 1 000 g - 1 499 g and ≥ 500 g during the COVID-19 period compared with the pre-COVID-19 period, but there were no statistically significant differences for any

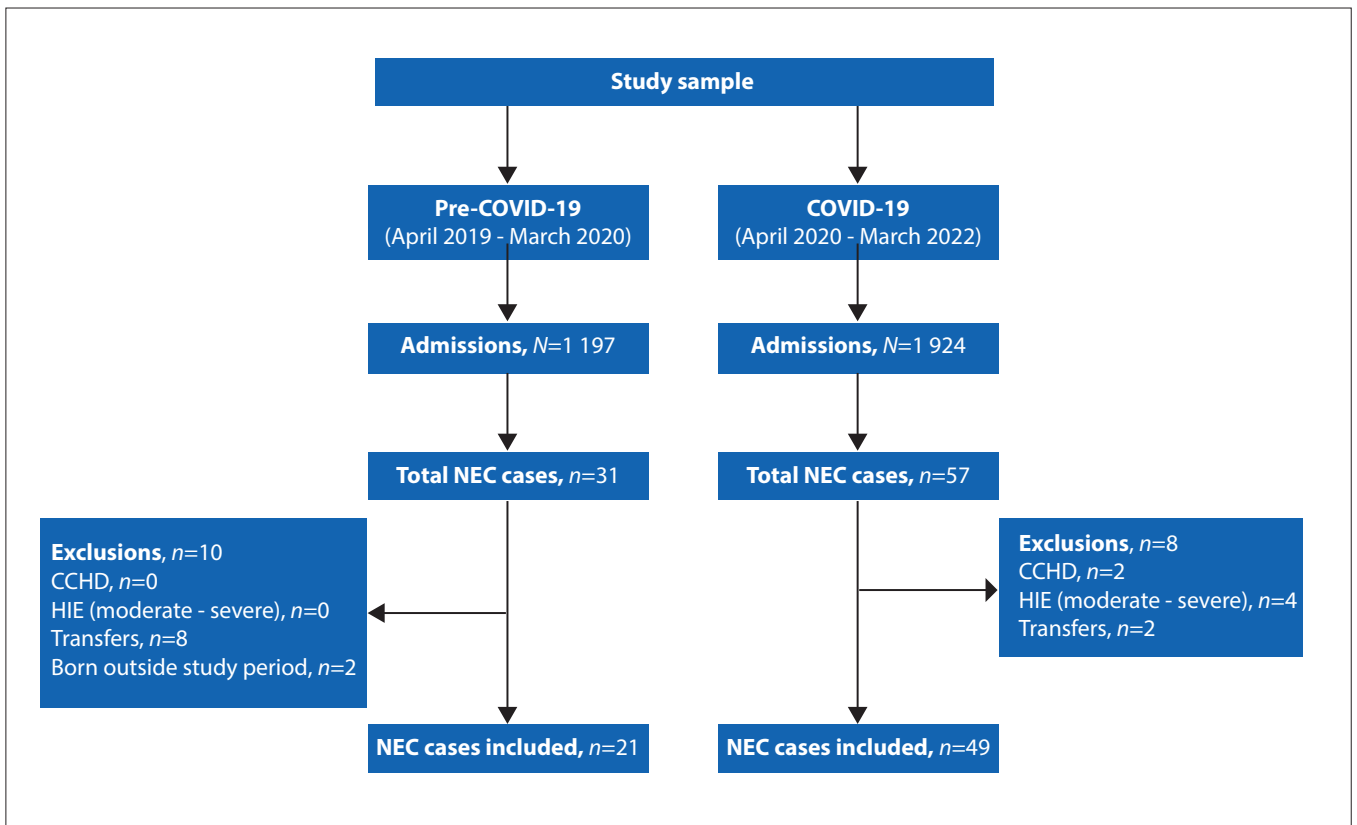


Fig. 1. Study sample for the pre-COVID-19 and COVID-19 periods, with exclusions shown. (CCHD, n=2; HIE, n=4; transfers, n=10; born outside study period, n=2.) (CCHD = congenital cyanotic heart disease; NEC = necrotising enterocolitis; HIE = hypoxic-ischaemic encephalopathy)

of the birthweight categories (≥ 2500 g: 6.1% v. 0.0% ($p=0.246$); 1500 g - 2499 g: 28.6% v. 28.6% ($p>0.999$); 1000 g - 1499 g: 40.8% v. 33.3% ($p=0.555$); <1000 g: 24.5% v. 38.1% ($p=0.250$)).

Day of life at diagnosis of necrotising enterocolitis and distribution by two time frames

The age of onset of NEC per infant is shown in Fig. 2. The median day of life of NEC diagnosis was 12 days for both the pre-COVID-19 and COVID-19 periods (IQR 9 - 25 and 7 - 21, respectively). Analysis was performed for the distribution of NEC cases pre-COVID-19 compared with the distribution of NEC cases during the COVID-19 period for two-time frames: <7 days of life (very early) v. ≥ 7 days of life, and <14 days of life (early) v. ≥ 14 days of life (Table 2).

The diagnosis of very early NEC <7 days during the COVID-19 period increased compared with the pre-COVID-19 period (22.4% v. 4.8%), but this was not statistically significant ($p=0.072$). Additionally, no statistically significant difference was found for the time frame <14 days v. ≥ 14 days ($p=0.960$). The same data were analysed per weight category, with none showing any significant difference (Table 2). During the pre-COVID-19 period, no infants weighing ≥ 2500 g developed NEC, compared with three infants during the two-year COVID-19 period (statistical significance not calculated owing to low numbers).

Sex comparison in all infants with necrotising enterocolitis

Analysis of a change in sex distribution during the pre-COVID-19 period compared with the COVID-19 pandemic showed no statistically significant differences (males 47.6% v. 36.7%, $p=0.395$ and females 52.4% v. 63.3%, $p=0.395$, respectively).

Outcome of infants with necrotising enterocolitis

The outcome of death before discharge in infants with NEC during the pre-COVID-19 and COVID-19 periods was also compared. However, there was no statistically significant difference (38.1% v. 36.7%, respectively) ($p=0.912$).

Discussion

The present study did not find an association between *in-utero* SARS-CoV-2-exposure and NEC. There was also no difference in the incidence of NEC during the pre-COVID-19 and COVID-19 periods.

Incidence of necrotising enterocolitis in SARS-CoV-2-exposed infants

As reported in the ten available publications regarding NEC and COVID-19 infection, a total of 16 infants developed NEC, which was believed to be related to SARS-CoV-2 exposure or infection.^[3-12] In the present study, the incidence of NEC was higher in the *in-utero* SARS-CoV-2-exposed infants, however, this was not statistically significant (3.1% v. 2.5%; $p=0.834$). This is however much higher than the incidence of NEC in SARS-CoV-2-exposed infants reported by Nayak *et al.* of 0.6% overall ($n=1/165$) and 2.6% in infants admitted to the NICU ($n=1/38$).^[8] In low-birthweight infants (birthweight <2500 g) Angelika *et al.*^[7] reported an incidence of NEC in SARS-CoV-2-exposed infants of 4.0% ($n=5/125$), whereas there was a higher incidence of NEC in SARS-CoV-2-exposed infants in the same weight category (5.6%) in the present study.

However, it is very likely that *in-utero* SARS-CoV-2 exposure was grossly underreported in this study, as George *et al.*^[14] reported a seroprevalence of SARS-CoV-2 antibodies of 30.8% (95% CI 25.6 - 36.3) in 2020 in women attending antenatal care in

Table 1. Necrotising enterocolitis by *in utero* COVID-19 exposure including weight categories (N=1 924)*

	Documented <i>in-utero</i> SARS-CoV-2 exposure (n=32) [†]	No documented <i>in-utero</i> SARS-CoV-2 exposure (n=1 892) [†]	p-value
All infants			
NEC (n)	1	48	
No NEC (n)	31	1 844	
Incidence (%)	3.1	2.5	0.834
<2 500 g (n=1 213) [‡]	n=18	n=1 195	
NEC (n)	1	45	
No NEC (n)	17	1 150	
Incidence (%)	5.6	3.8	0.697
< 1 500 g (n=398) [‡]	n=5	n=393	
NEC (n)	1	31	
No NEC (n)	4	362	
Incidence (%)	20.0	7.9	0.322
< 1 000 g (n=115)	n=4	n=111	
NEC (n)	1	11	
No NEC (n)	3	100	
Incidence (%)	25.0	9.9	0.332

NEC = necrotising enterocolitis.

*11 infants had no weight recorded on the neonatal unit's electronic database and were excluded from the weight category analysis (all infants with NEC had weight data available).

[†]Includes category < 1 500 g.

[‡]Includes category < 1 000 g.

Table 2. Necrotising enterocolitis onset during the pre-COVID-19 period and the 2-year COVID-19 period

	Pre-COVID-19 period, n/N (%)	COVID-19 period, n/N (%)	p-value
All infants			
NEC <7 days	1/21 (4.8)	11/49 (22.4)	
NEC ≥7 days	20/21 (95.2)	38/49 (77.6)	0.072
NEC <14 days	11/21 (52.4)	25/49 (51.0)	
NEC ≥14 days	10/21 (47.6)	24/49 (49.0)	0.960
≥ 2 500 g			
NEC <7 days	0	3/3 (100)	
NEC ≥7 days	0	0	-
NEC <14 days	0	3/3 (100)	-
NEC ≥14 days	0	0	
1 500 - 2 499 g			
NEC <7 days	1/6 (16.7)	6/14 (42.9)	
NEC ≥7 days	5/6 (83.3)	8/14 (57.1)	0.259
NEC <14 days	4/6 (66.7)	11/14 (78.6)	
NEC ≥14 days	2/6 (33.3)	3/14 (21.4)	0.576
1 000 - 1 499 g			
NEC <7 days	0	1/20 (5.0)	
NEC ≥7 days	7/7 (100)	19/20 (95.0)	0.549
NEC <14 days	6/7 (85.7)	8/20 (40.0)	
NEC ≥14 days	1/7 (14.3)	12/20 (60.0)	0.062
< 1 000 g			
NEC <7 days	0	1/12 (8.3)	
NEC ≥7 days	8/8 (100)	11/12 (91.7)	0.401
NEC <14 days	1/8 (12.5)	3/12 (25.0)	
NEC ≥14 days	7/8 (87.5)	9/12 (75.0)	0.497

NEC = necrotising enterocolitis.

9.4% of NEC cases pre-COVID-19 were in term infants. All three SARS-CoV-2-exposed infants who developed NEC in the study by Wu *et al.*^[4] were of term gestation. This was similar to findings in the present study, where three infants weighing ≥ 2 500g (GA 37 weeks, 38 weeks and 39 weeks) developed NEC during the COVID-19 period (6.1%; $n=3/49$), compared with no infants ≥ 2 500 g developing NEC in the pre-COVID-19 period. Although we cannot confirm SARS-CoV-2 exposure in these infants, the number of NEC cases in larger infants in this unit was higher than expected when compared with current data on NEC in inborn infants > 2 500 g without additional risk factors in this unit over the past two years (2023 and 2024) (4.2%; $n=1/24$).^[17]

Overall incidence of necrotising enterocolitis during the COVID-19 period

A Swedish study^[18] demonstrated an overall decreased incidence of NEC during the COVID-19 pandemic ($p=0.037$). However, when infants receiving probiotics (which were introduced during the COVID-19 pandemic) were removed from the analysis, the difference in incidence was no longer significant ($p=0.090$). The authors attribute this to the possible protective effects of probiotics against the development of NEC, but do not exclude improved infection control measures during the COVID-19 pandemic contributing to the overall decreased incidence of NEC.^[18] An Italian study,^[19] including four NICUs, reported a similar incidence of NEC in VLBW infants during the COVID-19 pandemic compared with before the pandemic (4.5% v. 4.6%; $p=1.000$). However, in one NICU the incidence of NEC decreased significantly during the COVID-19 pandemic (5.4% v. 0.0%; $p=0.009$), possibly attributed to COVID-19 infection control measures.^[19] Conversely, the present study showed an increased incidence of NEC overall during the COVID-19 period compared with the pre-COVID-19 period despite a similar improvement in infection control measures, although these differences were not significant (2.5% v. 1.8%; $p=0.147$).

Post-COVID-19 NEC data from 2023 and 2024 for inborn infants in this neonatal unit, without additional risk factors for NEC, show an overall incidence of 1.4% and 1.6%, respectively.^[17] This is similar to the data from the pre-COVID-19 period (1.8%), but is lower than the incidence found during the COVID-19 period (2.5%). However these differences in incidence are not significant ($p=0.067$ and $p=0.124$, respectively).

Timing of necrotising enterocolitis

In this study overall, only 22.4% of infants with NEC were diagnosed within the first 7 days of life. This was in contrast to most ($n/N=7/9$) published studies regarding SARS-CoV-2 exposure and the diagnosis of NEC where the age of onset of NEC was reported as being within the first 7 days of life in ~two-thirds (63.6%; $n=7/11$) of the infants, which was unexpected.^[3-6,8-12] This may be related to the fact that 54.5% of the infants in these nine studies were born at term,^[3-6,8-12] and term infants tend to develop NEC earlier than preterm infants.^[1] However, this may also represent a difference in the timing of presentation of NEC in SARS-CoV-2-exposed or infected infants, although we did not demonstrate this overall in this study. In support of this argument however, all three infants ≥ 2 500 g in this study who developed NEC during the COVID-19 period had an early age of onset (<7 days).

Sex predominance in necrotising enterocolitis cases

Numerous studies have shown a higher occurrence of NEC in males, suggesting that male sex may be a potential risk factor for its development.^[20] Carter and Holditch-Davis^[20] reported an overall

male predominance in NEC cases in three neonatal units in the United States ($n=15/24$; 62.5%). However, this was not statistically significant. Similarly, Llanos *et al.*^[21] reported a non-significant male predominance in NEC cases in an 8-year population-based survey in New York. Conversely, in this study, male infants accounted for fewer than half of the NEC cases during the pre-COVID-19 and COVID-19 periods (47.6% and 36.7%, respectively).

Study limitations

Infants exposed to SARS-CoV-2 may not have been identified owing to limitations in the detection methods outlined in the methods section. Furthermore, the SARS-CoV-2 exposure in this study is likely under-reported owing to the SARS-CoV-2 testing policy employed during the pandemic – testing was only performed in symptomatic mothers and mothers who delivered by caesarean section. One SARS-CoV-2-exposed neonate with NEC was excluded from the analysis, as the neonate also suffered moderate to severe HIE, which is an additional risk factor for the development of NEC. Had this infant been included in the analysis, the study results may have been different. Additionally, 5 more neonates with NEC during the COVID-19 period were excluded owing to the presence of additional risk factors, namely moderate to severe HIE ($n=3$) and CCHD ($n=2$), which may have unintentionally altered the results. More data regarding the possible association between *in-utero* SARS-CoV-2 exposure and the development of NEC are therefore still required.

Conclusion

The present study did not demonstrate a significantly increased incidence of NEC in SARS-CoV-2-exposed infants, nor an overall increased incidence of NEC during the COVID-19 pandemic period. However, the possibility of an association cannot be excluded, given the potential pathophysiological mechanisms to support this hypothesis.

Declaration. None.

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