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abstract

CONTEXT: Necrotizing enterocolitis (NEC) is a devastating intestinal disease affecting preterm infants. Studies implicate viral infections in etiopathogenesis.

OBJECTIVE: To summarize the association of viral infections with NEC by systematic review and meta-analysis.

DATA SOURCES: We searched Ovid-Medline, Embase, Web of Science, and Cochrane databases in November 2022.

STUDY SELECTION: We included observational studies that examined the association between viral infections and NEC in newborn infants.

DATA EXTRACTION: We extracted data regarding the methodology, participant characteristics, and outcome measures.

RESULTS: We included 29 and 24 studies in the qualitative review and meta-analysis, respectively. The meta-analysis demonstrated a significant association between viral infections and NEC (odds ratio [OR], 3.81, 95% confidence interval: 1.99–7.30, 24 studies). The association remained significant after excluding the outliers (OR, 2.89 [1.56–5.36], 22 studies) and studies with poor methodology (OR, 3.33 [1.73–6.43], 22 studies). In subgroup analysis based on participants' birth weight, studies including very low birth weight infants only (OR, 3.62 [1.63–8.03], 8 studies) and non-very low birth weight infants only (OR, 5.28 [1.69–16.54], 6 studies) showed a significant association. In subgroup analysis based on specific viruses, infection with rotavirus (OR, 3.96 [1.12–13.95], 10 studies), cytomegalovirus (OR, 3.50 [1.60–7.65], 5 studies), norovirus (OR, 11.95 [2.05–69.84], 2 studies), and astrovirus (OR, 6.32 [2.49–16.02], 2 studies) was significantly associated with NEC.

LIMITATIONS: Heterogeneity of the included studies.

CONCLUSIONS: Viral infection is associated with an increased risk of NEC in newborn infants. We need methodologically sound prospective studies to assess the effect of preventing or treating viral infections on NEC incidence.

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Neonatal necrotizing enterocolitis (NEC) is a spectrum of gastrointestinal pathologies most commonly affecting preterm infants that lead to the endpoint of intestinal necrosis when left untreated.[1] The pathogenesis of this disease is unclear but probably multifactorial.[2] Prematurity, enteral feeding with formula, altered intestinal immune response, and intestinal dysbiosis are significant factors in the pathophysiology of NEC. Infection may have a contributory role in the pathogenesis of NEC.[3] The intestinal microbiome comprises bacteria, viruses, archaea, and fungi. Bacteria are the most extensively studied component of the microbiome and to a lesser extent, the mycobiome or the fungal microbiome.[4] Despite the virome being more extensive than bacteria and they both interact, the role of the intestinal virome in NEC has not been well studied.

Viral infections with gastrointestinal manifestations have been suspected to be associated with the development of NEC. The hypothesized role of viral infections as an etiologic factor in NEC was based on observations made in case reports and case series describing the NEC outbreaks in neonatal intensive care units.[5] Several enteric viruses like torovirus, rotavirus, norovirus, cytomegalovirus, coxsackievirus, parecho virus, astrovirus, and HIV have been implicated in the development of NEC.[3]

Observational studies that ensued have reported conflicting results on the association of NEC with viral infections.[6,7] Most studies on this topic were limited by a small sample size, preventing us from drawing definitive conclusions.[8] We aimed to resolve this ambiguity by summarizing evidence in a systematic review and meta-analysis of the studies evaluating viral infections in NEC. Our review's primary objective was to determine whether infection with viral pathogens is associated with the occurrence of NEC in preterm and term infants compared with those without viral infection. The secondary objective was to determine whether birth weight or infection with any specific virus was associated with necrotizing enterocolitis in preterm and term infants.

METHODS

We conducted this systematic review based on the Meta-analysis of Observational Studies in Epidemiology reporting guidelines and the Preferred Reporting Items for Systematic Reviews[9] (PRISMA) and Meta-analyses reporting guidelines. We developed a review protocol including the objectives, eligibility criteria, information sources, and search strategy.[10] We registered the protocol in the International Prospective Register of Systematic Reviews (ID No. CRD42021281630). The protocol can be accessed by the following link: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=281630.

Eligibility Criteria

We included cohort and case-control studies that examined the association of viral infections with NEC in preterm and term infants. Reviews, case reports, letters, and editorials were excluded. We reviewed the reference list of the included studies to identify additional studies. Our PECO (participants, exposure and risk factor, comparator, and outcome) question for the study was as follows.

Participants

We included neonates and infants under 6 months of age admitted to the neonatal unit. We included infants of all gestational ages. We excluded all patients with known congenital or structural abnormalities of the gastrointestinal tract.

Exposure and Risk Factor

Infants infected with a viral enteric pathogen detected at the time of diagnosis of NEC. We defined infection as isolation or molecular detection of DNA or RNA of a pathogenic virus known to affect the intestine from the infant's laboratory specimen.

Comparator

All preterm and term infants under 6 months of age diagnosed with NEC and not infected with a virus.

Outcome

Our primary outcome measure was NEC, diagnosed by Bell staging criteria as stage 2 or greater.[11,12]

Information Sources

We searched for eligible studies using the Cochrane Neonatal Review Group's search strategy without language or publication date restriction in August 2021, which was updated in November 2022. We searched the following databases: Medline OVID (1946 to November 2022), Embase database (1974 to November 2022), Web of Science (1823 to November 2022), and the Cochrane library (data inception to November 2022). We manually checked the references of narrative reviews, systematic reviews, and original research papers included in the study.

Search Strategy

The search strategy for Medline OVID was developed by a librarian (A.S.) and is provided in Appendix 1 in Supplemental Information. We adapted the same strategy to suit Embase, Web of Science, and COCHRANE databases.

Selection Process

Two reviewers (S.M. and S.H.) independently reviewed the abstracts and included the studies based on the inclusion criteria following standard methods. The conflicts were resolved by a third author (M.P.). Rayyan.ai, a web-based software, was used in the selection process.[13]

We used 2 freely available online tools, namely Google translate and Reverso.net, for assessing potentially eligible studies in languages other than English.

Data Collection Process

Two reviewers (S.M. and S.H.) collected data independently using a data extraction form by manually reviewing the selected articles. S.H. and S.M. collected data from articles with the first author's last name, starting with the alphabet A to N and O to Z, respectively. M.P. verified the data collected by both the reviewers for any human errors. A list of all the data items collected is provided in Appendix 2 in Supplemental Information.

Study Risk of Bias Assessment

We used the Newcastle-Ottawa scale to assess the risk of bias in the included cohort and case-control studies.[14] Two reviewers (S.M. and S.H.) independently scored their respective studies during the data extraction in 3 domains, namely selection, comparability, and outcome, with a maximum score of 4, 2, and 3 for each domain, respectively. The possible maximum total score for each study was 9.

Data Analyses and Effect Measures

We performed a meta-analysis of studies that reported viral infection data for the NEC and control groups. A random effects model with an inverse variance method was used to compute the odds ratio (OR) for the association between viral infection and NEC across studies. A forest plot was created to visualize the results. Between-study heterogeneity was assessed using Cochran's Q test and the I^2 statistic. Publication bias was assessed by funnel plot asymmetry and Egger's test. Review Manager 5.4 was used to perform the meta-analysis.[15] The "metaphor" package in R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform Egger's test and create the funnel plots.[16]

RESULTS

Description of Studies

One thousand one hundred and two potentially relevant study abstracts were screened, 130 were selected for retrieval, and 29 studies were included in the review. The PRISMA flow diagram of the search process is shown in Fig 1. Included studies and their baseline characteristics are summarized in Table 1.[7,8,17–43] Excluded studies and the reasons for exclusion are presented in Supplemental Table 3. Twenty-one studies were published after the year 2000, and 41% were published in the last 12 years (Supplemental Fig 5A). The included studies were done

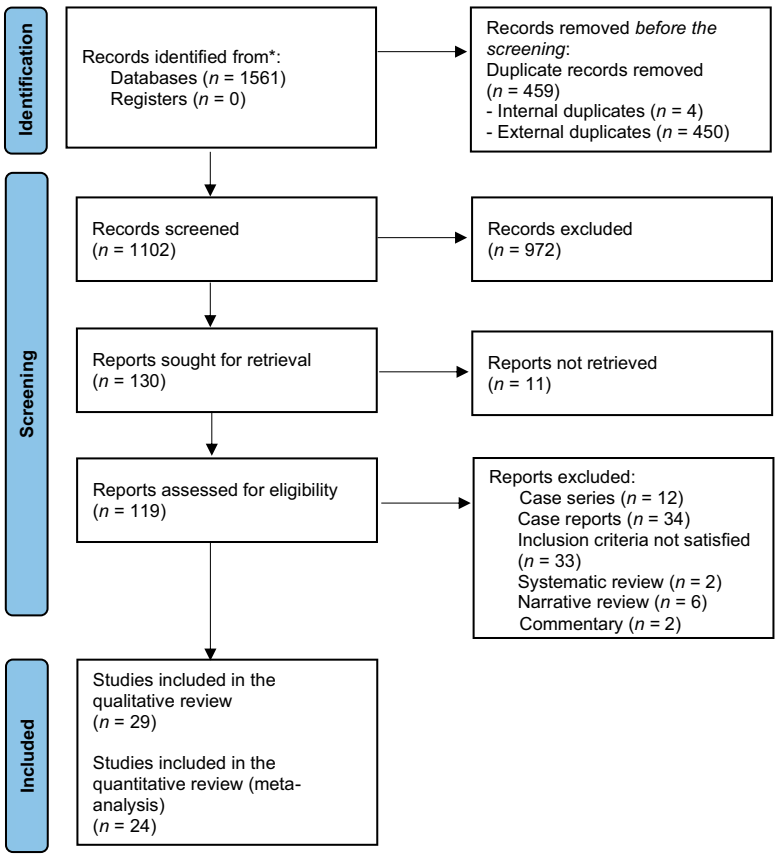

FIGURE 1
PRISMA flowchart shows the systematic search of the literature.

TABLE 1 Baseline Characteristics of the Included Studies

Study No.	Study ID	Study Period	Study Design	Location	Inclusion Criteria	GA (Weeks), Mean (SD), Median (IQR), or Range	BW (Grams), Mean (SD), Median (IQR), or Range	Exposure Ascertainment	Sample Size
1.	Akinci[17] (1991)	Jan–Mar 1990	Retrospective cohort	Turkey	All infants admitted to the NICU	≥37	2500–3500	Stool ELISA positive for rotavirus	70
2.	Angelika[18,75] (2022) (includes 2 reports)	May 2020–Mar 2021	Prospective cohort	Indonesia	All neonates hospitalized with positive maternal SARS-COV- 2 status	All	2867.6 (698.75)	Nasal or throat swab PCR positive for SARS-CoV- 2	125
3.	Bagci[19] (2008)	2002–2006	Case-control	Germany	Preterm infants with or without NEC	NR	750–1800	Stool ELISA for astrovirus	160
4.	Bagci[20] (2010)	1998–2007	Retrospective cohort	Germany	Preterm and late preterm with NEC or Gastroenteritis	25–40	618–3790	Stool ELISA for rotavirus, adenovirus, and astrovirus. PCR for norovirus	220
5.	Birenbaum[21] (1997)	Oct–Nov 1992	Case-control	Israel	Preterm and term babies with diarrhea	26–40	605–2145	Stool viral culture. Viral isolates typed by microneutralization assay.	57
6.	Chany[22] (1982)	Sep 1979–Mar 1980	Case-control	France	Preterm and term babies during the study period	37.4 (2.13)	2597 (642)	Stool immunoelectron microscopy, viral culture, and paired serology to see fold changes in antibody titers against coronavirus	79
7.	Chappé[23] (2011)	Jan 2005–Dec 2006	Retrospective cohort	France	Hospitalized term and preterm newborns with digestive symptoms or suspicion of neonatal infection	31.4 (28–34)	1445 (1030–2051)	Stool astrovirus by qualitative ELISA	136
8.	Cheng[8] (2020)	Jul 2014–Sep 2015	Case-control	China	Newborns with NEC stage 2/3 (case) and infants with pneumonia/jaundice (control)	37.39 (2.78)	2869	RT PCR to detect RV, ASV, sappovirus, EV, ADV, EBV, CMV, and HBoV	90
9.	de Villiers[24] (2012)	Over 14 mo, but not mentioned from which year to year	Case-control	South Africa	Term and preterm infants <4 wk requiring hospitalization for sepsis screen	24–38	801 (705–978)	Stool ELISA positive for rotavirus	107
10.	Keller[25] (1991)	1987–1990	Case-control	Germany	Infants with NEC stage 2/ 3	27–40	1360–2130	ELISA to detect Rota viral antigen	32
11.	Lodha[26] (2005)	1996–2001	Case-control	Canada	Infants with NEC stage 2/3	31.6 (4.7)	1764 (988)	Direct Electron microscopy	508
12.	Neuberger[27] (2006)	1995–2003	Case-control	Germany	Infants with CMV viruria	24.1–31.7	495–1670	CMV PCR and culture from throat, urine and milk	80

TABLE 1 Continued

Study No.	Study ID	Study Period	Study Design	Location	Inclusion Criteria	GA (Weeks), Mean (SD), Median (IQR), or Range	BW (Grams), Mean (SD), Median (IQR), or Range	Exposure Ascertainment	Sample Size
13.	Omarsdottir[28] (2017)	1990–2009	Case-control	Sweden	Postmortem intestinal specimens with NEC, SIP, or surgical complications after these conditions (case); postmortem intestinal samples from neonates without pathologic bowel morphology (control)	27.9 (7.3)	1015 (1220)	Detection of CMV immediate early antigen, late antigen by immunohistochemistry – confirmed by TaqMan PCR and In situ hybridization	80
14.	Panesso-Gomez[29] (2019)	2000–2016	Retrospective cohort	United States	Histopathological confirmed NEC with sufficient paraffin-embedded tissue for CMV testing	24 (23–27)	649 (601–896)	CMV DNA by PCR and CMV detection by IHC	143
15.	Patel[7] (2020)	Jan 2010–Jun 2013	Prospective cohort	United States	Infants with birth wt ≤ 1500 g and age ≤ 5 d	27.9 (2.6)	1016 (273)	Positive CMV NAT or viral culture result in blood or urine after 2 weeks of life with a previously documented negative result	596
16.	Ramani[30] (2008)	Jan 2003–Dec 2006	Prospective cohort	India	Neonates admitted for >48 h with GI symptoms (case); neonates admitted for >48 h with non-GI pathology (control)	NR	NR	Stool detection of rotavirus by EIA and PCR	375
17.	Rotbart[31] (1983)	Aug 1982	Case-control	United States	All patients admitted to the nursery in August 1982	28–37	820–2500	Rotavirus positive by ELISA test using a stool sample	27
18.	Rotbart[32] (1988)	May–Aug 1983	Case-control	United States	Infants with NEC 2/3	NR	NR	Rotavirus positive by Rotazyme assay by rectal swab and confirmed by 3 EIAs	154
19.	Rudd[33] (1984)	Aug 1981–Dec 1982	Prospective cohort	UK	All infants admitted to the NICU	32	1620	Rotavirus detected by the Rotazyme system in the stool sample and confirmed by electron microscopy using the negative stain phosphotungstic acid	170
20.	Sharma[34] (1996)	NR	Prospective cohort	United States	Neonates with gastrointestinal disturbances and sepsis	NR	NR	Rotavirus positive by EIA and EM	89
21.	Sharma[35] (2002)	Dec 1991–Nov 1995	Prospective cohort	United States	Infants with predefined GI signs or symptoms or NEC stage ≥2	31.6 (4.7)	1785 (961)	Rotavirus positive by EIA and IEM tested on a fecal specimen	194

TABLE 1 Continued

Study No.	Study ID	Study Period	Study Design	Location	Inclusion Criteria	GA (Weeks), Mean (SD), Median (IQR), or Range	BW (Grams), Mean (SD), Median (IQR), or Range	Exposure Ascertainment	Sample Size
22.	Sharma[36] (2004)	63 mo–year not reported	Prospective cohort	United States	All neonates with NEC stage ≥ 2	31 (3.4)	1569 (926)	Rotavirus positive by EIA and confirmed by immunoelectron microscopy	129
23.	Sizmaz[37] (2012)	May 2007–Nov 2009	Prospective cohort	Turkey	All patients with NEC	30.6 (3.5)	1492 (647.8)	Rotavirus positive by a stool antigen test	31
24.	Stuart[38] (2010)	Jan 2008–Jun 2008	Case-control	Australia	Neonates with NEC stage ≥2 (case); neonates without NEC during the same period (control)	27.3 (2.7)	1062.1 (725.9)	Norovirus RNA detected by nested RTPCR of 266 bp section of the capsid gene VP1	55
25.	Tai[39] (2012)	Oct 2008–Sep 2010	Retrospective cohort	Taiwan	Hospitalized neonates with stool specimens positive for rotavirus	33.7 (24–40)	2137 (460–4800)	Rotavirus positive stool by EIA	104
26.	Turcios-Ruiz[40] (2008)	Jan 1998	Case-control	United States	Newborns < 34 wk with NEC in Jan 1998	28 (24–34)	1073 (763–2106)	Norovirus identified by virus molecular diagnostics	33
27.	Turner[41] (2014)	1993–2008	Retrospective cohort	United States	All infants born ≤ 1500 g	28.7	966	Congenital- CMV positive by rapid culture from urine or saliva within 2 wk; acquired – CMV positive by rapid culture from urine or saliva after 2 wk	374
28.	Ullrich[42] (2011)	2007–2011	Case-control	United States	Surgically resected ileum from infants with NEC (case); surgically resected ileum from infants with non-NEC diagnosis (control)	28.2 (23–39)	NR	RT PCR positive for Adenovirus, rotavirus, norovirus	23
29.	Weimer[43] (2020)	Jan 2002–Dec 2016	Retrospective cohort	United States	Infants hospitalized on D21 with a diagnosis of postnatal CMV and hearing screen results after PMA 34 wk	25–28	500–999	CMV positive by culture or PCR on or after D21	546

ADV, adenovirus; ASV, astrovirus; bp, base pair; BW, birth wt; CMV, cytomegalovirus; EIA, enzyme immunoassay; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; EV, enterovirus; GA, gestational age; GI, gastrointestinal; HBoV, human bocavirus; IEM, immunoelectron microscopy; NAT, nucleic acid amplification test; NR, not reported; RT PCR, reverse transcriptase polymerase chain reaction; RV, rotavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SIP, spontaneous intestinal perforation; UK, United Kingdom; VP, viral protein.

across 14 countries all around the world. United States, Germany, France, and Turkey were the predominant locations where 65.5% of the studies were conducted (Supplemental Fig 5B). Forty-eight percent were cohort studies (27% prospective and 21% retrospective), and 52% were case-control studies. Twenty-four percent of the included studies were done during a particular period when a viral outbreak occurred. The included studies evaluated 4787 newborn infants for an association between 11 different viruses with NEC. Rotavirus and cytomegalovirus (CMV) were the most studied viruses, including 62% of studies (Supplemental Fig 5C).

Fourteen reviewed studies were designed to recruit infants with confirmed NEC and observe if there was an association with an enteric viral pathogen. Among those studies, 3 studies examined pathologic specimens obtained during autopsy or surgical procedures for viral infection. Thirteen studies used nucleic acid amplification tests, viral culture, or both to confirm the viral infection. One of those studies did genotype in addition to nucleic acid amplification tests. Sixteen studies did immunologic methods to identify viral pathogens. Six studies included preterm infants with gestational age at birth $<$ 32 weeks. Nine studies included only preterm infants with gestational age at birth $<$ 37 weeks. Combined, 52% of reviewed studies included preterm infants only. Thirteen studies included a combination of term and preterm infants. One study included term infants only. Ten studies included infants with birth weight $<$ 1500 g. Six studies included infants with birth weight $\geq$ 1500 g. Twelve studies included very low birth weight (VLBW) and non-VLBW infants. One study did not report the birth weight of the infants studied.

Twelve studies reported information about the type of milk fed in the baseline characteristics of the infant. Among them, only a few studies in the recent decade have reported the use of breast milk or infant formula and the type of milk fortifier. Seventeen studies did not report the type of milk the infants were fed. Three studies concluded no association between viral infections and NEC. The authors of 26 studies included in the review concluded a positive association between exposure to a virus and NEC.

Quality Assessment

The quality of each study according to the Newcastle-Ottawa Scale is summarized in Supplemental Table 4. Studies with NOS scores 0 through 3 were considered poor methodological quality, whereas 4 through 6 and 7 through 9 were considered fair and good quality studies. The 2 independent reviewers (S.M. and S.H.) had a 93% agreement among each other. Disagreement in 2 studies was resolved by discussion and consensus. Fourteen studies received 7 through 9 points out of a possible 9 points (Fig 2). The studies with higher quality scores had succinct criteria

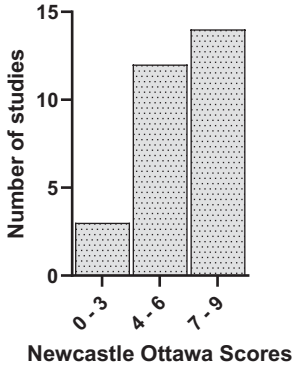

FIGURE 2

Risk of bias assessment. The column chart shows the Newcastle Ottawa scale scores along the x-axis divided into 3 groups high risk of bias (0–3), moderate risk of bias,[4–6] and low risk of bias.[7–9] The number of studies included in the review with those scores on the y-axis. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

for selecting participants, ascertaining exposure, and assessing the outcome. The participants of the comparison groups were similar in their gestational age and birth weight. The most common reason for lower scores in the quality assessment was that the study did not control gestational age and birth weight (15 studies). We found asymmetry on visual inspection of the funnel plot confirmed by a significant Egger's test, indicating potential publication bias in the meta-analysis ($Z = 3.6$, $P <$ .001, Supplemental Fig 6).

Meta-analysis

We included 24 studies that provided data for the meta-analysis. Five included studies did not have a comparator and hence were not included in the meta-analysis.[25,29,36,39,42] Viral infection significantly increased the odds of NEC (OR, 3.81, 95% CI: 1.99–7.30) (Fig 3), and there was significant between-study heterogeneity ($I^2 = 82\%$, $P <$ .0001).

Sensitivity Analyses

The association between viral infections and NEC remained significant after 2 sensitivity analyses. For the first sensitivity analysis, we excluded 2 of the 24 studies with outlying odds ratios $>$ 100.[32,33] This meta-analysis of 22 studies showed that viral infection significantly increased the odds of NEC (OR, 2.89, 95% confidence interval [CI]: 1.56–5.36, $I^2 = 80\%$). (Supplemental Fig 7A) For the second sensitivity analysis, we excluded 2 studies with the lowest score in the risk of bias assessment (NOS score = 3).[17,40] This analysis showed a significant association between viral infection and the occurrence of NEC (OR = 3.33, 95% CI: 1.73–6.43, $I^2 = 83\%$) (Supplemental Fig 7B).

Subgroup Analyses

We performed a subgroup analysis based on birth weight with participants $<$ 1500 g (VLBW) and those with participants $\geq$

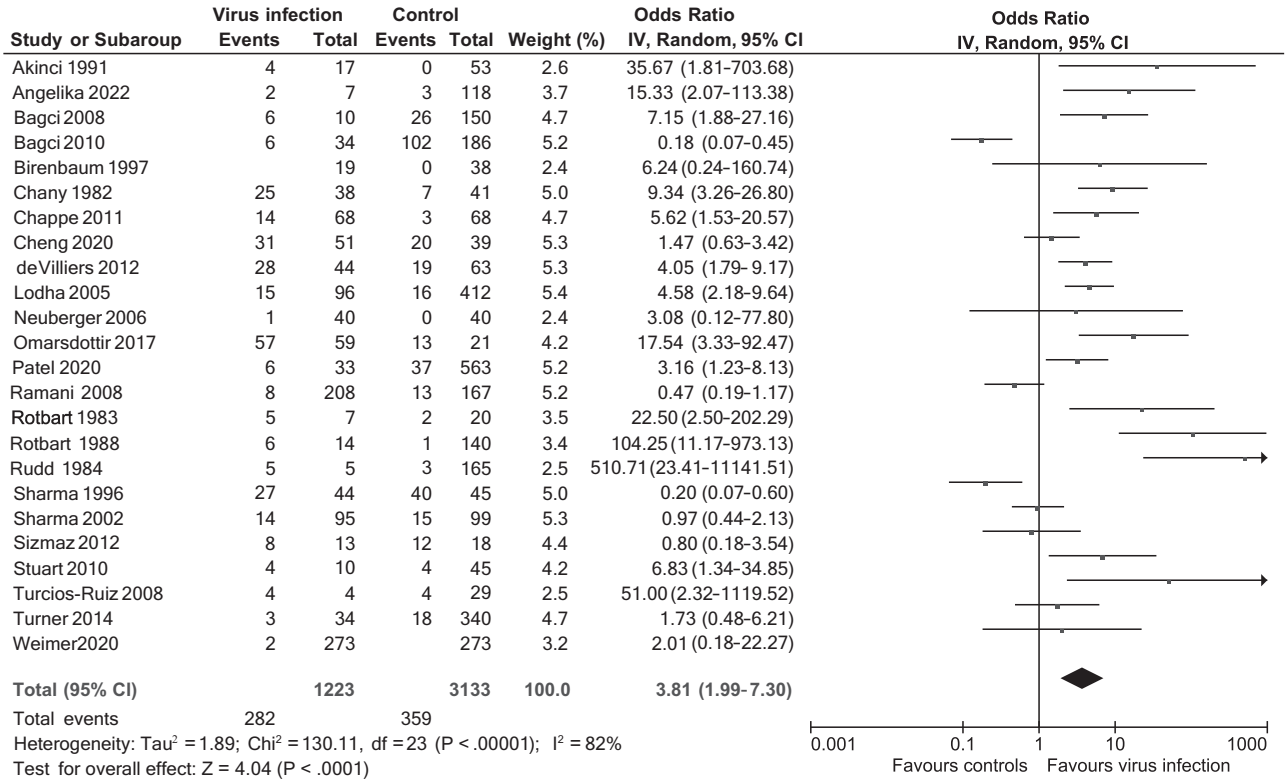

FIGURE 3

Meta-analysis. Forest plot shows the meta-analysis using inverse-variance weighting and random effects model testing for an association between viral infection and NEC.

1500 g. When 8 studies, including infants < 1500 g, were analyzed, a significant association between viral infection and occurrence of NEC (OR, 3.62, 95% CI: 1.63–8.03, I^2 = 43%) was observed. Similarly, analysis of 6 studies, including infants ≥ 1500 g, showed a significant association between viral infection and occurrence of NEC (OR = 5.28, 95% CI: 1.69–16.54, I^2 = 83%). Analysis of 10 studies that included a combination of VLBW and non-VLBW infants did not show a significant association between viral infection and NEC (OR = 3.19, 95% CI: 0.92–11.04, I^2 = 89%) (Fig 4A).

We performed another subgroup analysis based on the type of virus. Rotavirus (OR, 3.96, 95% CI: 1.12–13.95, I^2 = 88%), CMV (OR, 3.50, 95% CI: 1.60–7.65, I^2 = 20%), norovirus (OR, 11.95, 95% CI: 2.05–69.84, I^2 = 21%), astrovirus (OR, 6.32, 95% CI: 2.49–16.02, I^2 = 0%) showed a significant association between viral infections and NEC. (Fig 4B).

The summary of the findings of our review is shown in Table 2. We used GRADEpro Guideline Development Tool to classify the certainty of evidence into very low, low, moderate, or high based on the risk of bias, imprecision, inconsistency, indirectness, and publication bias[44] and found the certainty of evidence to be low. The reasons for downgrading the evidence found in our analysis were because of serious risk of bias and significant publication bias among the included studies. GRADEpro, a web-based software for synthesizing and grading evidence used worldwide by medical organizations like the World Health Organization, Cochrane Database, and American Thoracic Society, was used to make this conclusion.

DISCUSSION

Viruses have been associated with NEC, but the existing literature is inconclusive. Summarized evidence from this systematic review and the meta-analysis shows that viral infection is significantly associated with NEC. The sensitivity analyses showed that the association remained significant after excluding the outliers and studies with poor methodology. Based on the subgroup analyses, the association is significant in VLBW infants, non-VLBW infants and in those with rotavirus, CMV, norovirus, and astrovirus infection.[44] In our meta-analysis, we included only studies with unambiguous definitions of the outcome and reported all relevant data in pertinent comparison groups. We used NOS, a widely accepted validated tool for quality assessment of observational studies. Fourteen out of 24 studies included in our meta-analysis had a good methodological quality based on the NOS scores. We found that the

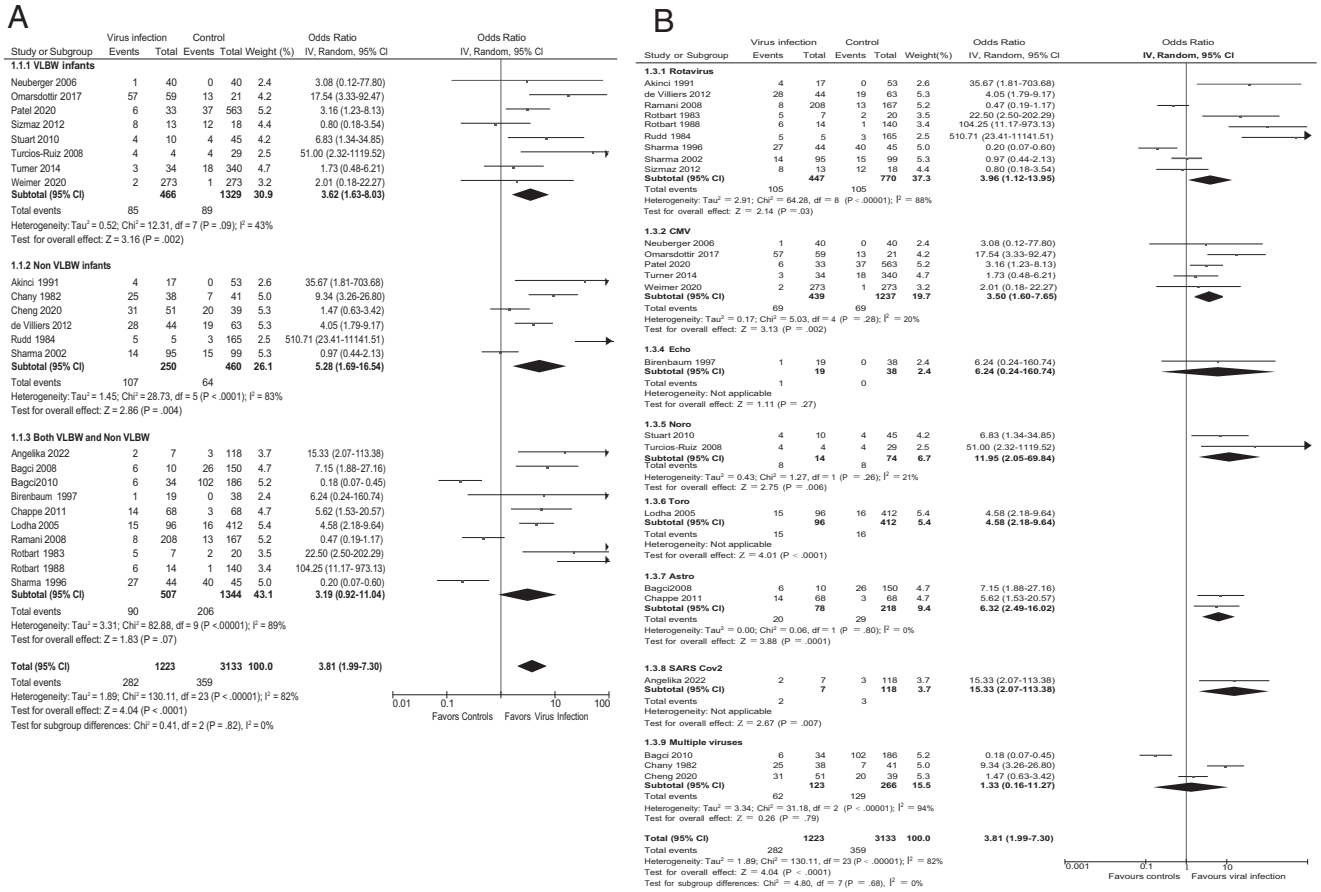

FIGURE 4

Subgroup analysis. (A) Forest plot shows the subgroup analysis by birth weight. (B) Forest plot shows the subgroup analysis by type of virus.

certainty of evidence assessed by GRADE was low for the association between viral infection and NEC.

Our meta-analysis with 4356 infants is the largest sample to date to analyze the association between viral infections and NEC. This is the first review to combine all the data for viruses hypothesized to be associated with NEC. Our results are congruent with a systematic review of 8 studies (2 case-control, 2 cohorts, and 4 case series) on norovirus infection, which showed that norovirus infection is associated with NEC in preterm infants.[45] A systematic review that evaluated the long-term effects of postnatal CMV infection in VLBW infants noted an association of CMV infection with feeding intolerance but not with NEC in preterm infants.[46] The difference between the results of our subgroup analysis and the systematic review by Stark et al could be because of the inclusion of studies with NEC as a secondary outcome in their review. Evaluating stool samples in conjunction with blood samples for CMV infection is essential because viremia may be absent in exclusive intestinal pathology.[5,47] The studies included in our review predominantly tested stool samples or intestinal histopathological specimens. The study by Omarsdottir et al, which showed a significant association in our analysis, was not included in the review by Stark et al.

We used the Bradford Hill causality score to check if the association between viral infections and NEC seen in our meta-analysis could establish causation.[48] The strong association in our meta-analysis satisfies the first Hill criterion (strength). Greater than three-fourths of the studies in our review conducted across 14 countries observed a positive association between viral infection and NEC (consistency). In some instances, exposure to viral infection precedes the development of NEC, which is inferred from the prospective studies reviewed (temporality). As discussed above, the emerging knowledge from viral metagenomic studies that reduced viral β diversity precedes NEC supports biological plausibility (plausibility). Our knowledge of the role of gram-negative bacteria in NEC is analogous to the possible role of viral infections. We can make conclusions about the role of viruses similar to bacteria with some confidence (analogy). Available evidence supports moderate coherence in explanation (coherence). There is some evidence supporting dose-response effect (biological gradient), especially related to CMV infection. However, viral infection is not specific to

TABLE 2 Summary of Data Analysis

Outcome	Number of Studies (Number of Participants)	Quality Assessment					Statistical Method	Effect Estimate	Certainty of Evidence (GRADE)
		Risk of Bias	Imprecision	Inconsistency	Indirectness	Publication Bias			
Meta-analysis									
NEC	24 (4356)	Serious	Not serious	Not serious	Not serious	Strongly suspected	OR (IV, random, 95% CI)	3.81 (1.99–7.30)	Low[a] (++)
Sensitivity analysis									
After excluding outliers									
NEC	22 (4032)	Serious	Not serious	Not serious	Not serious	Strongly suspected	OR (IV, random, 95% CI)	2.89 (1.56–5.36)	Low[a] (++)
After excluding poor-quality studies									
NEC	22 (4253)	Serious	Not serious	Not serious	Not serious	Strongly suspected	OR (IV, random, 95% CI)	3.33 (1.73– 6.43)	Low[a] (++)

GRADE, grading of recommendations, assessment, development, and evaluation; IV, inverse variance method; NEC, necrotizing enterocolitis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a] Downgraded because of risk of bias and publication bias.

cause NEC (specificity). Viral infections as a cause of NEC will be challenging to prove in an experimental study design, and no experimental data are available (experiment). Since treatment options for viral infections are limited, it is hard to prove reversibility, and there is no current data (reversibility). So, the current evidence of viral association with NEC falls short of proving causation.

Our review raises 2 critical questions – were the viruses identified in the infants with NEC inherent to their intestinal microenvironment, or was it because of a new infection occurring in the postnatal period? Can specific viruses affect intestinal microbial homeostasis and result in the phenotype seen in NEC? These questions stem from our understanding that intestinal dysbiosis is the final common pathway for risk factors, such as lack of enteral feed, use of TPN, metabolic acidosis, exposure to antibiotics, and formula feeds, leading to NEC.[49] Increased relative abundances of Proteobacteria and decreased relative abundances of Firmicutes and Bacteroidetes have been shown to precede the development of NEC.[50]

Prenatal fetal inflammatory response induced by histologic chorioamnionitis with fetal involvement is strongly associated with NEC.[51] So, intestinal dysbiosis may result from gut inflammation and be involved in the pathophysiology of NEC. This hypothesis is supported by a recent single-center case-control study from Italy that showed abnormal antenatal umbilical artery flow, clinical chorioamnionitis, and histologic chorioamnionitis are independent predictors of NEC and its severity.[52]

Gut virome, including the phageome, labeled by some authors as the "dark matter" of the microbiome, is increasingly recognized to play a role in dysbiosis.[53,54] Gut virome development in infants shows unique characteristics compared with bacteriome. A metagenomic study showed that the meconium of a newborn infant is free of virus-like particles. Viruses colonize the infant's gut during the first week of life and change significantly in composition between 1 and 2 weeks of life.[55] This finding was reproduced in a recent prospective study of 4 pairs of twin infants from birth to 2 years. The authors noted that the genetically identical twins had a similar virome modified by the diet and environment during development.[56] The development of virome in the neonate's gut occurs stepwise; bacteria colonizing the gut soon after birth induces the growth of bacteriophages present in their genome, followed by colonization with eukaryotic viruses. The type of feeding modulates both steps of virome development.[57] Breastfeeding has been shown to reduce and formula feeding to promote colonization with eukaryotic viruses.[58]

Eukaryotic viruses in the neonatal gut include definitive pathogenic viruses like rotavirus, norovirus, astrovirus, adenovirus (serotypes 40 and 41), and enterovirus.[59] Respiratory viruses such as rhinovirus, bocavirus, and coronavirus can be swallowed and seen in fecal samples.[60,61] HIV has been isolated from stool in HIV-infected patients.[62] However, identifying these viruses does not imply replication in the human intestinal cells.[63]

The components of enteric virome that have pathogenic potential, also called pathobionts, can proliferate in response to exposomes, such as formula feed and antibiotics. Virome interacts with the bacteriome and the host genome, modulating gut mucosal immunity.[64] These interactions can be beneficial or harmful.[65–67] A new viral infection or richness of the viral pathobionts in infants with susceptible gene polymorphisms can induce inflammation.[68,69] The

bacteriome responds to the crosstalk by propagating inflammation, resulting in bacterial dysbiosis, which triggers further inflammation and mucosal injury.[70] A recent study using metagenomic next-generation sequencing in 23 preterm infants during the first 11 weeks of life found reduced viral β diversity ten days before the onset of NEC. This prospective study identified specific viral signatures and viral-bacterial interactions involved in the process.[71]

Viral infections in the NICU can be sporadic or occur as an outbreak. The possible sources of viral infection in NICU include droplet transmission from the respiratory tract of infected adults, hand transmission from infected healthcare personnel, fomite transmission from infected medical equipment, contaminated blood products or formula milk, and vertical transmission through the placenta or breast milk. A study examining the sources of viral infection outbreaks in NICU found that the infected infant (index case) is the most commonly identified source.[72] Although the first case identified during an outbreak (index case) could be the source of the outbreak (primary case), it may not be possible to identify the primary case in many NICU outbreaks, which are either propagated or have a common source. The study reported that the source is unknown in approximately 40% of outbreaks. Gastrointestinal and respiratory tract viral infections are the major cause of outbreaks. Among the individual viruses, rotavirus and RSV are the most frequently reported. Major infection control measures used to control the outbreaks were hand hygiene, patient screening, isolation and cohorting, and the use of personal protective equipment. Screening or surveillance and training of healthcare personnel, modification of care to restrict workload, choice of equipment, closure of affected location, disinfection, sterilization, and vaccination are other prevention or containment measures. The universal use of leukoreduced blood component therapy in the NICU population and pasteurization of donor human milk also aid in prevention of viral infection.

Strengths and Limitations of This Review

Our review included observational studies with a high degree of heterogeneity. Similar to any other review of observational studies, the association cannot be translated to causation. Our funnel plot and Egger's test showed potential publication bias. Ten (37%) of the studies' log ORs fall outside of the triangular region, which should contain 95% of studies' effect estimates when there is no sample bias. Visual inspection of the funnel plot reveals a large degree of variability in the effect estimates across studies, with a disproportionate number of the larger studies having log ORs less than 0. There were significant differences in the study designs and heterogeneity in the study enrollment criteria. Less than 30% of the included studies were conducted prospectively. Approximately one-third of the included studies described the outbreaks that occurred in a particular period. This could have introduced potential selection bias. Approximately two-thirds of included studies did not report the type of infant feeding (human milk or formula), which is an important confounder. In addition to potential publication bias, we speculate that much of the observed heterogeneity and funnel plot asymmetry could be attributable to these factors and other sources of variability across studies, such as study quality and differences in virus types studied. Publication bias would be expected to lead to an underrepresentation of studies with results that did not achieve statistical significance, which may lead to an inflated estimate of the association between viral infection and NEC in this meta-analysis.

A randomized study design would be necessary to establish causality, but randomization of infants to viral exposure would be unethical. Therefore, confounding can never be completely ruled out in observational studies of the association of viruses with NEC, however future studies should employ triangulation of multiple approaches with differing sources of potential bias to facilitate causal inference.[73] For example, statistical methods (eg, using logistic regression analysis to compare odds of NEC for virus exposed versus unexposed infants after controlling for potentially confounding covariates) and design-based methods (eg, use of a natural experiment study design such as comparison of NEC incidence before versus after a virus outbreak) could be employed within the same study to examine whether the results from both approaches are consistent with increased risk of NEC because of viral infection, which would provide stronger evidence of causality than results from either approach alone. Another strategy to prove causality would be to use alternative trial designs to RCTs, including the randomized encouragement trials to manipulate the mediator.[74] For example, treating asymptomatic infants with CMV infection is not currently recommended. A randomized encouragement trial design can be employed in this situation where infants with asymptomatic CMV infection may randomly be assigned an opportunity or an encouragement to receive specific treatment. Parents can choose whether the infant receives the intervention or not, and NEC as an outcome can be studied. Such an analysis could provide causal inference.

Despite these limitations, our review is the first to study the association of exposure to viral infections with NEC in a systematic manner. We followed the standard guidelines for a systematic review of observational studies. We used a broad search strategy and selected relevant articles based on predefined inclusion criteria. Our systematic review included both term and preterm infants studying 12 different viruses for their association with NEC in 14 countries worldwide. We included only infants with definite NEC (≥ Bell stage 2) as diseased. Nearly two-thirds of the studies included in our meta-analysis were of good quality (NOS scores 6–9) based on quality assessment.

CONCLUSIONS

Implications for Practice

Summarized evidence from observational studies shows that viral infection is associated with an increased risk of NEC in newborn infants (low certainty evidence, 24 studies, 4356 participants). This risk may be more significant in VLBW infants exposed to CMV, norovirus, and astrovirus. Clinicians should suspect the possibility of virus infections as triggers for developing NEC. This may be especially important when VLBW infants presenting with NEC are clustered in time and place.

Implications for Research

Further research is needed to confirm the findings of our meta-analysis in methodologically sound prospective studies and to investigate whether prevention or treating viral infections might be associated with decreased risk for NEC. Developing a multiplex enteric panel of viruses to test from the stool or blood will be helpful. Future prospective clinical studies should aim to demonstrate the absence of candidate viruses in infants before the onset of NEC and the presence of viral infection during the diagnosis of NEC. Such studies should report the known confounders associated with NEC: gestational age, birth weight, type of enteral feed, history of chorioamnionitis, and exposure to antibiotics and probiotics.

Developing experimental humanized animal models of NEC induced by viruses similar to lipopolysaccharide-induced NEC models will help identify and elucidate the metabolic and immunologic pathways involved in NEC caused by viruses. Such animal models can facilitate experiments to establish a dose-response relationship and test the reversibility of NEC with appropriate treatment strategies. Intestinal organoid models with NEC induced by viruses are another strategy to help us ascertain the causality of viral infection in NEC.

Future microbiome research should use metagenomic techniques to characterize the dynamic intestinal virome "dark matter" of the intestinal microbiome in preterm infants at various gestational ages. This will be the essential first step in understanding perturbations in the diseased state. Recent initiatives like the human virome program undertaken by the National Institutes of Health are promising forward steps toward bridging our knowledge gaps related to the gut virome. Such efforts should simultaneously delineate the interlink between the virome, bacteriome, and host genome.

ABBREVIATIONS

CMV: cytomegalovirus
NEC: necrotizing enterocolitis
OR: odds ratio
PRISMA: preferred reporting items for systematic reviews and meta-analysis
VLBW: very low birth weight

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REFERENCES

1. Fanaroff AA, Martin RJ, Walsh MC. *Fanaroff and Martin's Neonatal–perinatal Medicine: Diseases of the Fetus and Infant.* Amsterdam, the Netherlands: Elsevier; 2019
2. Neu J, Modi N, Caplan M. Necrotizing enterocolitis comes in different forms: Historical perspectives and defining the disease. *Semin Fetal Neonatal Med.* 2018;23(6):370–373
3. Coggins SA, Wynn JL, Weitkamp JH. Infectious causes of necrotizing enterocolitis. *Clin Perinatol.* 2015;42(1):133–154, ix
4. Cresci GAM, Izzo K. Gut microbiome. In: Corrigan ML, Roberts K, Steiger E, eds. *Adult Short Bowel Syndrome*, Chapter 4. Washington, DC: Academic Press; 2019:45–54
5. Shimamura M. Is there a link between viruses and necrotising enterocolitis? *Acta Paediatr.* 2016;105(1):15–16
6. Skeath T, Stewart C, Waugh S, Embleton N, Cummings S, Berrington J. Cytomegalovirus and other common enteric viruses are not commonly associated with NEC. *Acta Paediatr.* 2016;105(1):50–52

7. Patel RM, Shenvi N, Knezevic A, et al. Observational study of cytomegalovirus from breast milk and necrotising enterocolitis. *Arch Dis Child Fetal Neonatal Ed.* 2019;105(3):259–265
8. Cheng C, He Y, Xiao S, Ai Q, Yu J. The association between enteric viruses and necrotizing enterocolitis. *Eur J Pediatr.* 2020;180(1): 225–232
9. Brooke BS, Schwartz TA, Pawlik TM. MOOSE reporting guidelines for meta-analyses of observational studies. *JAMA Surg.* 2021;156(8): 787–788
10. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 2021;372:n71
11. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg.* 1978;187(1):1–7
12. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am.* 1986;33(1): 179–201
13. Mourad O, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan — a web and mobile app for systematic reviews. *Syst Rev.* 2016; 5(1):210
14. Wells GA, Wells G, Shea B, et al, eds. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* Ontario, CA: The Ottowa Hospital; 2014.
15. Manager R. (RevMan) [computer program]. Version 5.4 ed: The Cochrane Collabration. Available at: https://training.cochrane.org/online-learning/core-software/revman. Accessed July 15, 2022
16. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw.* 2010;36(3):1–48
17. Akinci A, Teziç T, Gür I, Cetin H, Hatun S. Rotavirus diarrhea in newborn infants. *Turk J Pediatr.* 1991;33(3):153–157
18. Angelika D, Etika R, Kusumawardani NN, Mithra S, Ugrasena IDG. Observational study on necrotizing enterocolitis in neonates born to SARS-CoV-2-positive mothers. *Ann Med Surg (Lond).* 2022;78: 103711
19. Bagci S, Eis-Hübinger AM, Franz AR, et al. Detection of astrovirus in premature infants with necrotizing enterocolitis. *Pediatr Infect Dis J.* 2008;27(4):347–350
20. Bagci S, Eis-Hübinger AM, Yassin AF, et al. Clinical characteristics of viral intestinal infection in preterm and term neonates. *Eur J Clin Microbiol Infect Dis.* 2010;29(9):1079–1084
21. Birenbaum E, Handsher R, Kuint J, et al. Echovirus type 22 outbreak associated with gastro-intestinal disease in a neonatal intensive care unit. *Am J Perinatol.* 1997;14(8):469–473
22. Chany C, Moscovici O, Lebon P, Rousset S. Association of coronavirus infection with neonatal necrotizing enterocolitis. *Pediatrics.* 1982;69(2):209–214
23. Chappé C, Minjolle S, Dabadie A, Morel L, Colimon R, Pladys P. Astrovirus and digestive disorders in neonatal units. *Acta Paediatr.* 2012;101(5):e208–e212
24. de Villiers FP, Driessen M. Clinical neonatal rotavirus infection: association with necrotising enterocolitis. *S Afr Med J.* 2012; 102(7):620–624
25. Keller KM, Schmidt H, Schumacher R. Rotavirus positive (RV+) and non-rotavirus (RV-) necrotizing enterocolitis (NEC) in 32 infants. *Pediatr Radiol.* 1991;21(8):606–607
26. Lodha A, de Silva N, Petric M, Moore AM. Human torovirus: a new virus associated with neonatal necrotizing enterocolitis. *Acta Paediatr.* 2005;94(8):1085–1088
27. Neuberger P, Hamprecht K, Vochem M, et al. Case-control study of symptoms and neonatal outcome of human milk-transmitted cytomegalovirus infection in premature infants. *J Pediatr.* 2006; 148(3):326–331
28. Omarsdottir S, Agnarsdottir M, Casper C, et al. High prevalence of cytomegalovirus infection in surgical intestinal specimens from infants with necrotizing enterocolitis and spontaneous intestinal perforation: a retrospective observational study. *J Clin Virol.* 2017;93:57–64
29. Panesso-Gómez S, Shimamura M, Conces M, et al. Detection of cytomegalovirus in intestinal tissue of infants with necrotizing enterocolitis or spontaneous intestinal perforation. *J Pediatr.* 2019;214:34–40
30. Ramani S, Sowmyanarayanan TV, Gladstone BP, et al. Rotavirus infection in the neonatal nurseries of a tertiary care hospital in India. *Pediatr Infect Dis J.* 2008;27(8):719–723
31. Rotbart HA, Levin MJ, Yolken RH, Manchester DK, Jantzen J. An outbreak of rotavirus-associated neonatal necrotizing enterocolitis. *J Pediatr.* 1983;103(3):454–459
32. Rotbart HA, Nelson WL, Glode MP, et al. Neonatal rotavirus-associated necrotizing enterocolitis: case control study and prospective surveillance during an outbreak. *J Pediatr.* 1988;112(1):87–93
33. Rudd PT, Carrington D. A prospective study of chlamydial, mycoplasmal, and viral infections in a neonatal intensive care unit. *Arch Dis Child.* 1984;59(2):120–125
34. Sharma R, Premachandra BR, Mollitt DL, Tepas JJ. Necrotizing enterocolitis (NEC) associated with and without rotavirus infection (RVI): a comparative evaluation. *Pediatr Res.* 1996;39(Suppl 4):302
35. Sharma R, Hudak ML, Premachandra BR, et al. Clinical manifestations of rotavirus infection in the neonatal intensive care unit. *Pediatr Infect Dis J.* 2002;21(12):1099–1105
36. Sharma R, Garrison RD, Tepas JJ III, et al. Rotavirus-associated necrotizing enterocolitis: an insight into a potentially preventable disease? *J Pediatr Surg.* 2004;39(3):453–457
37. Sizmaz E, Satar M, Ozlü F, Yaman A, Yõldõzdaş HY, Ozcan K. The coincidence of necrotizing enterocolitis and rotavirus infections and potential associations with cytokines. *Can J Infect Dis Med Microbiol.* 2012;23(4):e103–e105
38. Stuart RL, Tan K, Mahar JE, et al. An outbreak of necrotizing enterocolitis associated with norovirus genotype GII.3. *Pediatr Infect Dis J.* 2010;29(7):644–647
39. Tai IC, Huang YC, Lien RI, Huang CG, Tsao KC, Lin TY. Clinical manifestations of a cluster of rotavirus infection in young infants hospitalized in neonatal care units. *J Microbiol Immunol Infect.* 2012; 45(1):15–21
40. Turcios-Ruiz RM, Axelrod P, St John K, et al. Outbreak of necrotizing enterocolitis caused by norovirus in a neonatal intensive care unit. *J Pediatr.* 2008;153(3):339–344

41. Turner KM, Lee HC, Boppana SB, Carlo WA, Randolph DA. Incidence and impact of CMV infection in very low birth weight infants. *Pediatrics*. 2014;133(3):e609–e615

42. Ullrich T, Tang YW, Correa H, et al. Absence of gastrointestinal pathogens in ileum tissue resected for necrotizing enterocolitis. *Pediatr Infect Dis J*. 2012;31(4):413–414

43. Weimer KED, Kelly MS, Permar SR, Clark RH, Greenberg RG. Association of adverse hearing, growth, and discharge age outcomes with postnatal cytomegalovirus infection in infants with very low birth weight. *JAMA Pediatr*. 2020;174(2):133–140

44. GRADEpro GDT. *GRADEpro Guideline Development Tool*. Hamilton, Canada: McMaster University and Evidence Prime; 2022

45. Petrignani M, Verhoef L, de Graaf M, Richardus JH, Koopmans M. Chronic sequelae and severe complications of norovirus infection: a systematic review of literature. *J Clin Virol*. 2018;105:1–10

46. Stark A, Cantrell S, Greenberg RG, Permar SR, Weimer KED. Long-term outcomes after postnatal cytomegalovirus infection in low birthweight preterm infants: a systematic review. *Pediatr Infect Dis J*. 2021;40(6):571–581

47. You DM, Johnson MD. Cytomegalovirus infection and the gastrointestinal tract. *Curr Gastroenterol Rep*. 2012;14(4):334–342

48. Fundora JB, Guha P, Shores DR, Pammi M, Maheshwari A. Intestinal dysbiosis and necrotizing enterocolitis: assessment for causality using Bradford Hill criteria. *Pediatr Res*. 2020;87(2):235–248

49. Neu J. Necrotizing enterocolitis: the mystery goes on. *Neonatology*. 2014;106(4):289–295

50. Pammi M, Cope J, Tarr PI, et al. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. *Microbiome*. 2017;5(1):31

51. Been JV, Lievense S, Zimmermann LJ, Kramer BW, Wolfs TG. Chorioamnionitis as a risk factor for necrotizing enterocolitis: a systematic review and meta-analysis. *J Pediatr*. 2013;162(2):236–42.e2

52. Duci M, Frigo AC, Visentin S, Verlato G, Gamba P, Fascetti-Leon F. Maternal and placental risk factors associated with the development of necrotizing enterocolitis (NEC) and its severity. *J Pediatr Surg*. 2019;54(10):2099–2102

53. Cao Z, Sugimura N, Burgermeister E, Ebert MP, Zuo T, Lan P. The gut virome: a new microbiome component in health and disease. *EBioMedicine*. 2022;81:104113

54. Shkoporov AN, Hill C. Bacteriophages of the human gut: the "known unknown" of the microbiome. *Cell Host Microbe*. 2019;25(2):195–209

55. Breitbart M, Haynes M, Kelley S, et al. Viral diversity and dynamics in an infant gut. *Res Microbiol*. 2008;159(5):367–373

56. Lim ES, Zhou Y, Zhao G, et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. *Nat Med*. 2015;21(10):1228–1234

57. Liang G, Zhao C, Zhang H, et al. The stepwise assembly of the neonatal virome is modulated by breastfeeding. *Nature*. 2020;581(7809):470–474

58. Bushman F, Liang G. Assembly of the virome in newborn human infants. *Curr Opin Virol*. 2021;48:17–22

59. Mukhopadhya I, Segal JP, Carding SR, Hart AL, Hold GL. The gut virome: the 'missing link' between gut bacteria and host immunity? *Therap Adv Gastroenterol*. 2019;12:1756284819836620

60. Harvala H, McIntyre CL, McLeish NJ, et al. High detection frequency and viral loads of human rhinovirus species A to C in fecal samples; diagnostic and clinical implications. *J Med Virol*. 2012;84(3):536–542

61. Kapoor A, Slikas E, Simmonds P, et al. A newly identified bocavirus species in human stool. *J Infect Dis*. 2009;199(2):196–200

62. van der Hoek L, Boom R, Goudsmit J, Snijders F, Sol CJ. Isolation of human immunodeficiency virus type 1 (HIV-1) RNA from feces by a simple method and difference between HIV-1 subpopulations in feces and serum. *J Clin Microbiol*. 1995;33(3):581–588

63. Oude Munnink BB, van der Hoek L. Viruses causing gastroenteritis: the known, the new and those beyond. *Viruses*. 2016;8(2):42

64. Neil JA, Cadwell K. The intestinal virome and immunity. *J Immunol*. 2018;201(6):1615–1624

65. Yang JY, Kim MS, Kim E, et al. Enteric viruses ameliorate gut inflammation via toll-like receptor 3 and toll-like receptor 7-mediated interferon-β production. *Immunity*. 2016;44(4):889–900

66. Smith PD, Shimamura M, Musgrove LC, et al. Cytomegalovirus enhances macrophage TLR expression and MyD88-mediated signal transduction to potentiate inducible inflammatory responses. *J Immunol*. 2014;193(11):5604–5612

67. Brunse A, Deng L, Pan X, et al. Fecal filtrate transplantation protects against necrotizing enterocolitis. *ISME J*. 2022;16(3):686–694

68. Cadwell K, Patel KK, Maloney NS, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell*. 2010;141(7):1135–1145

69. Sampath V, Bhandari V, Berger J, et al. A functional ATG16L1 (T300A) variant is associated with necrotizing enterocolitis in premature infants. *Pediatr Res*. 2017;81(4):582–588

70. Casaburi G, Wei J, Kazi S, et al. Metabolic model of necrotizing enterocolitis in the premature newborn gut resulting from enteric dysbiosis. *Front Pediatr*. 2022;10:893059

71. Kaelin EA, Rodriguez C, Hall-Moore C, et al. Longitudinal gut virome analysis identifies specific viral signatures that precede necrotizing enterocolitis onset in preterm infants. *Nat Microbiol*. 2022;7(5):653–662

72. Civardi E, Tzialla C, Baldanti F, Strocchio L, Manzoni P, Stronati M. Viral outbreaks in neonatal intensive care units: what we do not know. *Am J Infect Control*. 2013;41(10):854–856

73. Hammerton G, Munafò MR. Causal inference with observational data: the need for triangulation of evidence. *Psychol Med*. 2021;51(4):563–578

74. West SG, Duan N, Pequegnat W, et al. Alternatives to the randomized controlled trial. *Am J Public Health*. 2008;98(8):1359–1366

75. Angelika D, Etika R, Vita AD, Mithra S, Ugrasena IDG. Necrotizing enterocolitis in preterm infants born to mother with COVID-19. *Open Access Maced J Med Sci*. 2021;9(B):1499–1504