

POINT OF VIEW

Contaminated milk is a common cause of necrotising enterocolitis: A hypothesis

Nem-Yun BOO *MBBS, FRCPCH*

Department of Population Medicine, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Malaysia

Abstract

Necrotising enterocolitis (NEC) is the most commonly acquired gastrointestinal disease of neonates, particularly the very preterm (gestation <32 weeks) and/or very low birth weight (<1500g). It is associated with high morbidity and mortality. Despite improvement in neonatal care and increased use of expressed breast milk (EBM), the incidence remains high in many neonatal intensive care units (NICU), and even shows increasing trend in some countries. Numerous studies have pointed to the infective nature of NEC. Some investigators have reported an increase in the incidence of NEC in their NICU when the percentage of infants with pathogens isolated from their gut increased, and decreased when gut colonisation rate was low. Both bacteria and viruses have been reported to be associated with outbreaks of NEC. The majority (>90%) of the NEC cases occurred in neonates on enteral feeding. Studies have shown that milk (whether EBM or formula) fed to neonates was not sterile and were further contaminated during collection, transport, storage and/or feeding. Other investigators have reported a reduction in the incidence of NEC when they improved infection control measures and hygienic procedures in handling milk. It is, therefore, hypothesised that the most common cause of NEC is due to the feeding of neonates, particularly the vulnerable very preterm small neonates, with milk heavily contaminated during collection at source, transport, storage and/or feeding. Because of the immaturity of the immune system of the neonates, excessive inflammatory response to the pathogen load in the gut leads to the pathogenesis of NEC.

Keywords: necrotising enterocolitis, NEC, neonates, milk

THE BURDEN OF NECROTISING ENTEROCOLITIS

Necrotising enterocolitis (NEC) is the most commonly acquired gastrointestinal disease of neonates. It is characterised by intestinal necrosis and pneumatosis intestinalis, which often leads to gangrene and perforation of the bowel wall, septicaemia and death. It is associated with high morbidity among survivors, such as short gut syndrome or stricture.¹ In extremely low birth weight (<1000g) neonates with NEC, a mortality of 35-45% has been reported.^{2,3} NEC affects mainly preterm neonates born at \leq 35 weeks of gestation.⁴⁻⁶ Majority (85%) of the NEC occurred in preterm neonates of very low birth weight (VLBW or <1500g) or very preterm gestation (<32 weeks). Only 7 to 15 % occurred in later preterm and term neonates.^{7,8} The incidence of NEC, ranging from 3% to 12%, increases

markedly with decreasing birth weight and gestation.^{1,4,6,9} Despite improvement in neonatal intensive care, the incidence remains high in neonatal intensive care units (NICU), and in some countries like Sweden the incidence of NEC was reported to be increasing over an 8-year period from 2.6 per 10,000 live births in 1987 to 5.7 per 10,000 live births in 2009.¹⁰

Intestinal immaturity, inappropriate microbial colonization and enteral feedings have been identified to be important risk factors associated with NEC.^{1,11-13} However, despite more than 150 years of research, the exact cause of NEC has not been identified.^{1,14}

EVIDENCE SUGGESTIVE OF AN INFECTIVE NATURE OF NEC

Numerous studies have pointed to the infective nature of NEC. Bell *et al* in 1979 observed that

Address for correspondence: Professor Nem-Yun BOO, Department of Population Medicine, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Jalan Sungai Long, Bandar Sungai Long, 43000 Kajang, Selangor, Malaysia. Tel: +603 90860288. Email: boony@utar.edu.my

the incidence of NEC in their NICU increased when the percentage of infants with pathogenic organisms isolated from their gut increased, and decreased when gut colonisation rate was low.¹⁵ Other investigators reported that neonates with confirmed NEC had abundance of faecal *Clostridium perfringens* type A and *Klebsiella* species isolated.¹⁶ No NEC has been reported in gnotobiotic (germ-free) animals.¹⁷ Furthermore, both bacteria¹⁸⁻²⁰ and viruses^{21,22} have been reported to be associated with outbreaks of NEC. The pathological findings of NEC reveal excessive inflammatory response to luminal microbial stimuli in the affected segment of the gastrointestinal tract, further supporting the infective nature of this condition. Cochrane review reported that no particularly effective antibiotic regimen for treatment of NEC has been identified.²³

CONTAMINATED MILK AS THE MOST LIKELY COMMON CAUSE OF NEC

Milk is a rich culture medium. Both formula milk powder and mother's milk are not sterile and the pathogens present in the milk can multiply under a conducive environment. Many investigators have reported high rates of heavy bacterial contamination of expressed breast milk (EBM), with colony counts exceeding 10^5 colony forming units (CFU)/millilitre of milk.^{15,24-26} Boo *et al* found that EBM collected at home were more likely to be contaminated with faecal pathogens than those collected in the hospital, where contamination was more common with skin organisms than faecal pathogens.²⁴ Both formula milk and EBM can be contaminated during collection at source, transport, storage, and/or feeding.^{27,28} During the process of collection from the mother's breast, EBM can be contaminated by a contaminated breast pump, or the milk container/bottle. High temperature during storage ($>7^{\circ}\text{C}$) will promote multiplication of bacteria in the milk. When human milk fortifier is added to the EBM for VLBW neonates to increase the nutrient content and calories, pathogens from the hands of the care provider can be transferred to the milk. Jocson *et al*²⁹ also showed that human milk fortifiers enabled bacterial growth in EBM by providing free iron. Pasteurisation of milk has been shown to be effective to eliminate most pathogens.³⁰ In a population study carried out over a six-year period, Kantorowska *et al* showed that increased use of donor milk which

were routinely pasteurised in the milk bank was associated with a lower NEC rate.³¹

CONTAMINATED ORAL GASTRIC TUBES POSSIBLY CONTRIBUTES TO INCREASED RISK OF NEC

In the NICU, preterm infants are usually fed via oral gastric (OG) tubes during the first few weeks of life. Some investigators have reported that gram-negative bacteria contamination of OG tubes increased with the duration of use of these indwelling tubes.^{25,32} In a study to determine whether neonatal nasogastric enteral feeding tubes were colonised by opportunistic pathogens, Hurrell *et al*³² reported isolation of infective pathogens in 76% of enteral feeding tubes, either from biofilms (up to 10^7 CFU/tube from neonates fed fortified EBM and reconstituted powdered infant formula) or from the residual lumen liquid of OG tubes. They also found that *Enterobacteriaceae* were isolated from OG tubes of all feeding regimens, and that the frequency of isolation of this pathogen was lower in the OG tubes of neonates on EBM (52%) than those of neonates on mixed feeding regimens (78%) or given reconstituted powdered infant formula (80%). In the same study, they also found that, when compared with those OG tubes remaining in-situ for <6 hours in the neonates, OG tubes remaining in-situ for 6-12 hours, 19- <24 hours, 24-48 hours and >48 hours all had significantly greater bacterial counts, with the maximum at 48 hours. They reported that besides *Enterobacteriaceae* spp, other gram positive and gram negative bacteria and candida were also isolated from the OG tubes.

NEC was reported most commonly ($>90\%$ of NEC cases) in neonates on enteral feeding, irrespective of whether they were on formula or expressed breast milk.^{33,34} Outbreaks of NEC have been reported to be associated with milk powder contaminated with infective pathogens.²⁰ Although the incidence of NEC among EBM-fed preterm neonates was reported to be lower than those on formula milk, the incidence of NEC in EBM-fed neonates remains high. A Swedish population study over an 8-year period reported progressive increase in the incidence of NEC despite high rates of infants fed EBM.¹⁰ This increasing trend of NEC among preterm neonates in NICUs with high EBM-feeding rates of $>90\%$ was similarly reported by other investigators.^{35,36} In most NICUs where larger (and generally stronger) preterm neonates were

usually commenced on enteral feeds earlier and with larger volume of milk than the smaller and/or more preterm neonates, onset of NEC occurred earlier in the more mature preterm neonates.⁹

PATHOLOGICAL CHANGES OF NEC

Neonates, particularly the preterm ones, have immature innate immunity. When compared with older children, neonatal intestine is weaker and more permeable to macromolecules.³⁷ The gel-like protective mucus in the premature gut is also more immature, and the anti-inflammatory pathway is inadequate.³⁸ These predispose the preterm neonates to develop excessive intestinal inflammation in response to injuries, including those induced by pathogens, with decreased enterocyte migration and proliferation, and increased bacterial translocation.³⁹ The typical pathological findings in the gut of neonates with NEC consist of inflammation of the intestinal mucosa, invasion of the immature gut by enteric gas-forming bacteria, dissection of the gut wall and portal veins by this gas, and ischemic necrosis of the intestine in severe cases. Animal models of NEC have been produced for research by feeding preterm pups with large doses of infective pathogens to produce similar findings.^{40,41}

NEC INCIDENCE DECREASED IN NICUS IMPLEMENTING STRATEGIES TO IMPROVE INFECTION CONTROL AND HYGIENIC HANDLING OF MILK

The World Health Organisation (WHO) has produced very clear guidelines on the safe preparation, storage and handling of powdered infant formula (PIF) to prevent contamination by pathogens.⁴² WHO recommends that, whenever possible, sterile liquid infant formula should be used for high risk infants instead of PIF. However, WHO has yet to produce any guidelines on the safe collection, storage and handling of EBM. The incidence of NEC varied in different NICU worldwide.^{6,10,43-45} One possible explanation could be the different levels of milk contamination in different NICU, as there is no standard protocol used by all NICUs regarding the handling of EBM.

Recognising the need to address the hazards in EBM, Cossey *et al* proposed using the hazard analysis and critical control points (HACCP) method to improve safety of EBM in the NICU.⁴⁶ Under this method, all stages of handling of EBM (from milk expression and collection, transfer of EBM to NICU, storage in NICU, preparation

for use and feeding), except use of OG tubes, were addressed to reduce contamination.

In a study by Patel *et al* where the authors initiated a series of quality improvement initiative steps, the incidence of NEC in their NICU fell from 19% to 3%. The steps implemented included improving infection control policies, hygienic handling of human milk, reduction of duration of in-dwelling nasogastric tubes, changing the extension feeding tubing between each feed in the preterm neonates, and re-education of mothers on hygienic handling and cleaning of breast pumps.³⁶ Their findings were concordant with those reported in the Cochrane review⁴⁷ that showed that slowing the increase in feeding volume did not bring about a reduction in NEC incidence. Other investigators have similarly reported a reduction in the incidence of NEC with the implementation of infection control measures with emphasis on good hand hygiene.^{48,49}

HYPOTHESIS ON THE CAUSATION OF NEC

Based on the evidence above, it is hypothesised that the most common cause of NEC is due to the feeding of neonates, particularly the vulnerable very preterm small neonates, with milk heavily contaminated during collection at source, transport, storage or/and feeding. Because of the immaturity of the immune system, excessive inflammatory response to the pathogen load in the gut leads to the pathogenesis of NEC. Various authors have shown that the incidence of NEC in NICU can be reduced drastically by instituting infection control measures and procedures to prevent contamination of milk in all these stages, irrespective of whether it is EBM or formula milk.

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