The temporal relationship between exposure to bovine milk products and surgical NEC in preterm infants

Necrotising enterocolitis (NEC) continues to be a major cause of neonatal mortality and morbidity. It is less common in breastfed infants suggesting a protective role for breast milk. However NEC is now being seen in fully breast milk fed infants following introduction of fortifiers derived from bovine milk, which suggests a possible causative role for bovine products. This study investigates the relationship between the commencement of feed containing bovine milk products and the development of surgical NEC in preterm infants.

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Keywords

necrotising enterocolitis; infant feeding; cows' milk protein

Key points

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1. NEC is less common in breastfed infants but may occur following introduction of bovine milk product fortifiers.
2. In this study half of the infants with surgical NEC developed the condition within seven days of exposure to bovine products, sometimes after several weeks of successful breast feeds.
3. Bovine products appear to have a causative role in surgical NEC and this may be via non-IgE mediated cows' milk protein allergy.

Necrotising enterocolitis (NEC) continues to be a major cause of morbidity and mortality in preterm infants.[1] Although the causes of NEC remain unclear, a relationship between enteral feeding and the development of NEC is well established. This was first highlighted by Lucas who observed that NEC occurred six times more frequently in infants receiving formula feeds than infants who were breastfed and three times more frequently if mixed feeds were used.[2] In recent years there has been considerable focus on how different feeding strategies may reduce the risk of NEC, mostly concentrating on the timing of feed introduction and the speed of progressing feeds in the early postnatal period.[3-5]

Although it has been noted that NEC typically occurs in the second week of life,[6] it may also occur when the infant is several weeks old and well established on full feeds[7] and may be seen following a transition from one type of feed to another, particularly exposure to preterm formula feeds.[8] There has been recent interest in the relationship between feed type and the development of NEC particularly in the context of bovine versus human derived milk proteins.[9-11] These studies suggest that bovine milk protein (BoMP) might be a causative factor in the development of NEC. We undertook this study to examine the temporal relationship between the introduction of feeds containing BoMP and the development of surgical NEC in preterm infants referred to our centre.

Materials and methods

A retrospective observational study of all infants with surgical NEC treated in a single neonatal surgical centre from April 2007 to April 2017 was performed. Infants are admitted to a combined neonatal medical/surgical intensive care unit that serves a neonatal network with 33,000 live births annually. Cases were identified from a prospectively maintained neonatal surgical database and only infants in whom NEC had been confirmed at laparotomy or post-mortem were included in the study. Infants born at term, those with congenital cardiac abnormalities and infants who had undergone previous intestinal surgery were excluded. The feed history for each infant was reviewed using data available on the electronic Badgernet system and by review of the individual patient's hospital records, both at their referring hospital (by a local clinician) and at the surgical centre.

The type of feed was recorded for each day of life from birth until the diagnosis of NEC. Feed types were classified as:

- breast milk (BM), which might be maternal or donor in origin
- preterm formula (PTF)
- extensively hydrolysed formula (EHF)
- amino acid formula (AAF)
- breast milk fortifier (BMF).

BMF and all formula feeds (other than AAF) contain, or are derived from, bovine milk. Data are presented as median (range). The study had institutional approval.

Results

Eighty infants with surgical NEC were managed during the study period of whom 67 met the inclusion criteria. Demographic data relating to the patient population is shown in **TABLE 1**.

Twenty-four infants (36%) died during the surgical admission episode. The postnatal age at diagnosis of surgical NEC is shown in **FIGURE 1**. All infants in this study had received enteral feeds prior to developing NEC. The feed type at diagnosis of surgical NEC is shown in **TABLE 2**. Overall there were 22 infants (33%) who were receiving full BM feeds, either alone (n=7) or in combination with BMF (n=15) who developed NEC.

Parenteral nutrition (PN) was provided in the early days of life in 63 infants and 19 infants were still receiving some PN at the time they developed NEC. The 48 infants who had reached full feeds prior to developing surgical NEC had been fully enterally fed for 14 days (range 2-42).

Sixty infants received BoMP, with or without BM, introduced at a median age of 17 (range 1-55) days. Five infants only ever received BoMP with the other 55 starting feeds on BM prior to exposure to BoMP 16 (range 1-52) days later. **FIGURE 2** shows the time in days between first exposure (or in one case re-exposure) to BoMP and the development of surgical NEC in these 55 infants. Surgical NEC developed within a week of exposure in 28 infants (51%) and within five days in 21 (38%) (range 1-38).

FIGURE 3 shows the timeline from initiation of enteral feeds to introduction of BoMP and subsequent development of surgical NEC for each of the 55 infants who received BM prior to BoMP. Four infants who had tolerated BM feeds for over one month before being exposed to BoMP developed NEC at an average of five days (range 2-9) after exposure.

One specific case history is of particular interest. A female infant born at 26 weeks' gestation weighing 680g had BM feeds introduced at 10 days of age, BMF added on day 20 and PTF added on day 27. On day 33 the infant developed abdominal symptoms including distention and rectal bleeding and a diagnosis of possible NEC or milk intolerance was made. The infant was kept nil-by-mouth for seven days and treated with antibiotics. Feeds were restarted on day 39 using an AAF. Two weeks later PTF was re-introduced and within 48 hours she developed NEC requiring laparotomy and intestinal resection.

Birth gestation (weeks)	27 (23-33)
Birth weight (g)	877 (458-1995)
Age at surgical NEC (days)	28 (4-61)
Acute NEC-related mortality (n)	24 (36%)

TABLE 1 Demographic detail of 67 infants with surgical NEC. Data are median (range).

Feed type	n	%
BM only	7	10%
BM + other feed	38	57%
BM+BMF	15	
BM+ PTF	14	
BM + EHF	3	
BM+BMF+PTF	6	
PTF	17	25%
EHF	5	7%
Total	67	

TABLE 2 Feed type at the time of development of surgical NEC in 67 infants. Key: BM = breast milk, BMF = breast milk fortifier, PTF = preterm formula, EHF = extensively hydrolysed formula.

Discussion

The results confirm the serious nature of advanced NEC with a mortality approaching 40%, similar to that reported in a recent large UK study.[12] This, combined with serious neurological consequences in survivors,[13] makes it one of the most devastating consequences of premature birth. Much attention and research has focused on the pathological mechanisms

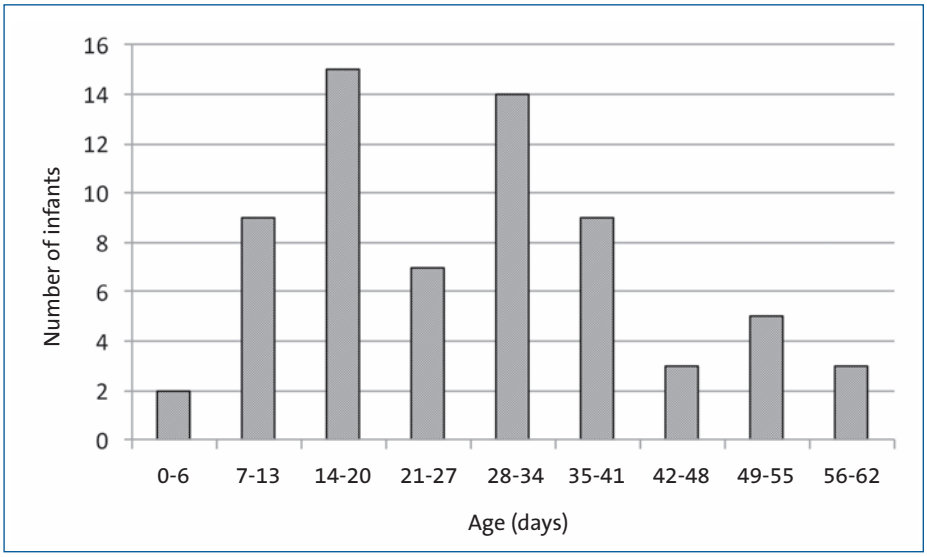

FIGURE 1 Age at presentation in 67 infants with surgical NEC.

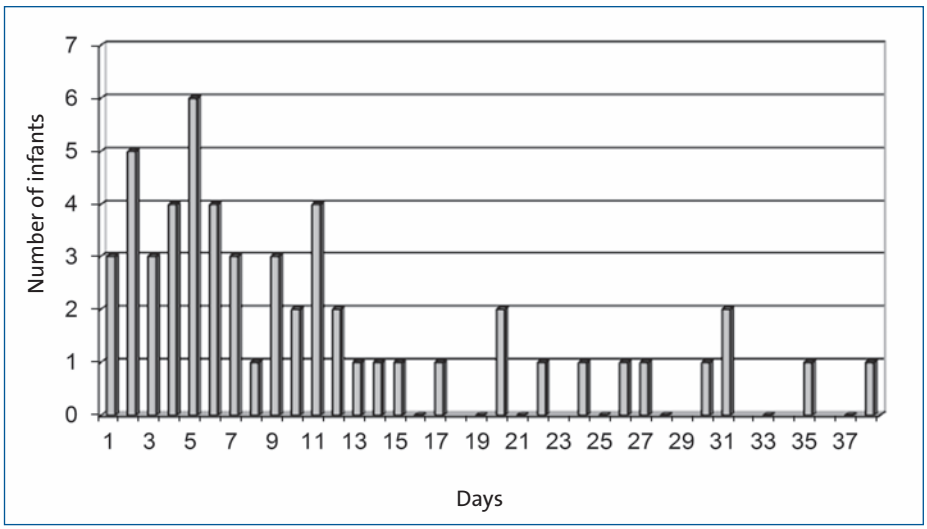

FIGURE 2 Time in days from first or re-exposure to BoMP to development of surgical NEC in 55 infants who had previously received BM.

that occur in NEC in the hope of finding ways to prevent the disease, enable earlier diagnosis or reduce its consequences.

The stimulus for this study was the clinical observation that surgical NEC may follow a recent change in feed type. Our findings that surgical NEC developed within seven days of introduction of BoMP in half of the cases and within five days in 38% and that 55 of these infants had been enterally fed with BM for an average of 16 days prior to BoMP exposure provides data to support this observation. Analysis of the relationship between feed type and NEC has in the past usually focused on the protective effect of BM compared to formula feeds.[2] Recently it has been postulated that there are a number of subsets of NEC including term NEC, transfusion related NEC and cows' milk related NEC,[14] suggesting that BoMP may have a role in NEC development.

There is some evidence in the literature that BoMP may be associated with NEC. Hammond reported nine preterm infants who developed fulminant NEC within 24 hours of exposure to a PTF.[8] BMF has been implicated in cows' milk protein (CMP) sensitisation in preterm infants.[15] Reduced rates of NEC have been observed in a randomised controlled trial of human BM-derived BMF (hBMF) versus bovine BMF (bBMF).[9] A number of other studies have shown reduction in rates of NEC in infants receiving hBMF compared to bBMF or PTF.[10,11] In a UK-wide study of infants born before 32 weeks' gestation, avoidance of BoMP in the first 14 days of life reduced the relative risk of developing severe NEC.[12] A recent randomised study comparing supplementation of BM with either donor BM or BoMP in the first 10 days of life found no difference in the incidence of Bell stage II or above NEC by 60 days of age. However, nearly half of the infants in both treatment groups in this study subsequently received BoMP after 10 days of age and most NEC occurred after this age.[16]

The precise mechanism by which BoMP might cause NEC is not clear. A number of case reports suggest that NEC may occur as a result of immunoglobulin E (IgE)-mediated CMP allergy (CMPA).[17-19] However there are many similarities between NEC and non-IgE mediated CMPA in infants and children, the effects of which are largely intestinal[20,21] and may be severe enough to merit emergency laparotomy.[22] Of note, non-IgE mediated CMPA is not confined to infants receiving formula feeds; it may occur in infants receiving BM or EHF.[21,23,24] Increased intestinal permeability and dysmotility of the preterm bowel may further predispose to sensitisation to CMP.[25]

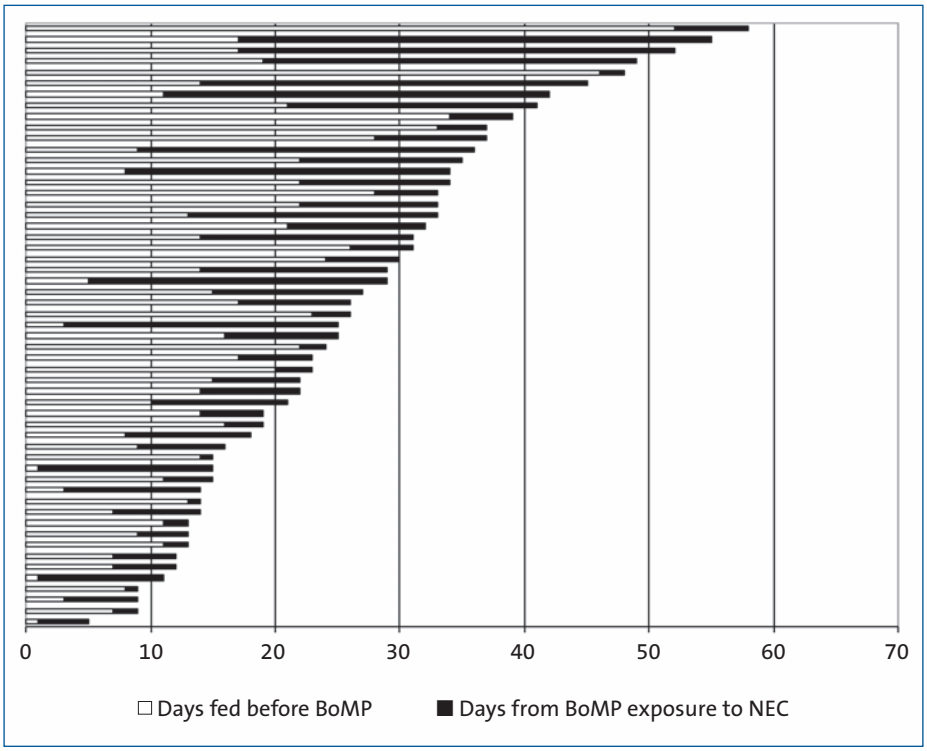

FIGURE 3 Duration of enteral feeding (days) before (white bar) and after (black bar) first or re-exposure to BoMP. Development of surgical NEC is depicted by the end of the bar. Each bar relates to an individual infant (n=55).

Laboratory data also support the concept of a non-IgE mediated CMPA mechanism in NEC. Analysis of peripheral blood mononuclear cells in infants with NEC showed evidence of a strong cytokine response to beta lactoglobulin.[26] This was taken to indicate significant CMP sensitisation. This response was increased with reintroduction of feeds following NEC and increased further when full feeding was achieved.[27] In an invited commentary on these two papers, Murch commented: "These studies have been valuable in raising the profile of cows' milk as a trigger of intestinal inflammation in preterm neonates. Similar enhanced lymphocyte response to cows' milk has been identified in infants with food protein intestinal enteropathy syndrome."[28]

There are a number of limitations to our study. It was retrospective, which may lead to concerns over data quality or missing data. We were clear to only include advanced NEC, diagnosed at either surgery or post-mortem so as to be completely sure of the diagnosis. Despite the retrospective nature of the study we were able to ascertain feed data on all infants using our methodology. It is possible that some infants may have been at greater risk of NEC at the time BoMP was introduced due, for instance, to poor nutrition.

Despite these limitations, this study supports the concept that the incidence of NEC in preterm infants may be reduced by the avoidance of BoMP. This might be achieved using hBMF in infants receiving BM in whom additional supplementation is needed and using an AAF in infants requiring formula feeds. Currently hBMF is not available in the UK and there is no AAF formula designed for preterm infants. We are hopeful that this study, and others that may follow it, may be a stimulus to manufacturers to consider providing such products. Any reduction in the incidence of NEC would have a huge impact on preterm mortality and morbidity as well as financial cost to health services.[29]

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Discussing cows' milk protein and NEC

Re: **Burge DM, Drewett M, Hall NJ.** The temporal relationship between exposure to bovine milk products and surgical NEC in preterm infants. *Infant* 2018;14(2):58-61.

Thank you for raising this issue, which is often debated but poorly researched, namely the role of cows' milk protein in the pathogenesis of necrotising enterocolitis (NEC). Confusion arises when all products containing cows' protein are grouped together; this includes preterm formula, breast milk fortifier (BMF) containing whole cows' milk protein, and BMF that contains hydrolysed cows' milk protein. They are not all equal and the differences between them have not been tested in any of the papers published so far. In particular, the effects of a whole protein-based BMF vs one that is hydrolysed.

An association with use of formula and NEC is well established and the case study used by Burge et al illustrates this. However, our concern is the suggestion that the association with BMF is now also confirmed – we feel it is not. In correspondence with some of the authors of the Sullivan et al 2010 article,[1] it was confirmed that a significant number of the babies with NEC had received both formula and BMF and therefore it was not possible to distinguish which was associated with NEC (King C, personal communication). Subsequently a letter was published by Embleton et al[2] outlining other concerns around the Sullivan et al paper. In particular it was noted that the background rate of NEC in infants fed according to standard guidelines with bovine milk-based fortifiers (16% all NEC, 12% NEC surgery) is much higher than in many units.

Of the other articles referred to by Burge et al:

- The study by Cristofalo et al 2013[3] was underpowered to look at incidence of NEC.
- Hair et al 2016[4] was not a randomised controlled trial but a retrospective cohort study and therefore very weak evidence.
- Battersby et al 2017[5] noted that >50% of their cohort who developed NEC received breast milk alone and that it gave only a small protective effect. Interestingly, although there was a wide variation in feeding practices across the neonatal networks in the UK, Battersby et al noted that there was no unusual variation in the incidence of NEC.

Regarding Burge et al's article, the aim was stated as to examine the temporal relationship between the introduction of feeds containing cows' milk products and the development of surgical NEC in preterm babies in their centre. We wonder how an association with cows' milk protein products could be made when we do not know what the denominator population were fed? In some units, use of BMF or formula is high; do they have a higher incidence of surgical NEC? This would be a very interesting question to answer and would build on the work of Battersby et al.

Also, as this is observational, we do not know the incidence of other known risk factors among the population. For example, intrauterine growth restriction, gut dysbiosis, proton pump inhibitors, antenatal steroids and feeding protocols.

Lucas and Cole 1990[6] found that breast milk appeared more protective in babies born at ≥30 weeks' gestation whereas the younger gestation babies appeared to be more at risk of NEC, whatever their feed. This would suggest an increased importance of other risk factors for the less mature babies.

It would be really interesting to see the temporal relationship between days from cows' milk exposure to development of NEC subdivided by origin of the protein, ie formula, whole protein fortifier or hydrolysed protein fortifier.

In summary, we wonder if keypoint 3 is made before adequate evidence and we call for research to try and obtain that evidence. A simple start would be to carry out an *in vitro* study of peripheral blood mononuclear cell response to cows' milk-based formula, BMF containing whole protein, and BMF containing hydrolysed protein. There is a good theoretical basis to suggest that hydrolysed protein will not elicit an adverse cytokine response.

Yours sincerely

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Authors' response

We thank you for your interest in our article and agree that this is a complex issue, not simplified by the wide variety of different milk products (all with different constituent proteins, peptides or amino acids) given to newborn infants. Our main aim was to highlight the potential relationship between feed type and development of NEC. We do not suggest that our data can be used to definitively prove causality. We completely support your calls for further well-designed research in this field since the incidence of NEC remains unacceptably high.

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