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Cytomegalovirus and other common enteric viruses are not commonly associated with Necrotizing Enterocolitis

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ABSTRACT

Aim: Changes in gut microbiota may contribute to NEC but most studies focus on bacteria. Case reports suggest a link between cytomegalovirus (CMV) or other enteric viruses and NEC, but there are few case series systematically looking at common potential viral causes. We aimed to assess the presence of candidate viruses in blood or stool of a case series of infants with NEC managed in one surgical centre.

Methods: We identified 22 infants diagnosed with NEC (from 11/11 to 03/14): 17 had suitable blood stored, of whom 14 also had suitable stool samples stored. Blood was analysed with polymerase chain reaction (PCR) for CMV, Ebstein-Barr virus(EBV) and adenovirus, and stool by PCR for norovirus, sappovirus, astrovirus, adenovirus and rotavirus.

Results: All samples were negative.

Conclusion: Although case reports indicate an episodic association of enteric viruses in NEC, the inability to detect any of these viruses in our 17 NEC infants suggests that a viral aetiology is unlikely to be causative for most sporadic forms of NEC.

Key Notes:

- The aetiology of NEC remains unknown, clustering of cases of NEC and case reports of concurrent infection suggest viruses potentially play a part in the onset of NEC.
- In a 17 case series of definite NEC, no cases of CMV or common enteric viruses were identified

- Our series identified no viral pathogens contributing to the onset of NEC

BACKGROUND

The gut microbiota is increasingly implicated in the aetiological pathway in NEC.(1) Most work to date focuses on the large and diverse bacterial communities as they are most readily studied and currently documented in greatest numbers and type. However other non-bacterial members of the gut microbiota may also be important either in terms of direct 'causation' or by disturbing the environmental 'status quo' of gut microbial community structures, including fungi (2) and viruses.

Torovirus (3) was found in 48% of a series of 44 infants with NEC in a single unit over five years, a rate higher than in non-NEC controls where 17% harboured torovirus. Astrovirus (4) was similarly identified in 19% of a cohort of 32 cases of NEC in a single unit case series over 4 years. Cytomegalovirus (CMV) has featured in case reports as playing a possible role in NEC development. Tran *et al* (5) reported a single infant of 25weeks gestation who was taking part in a study of the gut microbiome when it developed fulminant lethal NEC. At post mortem inclusion bodies were noted, and CMV confirmed with immunohistochemical techniques on resected intestinal tissue. An associated bloom in Proteobacteria was noted. CMV contributes to direct mucosal injury in inflammatory bowel disease, so may have been directly causal in NEC development, or the presence of the virus could have destabilised gut bacteria community structures. Similarly a case of a twin developing NEC in association with CMV infection is reported. (6) Despite these reported associations, others have failed to identify a viral link - a study of resected paraffin embedded tissue samples from 27 cases of NEC using PCR testing did not identify any common viral causes of paediatric gastroenteritis, but CMV specifically was not tested for (7).

To further explore the possible role of CMV and other enteric viruses not previously well explored in NEC development we utilised blood and stool samples from our previously

reported sample salvage study SERVIS (8) to look for evidence of either systemic or enteric viral infections.

METHODS

The SERVIS Study was approved by the NRES Committee North East Co Durham and Tees Valley in July 2010, and written consent obtained from parents (ref 8). Infants included in this analysis were identified from the SERVIS database. *177 infants <1500g or <32 weeks gestation entered the SERVIS study of which 22 infant had NEC.*In this infants are classified as having NEC if they had either surgically or post mortem confirmed disease, or definite pneumatosis with associated clinical signs after independent verification by two clinicians. Samples from infants with NEC (a stool sample and salvaged EDTA) in close proximity to disease onset were selected for analysis. Stool samples were selected ideally from a time period from as close to diagnosis as possible. Optimal stool sampling was defined as from 7 days before diagnosis to 14 days after, based on accepted incubation/excretion timescales, and optimal blood sampling was defined as from 4 days before onset of NEC to 12 days after. *17 infants* were identified with suitable EDTA samples: of these 14 infants also had stool suitable stool samples available for analysis. Gestationally matched control infants with postnatally aged matched samples were also selected from the SERVIS database. For financial reasons cases were tested initially, with a plan to test controls if cases tested positive for the viral panel. Whole blood samples were salvaged as previously described (8) and tested for CMV using a real time multiplexed polymerase chain reaction (PCR) assay including EBV and adenovirus performed by the Public Health England North East Laboratory. Faecal samples were salvaged as previously described (8) and tested by multiplexed reverse transcription PCR (RT-PCR) for the detection of viral RNA (norovirus, sappovirus, astrovirus, rotavirus) and by PCR for the detection of viral DNA (adenovirus) performed by Microbiology Laboratory, Leeds General Infirmary. *A total of 10 separate PCRs*

for 8 viral targets and 2 internal controls, each with 3 separate primer/probe sequences. Both laboratories are UK accredited laboratories and participate in available external quality assurance schemes. Internal controls were added to samples prior to extraction to control for PCR inhibition, in addition to positive and negative controls on each run. Further details, including primer/probe sequences are available from A. Sails (Newcastle PHE)

RESULTS

The 17 contributing NEC infants had median birth weight 830g (range 500 -1350g) and median gestation 26 weeks (range 23-29) (Table 1).

The stool samples analysed were from a median of 3 days after NEC onset: all but two were within the ideal range (two exceeded the 7 days before by 3 and 7 days respectively). EDTA samples ranged from 2 days before onset to 14 days after (median 4 days after onset) (Table). NEC onset was at median 16 days of life (range 2 to 46 days): clinical features of each individual baby's disease are given in the Table. Of the 17 infants and 31 samples analysed, no baby or sample was identified as having CMV or EBV DNA present. Additionally, adenovirus, norovirus, sappovirus, astrovirus, rotavirus was not identified in any samples. There was no evidence of active or recent viral infection in any of the samples assayed. Given the global negative results in cases, control samples were not tested.

DISCUSSION

NEC is likely to represent the final common pathway of varying pathological processes including mucosal ischaemia, immune-mediated responses both to gut microbes and dietary components, and microbial dysbiosis and genetic factors. Thus key precipitants are likely to

differ between babies. However, understanding individual risk factors may offer preventive or treatment strategies for consideration and are thus worthy of exploration. Historical (from as early as 1975) and case report suggestions of viral associations in some cases of NEC, supported by the apparent seasonal variation occasionally seen in NEC and the clustering of cases that sometimes occurs, are all also seen in virally mediated phenomena. We have then been able to explore possible links to some viruses using our salvaged samples. This comparatively large case series of viral aetiology in NEC has been comprehensively and prospectively examined as part of the SERVIS study: all these cases represent definite NEC with the majority requiring surgical intervention. Our SERVIS study methodology enabled consecutive cases of definite NEC to be captured covering monthly variation throughout the year and explored possible association with common viral causes of gastroenteritis not previously well explored, as well as further exploring the hypothesised role of CMV. In the UK the most common viral causes of gastroenteritis are those explored here: adenovirus, norovirus, sappovirus, astrovirus and rotavirus with rotavirus being the most prevalent in the paediatric population prior to introduction of vaccination. Whilst not exhaustive, this viral panel is thus a sensible panel to have explored. We used blood from as close to diagnosis as possible. Given the range of sampling before and after NEC onset and the persistence of viral DNA it is unlikely that we missed many cases where the examined viruses were causative. Evidence suggests that viral RNA or DNA can be detectable in the stool weeks after infection (9): we used a 2 week cut off to maximise the identification of any infection, again meaning it is unlikely that we missed causative viruses of these types. Likewise CMV which was of aetiological importance should have been detected by blood PCR in the studied time frame, but it is possible that we have missed purely enteric disease, which for future studies could be further explored using either stool CMV assays or tissue histochemical analysis where resection occurs. In other units with differing bacterial gut communities it is possible that

these viruses may still play an important part, or other viruses not studied here may be important. Similarly bacteriophages (viruses that infect bacteria) may be important, and have recently been recognised in relation to infecting bacteroides (10) but remain unexplored in the context of NEC development. Future efforts to better understand the role of the gut microbiome in promoting health or disease in preterm infants need to consider bacterial, fungal, viral and archael elements, as well as functional aspects of these community constituents.

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Gestation (weeks)	Birth weight (g)	NEC details SB =small bowel LB = large bowel	Age in days of:			Month of diagnosis
			NEC onset	Stool sample	EDTA sample	
26	804	SB resection, ileostomy	9	18	14	December
28	1195	Extensive SB and LB resection	15	-	16	December
27	1060	Medically managed:definite pneumatosis	21	25	31	February
25	700	Ileostomy no resection	31	32	29	March
26	790	Medically managed:definite pneumatosis	19	19	26	April
29	1350	Extensive SB and LB resection: ileostomy and colostomy	26	12	28	May
23	500	SB disease, perforation, ileostomy	16	13	20	May
28	1250	Panenteric NEC	10	-	11	June
25	725	Extensive SB resection, ileostomy	25	23	26	July
28	1120	SB resection, ileostomy	16	25	21	August
25	890	Terminal ileal disease and perforation - 15cm resection	2	14	4	January
25	750	SB resection and ileostomy	3	8	4	January
24	830	Medically managed initially, later surgery for adhesions	36	-	48	January
26	880	Colonic perforation, histology NEC	3	10	9	January
24	630	SB resection and ileostomy	46	36	48	March
28	1120	Right hemi-colectomy	22	25	27	April
24	770	Extensive SB resection	10	9	15	April

Table 1 – Patient demographic