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Clinical characteristics of viral intestinal infection in preterm and term neonates

S. Bagci, A. M. Eis-Hübinger, A. F. Yassin, A. Simon, P. Bartmann, A. R. Franz, A. Mueller

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Abstract: Objectives: The clinical presentation of the viral enteric pathogens in newborn infants has not been adequately examined. The aim of this study was to evaluate the clinical characteristics of viral intestinal infections in newborn infants.

Methods: Clinical data of all term and preterm infants admitted to our tertiary neonatal intensive care unit from 1998 to 2007 with clinical signs of gastroenteritis (GE) or necrotizing enterocolitis (NEC) were retrospectively reviewed and compared between infants with different viral enteric pathogens in stool specimens.

Results: In thirty-four infants with signs of GE or NEC, enteropathogenic viruses were found in stool specimens. Rotavirus was detected in 12 cases, of which 2 infants had NEC. Compared with infants with rotavirus or norovirus, infants with astrovirus more frequently suffered from NEC ($p < 0.05$). In addition, an acute systemic inflammatory response was significantly more common in patients with astrovirus infection (astrovirus vs. rotavirus and astrovirus vs. norovirus $p < 0.01$ and $p < 0.05$, respectively). Of 8 children infected with norovirus, one infant had a systemic acute inflammatory response and NEC.

Conclusions: This study demonstrates that in newborn infants, intestinal rotavirus, norovirus, and astrovirus infections may be associated with severe illness such as hemorrhagic enteritis resulting in bloody diarrhea or even NEC.

Response to Reviewers: Ms. Ref. No.: EJCMIID-D-10-00107

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Answer 1: According the reviewers' suggestions we revised the objectives in the abstract. Because of "200 words-limit in abstract" we revised the abstract, too (all changes made to the manuscript text are underlined).

Reviewers' Comment 2: In the Results section (p.2, line 19) it should be mentioned that enteropathogenic viruses were found in 34 infants out of 220 (15.45%) infants with gastroenteritis (GE) and NEC (necrotizing enterocolitis).

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Reviewers' Comment 3: Among 112 infants with gastroenteritis, enteric viruses were detected in 22 patients (19.64%), and in 12 cases (11.11%) out of 108 infants with NEC. That means that approximately only in one case out of every 10 infants suffering of NEC was an enteric virus detected. The majority of cases with RV infection (10/12) were mild and self-limiting GE, but 2 preterm infants had signs of NEC. In this study, no enteric viruses were detected among 186 infants with the diagnosis of GE or NEC. Despite these observations, the authors conclude (Abstract, p. 2, line 39) that this study demonstrates that in newborn infants, intestinal rotavirus, norovirus, and astrovirus infections may be associated with severe illness such as hemorrhagic enteritis resulting in bloody diarrhea or even NEC. The important question that needs to be answered is whether this association is significant or not, and this answer is not given.

Answer 3: This topic is extensively discussed in the discussion on pages 9 and 10 (p.3, about our patients with HAstV and p.4, about NV).

Reviewers' Comment 4: It is remarkable that the virus most frequently detected in cases of NEC is human astrovirus (9 infants), whereas in only 5 infants with GE was this virus found. This topic was already addressed by the authors of the present manuscript in a previously published paper (Bagci et al., 2008: Detection of astrovirus in premature infants with necrotizing enterocolitis. *Pediatr Infect Dis J* 27:347-50). In fact, 6 infants with NEC and astrovirus infection were already reported in that paper.

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Answer 7: We corrected these errors which resulted from an error of the reference manager.

We thank you for reviewing the manuscript.

Yours sincerely,
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CLINICAL CHARACTERISTICS OF VIRAL INTESTINAL INFECTION IN PRETERM
AND TERM NEONATES

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Keywords: Norovirus, rotavirus, astrovirus; necrotizing enterocolitis; neonates

Abbreviated title: Outcome of viral intestinal infection in newborn infants

Running title: Viral intestinal infection in neonates

ABSTRACT

1
2 **Objectives:** The clinical presentation of the viral enteric pathogens in newborn infants
3 has not been adequately examined. The aim of this study was to evaluate the clinical
4 characteristics of viral intestinal infections in newborn infants.
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10 **Methods:** Clinical data of all term and preterm infants admitted to our tertiary neonatal
11 intensive care unit from 1998 to 2007 with clinical signs of gastroenteritis (GE) or
12 necrotizing enterocolitis (NEC) were retrospectively reviewed and compared between
13 infants with different viral enteric pathogens in stool specimens.
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20 **Results:** In thirty-four infants with signs of GE or NEC, enteropathogenic viruses were
21 found in stool specimens. Rotavirus was detected in 12 cases, of which 2 infants had
22 NEC. Compared with infants with rotavirus or norovirus, infants with astrovirus more
23 frequently suffered from NEC (p<0.05). In addition, an acute systemic inflammatory
24 response was significantly more common in patients with astrovirus infection (astrovirus
25 vs. rotavirus and astrovirus vs. norovirus p<0.01 and p<0.05, respectively). Of 8 children
26 infected with norovirus, one infant had a systemic acute inflammatory response and
27 NEC.
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40 **Conclusions:** This study demonstrates that in newborn infants, intestinal rotavirus,
41 norovirus, and astrovirus infections may be associated with severe illness such as
42 hemorrhagic enteritis resulting in bloody diarrhea or even NEC.
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INTRODUCTION

Viral pathogens are the most frequent cause of endemic and outbreak-related GE and are responsible for significant morbidity and mortality in infants and children world-wide, both outbreak-related and endemic. Most episodes are due to infections with rotavirus (RV), human astrovirus (HAstV), norovirus (NV), and adenovirus (AdV) [1-2].

Etiologic studies of acute GE showed that RV is the major cause of clinically severe, epidemic and sporadic viral GE in young children, immunocompromized patients, and in the elderly [1]. NV and HAstV are usually associated with cases of mild and self-limiting GE in children [3-6].

In contrast to the usually self-limiting course of GE in healthy children, NEC, a major and life-threatening gastrointestinal emergency, has occasionally been reported associated with RV infection or colonization in preterm infants [7-9]. Moreover, we have previously shown that colonization or infection with HAstV may also be associated with severe confirmed NEC in preterm infants [10], and recently, Armbrust et al. [11] reported a NV outbreak in 11 premature infants in a tertiary Neonatal Intensive Care Unit (NICU), of whom 2 developed NEC.

With more sensitive virus identification techniques, viral GE has been increasingly reported in infants, especially in pediatric hospitals and child-care centers. The aim of this retrospective investigation was to evaluate the clinical presentation of viral intestinal infections especially with NV in newborns admitted to a NICU.

METHODS

1
2 Medical records of 3402 newborn infants admitted between January 1998 and
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4 December 2007 to a tertiary NICU of the Department of Neonatology, University
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6 Hospital of Bonn were retrospectively reviewed for the diagnoses of GE or NEC. As
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8 several studies showed the relationship between pathogenic microorganisms and NEC
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10 [12] , it has been routine care at our institution during this 10 year period to examine
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12 fecal specimens of all infants with one or more acute gastrointestinal symptoms such as
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14 abdominal distension, feeding difficulties, increased gastric residuals (> 5 ml/kg per
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16 feeding), vomiting, bloody stools, loose stools, or diarrhea by culture for enteric bacterial
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18 pathogens, by cell culture test (human foreskin fibroblasts) for *Clostridium difficile*
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20 cytotoxin, and by ELISA or PCR for a series of viral pathogens including RV, AdV, NV,
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22 and HAstV. When a new case with viral pathogen was identified, all patients who
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24 shared a room with the infected infant were immediately tested. All infants who were
25
26 diagnosed to have NEC or GE were eligible for analysis. Infants without NEC or GE
27
28 were excluded from the study if no viral or bacterial pathogens were detected in their
29
30 stool specimens.

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32 **Case Definitions and Laboratory Methods:** NEC was staged according to modified
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34 Bell's criteria [13], a definite case of NEC (stage \geq IIA) is an infant with one of the
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36 following intestinal or systemic signs: visible blood from rectum, apnoea, shock,
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38 hypotension or X-rays with positive signs of pneumatosis intestinalis; a suspected case
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40 of NEC (stage I) is a infant with at least two of the following clinical signs: abdominal
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42 distension, gastric residual (> 5 ml/kg per feeding), occult blood in stool or a radiological
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44 finding of persistent intestinal distension [14].
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55 Although there was no clear definition to differentiate between NEC stage I and GE, we
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57 defined viral GE in this study by the presence of diarrhea defined as loose, watery
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1 stools that occur more than three times per day for at least three consecutive days and
2 a positive ELISA or RT-PCR for a viral enteric pathogen but negative culture results for
3 bacterial enteric pathogens and *Clostridium difficile* cytotoxin [14].
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7 From all infant records, that contained the diagnoses GE or NEC with positive ELISA or
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9 RT-PCR for an enteric virus, the following data were extracted: Baseline characteristics;
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11 clinical features of the acute episode, standard laboratory tests, including complete
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13 blood cell count, basic metabolic profile, acute phase reactant including C-reactive
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15 protein and interleukin 6, reports of stool cultures, virus detection, therapeutic
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17 interventions, and complications. An acute systemic inflammatory response was defined
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19 as either C-reactive Protein >10 mg/l [15] or Interleukin 6 >100 pg/ml [16] and lactic
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21 acidosis was defined as lactate >1.8 mmol/l.
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26 Examinations of viral pathogens were done by antigen-specific enzyme-linked
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28 immunabsorbent assay (ELISA) for RV (Premier Rotaclone, Meridian Bioscience, Inc.
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30 Cincinnati, USA), AdV (Premier Adenoclone, Meridian Bioscience, Inc. Cincinnati, USA)
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32 and HAstV (Amplified IDEIA Astrovirus, DakoCytomation, Carpinteria, CA, USA). All
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34 ELISAs were performed according to the manufacturer's instructions. NV was detected
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36 by RT-PCR as previously described [2, 17-19].
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41 **Statistical Analyses:** Statistical analysis was performed by SPSS 17.0 software
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43 package for windows. Infants with evidence of different viral pathogens were compared
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45 using Fisher's Exact Test for dichotomous variables, Mann Whitney U test for
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47 continuous variables which were not normally distributed and t-test for continuous
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49 variables with normal distribution. $P < 0.05$ was considered statistically significant.
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RESULTS

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2 A total of 906 stool specimens from 567 infants were examined for gastrointestinal
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4 viruses and enteropathogenic bacteria. Of 220 infants, 108 (3.2%) had NEC and 112
5
6 (3.3%) had GE. In the other 347 infants, no viral or bacterial pathogens were detected
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8 and these infants were excluded from analysis.
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10
11 Gastrointestinal viral agents were detected in 34 infants out of 220 (15.5%) infants with
12
13 GE and NEC; of these, 26 (77 %) were preterm infants at the onset of clinical signs. All
14
15 patients with virus detection showed signs of GE (n=22) or NEC (n=12), of whom 6 had
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17 Bell's stage \geq IIA NEC. There was no statistically significant difference in the gestational
18
19 age between infants with and without viral enteric pathogens both in the GE group
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21 (median [range]: 34 [26-40] vs. 31 [23-40] weeks; $p=0.064$), and in the NEC group (29
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23 [25-35] vs. 28 [23-41] weeks; $p=0.207$), respectively.
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29 RV was detected in stool specimens of 12 infants, HAstV in 14, and NV in 8 infants. No
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31 adenovirus was identified. RV could be identified only during an outbreak of
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33 gastrointestinal rotavirus infections between January 1998 and June 1998. Moreover,
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35 RV was detected in the second stool specimen of an infant with HAstV who was
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37 admitted to the group of HAstV. The majority of cases with RV infection have been mild
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39 and self-limiting GE, but 2 preterm infants had signs of NEC.
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44 Infants with detection of HAstV more frequently suffered from NEC \geq IIA if compared
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46 with infants with detection of RV or NV (RV n=2/12. NV n= 1/8, HAstV n=9/14; HAstV
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48 vs. RV and HAstV vs. NV $p < 0.05$, respectively). In addition, analysis concerning the
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50 infants with GE (without NEC), HAstV GE showed more frequently an acute systemic
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52 inflammatory response (RV n=0/10, NV n=1/7, HAstV n=4/5; HAstV vs. RV and HAstV
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54 vs. NV, $p < 0.01$ and $p < 0.05$, respectively). The clinical features of all neonates with
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56 clinical GE or NEC in whom viral pathogens were detected are summarized in Table 1.
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1 NV was detected in 8 newborns with a median gestational age of 29 weeks (range 26-
2 36 weeks). Of these 8 children, 5 (63%) were still preterm at the onset of illness.
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4 Predominant clinical signs in infants in whom NV was detected were abdominal
5 distension, diarrhea, and apnea. Four neonates (n=4/8, 50%) with detection of NV
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7 showed bloody stools and one of them displayed an acute inflammatory response with
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9 elevated values for C-reactive protein (maximum value 42.1 mg/l), lactate (4.5 mmol/l)
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11 and clinical findings of NEC stage IB. In this infant oral feedings were withheld and
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13 antibiotics were administered for seven days. In one infant, repetitive stool specimens
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15 revealed a positive result for NV for 21 days after the onset of illness. The clinical
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17 features of infants in whom NV was detected are shown in Table 2.
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DISCUSSION

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2 Although viral pathogens have been identified as cause of epidemics of hospital-
3 acquired GE for many years, the association of viral agents with NEC in preterm infants
4 has only recently been recognized [8-10, 20-21]. Our study demonstrates that intestinal
5 RV, NV and HAstV infection in preterm and term neonates may be associated with
6 severe illness such as hemorrhagic enteritis resulting in bloody diarrhea or even NEC
7 and provides further evidence for the role of gastrointestinal viral infections in this most
8 common gastrointestinal emergency in premature infants. Nevertheless, the etiologic
9 role of gastrointestinal viral agents in the pathogenesis of hemorrhagic enteritis or NEC
10 remains largely unknown [1, 22-23].
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24 Rotavirus is the main viral pathogen that causes severe diarrheal diseases in infants
25 and young children up to two years. In addition, several reports have described the
26 association of NEC with RV infection or colonization [7-9]. In our cohort we found two
27 patients with RV infection and NEC. Hypotheses of the mechanism of diarrhea include
28 reduced absorptive surface, functionally impaired absorption, cellular damage impairing
29 absorption, enterotoxic effects of rotavirus protein NSP4, and stimulation of the enteric
30 nervous system [1, 22]. However, the role of RV in the pathogenesis of NEC has not
31 been established. The authors suggested that a possible synergism between RV and
32 other microorganism can worsen the infection in the intestine and cause necrotizing
33 enterocolitis [1, 7-8]. Although all patients in our NICU received a regular screening for
34 viral agents and enteric bacterial pathogens during an outbreak of RV infections, no
35 other gastrointestinal pathogen microorganisms were detected. Furthermore, we found
36 no asymptomatic RV carriers in our NICU at that time.
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55 Although HAstV is usually associated with cases of mild and self-limiting GE in older
56 children, we previously reported that colonization or infection with HAstV may be
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1 associated with the development of severe NEC in preterm infants [10]. Previously,
2 Sirinavin et al. [24] described a HAstV outbreak in healthy neonates in a nursery of a
3 maternity ward. However, so far neonatal cases are rare and outbreaks have not been
4 reported from NICUs. Enteropathogenic viruses generally infect epithelial cells and
5 result in cytolysis and cell destruction [25]. In symptomatic HAstV infection, viral
6 particles have been detected in the low villous, and on the surface of the intestinal
7 epithelium as well as in macrophages of the lamina propria. Investigations in an animal
8 astrovirus model revealed vacuolization followed by degeneration and cell death leading
9 to villous atrophy [26]. However, it is unclear which pathogenetic mechanisms are
10 involved in the occurrence of acute systemic inflammatory response and intestinal
11 ischaemia, i.e. in NEC, in infants with HAstV infection / colonization. Of our 14 infants
12 infected with HAstV, 5 (36%) had Bell's stage \geq IIA NEC and 4 (80%) of 5 infants with
13 clinical GE showed an acute systemic inflammatory response. In contrast to older
14 children, the intestinal epithelium in premature infants may not have the same
15 regenerative capacity. Exaggerated inflammation caused by immature intestinal innate
16 immunity may lead to the generation of toxic metabolites, enhance the epithelial
17 damage and result in intestinal lesions recognized as necrosis. On the other hand, one
18 could speculate that NEC or the systemic inflammatory response may result from
19 bacterial translocation after mucosal damage as a result of a possible synergism
20 between HAstV and bacteria or from the systemic spread of virus particles. However,
21 we are unable to identify another bacterial enteropathogenic agent in stool and blood
22 cultures in these infants.

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Generally, NV infections are mild and self-limiting and diarrheal stool during NV
infection is non-bloody, lacks mucus, and may be loose or watery [4, 6, 27]. In contrast,
four of our 8 newborn infants with NV infection (50%) had bloody stools and one of them

1 had NEC [6]. Human noroviruses are thought to have an enteric tropism limited to the
2 upper intestinal tract, because intestinal biopsies from volunteers revealed villous
3 broadening and atrophy, crypt cell hyperplasia and cytoplasmic vacuolization in the
4 jejunum and duodenum and an infiltration of polymorphonuclear and mononuclear cells
5 into the lamina propria [28-31]. Similar pathology is seen in biopsies from pediatric
6 transplant patients with diarrhea associated with human NV infection [32].
7 Histopathologic examination in the intestine of gnotobiotic pig's also showed mild
8 lesions in the upper intestinal tract, but no cytopathic lesions [33]. Cheetham et al. [33]
9 speculated that extensive histopathologic lesions may not be apparent in the intestine
10 because the NV-infected enterocytes may be dying by apoptosis rather than necrosis.
11 Recently, Turcios-Ruiz et al. [21] and Armbrust et al. [11] reported cases of NEC
12 associated with NV in newborn infants similar to the case we describe in this report.
13 Unfortunately none of these studies provided information on the histopathologic
14 examination of colons to establish the involvement of the colon with NV infection /
15 colonization in premature newborn infants.

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17 In summary, just like other authors [34-36], we can only speculate that viral infections in
18 preterm infants may increase epithelial cell damage leading to weakening of the
19 mucosal barrier and occasionally resulting in NEC. The limitations of our study are the
20 retrospective design, the lack of data on the prevalence of gastrointestinal viruses in
21 stool specimen of infants without gastrointestinal symptoms and the lack of data
22 concerning the time course of the development of GE or NEC with regard to the onset
23 of colonization / infection with viruses. In order to understand the prevalence of enteric
24 viruses in neonatal intensive care units and their role in the pathophysiology of NEC,
25 gastrointestinal viruses should be included in the microbiological examination of stool
26 specimens in patients with gastrointestinal symptoms and further studies are necessary.
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REFERENCES

- 1
2 [1] Clark B, McKendrick M (2004) A review of viral gastroenteritis. *Curr Opin Infect*
3
4 *Dis* 17 (5):461-469
5
6
7 [2] Oh DY, Gaedicke G, Schreier E (2003) Viral agents of acute gastroenteritis in
8
9 German children: prevalence and molecular diversity. *J Med Virol* 71 (1):82-93
10
11 [3] Walter JE, Mitchell DK (2003) Astrovirus infection in children. *Curr Opin Infect Dis*
12
13 16 (3):247-253
14
15
16 [4] Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD (2008)
17
18 Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg*
19
20 *Infect Dis* 14 (8):1224-1231
21
22
23 [5] Simon A, Schildgen O, Maria Eis-Hubinger A, Hasan C, Bode U, Buderus S,
24
25 Engelhart S, Fleischhack G (2006) Norovirus outbreak in a pediatric oncology unit.
26
27 *Scand J Gastroenterol* 41 (6):693-699
28
29
30 [6] Thornton AC, Jennings-Conklin KS, McCormick MI (2004) Noroviruses: agents in
31
32 outbreaks of acute gastroenteritis. *Disaster Manag Response* 2 (1):4-9
33
34
35 [7] Rotbart HA, Levin MJ, Yolken RH, Manchester DK, Jantzen J (1983) An outbreak
36
37 of rotavirus-associated neonatal necrotizing enterocolitis. *J Pediatr* 103 (3):454-459
38
39
40 [8] Sharma R, Garrison RD, Tepas JJ, 3rd, Mollitt DL, Pieper P, Hudak ML,
41
42 Bradshaw JA, Stevens G, Premachandra BR (2004) Rotavirus-associated necrotizing
43
44 enterocolitis: an insight into a potentially preventable disease? *J Pediatr Surg* 39
45
46 (3):453-457
47
48
49 [9] Herruzo R, Omenaca F, Garcia S, Diez J, Sanchez-Fauquier A (2009)
50
51 Identification of risk factors associated with nosocomial infection by rotavirus P4G2, in a
52
53 neonatal unit of a tertiary-care hospital. *Clin Microbiol Infect* 15 (3):280-285
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61
62
63
64
65
- [10] Bagci S, Eis-Hubinger AM, Franz AR, Bierbaum G, Heep A, Schildgen O, Bartmann P, Kupfer B, Mueller A (2008) Detection of astrovirus in premature infants with necrotizing enterocolitis. *Pediatr Infect Dis J* 27 (4):347-350
- [11] Armbrust S, Kramer A, Olbertz D, Zimmermann K, Fusch C (2009) Norovirus infections in preterm infants: wide variety of clinical courses. *BMC Res Notes* 2:96
- [12] Caplan MS, Jilling T (2001) New concepts in necrotizing enterocolitis. *Curr Opin Pediatr* 13 (2):111-115
- [13] Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T (1978) Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg* 187 (1):1-7
- [14] Faustini A, Forastiere F, Giorgi Rossi P, Perucci CA (2004) An epidemic of gastroenteritis and mild necrotizing enterocolitis in two neonatal units of a University Hospital in Rome, Italy. *Epidemiol Infect* 132 (3):455-465
- [15] Weitkamp J-H, Aschner JL (2005) Diagnostic use of C-reactive protein (CRP) in assessment of neonatal sepsis. *NeoReviews* 6 (11):e508-e515
- [16] Krueger M, Nauck MS, Sang S, Hentschel R, Wieland H, Berner R (2001) Cord blood levels of interleukin-6 and interleukin-8 for the immediate diagnosis of early-onset infection in premature infants. *Biol Neonate* 80 (2):118-123
- [17] Ando T, Monroe SS, Noel JS, Glass RI (1997) A one-tube method of reverse transcription-PCR to efficiently amplify a 3-kilobase region from the RNA polymerase gene to the poly(A) tail of small round-structured viruses (Norwalk-like viruses). *J Clin Microbiol* 35 (3):570-577
- [18] Hohne M, Schreier E (2004) Detection and characterization of norovirus outbreaks in Germany: application of a one-tube RT-PCR using a fluorogenic real-time detection system. *J Med Virol* 72 (2):312-319

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52
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65
- [19] Schreier E, Doring F, Kunkel U (2000) Molecular epidemiology of outbreaks of gastroenteritis associated with small round structured viruses in Germany in 1997/98. *Arch Virol* 145 (3):443-453
- [20] Lodha A, de Silva N, Petric M, Moore AM (2005) Human torovirus: a new virus associated with neonatal necrotizing enterocolitis. *Acta Paediatr* 94 (8):1085-1088
- [21] Turcios-Ruiz RM, Axelrod P, St John K, Bullitt E, Donahue J, Robinson N, Friss HE (2008) Outbreak of necrotizing enterocolitis caused by norovirus in a neonatal intensive care unit. *J Pediatr* 153 (3):339-344
- [22] Lundgren O, Svensson L (2001) Pathogenesis of rotavirus diarrhea. *Microbes Infect* 3 (13):1145-1156
- [23] Mumhrey SM, Changotra H, Moore TN, Heimann-Nichols ER, Wobus CE, Reilly MJ, Moghadamfalahi M, Shukla D, Karst SM (2007) Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon responses. *J Virol* 81 (7):3251-3263
- [24] Sirinavin S, Techasaensiri C, Okascharoen C, Nuntnarumit P, Tonsuttakul S, Pongsuwan Y (2006) Neonatal astrovirus gastroenteritis during an inborn nursery outbreak. *J Hosp Infect* 64 (2):196-197
- [25] Lundgren O, Peregrin AT, Persson K, Kordasti S, Uhnöo I, Svensson L (2000) Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* 287 (5452):491-495
- [26] Mitchell DK (2002) Astrovirus gastroenteritis. *Pediatr Infect Dis J* 21 (11):1067-1069

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- [27] Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK (1994) Norwalk virus infection of volunteers: new insights based on improved assays. *J Infect Dis* 170 (1):34-43
- [28] Dolin R, Blacklow NR, DuPont H, Buscho RF, Wyatt RG, Kasel JA, Hornick R, Chanock RM (1972) Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proc Soc Exp Biol Med* 140 (2):578-583
- [29] Guo M, Hayes J, Cho KO, Parwani AV, Lucas LM, Saif LJ (2001) Comparative pathogenesis of tissue culture-adapted and wild-type Cowden porcine enteric calicivirus (PEC) in gnotobiotic pigs and induction of diarrhea by intravenous inoculation of wild-type PEC. *J Virol* 75 (19):9239-9251
- [30] Schreiber DS, Blacklow NR, Trier JS (1973) The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. *N Engl J Med* 288 (25):1318-1323
- [31] Wobus CE, Thackray LB, Virgin HWt (2006) Murine norovirus: a model system to study norovirus biology and pathogenesis. *J Virol* 80 (11):5104-5112
- [32] Morotti RA, Kaufman SS, Fishbein TM, Chatterjee NK, Fuschino ME, Morse DL, Magid MS (2004) Calicivirus infection in pediatric small intestine transplant recipients: pathological considerations. *Hum Pathol* 35 (10):1236-1240
- [33] Cheetham S, Souza M, Meulia T, Grimes S, Han MG, Saif LJ (2006) Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. *J Virol* 80 (21):10372-10381
- [34] Kliegman RM, Walker WA, Yolken RH (1993) Necrotizing enterocolitis: research agenda for a disease of unknown etiology and pathogenesis. *Pediatr Res* 34 (6):701-708
- [35] Lin PW, Stoll BJ (2006) Necrotising enterocolitis. *Lancet* 368 (9543):1271-1283

[36] Markel TA, Crisostomo PR, Wairiuko GM, Pitcher J, Tsai BM, Meldrum DR
(2006) Cytokines in necrotizing enterocolitis. Shock 25 (4):329-337

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Table 1. Neonatal Characteristics and Clinical Findings of Newborns with Enteric Viral Infection

Characteristics	Total (n=34)	Rotavirus (n=12)	Astrovirus (n = 14)	Norovirus (n = 8)	P Value*
Birth weight (g) [#]	1620 (618-3790)	1700 (940-3080)	1690 (618-3340)	1335 (670-3790)	n.s.
Gestational age (weeks) [#]	32 (25-40)	33 (27-40)	33 (25-40)	29 (26-36)	n.s.
Male gender (%)	20 (59)	7 (58)	11 (79)	2 (25)	p <0.05 ‡
Surfactant treatment (%)	8 (24)	3 (25)	2 (14)	3 (38)	n.s.
Postnatal age at onset of illness (d) [#]	15 (2-92)	26 (6-45)	13 (2-45)	25 (8-92)	p <0.05 § ‡
Gastroenteritis (%)	22 (65)	10 (83)	5 (36)	7 (88)	p <0.05 § ‡
NEC (%)	12 (35)	2 (17)	9 (64)	1 (12)	p <0.05 § ‡
NEC Bell's stage ≥ IIA (%)	6 (18)	1 (8)	5 (36)	-	n.s.
Surgery (%)	5 (15)	1 (8)	4 (29)	-	n.s.
Duration of illness (d) [#]	3 (2-21)	3 (2-21)	2 (2-3)	5 (2-11)	p <0.05 ‡
Mortality (%)	-	-	-	-	
Symptoms of newborns with gastroenteritis^{##}	22	10	5	7	
Diarrhea (% of those with gastroenteritis)	22 (100)	10 (100)	5 (100)	7 (100)	n.s.
Gastric residue ((% of those with gastroenteritis))	7 (32)	2 (20)	3 (60)	2 (29)	n.s.
Vomiting (% of those with gastroenteritis)	9 (41)	5 (50)	1 (20)	3 (43)	n.s.
Abdominal distension (% of those with gastroenteritis)	11 (50)	3 (30)	2 (40)	7 (100)	p <0.05 ‡ p <0.01 †
Blood in stool (% of those with gastroenteritis)	4 (18)	1 (10)	-	3 (43)	n.s.
Apnea (% of those with gastroenteritis)	9 (41)	3 (30)	3 (60)	4 (57)	n.s.
Acute systemic inflammatory response (% of those with gastroenteritis)	5 (23)	-	4 (80)	1 (14)	p <0.05 ‡ p <0.01 §

[#] Data are expressed as median (range), ^{##} n= Gastroenteritis

n.s. not significant (p>0,05) (Rotavirus vs. Astrovirus, Rotavirus vs. Norovirus, Astrovirus vs. Norovirus)

§ Rotavirus vs. Astrovirus; † Rotavirus vs. Norovirus; ‡ Astrovirus vs. Norovirus

Table 2

Table 2. Neonatal Characteristics and Clinical Findings in Patients with Gastroenteritis or NEC and Detection of Norovirus

Characteristics	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Birth weight (g)	1830	885	670	2800	1620	1050	3790	910
Gestational age (weeks)	33+3	26+5	27+1	34+0	29+5	28+4	36+1	26+1
Gender	F	F	F	F	F	F	M	M
Age at onset of illness (d) (W)	11 (34+6)	92 (39+5)	59 (35+4)	36 (39+1)	17 (32+1)	23 (31+6)	28 (40+1)	7 (27+1)
Month/ year at onset of illness	March 04	August 04	August 04	August 04	August 04	September 04	January 05	October 07
Increased gastric residue (>5 ml/kg)	-	-	-	-	+	-	+	-
Vomiting	+	-	-	+	-	-	+	-
Abdominal distension	+	+	+	+	+	+	+	+
Diarrhea	+	+	+	+	+	+	+	-
Duration of occult blood in stool	3	11	-	-	-	-	8	1
Apnoea	-	+	+	-	+	+	-	+
Acute systemic inflammatory response*	-	-	-	-	-	-	-	+
Lactic acidosis [#]	-	-	+	-	-	-	-	+
Diagnosis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	NEC

* CRP >10mg/l and/or IL-6 >100pg/ml, [#] Lactate >1.8mmol/l, NEC: Necrotizing enterocolitis