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An epidemic of gastroenteritis and mild necrotizing enterocolitis in two neonatal units of a University Hospital in Rome, Italy

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SUMMARY

In the summer of 1999 a cluster of 18 cases of necrotizing enterocolitis (NEC) occurred in a University Hospital in Rome, Italy. The cases presented with mild clinical and radiological signs, and none died. Seventy-two per cent had a birth weight of >2500 g, 66·7% had a gestational age of >37 weeks, 30% presented with respiratory diseases and/or hypoglycaemia. All cases occurred within 10 days of birth and between 5 and 7 days after two clusters of diarrhoea (14 cases). The NEC outbreak had two phases; most cases in the first phase occurred in the at-risk unit, whereas those in the second phase occurred in the full-term unit. In the multivariate analysis, invasive therapeutic procedures, pathological conditions and formula feeding were associated with NEC. Although no predominant common bacteria were isolated, we suggest an infective origin of this outbreak.

INTRODUCTION

Neonatal necrotizing enterocolitis (NEC) is a serious gastrointestinal disease characterized by abdominal distension, gastrointestinal haemorrhage, ileus (gastric residuals), intestinal necrosis and pneumatosis intestinalis [1]. The incidence of the disease in the neonatal period has been reported as between 1 and 5% of admissions in neonatal intensive care units, and between 0·3 and 2·4 cases per 1000 live births in population-based studies [2]. NEC generally occurs as sporadic cases, but it also occurs in temporal and geographic clusters, and a few important outbreaks have been reported [3]. The mortality rate varies between 9 and 28% [2].

The aetiology and pathogenesis of the disease are still unclear. Most studies indicate that prematurity is the most important risk factor [4–9], probably due to the immaturity of the gastrointestinal tract or to a host-defence mechanism [10]. Another factor associated with NEC is modality of feeding: NEC occurs less frequently in infants who are fed, those fed human breast milk or those on a careful feeding regimen (i.e. a diluted formula slowly increased in concentration) [9, 11–14]. The third important factor in the pathogenesis of this disease is the presence of pathogens or imbalance in the intestinal microbial flora. While it is generally agreed that NEC does not occur without bacterial colonization of the gastrointestinal tract, no single bacterial agent, bacterial product, or virus has been consistently identified as a cause of NEC [15], nor is the relationship between gastroenteritis and NEC clear [16–20].

In the summer of 1999, 14 cases of NEC were reported in a University Hospital in Rome, the first case occurred on 6 June in a 2-day-old child and the last case occurred on 8 July in a child born on the 1 July. The results of the epidemiological investigation of this outbreak follow.

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MATERIALS AND METHODS

Setting of the outbreak

Forty cases of NEC were diagnosed in newborns (45 000 births per year) between January 1997 and June 1999 in the Lazio region (including the city of Rome) with an incidence of 0·1/1000 per year. The incidence rate ranged from 0·04 to 0·37/1000 per year in the different hospitals of the region; no epidemic was detected before July 1999. At the time of the outbreak there were three neonatal units at the University Hospital: the Neonatal Intensive Care Unit (NICU), the At-Risk Unit (ARU) for low birth weight and low gestational age babies who were not acutely ill, and the Full-Term Unit (FTU). The NICU was in a separate building, the other two units were next to each other, divided only by a double glass door, beyond which disposable aprons, masks and gloves were required before entering the ARU. The same paediatric staff cared for babies in the two units. All babies born in the hospital were admitted to the FTU or ARU. Sick children from other hospitals were admitted to the NICU but not to the other units.

Early in July 1999 a cluster of NEC cases was observed, a few of these presented diarrhoea. There were no suspected cases diagnosed in the NICU. On 3 July the maternity ward stopped admitting new patients, but those already hospitalized delivered there. On this date 32 babies were already hospitalized in non-critical units (14 in the FTU and 18 in the ARU). A total of 221 live births occurred during the period 1 June to 5 July 1999, of these 161 were in the FTU and 54 in the ARU (in six cases the information was missing).

All hospital records of the 221 newborns, those of their mothers, and registers reporting delivery, nursing and feeding practices were reviewed to find all cases of NEC and gastroenteritis and to analyse the possible associated factors. For children discharged in the period 1 June to 5 July, a follow-up was done through a telephone questionnaire after 5 July; the questionnaire requested information about the child's health and gastroenteritis in other family members since the mother's hospitalization.

Case definitions

According to the case definition proposed by Bell [21], a definite case of NEC (stage II) is a baby with one of the following intestinal or systemic signs: frank blood from rectum, apnoea, shock, hypotension or radiographs positive for pneumatosis intestinalis; a suspected case of NEC (stage I) is a baby with at least two of the following clinical signs: abdominal distension, gastric retention, haemoglobin-positive stools or a radiological finding of persistent intestinal distension. The onset date of NEC was defined as the date in which the first clinical or radiological sign was detected. A case of gastroenteritis (or diarrhoea) was defined as a baby with more than three stool emissions per day for at least three consecutive days [22].

Microbiological tests

Stool samples from babies with diarrhoea were collected in only three cases and were tested for *Salmonella* and *Shigella*; stool samples from babies with NEC were cultured for enteropathogen bacteria, only after antibiotic therapy was administered. Blood cultures were not done. The follow-up of discharged newborns also included cultures tested for enterobacteria.

Reliability study of radiological diagnosis

In a separate study on reliability of radiological diagnosis, the radiographs of 13 children diagnosed with NEC were reviewed independently by three expert radiologists blind with regard to their clinical condition and previous radiological diagnosis. These data were compared with the original radiological findings. When two reviewers reported the presence of intramural gas, the radiological diagnosis was confirmed as pneumatosis or stage II NEC; when two reviewers reported the presence of at least two signs among meteorism, loops distension and oedema in the bowel wall, the radiological diagnosis of NEC was confirmed as stage I.

Data analysis

Both overall and unit-specific daily attack rates were calculated for diarrhoea and for NEC, according to onset date. Among the 221 live births in the University Hospital from 1 June 1999, newborns with a diagnosis of NEC or gastroenteritis were compared with their contemporaries in the neonatal unit who did not present any sign of these syndromes.

Among the variables potentially associated with NEC, we analysed maternal factors (nationality, age at delivery, pre-eclampsia, hypertension, sepsis,

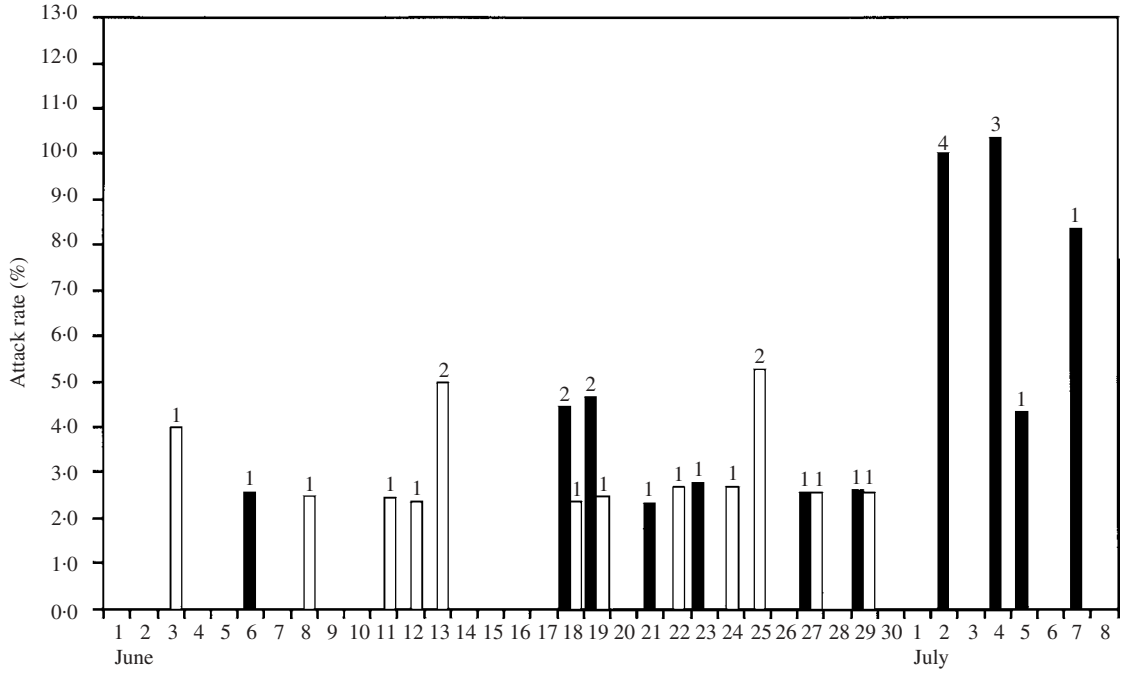

Fig. 1. Daily attack rate (%) for necrotizing enterocolitis (NEC) and diarrhoea, in live births in two neonatal units, from June to July 1999, Rome, Italy. Number of cases are shown on the top of columns. ■, NEC; □, diarrhoea.

genito-urinary tract infection), delivery and newborn characteristics (sex, birth weight, gestational age, delivery type, 5-min Apgar score, neonatal unit, and the first method of feeding), perinatal diseases (hypoglycaemia, asphyxia, respiratory distress, hyaline membrane disease, polycythaemia, congenital heart diseases), and invasive procedures before the onset of symptoms (tracheal intubation, intravenous infusions, surfactant administration, blood transfusion, use of umbilical vessel catheters or vesical catheters).

Crude odds ratios (OR) were calculated with their 95% confidence intervals (CI) for cases of NEC [21, 23] and for cases of gastroenteritis. A multivariable logistic regression was performed including all variables associated with the outcomes in the single variable analysis. Potential effect modification of any neonatal pathological condition on the risk estimates for invasive procedures, neonatal unit and formula feeding was evaluated with stratified analysis. Stata 6.0 was the software used [24].

RESULTS

Of the 221 live births, NEC was diagnosed in 14 cases, and 4 additional cases were identified by reviewing hospital charts, making the cumulative incidence 8 and 1% for the period 1 June to 5 July. The incidence was 18·5% (10/54) in the ARU and 5·0% (8/161) in the FTU. Nine of the 18 cases were male; the mean age of onset was 1·3 days (range 0–6 days); the mean delay of diagnosis was 3·4 days (range 0–14 days). There were 12 cases of gastroenteritis, 2 additional cases of NEC presented diarrhoea among the first symptoms, with a total cumulative incidence of 7·7%; diarrhoea lasted between 3 and 8 days; all cases of gastroenteritis except one occurred in the ARU; the single case which occurred in the FTU presented diarrhoea and NEC. No other cases of NEC or gastroenteritis were reported in the discharge follow-up, which was completed for 152 (80%) of the children who were not cases.

Figure 1 shows daily attack rates of gastroenteritis and NEC. Two peaks of incidence of gastroenteritis were observed, the first on 13 June and the second on 25 June. No additional cases occurred after 29 June. Two peaks of NEC were observed, a small one on 18 June, 5 days after the first peak of diarrhoea, and a larger one during 2–4 July, 7 days after the second peak of diarrhoea.

Table 1 shows the clinical and radiological characteristics of the NEC cases and Figure 2 illustrates date of onset of NEC, date of diagnosis, and length of patients' stay in neonatal units. The first nine cases occurred in June; among them, four cases presented

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Table 1. *Clinical and radiological characteristics of the newborns with necrotizing enterocolitis (NEC) from June to July 1999, Rome, Italy*

Case no.	Birth weight (g)	Gestational age (wk)	Signs and symptoms of NEC	Diarrhoea	Other pathological conditions	Radiological signs	Final diagnosis
1	2710	36	Blood in stool, abdominal distension	1 day	Respiratory distress	Pneumatosis	Definite
2	1325	33	Blood in stool, apnoea, gastric retention	Absent	Respiratory distress	Loops distension	Definite
3	1455	33	Apnoea, abdominal distension, gastric retention	Absent		Loops distension	Definite
4	1700	34	Blood in stool, apnoea, abdominal distension, gastric retention	3 days	Hypoglycaemia	Loops distension	Definite
5	1725	34	Blood in stool, apnoea, abdominal distension, gastric retention	1 day	Respiratory distress, hypoglycaemia	Pneumatosis	Definite
6	2660	37	Gastric retention	1 day		Pneumatosis	Definite
7	3240	38	Blood in stool, abdominal distension, gastric retention	1 day		Pneumatosis	Definite
8	2975	40	Blood in stool, abdominal distension	3 days		Pneumatosis	Definite
9	2305	33	Abdominal distension, gastric retention	1 day	Respiratory distress, hypoglycaemia	Loops distension	Suspect
10	3525	40	Blood in stool	Absent			Definite
11	3615	37	Abdominal distension	1 day	Respiratory distress	Pneumatosis	Definite
12	3570		Apnoea	1 day	Asphyxia	Pneumatosis	Definite
13	2930	37	Blood in stool	1 day	Hypoglycaemia	Pneumatosis	Definite
14	3030	38	Blood in stool	absent			Definite
15	3060	39	Blood in stool	1 day		Loops distension	Definite
16	3400		Blood in stool	Absent		Pneumatosis	Definite
17	3410	41	Blood in stool	Absent		Pneumatosis	Definite
18	3260	40	Abdominal distension	1 day		Loops distension	Suspect

signs of NEC at birth and seven of them were admitted to the ARU; in the second part of the outbreak, beginning 2 July, most of the cases (6/9) were received in the FTU and, on average, presented the first signs of disease by day 3 of life. Nine cases passed frank blood in their stool for between 1 and 4 days, two presented apnoea, and three presented both frank blood and apnoea. Among these 14 cases with important clinical signs, the radiographic findings were of loops distension for four children, while intramural gas was seen in eight cases. Gas was not reported in the portal venous system. For two cases, radiograph reports were missing from the hospital records. Four cases of NEC presented two or more milder clinical signs; the radiological findings showed loops distension in two cases and intramural gas in the other two. There were two sets of twins among the cases.

The pathological conditions detected at birth in NEC cases were: birth weight <2500 g and/or gestational age <37 weeks in six children, low Apgar score (<7) in three children, hypoglycaemia in four and asphyxia or respiratory distress in six cases. None presented shock, hypotension, polycythaemia, membrane hyaline or congenital heart disease. None received blood transfusion or had an umbilical or urinary catheter. Two acutely ill babies were transferred to the paediatric surgery unit. One case required massive small and large bowel resection, in the other case a shorter piece of the ileum was excised. Four other cases of NEC were transferred to the NICU. No fatalities occurred. All NEC cases received immediate antibiotic treatment; netilmycin, ampicillin, teicoplanin and metronidazole were used.

The radiological re-evaluation by the three expert reviewers was possible for 13 cases (12 of the original

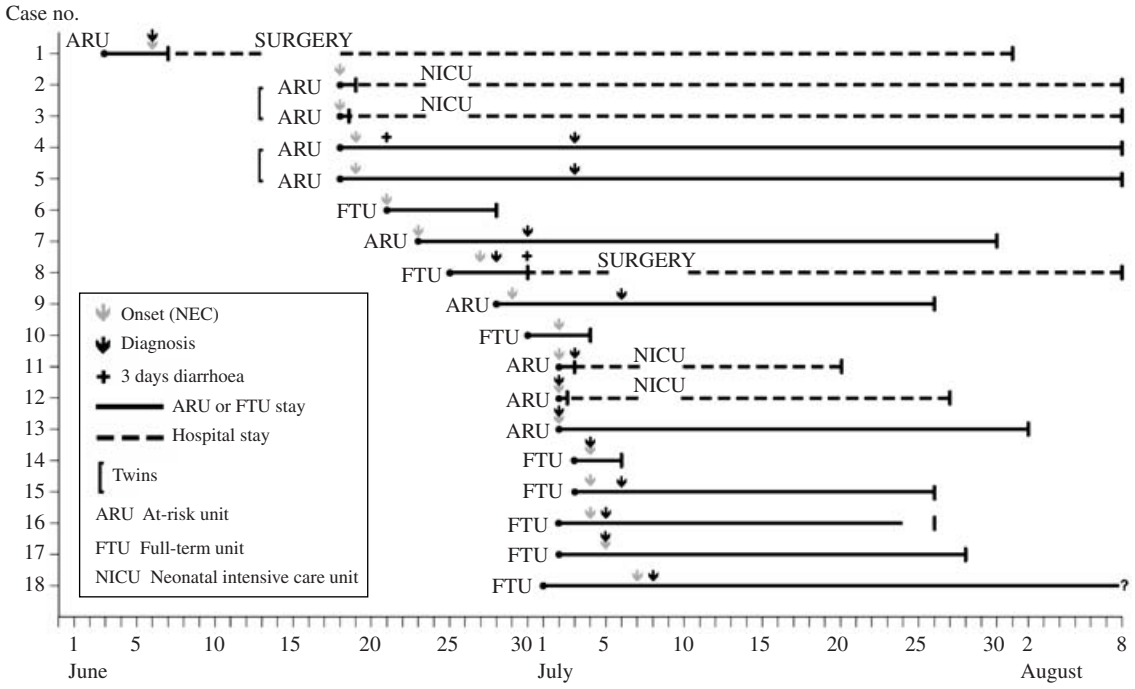

Fig. 2. Spatial and temporal relationship among 18 patients with necrotizing enterocolitis. Clinical characteristics of cases from June to July 1999, Rome, Italy. ?, for the last case the date of discharge was missing.

14 and 1 of the 4 identified afterwards). Three out of seven pneumatosis, originally diagnosed as stage II NEC were confirmed, the remaining four being evaluated as stage I. Four out of five intestinal distensions were confirmed as stage I, while one case was classified as negative. Finally, the reviewers also classified as negative the case where the radiological report was missing from the hospital chart.

No enterobacteria such as *Escherichia*, *Shigella*, *Proteus* or *Salmonella* were detected in NEC cases. The three cases of gastroenteritis tested by culture for *Salmonella* and *Shigella* gave negative results.

Table 2 presents the results of the univariate analysis for NEC and diarrhoea.

Factors associated with NEC were: pre-eclampsia, birth weight <2500 g, gestational age <37 weeks, hypoglycaemia, asphyxia or respiratory distress, having at least one invasive procedure such as tracheal intubation or intravenous therapy, being hospitalized in the ARU and formula feeding. Two babies with respiratory distress were not feeding at all. Only NEC cases received intravenous infusions. Time of first feeding was earlier in controls (11·3 h of life; range 3–96 h) than in NEC cases (23·0 h of life; range 7–56 h), but the ORs were not estimated because the information was available for only 43% of the children. We repeated all our analyses considering only 10 radiologically confirmed NEC cases. The results did not differ either in direction or in strength from those estimated for the total 18 cases, except for larger CIs (data not shown, available on request).

Factors associated with gastroenteritis were birth weight <2500 g, gestational age <37 weeks, hypoglycaemia, being hospitalized in the ARU and formula feeding. Nationality and age of mother, maternal hypertension, urinary or systemic infection, sex of newborn, type of delivery, and Apgar score were not associated with any outcome.

In the multivariate analysis, invasive procedure (OR equal to infinity), pathological conditions (OR 3·3, 95% CI 0·8–12·7) and first time feeding with formula (OR 15·0, 95% CI 1·8–125·7) were independently associated with NEC. For cases of gastroenteritis the only factor associated was the type of neonatal unit of hospitalization. Babies fed with formula had a higher probability of gastroenteritis (OR 1·7, 95% CI 0·4–8·0), although it was not statistically significant.

Table 3 presents the results of analysis stratified by neonatal pathological conditions. Invasive procedures are associated with NEC both in premature or ill newborns, and in healthy full-term infants. Being

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Table 2. *Crude odds ratios of necrotizing enterocolitis (NEC) and diarrhoea among newborns, in two neonatal units, June to July 1999, Rome, Italy*

	NEC				Diarrhoea			
Variables	Cases	Non-cases	OR*	95% CI	Cases	Non-cases	OR†	95% CI
Pre-eclampsia								
No	15	185	1·0		13	185	1·0	
Yes	3	5	7·4	1·6–34·0	1	5	4·4	0·8–17·7
Gender								
Male	9	97	1·0		8	97	1·0	
Female	9	93	1	0·4–2·7	6	93	0·78	0·3–2·3
Gestational age (weeks)								
37+	10	155	1·0		9	155	1·0	
<37	6	19	4·9	1·6–15·0	4	19	3·6	1·0–12·9
Data missing	2	16				16		
Birth weight (g)								
3000+	9	137	1·0		2	137	1·0	
2500–2999	4	34	1·8	0·5–6·2	7	34	14·1	2·8–70·9
<2500	5	17	4·5	1·3–14·9	5	17	20·1	3·6–112
Data missing	—	2				2		
Hypoglycaemia								
No	14	188	1·0		12	188	1·0	
Yes	4	2	26·9	4·5–160	2	2	15·7	2·0–121
Asphyxia or respiratory distress								
No	13	188	1·0		14	188	1·0	
Yes	5	2	36·2	6·4–205	0	2	0	0·0–27·5
Any neonatal pathological condition‡								
No	8	151	1·0		7	151	1·0	
Yes	9	25	6·8	2·4–19·3	6	25	5·2	1·6–16·7
Data missing	1	14				14		
Tracheal intubation								
No	17	189	1·0		14	189	1·0	
Yes	1	1	11·1	0·7–186	0	1	0	0·0
Intravenous therapy								
No	10	190	1·0		13	190	1·0	
Yes	8	0	Infinite	35·1–∞	1	0	Infinite	0·0–∞
Any invasive procedures								
No	10	188	1·0		13	188	1·0	
Yes	8	2	75·2	14·1–401·5	1	2	7·2	0·6–85·1
Neonatal unit								
Full term	8	153	1·0		1	153	1·0	
At risk	10	32	6	2·2–16·3	13	32	62·2	7·8–492
Data missing		5				5		
First time feeding								
Maternal	1	121	1		3	121	1	
Formula	10	58	20·9	2·6–167	11	58	7·6	2·1–28·5
None	2	0	Infinite	34·1–∞	0	0	0	
Data missing	5	11	55	5·9–514	0	11	0	0·0–15·4

OR, Odds ratio; CI, confidence interval.

* Thirteen babies with diarrhoea or other symptoms, not NEC cases, are excluded.

† Seventeen babies were excluded: NEC cases without diarrhoea or symptomatic children who did not fit the case definition.

‡ Hypoglycaemia, asphyxia or respiratory distress, birth weight <2500 g, gestational age <37 weeks.

hospitalized in the ARU presented a strong association with the presence of gastroenteritis and NEC only in low-birth-weight or ill newborns, while first-time feeding with formula was associated with both these outcomes in low-birth-weight and full-term newborns.

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Table 3. *Odds ratios of necrotizing enterocolitis (NEC) and diarrhoea stratified by neonatal pathological conditions, from June to July 1999, Rome, Italy*

	NEC				Diarrhoea			
	Cases	Non-cases	OR*	95% CI	Cases	Non-cases	OR†	95% CI
One or more invasive procedures‡								
Babies without neonatal pathological conditions								
No	6	151	1·0		7	151	1	
Yes	2	0	Infinite		0	0	nc¶	
Babies with neonatal pathological conditions								
No	3	23	1·0		5	23	1	
Yes	6	2	23	3·1–170·3	1	2	2·3	0·2–30·6
Neonatal unit§								
Babies without neonatal pathological conditions								
Full term	7	138	1·0		1	138	1·0	
At risk	1	10	2·0	0·2–17·6	6	10	82·8	9·1–756
Babies with neonatal pathological conditions								
Full term	0	2	1·0		0	2	1·0	
At risk	9	22	Infinite	0·19–∞	6	22	Infinite	0·1–∞
First time feeding‖								
Babies without neonatal pathological conditions								
Maternal	1	106	1		1	106	1·0	
Formula	5	42	12·6	1·4–111	5	42	15·1	1·8–130
Babies with neonatal pathological conditions								
Maternal	0	5			1	5	1·0	
Formula	5	12	Infinite	0·44–∞	6	12	2·1	0·2–22·7

OR, Odds ratio; CI, confidence interval.

* Thirteen babies with diarrhoea or other symptoms, not cases of NEC, are excluded.

† Seventeen babies were excluded: NEC cases without diarrhoea or symptomatic children who did not fit the case definition.

‡ Tracheal intubation and intravenous therapy.

§ Data missing for 5 non-cases.

‖ Data missing for 5 cases of NEC and 11 non-cases. Two NEC cases had never fed.

¶ nc, Not computable.

DISCUSSION

NEC cases had three characteristics: (1) they presented mild clinical and radiological signs, (2) they occurred 5–7 days after a cluster of diarrhoea, and (3) both prematurity and neonatal diseases were important associated factors; although they explained only a proportion of the cases. Feeding and therapeutic procedures were also relevant factors for outcome in full-term children.

The case definition

We found 18 cases of NEC (14 original and 4 identified by hospital record revision), 16 of which were classified as definitive on the basis of clinical signs like bloody stools and/or apnoea ($n=6$), or radiological findings of pneumatosis intestinalis ($n=10$). All the cases presented were not seriously ill. A mild form of NEC has been described in the literature as having minimal signs and symptoms followed by a complete recovery, and characterized by the presence of isolated sigmoid or colonic pneumatosis [25–27] in abdominal radiographs. Mild cases are generally reported in clusters and epidemics during which the affected infants have different characteristics than during endemic periods: they have higher birth weights, fewer perinatal complications, and lower case fatality rates (CFRs) [4, 27–31]. Nine cases in the outbreak reported here presented other neonatal diseases, low gestational age or low birth weight, none were born prior to 32 weeks or weighed less than 1300 g. There were no fatalities. The CFR for NEC is reported as increasing with decreasing birth weight and gestational age [5, 25, 32–34]. CFRs ranged from 10 to 44% in infants weighing less than 1500 g, while they ranged from 0 to 20% in those weighing more

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than 2500 g [5, 29, 32, 33]. The lower mortality observed in epidemics is probably due to the higher number of mature and healthy children affected than in endemic periods.

Our cases had a mean age at onset of 1·3 days and seven presented the first sign on the first day of life. Early onset is a further characteristic reported in mild cases, not associated with the more severe course of the disease. In fact, the age of NEC onset is inversely related to birth weight and gestational age [5, 12, 35–37]. Wiswell et al. [26] reported a median age of NEC onset of 2 days in full-term infants, 42% of whom presented symptoms on the first day of life. The characteristics of NEC cases described here are consistent with a less severe form of the disorder occurring in healthy, full-term infants who are affected more often during epidemic events than in endemic periods.

We included in the analysis the six cases classified as definitive on the basis of clinical signs, but for which radiographs did not report pneumatosis, and two other cases, classified as suspected, that had milder clinical signs and radiological findings of intestinal distension. The suspected cases may not have had NEC, as Bell himself believed [21], but their inclusion as cases is legitimate in the context of a cluster of affected babies [19, 29, 38–40]. Cases characterized only by frank blood in the stool for 1 day or less and abdominal distension with no abnormalities or only ileus distension diagnosed by radiographs were included in the analysis of two epidemics, one due to *Clostridium butyricum* [29], and the other due to *Clostridium difficile* [39]. Moreover, in a prospective study on NEC, a case was defined as an infant with pneumatosis intestinalis, or clinical signs such as bloody stools, abdominal distension, and toxic state, and the authors stated that these are very strict criteria. Therefore it is unlikely that infants without NEC were included in our study [34].

The detection of radiological signs can be difficult in the case of NEC, because of subjective variation in the radiological diagnosis [41]. In a study comparing radiological and histological signs [42], out of 12 cases confirmed histologically, 30% presented pneumatosis, while 70% presented intestinal distension. In this outbreak, a revision of the radiographs confirmed the diagnosis in 11 of 12 cases and in 8 cases even found stage I or II NEC.

Looking at hospital records, an additional four cases were identified, apart from the 14 previously identified by clinicians. This method, used in other studies during epidemics [17, 32, 40], was justified by the mildness of the cases and by the possibility that not all cases were recognized during the outbreak, as suggested by the long interval between onset and diagnosis dates for the early cases of June (range of interval between onset and diagnosis 1–11 days).

The infective hypothesis

We observed a cluster of diarrhoea occurring in the ARU 15 days before the beginning of NEC cases. The outbreak presented a temporal trend, with two distinct phases. Most cases in the earlier phase presented low birth weight, low gestational age or neonatal pathologies, in the later phase cases were essentially healthy, full-term children. These observations are congruent with the hypothesis of an infectious agent for NEC in the event reported here, even if no bacterium or virus was identified from cases in this epidemic. In fact, the occurrence of temporal clusters and outbreaks is the most important reason in support of the infective hypothesis of NEC [23, 31, 36]. Moreover, the association we observed between gastroenteritis and the ARU was not found with neonatal pathological conditions, including low birth weight and low gestational age. This suggests an infective hypothesis for diarrhoea, possibly due to the same agent as NEC. The sequence of gastroenteritis followed by NEC syndrome observed in newborns [17, 42] or in the NICU staff [43] or, their contemporary occurrence in newborns [16, 18–20] has been considered in other studies as a reason supporting the infective hypothesis of NEC. A final point is the interruption of NEC clustering by the use of antibiotics and by control measures of transmission [39, 44–46]. Control measures applied were the grouping of ill patients in the ARU and increasing sanitary measures for staff moving between neonatal units. Many pathogens associated with NEC are reported in the literature: *Staphylococcus aureus* [25], *Pseudomonas aeruginosa* [25, 47], *Serratia* [47], *Clostrida* [29, 39, 48], *Escherichia coli* [16, 19, 25, 46], *Klebsiella pneumoniae* [25, 49], *Salmonella* and enterobacter [31], coronavirus [18], rotavirus [50], echovirus [51], but no single bacterial agent, bacterial product, or virus has been consistently identified as a cause of NEC, nor has any pathogen been identified in clusters of NEC [40, 44, 45]. Nevertheless the infective hypothesis of NEC is still valid and speculation continues about a possible role of toxins [47] or infectious agents not yet identified [2].

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In this outbreak, the role of personnel as vectors of disease seems quite plausible as described in other studies [20]. Despite the fact that no one among the staff presented symptoms, it cannot be excluded that they could have played a role in transmitting the infection from one child to another within and between the different neonatal units since the same staff work in both the FTU and the ARU. Supporting this possibility is the time sequence of different phases of the outbreak, and a strong association of the location of cases with diarrhoea.

There are some possible explanations for not finding bacteria in stool cultures in this outbreak. A few of them are due to the limits of the epidemic investigation. Tests were performed after antibiotic therapy had begun. Stool cultures reflecting the bacteria colonizing the distal large bowel did not evidence those in the small bowel, i.e. the site of NEC lesions. Bacteria not normally recognized as pathogens could also cause NEC in premature infants [44]. Finally, blood cultures were not performed, and in a study in which *Clostridium butyricum* was isolated from blood in nine cases of NEC, the complete failure to isolate bacterium from stool cultures was attributable to inappropriate methods [29].

The associated factors

Prematurity is the primary risk factor for NEC [15]. It was the first factor associated with the disease [2], and the most relevant; between 62 and 94% of affected infants were premature, so NEC has typically been thought as a disease exclusively effecting premature infants [12, 23, 26, 31, 35, 37]. In our study, low birth weight, low gestational age and diseases at birth played an important role as risk factors of NEC. However these factors were present only among six cases, and three other cases had associated neonatal diseases. Half of the cases that occurred in healthy full-term children could not be explained by neonatal pathologies. In studies that compare NEC with birth weight and gestational age-matched controls, no risk factor except prematurity was identified [4–9]. It was suggested that risk factors might vary with birth weight and gestational age [5, 12, 42, 52, 53]. Risk factors of NEC in mature babies include congenital heart disease and polycythaemia [27, 42]. None of these diseases was observed in mature NEC cases reported here.

Formula feeding, already reported as a risk factor for NEC [9, 11–14], was associated with the outcome of both NEC and diarrhoea, independently from other factors, in both pathological and healthy full-term babies. It is possible to conclude that formula feeding contributed to the pathogenesis of cases of NEC and gastroenteritis in this outbreak, although none of the pathogenetic hypotheses were clear.

Finally, invasive procedures played a role in NEC onset independently from neonatal pathological conditions. Ischaemic damage to the gastrointestinal tract has been hypothesized to be the pathogenetic mechanism in causing NEC by invasive therapies, as it could lead to bacterial invasion [10]. In the outbreak reported here, it is difficult to confirm this hypothesis. One possible mechanism of intravenous therapy could be the mechanic transmission of an infection.

There are some unexplained issues in this outbreak: the time elapsed between the first case on 6 June and the others which occurred after 17 June, and the absence of gastrointestinal diseases in the FTU. In conclusion, however, we believe that an infectious agent responsible for the diarrhoea cluster in the ARU caused newborns of low birth weight and low gestational age to develop NEC. In a second phase of the outbreak, possibly due to staff, the disease was transmitted to children in the FTU, in which other factors were present, like formula feeding and invasive procedures. Although there was a high number of full-term and healthy newborns affected, the attack rates were higher in premature newborns with neonatal diseases, as expected on the basis of previous knowledge [26, 33].

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