ITAL J PEDIATR 2005;31:395-398

SHORT COMMUNICATION
COMUNICAZIONE BREVE

Surveillance of enteric virus infection in a neonatal intensive care unit

Sorveglianza delle gastroenteriti virali in una unità di terapia intensiva neonatale

P. DI CARLO, S. DE GRAZIA[1], D. CIPOLLA[2], S. ARISTA[1], G.M. GIAMMANCO[1], C. MAMMINA[1], C. COLOMBA, G. CORSELLO[2]
Istituto di Malattie Infettive e Virologia, [1] Dipartimento di Igiene e Microbiologia, [2] Dipartimento Materno Infantile, Università di Palermo, Italy

Summary

Objective. To investigate the epidemiology of neonatal viral gastroenteritis compared to the circulation of enteric viruses in children, 109 newborns in the NICU of Mother and Child Department and 214 children with enteritis admitted to the "G. Di Cristina" Children's Hospital in Palermo were monitored for Rotavirus, Adenovirus, Astrovirus and Norovirus infections.
Methods. Stool samples were examined by EIA to detect viral antigens. Rotavirus strains were subjected to P- and G-typing.
Results. A Norovirus strain was detected in one neonatal stool specimen whereas an astrovirus strain was dectected in two neonatal specimens. No Rotavirus or Adenovirus infection was identified among the newborn infants, while Rotavirus infections were detected in 24.8% of the symptomatic children. Type G4P[8] constituted 43.4% of the Rotavirus strains, followed by G2P[4] (18.9%), G3P[8] (17%), G1P[8] (13.2%) and G9P[8] (1.9%). Overall, Norovirus, Adenovirus and Astrovirus strains were responsible for 15.4% of infections in the paediatric population with diarrhoea.
Conclusions. Viruses are diffuse agents of infection in children with enteritis. Virological tests have to be performed to diagnose enteric infections in the paediatric population. Maternal immunity to common Rotavirus strains combined with the limited circulation of the emerging G9 Rotavirus type among our population may account for the absence of Rotavirus infections in newborn infants.

Riassunto

Obiettivo. *Per verificare se l'epidemiologia delle gastroenteriti virali in epoca neonatale sia comparabile con il tipo di virus identificati nelle epoche successive, gli Autori riportano i risultati di uno studio di sorveglianza sulle infezioni da Rotavirus, Adenovirus, Astrovirus e Norovirus condotto in 109 neonati degenti presso l'UTIN del Dipartimento Materno-Infantile e in 214 bambini con diarrea ospedalizzati nello stesso periodo di osservazione presso l'ospedale pediatrico "G. Di Cristina" di Palermo.*
Metodi. *La ricerca dei virus è stata condotta sui campioni fecali con il metodo EIA. I Rotavirus isolati sono stati sottoposti a P e G tipizzazione.*
Risultati. *Nella popolazione neonatale, un singolo campione è risultato positivo per Norovirus, due per Astrovirus e nessuno per Rotavirus ed Adenovirus; nella popolazione pediatrica, il 24,8% dei campioni era positivo per Rotavirus, di tipo G4P[8] nel 43,4% dei casi, G2P[4] nel 18,9%, G3P[8] nel 17%, G1P[8] nel 13,2% e G9P[8] nell'1,9%. Norovirus, Adenovirus ed Astrovirus erano nel complesso responsabili del 15,4% delle enteriti nella popolazione pediatrica.*
Conclusioni. *I virus sono diffusi agenti di infezione in bambini con enterite e test virologici nei confronti di Rotavirus, Adenovirus, Norovirus ed Astrovirus sono utili per diagnosticare infezioni enteriche nella popolazione pediatrica. L'immunità materna nei confronti dei comuni ceppi di Rotavirus combinata con la limitata circolazione del tipo emergente di Rotavirus G9 può aver contribuito alla mancata circolazione di Rotavirus nella popolazione neonatale.*

Key words

Rotavirus • Adenovirus • Astrovirus • Norovirus • NICU

Parole chiave

Rotavirus • Adenovirus • Astrovirus • Norovirus • UTIN

Submitted: June 24, 2005
Accepted: December 12, 2005

Correspondence:
Dr. Paola Di Carlo
Istituto di Patologia Infettiva e Virologia
Università di Palermo
p.zza Montalto 8
90133 Palermo, Italy
Tel. +39 091 6666071
Fax +39 091 6514634
E-mail: dicarlo19@libero.it

Introduction

Rotavirus is the leading cause of childhood diarrhoea, occurring most commonly among children aged from 6 months to 2 years. Neonatal infections, frequently documented in developing countries, are usually mild or asymptomatic and are caused by Rotavirus strains that differ from those responsible for diarrhoea in older infants. In general, neonatal Rotavirus strains are regarded as naturally attenuated. As they appear to protect against severe diarrhoea associated with subsequent Rotavirus infection, they are considered to be potentially useful vaccine candidates. However, rare cases of severe disease (necrotizing enterocolitis, bowel perforation) have been related to Rotavirus infection acquired soon after birth [1].

Although the correlates of protection against rotaviral diarrhoea are not fully understood, it is thought that type-specific immune response plays an important role. Two viral surface proteins, VP7 and VP4, both inducing neutralizing antibodies, are used to classify Rotavirus strains in G and P types, respectively VP7- and VP4-related. At least 15 G types and 23 P types have been identified to date. Ten of these G types and 11 P types have been detected in humans. Over the years, many different G/P combinations have been described, but most strains belong to types G1P[8], G2P[4], G3P[8] and G4P[8]. In 1995 a diffuse circulation of Rotavirus types G9P[6] and G9P[8] was reported throughout the world [2]. G9P[6] strains have frequently been isolated in Indian neonates [3]. The P6 type probably plays a role in rotaviral attenuated virulence, as strains with this VP4 have frequently been reported to cause asymptomatic infections in neonates [1].

Surveillance of neonatal rotaviral infections in Europe is poorly developed. In 2000, a protracted nosocomial outbreak of diarrhoea in neonates caused by G9P[6] Rotavirus strains was reported in the Netherlands [4]. This outbreak was attributed to the recent introduction of G9 Rotavirus strains into the country, and to the lack of protective homotypic immunity in newborn infants and their mothers. In Palermo, G9 strains were first detected as agents of childhood diarrhoea in the winter of 1999-2000 [2]. Sequence analysis of the VP7-coding gene of these strains revealed high nucleotidic homology with recently circulating G9 strains [5]. The emergence of G9 strains highlights the importance of ongoing Rotavirus surveillance in neonatal care units.

To understand the epidemiology of neonatal Rotavirus infections in our geographical area, we conducted a survey on newborn infants admitted to the neonatal care unit (NICU) of the Mother and Child Department of the University of Palermo in 2003. Simultaneously, Rotavirus strains responsible for infection in infants and children admitted to the "G. Di Cristina" Children's Hospital in Palermo with enteritis were detected and typed.

Materials and Methods

Patients

One hundred and nine patients admitted to the Neonatal Intensive Care Unit (NICU) of the Mother and Child Department of the University of Palermo for at least 48 h, from January 1 through December 30, 2003, were enrolled in the study. Approximately 200 patients are admitted to this unit each year, including 15% of VLBW newborns (birthweight < 1,500 g). Prematurity is the most common reason for admission to the NICU (approximately 70%), followed by respiratory distress, congenital malformation, surgical conditions and infections.

In the same period, all children aged ≥ 1 months who were hospitalized at the "G. Di Cristina" Children's Hospital for at least 48 h with vomiting and/or diarrhoea were also enrolled in the study. The definition of diarrhoea in neonates and children was considered to be the occurrence of three or more watery or loose stools in a period of 24 h, not corresponding to the usual stool pattern [6 7].

Microbiological surveillance

Four hundred and ninety-eight stool specimens were collected: 284 from 109 newborns and 214 from the same number of children. From 1 to 12 specimens were obtained from each patient. Neonatal rectal swabs were collected weekly every Tuesday from all patients throughout their NICU stay.

The older children's specimens were collected within 24 hours of admission to exclude the possibility of hospital infection.

Viral detection and typing

Stool specimens were screened by EIA (IDEIA, DakoCytomation, Angel Drove, UK) to detect Rotavirus, Adenovirus, Norovirus and Astrovirus. Rotavirus-positive stool specimens underwent G and P typing. As previously reported, viral strains were first G-serotyped by monoclonal antibody enzyme immunoassays; then, those not typeable were genotyped by semi-nested reverse transcription (RT)-PCR, using a pair of generic primers (Beg9 and End 9) and subsequently a pool of internal primers specific to G1-G4 and G9 [5]. For P typing, gene 4 was amplified using the generic primers Con 3 and Con 2 and subsequently the internal primers specific to P[4], P[6], P[8] and P[9] genotypes [2].

Statistical analysis

Results were reported as mean (SD) or percentage of observations. The chi-square test was used to compare rates among or between group. Two tailed p value < 0.05 was considered statistically significant. The SPSS package version 11.5 for Windows (SPSS Inc., Chicago, II USA) was used for statistical analysis.

Tab. I. Characteristics of the 109 patients at the time of admission to the neonatal intensive care unit (NICU).

Population		N° infants (%)
Gender		
Males		53 (48)
Female		56 (52)
Birthweight, g		
	500	2 (1.8)
	501-1000	9 (8.3)
	1001-1500	16 (14.7)
	1501-2000	32 (29.4)
	2001-2500	19 (17.4)
	> 2500	31 (28.4)
Gestational age, wks		
	24-29	9 (8.3)
	30-36	63 (58.3)
	> 36	36 (33.3)
Inborn		69 (63.3)
Age at admission > 24 h		7 (6.7)
Twin birth		17 (16)
Cesarean delivery		82 (77)
Apgar score at 5 min < 5		0
Invasive devices*		85 (78)
Enteric symptoms**		8 (7.3)
Length of stay-days***		31.9 (29.4)

* Endotracheal tube, central venous catheter, intravenous catheter, nasogastric tube, parenteral nutrition; ** Vomiting and/or diarrhea; *** expressed as mean (SD)

Results

Table I lists the clinical characteristics of the newborns on admission, and the invasive treatments they underwent during hospitalization.

Over 60% of them were born at less than 36 weeks gestational age, and 25% of them were very low birthweight babies (VLBW; ≤ 1,500 g). Two-thirds of the total newborns and 98% of the VLBW babies came to the neonatal care unit directly from the hospital's maternity unit; the others were referred by public health centres in Sicily. All VLBW inborn babies had immediate intensive care assistance and none showed an Apgar score of < 5 at 5 minute.

Most of the neonates (92%) were admitted within their first day of life. For at least 48% of them the duration of hospitalization exceeded 2 weeks, with an average length of stay in hospital of 31.9 days (median 18 days, range 4-158).

A feeding assessment showed that over 50% of patients received formula only.

Tab. II. Viral pathogens isolated from the two groups of patients.

Virus	Newborns (n = 109)	Children (n = 214)
Rotavirus	0	53 (24.8%)
Adenovirus	0	12 (5.6%)
Astrovirus	2 (1.8%)	8 (3.7%)
Norovirus	1 (0.9%)	13 (6.1%)

Table II shows the results of our enteric virus investigations: a single Norovirus infection (0.9%) and two Astrovirus infections (1.8%) were found in the neonatal population. The birth-weight of these neonates were 1,610, 1,780, and 890 g respectively; only one was breastfed. The three patients were inborn with a protracted hospital stay (average 50 days). None of these neonates showed enteric symptoms.

During their stay in the NICU, a total of eight newborns showed enteric symptoms, characterized by diarrhoea with mucus but without blood for approximately five days. In seven of these patients, *Escherichia coli* (1 case), *Pseudomonas aeruginosa* (4 cases) and *Klebsiella oxytoca* (2 cases) were detected in rectal swab cultures. These enterobacterial strains were proved by PFGE (pulsed field gel electrophoresis) analysis to belong to endemic clones circulating through extensive cross-colonization in the NICU during the surveillance period [8].

For the group of older symptomatic children (6-28 months of age), Rotavirus infection was detected in 53 (24.8%) specimens, Norovirus in 13 (6.1%), Adenovirus in 12 (5.6%) and Astrovirus in 8 (3.7%). Rotavirus strain typing revealed type G1P[8] in 7 (13.2%) children, G2P[4] in 10 (18.9%), G3P[8] in 9 (17%), G4P[8] in 23 (43.4%) and G9P[8] in 1 child (1.9%). Three strains were not typed.

Clinical features according to type of viral infection in the paediatric population are shown in Table III. No statistically significant differences were observed in

Tab. III. Clinical features according to type of infection in the paediatric population examined.

Characteristic	Rotavirus	Adenovirus	Astrovirus	Norovirus
No. (%) of children infected	53 (24.8)	12 (5.6)	8 (3.7)	13 (6.1)
Days of diarrhea*	4 (1-12)	4 (3-8)	4 (3-7)	3 (1-8)
Vomiting (%)	41 (77.4)	7 (58.3)	5 (62.5)	9 (69.2)
Fever (%)	34 (64.1)	6 (50)	5 (62.5)	6 (46.2)
No. of dehydrated children (%)	28 (52.8)	3 (25)	3 (37.5)	6 (46.2)

* median and (range)

the duration of diarrhoea, frequency of vomiting, fever and dehydration among the viral agents examined.

Conclusion

Various reservoirs have been documented during nosocomial outbreaks of viral infection, including the environment and infected hospital staff [4]. No environmental or staff sampling was carried out during our investigations. However, the main reservoir for Rotaviruses is thought to be sick infants excreting a large number of viral particles, as asymptomatic infections have a small role in the maintenance of Rotavirus infection [1]. For the eight newborn infants in our study showing enteric symptoms, viral infections were excluded by multiple stool sample tests. Considering that Rotaviruses remain viable on fomites for several weeks and resist to common detergents and disinfectants, and that the infectious dose can be as low as 10 viral particles, we can assume that Rotaviruses have not been circulating in our care unit. A similar result was reported by Linhares et al. in a neonatal, 2-year surveillance study on Rotavirus infection [6]. In developed countries, nosocomial Rotavirus strains transmission correspond usually to those circulating in the community [4]. In our area, the limited circulation, in infants and children, of diarrhoea caused by G9 Rotavirus infection, and the absence of P[6] type strains, may account for the absence of neonatal rotaviral infections. Moreover, because most of the neonates were inborn and directly admitted from the maternity unit, they escaped any contact with older children excreting Rotavirus.

Information on other neonatal viral infections is scarce. Astroviruses are usually detected in children between 2 and 3 years of age, and as agents of mixed infections they may increase the severity of illnesses caused by other enteropathogens, with important nutritional consequences for the child.

The importance of Norovirus as causative agent of acute gastroenteritis in young children has recently been highlighted. In France, Bon et al. [9] detected Astrovirus and Calicivirus infections in 10% and 6%, respectively, of symptomatic neonates. In the Netherlands, neonatal Astrovirus and Calicivirus infections were detected in 0.7% and 6.7%, respectively, of asymptomatic neonates [10]. We found a low circulation of Astrovirus and Norovirus in asymptomatic hospitalised newborns. The number of virus-positive patients in our study is too small to allow us to assess the correlation between risk factor and viral infection, although the observation that all infected neonates had a very prolonged hospital stay is significant in NICU infection. Our results on viral infections in the paediatric population are consistent with those of previous studies carried out in children with gastroenteritis in developed countries, such as France and Spain [9 11]. Norovirus has recently been recognized as the second most common enteropathogenic virus among children with gastroenteritis [11]. In our experience, it is responsible for cases of gastroenteritis that are clinically undistinguishable from those caused by Rotavirus. Routine testing for Norovirus, Astrovirus and Adenovirus would help reduce the diagnostic gap in infectious gastroenteritis.

In conclusion, we can confirm that Rotavirus is the leading agent of acute diarrhoea in the paediatric population in our geographical area. Its role in neonatal infection is related to several factors, the most important perhaps being the emergence of new viral strains and the presence of infected symptomatic children. While in 1999-2000 G9 strains represented 19% of Rotaviruses circulating in our paediatric population, in 2003 they accounted for 0.9% of total infections, making their introduction into neonatal intensive care units unlikely. However, only by continuously monitoring Rotavirus strains in the paediatric population can nosocomial outbreaks of gastroenteritis be effectively controlled.

References

1 Kapikian AZ, Hoshino Y, Chanock RM. *Rotaviruses*. In: Knipe DM, Howley PM, eds. *Fields virology: vol. 2*. Philadelphia: Lippincott-Raven Publishers 2001, pp. 1787-1833.

2 Arista S, Vizzi E, Migliore MC, Di Rosa E, Cascio A. *High incidence of G9P[8] rotavirus infections in Italian children during the winter season 1999-2000*. Eur J Epidemiol 2003;18:711-4.

3 Jain V, Parashar UD, Glass RI, Bhan MK. *Epidemiology of rotavirus in India*. Indian J Pediatr 2001;68:855-62.

4 Widdowson MA, van Doornum GJ, van der Poel WH, de Boer AS, van de Heide R, Mahdi U, et al. *An outbreak of diarrhea in a neonatal medium care unit caused by a novel strain of rotavirus: investigation using both epidemiologic and microbiological methods*. Infect Control Hosp Epidemiol 2002;23:665-70.

5 Arista S, Giammanco GM, De Grazia S, Migliore MC, Martella V, Cascio A. *Molecular characterization of the genotype G9 human rotavirus strains recovered in Palermo, Italy, during the winter of 1999-2000*. Epidemiol Infect 2004;132:343-9.

6 Linhares AC, Mascarenhas JD, Gusmao RH, Gabbay YB, Fialho AM, Leite JP. *Neonatal rotavirus infection in Belem, northern Brazil: nosocomial transmission of a P[6] G2 strain*. Med Virol 2002;67:418-26.

7 Mota-Hernández F, Calva JJ, Gutiérrez-Camacho C, Villa-Contreras S, Arias CF, Padilla-Noriega L, et al. *Rotavirus diarrhea severity is related to the VP4 Type in Mexican Children*. J Clin Microbiol 2003;41:3158-62.

8 Di Carlo P, Mammina C, Corsello G, Giuffrè M, Cipolla D, Tantillo M, et al. *Circolazione di Gram-negativi produttori di ESBL in una unità di terapia intensiva neonatale*. Giorn Ital Mal Infet 2004;10:162-3.

9 Bon F, Fascia P, Dauvergne M, Tenenbaum D, Planson H, Petion AM, et al. *Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France*. J Clin Microbiol 1999;37:3055-8.

10 Koopmans M, Vinje J, Duizer E, de Wit M, van Duijnhoven Y. *Molecular epidemiology of human enteric caliciviruses in The Netherlands.* Novartis Found Symp 2001;238:197-214.

11 Román E, Wilhelmi I, Colomina J, Villar J, Cilleruelo ML, Nebreda V et al. *Acute viral gastroenteritis: proportion and clinical relevance of multiple infections in Spanish children*. J Med Microbiol 2003;52:435-40.