

***NOROVIRUS* and *ROTAVIRUS* – two major causative agents of sporadic viral gastroenteritis in hospitalized Polish children**

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ABSTRACT

Purpose: The aim of the study was to establish the main etiologic agents of acute viral gastroenteritis and to assess the severity of illness associated with the different viral agents of gastroenteritis in children hospitalized during spring/summer season 2008.

Material and Methods: 181 stool specimens were collected from children under 5 years of age, hospitalized with acute viral diarrhea from April to September 2008. Commercial enzyme immunoassay kits were used to detect noroviruses. The immunochromatographic tests for combined detection of rotaviruses and adenoviruses were performed routinely in our department in all in-patients suspected for viral gastroenteritis.

Results: A viral etiologic agents were detected in 108 of 181 (59.7%) samples tested. Dual viral pathogens (rotavirus and norovirus) were found in 3 of 181 (1.7%) samples. Rotavirus was the most common viral pathogen found in the study group (86/181; 47.5%), followed by NV (19/181; 10.5%) and adenoviruses (3/181; 1.7%). Approximately, 60% of acute gastroenteritis episodes occurring in children less than 5 years of age were accounted for by infection due to rotavirus and/or norovirus. Norovirus cases were clinically indistinguishable from those of rotavirus origin in children aged less than 2 years whereas they were slightly milder in older group of patients.

Conclusions: Rotavirus infections are leading cause of children's hospitalization in spring months whereas Norovirus infections during spring/summer time. There is a great need to apply molecular diagnostic tools to determine the actual and monitoring the changing etiology of acute enteritis in Polish population.

Key words: acute gastroenteritis, children, norovirus, rotavirus

INTRODUCTION

Viral infections of gastrointestinal tract, resulting in the acute dehydrating diarrheal disease, affect children worldwide and remain a major problem of public health. Acute viral gastroenteritis is after respiratory tract infections, the second most common cause of childhood morbidity and hospitalizations in developed countries. Amongst numerous causative agents of acute viral gastroenteritis, rotavirus (RV) and norovirus (NV) have been presently identified as the most important. Both viruses are highly contagious and virulent pathogens. A small infectious dose (10-100 particles), resistance to environmental

factors, and long-term shedding of huge amounts of virus particles in feces, promote secondary cases of infection and their epidemic prevalence. The burden of RV gastroenteritis appears to be well recognized in developed and developing countries [1-3]. The global epidemiology of NV gastroenteritis and its impact on the national healthcare systems is recognized to a lesser extent. Until recently, NVs have been considered as the major cause of gastroenteritis outbreaks. Currently, the growing number of molecular and epidemiological studies of sporadic and outbreak cases of gastroenteritis show that NV is a common cause of disease, especially in the youngest and the oldest age groups of the community [4-7]. Within Europe,

norovirus infections are monitored by the *Food-borne Viruses in Europe* (FBVE) network, which had been collecting data since 1994 [8]. Poland has no surveillance system for viral gastroenteritis. The first pilot study on frequency of Norovirus infections in hospitalized Polish children with acute viral gastroenteritis was performed in our department in 2005 and reported in 2007 [9]. In that time, the seasonal peak of NV infections was seen on September through December. Since detection of a new genogroup II.4 variant in 2002, highly atypical spring or summer peaks of NV infections were noted in the Netherlands, Germany, Finland, and England and Wales [10].

The present study was undertaken to determine the frequency of sporadic rotavirus, adenovirus and norovirus infections in children up to five years old, hospitalized due to community-acquired acute viral gastroenteritis during spring/summer months, and to assess the severity of illness associated with the different viral agents of gastroenteritis.

MATERIAL AND METHODS

From April to September 2008, 181 stool specimens were obtained from 181 children (100 males, 81 females) with acute gastroenteritis within 36 hours of admission to the Department of Pediatric Infectious Diseases, Medical University of Białystok, Poland. Gastroenteritis was defined as three or more loose stools per 24 hours or one or more episode of vomiting. The inclusion criteria were: children under the age of five who presented to our department with symptoms of acute gastroenteritis, informed consent obtained from parents or legal guardians, no bacterial co-infection, no underlying diseases.

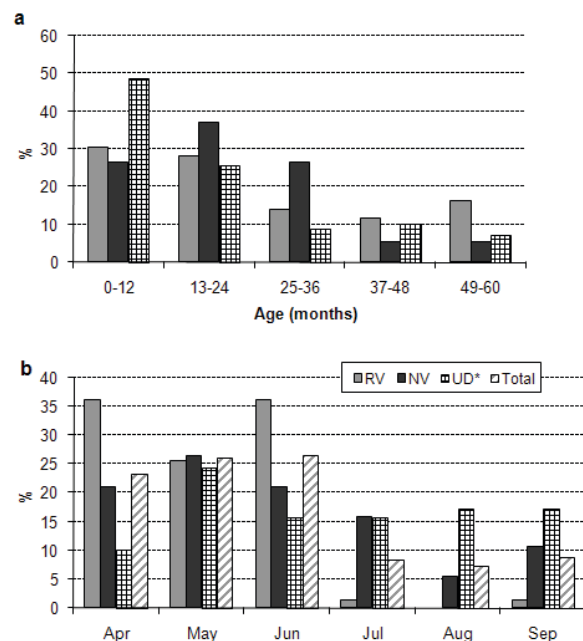
Commercial, immunochromatographic tests (Vikia Rota-Adeno, Biomerieux) for simultaneous detection of rotavirus group A and adenovirus were performed soon after the fecal specimens were obtained. Then, specimens for NV detection were frozen and stored in $<20^{\circ}\text{C}$ for future simultaneous testing with IDEA™Norovirus (DacoCytomation) according to the manufacturer's instruction.

Severity of gastrointestinal disease was determined by the numerical score of Ruuska and Vesikari, with a maximum of severity score of 20 points [11]. Occurrence of clinical symptoms of gastroenteritis in patients' close relatives were also recorded.

Quantitative variables were expressed as median, mean, standard deviation and range. Qualitative variables were calculated as frequency and percentage, and were compared by the χ^2 test. Statistical analysis was performed with Statistica Statsoft, version 8.0. All statistical analysis was performed with a significance level of 0.05.

The study protocol was approved by the Research Ethical Committee of Medical University in Białystok and informed consent of parents or guardians was obtained prior to enrolment.

Figure 1. Age-related [a] and monthly distribution [b] of acute rotavirus, norovirus and undetermined viral gastroenteritis in hospitalized children.



UD* undetermined

RESULTS

A viral etiological agent was detected in 108 of 181 (59.7%) samples tested. Dual viral pathogens (RV and NV) were found in 3 of 181 (1.7%) samples. Seventy of 181 cases (38.7%) of acute gastroenteritis remained undetermined. Rotavirus was the most common viral pathogen found in the study group (86/181; 47.5%), followed by NV (19/181; 10.5%) and adenoviruses (3/181; 1.7%). Thus, 59.7% of acute gastroenteritis episodes occurring in children less than 5 years of age were accounted for by infection due to rotavirus and/or norovirus.

Although samples were collected from children aged ≤ 5 years, they were predominantly from children aged ≤ 2 years (116/181; 64.1%). The numbers of children presenting with gastroenteritis to our department declined as age increased regardless of etiological agent (Fig. 1a). While rotavirus infection tended to peak in spring months, norovirus infection occurred more or less spring/summer round (Fig. 1b). A comparison of ages of children with single viral infections demonstrated that children with norovirus infection were younger (median age 16.5 months; range 2-58 months) than children with rotavirus infection (median age 20.1 months; range 2-59 months).

The severity score in rotavirus and norovirus infected children is shown in Tab. 1. In children under 2 years of age the clinical course of RV and NV gastroenteritis was similar according to the severity scoring. In older children RV infections were associated with higher severity score than that

Table 1. Demographic and clinical pattern of rotavirus and norovirus infections in hospitalized children.

Parameters	Rotavirus (86)			Norovirus (19)			p
	median	mean (s.d.)	range	median	mean (s.d.)	range	
age (months)	20	20.1 (17.1)	2-59	16	16.5 (14.1)	2-58	0.39
severity score total group	12.5	11.9 (2.4)	7-18	11	11.3 (2.2)	8-17	0.11
severity score children ≤24 months	12.5	11.9 (2.4)	7-16	12.5	11.5 (2.3)	8-17	0.6
severity score children >24 months	12	12.4 (2.4)	9-18	10	10.4 (2.0)	8-14	0.03

for NV single infections and the difference was statistically significant ($p = 0.03$)

Epidemic spread of RV infections in family members of our patients was slightly higher than epidemic spread of NV infections (34.9% vs 31.6% of affected families, respectively).

DISCUSSION

Since 2001, surveillance and laboratory diagnosis of norovirus in outbreaks of gastroenteritis has been routinely performed throughout the European Food-borne Viruses network [10,12]. Noroviruses are known as a common cause of acute gastroenteritis often in outbreaks and predominantly in winter. Food- and waterborne transmission play an important role in the spread of NV. Numerous studies had been carried out on the molecular epidemiology of norovirus in outbreaks but relatively few on sporadic cases. Poland, unlike other European countries has no surveillance system for viral gastroenteritis. Basic virus detection and typing methods are not routinely available in our country. Up to date, the cause of the majority of acute gastroenteritis cases remains undetermined. The present study was undertaken to establish the etiologic agents of acute gastroenteritis in children hospitalized during spring/summer season 2008, using commercial enzyme immunoassay kits for detection of noroviruses. The immunochromatographic tests for combined detection of rotaviruses and adenoviruses are performed routinely in our department in all in-patients suspected for viral gastroenteritis.

Noroviruses were established as the second after rotaviruses, the major cause of spring/summer acute viral gastroenteritis in north-eastern Poland. Nineteen studies, reviewed recently by Patel and co-workers, evaluated norovirus disease among hospitalized diarrhea cases in children under 5 years of age [7]. The proportion of norovirus disease in these studies ranged from 10% to 15%, similarly to our results. It should be emphasized, that all of reviewed studies used RT-PCR assays to diagnose norovirus in patients with diarrhea. ELISA testing reaches a sensitivity of about 64% in stool samples and specificity of 96%. Immunoassays fail to detect the large genetic diversity of the virus [13]. ELISA studies show variable results when compared with PCR based tests. In other words, the real frequency of childhood norovirus diarrhea in Poland may be higher than that reported in the present study.

The interesting observation of the present study is that rotavirus infections were leading cause of childhood hospitalization in spring months whereas Norovirus infections during spring and summer time. Our results are consistent with observations of FBVE network, reported since 2002, on atypical spring and summer peak of norovirus outbreaks in some European countries [10,12,14]. Increased number of norovirus outbreaks was preceded by detection of new genogroup II.4 variants [15].

The second objective of our study was to evaluate the severity of norovirus disease compared to rotavirus infections using a 20-point severity score of Ruuska&Vesikari [11]. Previously, it was admitted that noroviruses, unlike rotaviruses, cause a benign, short-time and self-limiting illness [16]. As it was calculated in the present study, norovirus cases were indistinguishable in view of clinical severity from those of rotavirus origin, in children aged less than 2 years. Norovirus infection were slightly milder in older group of patients. Our results are consistent with those presenting by Murata and co-workers in Japanese children [17].

CONCLUSIONS

Results of our study highlight a need to apply molecular diagnostic tools to determine the actual and monitoring the changing etiology of acute enteritis in Polish population. The molecular techniques allow to determine the microbial quality of drinking and recreational water as well as food products. The same measures that reduce the incidence of bacterial gastroenteritis may be ineffective in the case of noroviruses, because these are resistant to chlorination and freezing, they persist in the environment and need really small doses to be infective.

Noroviruses emerged in our region as the main cause of sporadic, spring/summer acute gastroenteritis in hospitalized children.

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