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## Viral agents of acute gastroenteritis in hospitalized children in Greece

S. Levidiotou, C. Gartzonika, D. Papaventsis, C. Christaki, E. Priavali, N. Zotos, E. Kapsali and G. Vrioni\*

Department of Microbiology, Medical School, University of Ioannina, Ioannina, Greece

### Abstract

A 6-year study of stool samples from 4604 children hospitalized for acute gastroenteritis was conducted to investigate the role of enteric viruses as a cause of gastroenteritis in north-west Greece. Rotaviruses, noroviruses, adenoviruses and astroviruses were detected in 21.35%, 4%, 3.5% and 2.35%, respectively, by enzyme immunoassays and molecular techniques. Molecular techniques enhanced overall diagnostic efficacy by 2.5%, and by c. 10% each for rotavirus and adenovirus. Rotavirus was the leading cause of viral gastroenteritis, usually associated with severe illness. Mixed infections were found in 4.4% of positive specimens, and rotavirus plus astrovirus represented the most frequent co-infection (55.5%). This first study on the epidemiology of viral gastroenteritis in Greece shows that recent advances in the diagnosis of viral enteropathogens may have only marginal effects on overall diagnostic efficacy, and thus the impact of viral agents causing sporadic gastroenteritis in public health cannot be fully evaluated.

**Keywords:** Adenovirus, astrovirus, child, gastroenteritis, Greece, norovirus, rotavirus

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**Corresponding author and reprint requests:** S. Levidiotou, Department of Microbiology, Medical School, University of Ioannina, Ioannina, 45110, Greece  
**E-mail:** sleveidi@uoi.gr

\*Present address: G. Vrioni, Department of Clinical Microbiology, General University Hospital 'Attikon', Medical School, University of Athens, Athens, Greece.

Viral enteropathogens are important causes of childhood gastroenteritis in both developed and developing countries [1,2]. The commonest aetiological agents include rotavirus, astrovirus, adenovirus and norovirus, with differences in prevalence depending on environmental, geographical or socio-economic factors [3,4]. The development of enzyme immunoassays (EIAs) and RT-PCR assays for such pathogens has led to increased sensitivity of viral detection and increasing knowledge of disease epidemiology.

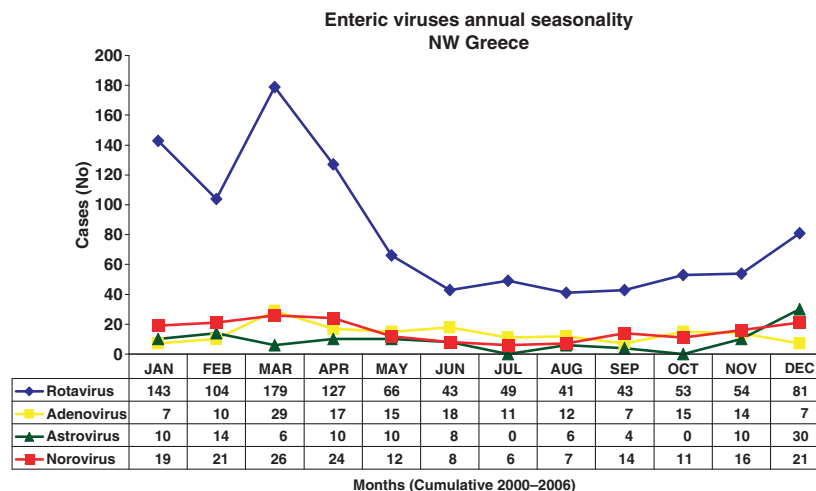
Limited data on enteropathogenic viruses are available from Greece, where there is no specific surveillance system for viral gastroenteritis. The objective of the present study was to evaluate the aetiological implications of rotavirus, adenovirus, astrovirus and norovirus in young children hospitalized for acute gastroenteritis in Greece.

Faecal samples from children under 5 years of age (median age: 14 months) were collected from five hospitals in north-west Greece. During the 2000–2006 study period, no acute gastroenteritis outbreak was recorded. Informed consent from all parents, as well as approval by the local hospital scientific committees, was obtained prior to the study. Clinical features were documented. Samples were obtained during the first 48 h of hospitalization, and were stored at  $-70^{\circ}\text{C}$  until testing.

Stool samples were screened for the presence of rotavirus (group A), adenovirus, astrovirus and norovirus antigens by EIA (IDEIA; DAKO Cytomation, Angel Drove, UK). Rotavirus presence was further investigated by an 'in-house' RT-PCR, which has been previously described [5]. Adenovirus presence was also molecularly evaluated by a PCR-microplate hybridization assay (PCR Adenovirus consensus; Argene, Biosoft, France). To validate PCR results, control stool specimens were obtained from persons without symptoms of diarrhoea for at least 3 weeks prior to testing. All samples were examined for parasites and bacterial pathogens by conventional laboratory methods.

A total of 4604 stool samples were examined, and aetiological agents were detected in 1789 cases (38.9%). Monobacterial infections were detected in 389 cases (8.4% of the total) (*Salmonella* spp. in 258, *Shigella* spp. in five, *Campylobacter jejuni* in 118, *Yersinia enterocolitica* in four, *Escherichia coli* in two, and *Aeromonas hydrophila* in two), and single viral infections were identified in 1338 children (29.1% of the total). Viral–bacterial co-infection was found in 26 cases, and viral–viral co-infection in 36 cases. No sample was positive for parasites.

The majority of the 1400 cases of viral pathogen isolation were in children <24 months of age (75%). Early spring and late winter seasonality was observed, particularly for rotavirus (Fig. 1). There were no significant differences in the



**FIG. 1.** Annual seasonality of enteric viruses in north-west Greece.

**TABLE I.** Viral enteropathogens detected by enzyme immunoassay

Pathogen	Mono-infection Cases (% of total)	Co-infection	
		Viral-viral co-infections	Viral-bacterial co-infections
Rotavirus	941 (20.4)	32 viral-viral (adenovirus, 10; astrovirus, 20; adeno-astrovirus, 2)	10 viral-bacterial ( <i>Salmonella</i> spp., 3; <i>Yersinia enterocolitica</i> , 2; <i>Campylobacter jejuni</i> , 5)
Adenoviruses	142 (3.1)	16 viral-viral (rotavirus, 10; astrovirus, 4; rota-astrovirus, 2)	4 viral-bacterial (all <i>Salmonella</i> spp.)
Astroviruses	70 (1.5)	26 viral-viral (rotavirus, 20; adenovirus, 4; rota-adenovirus, 2)	12 viral-bacterial (all <i>Salmonella</i> spp.)
Noroviruses	185 (4)	None	None

seasonal distribution of admissions, and no peak of viral detection rate was found, excluding the aforementioned season. Table I depicts the frequency of viral isolation with EIA. As noted, group A rotavirus was responsible for the majority of cases, followed, in order of frequency, by noroviruses, adenoviruses, and astroviruses. The adenovirus strains detected belonged to subgenus F (69.8%), subgenus C (16%) and subgenus A (14.2%).

Molecular testing clarified an additional 110 positive samples: 92 rotavirus cases and 18 adenovirus cases. Negative results were found in all control group samples.

To our knowledge, this is the first extended report on the epidemiology of viral gastroenteritis in hospitalized children under 5 years of age in Greece. In a 1999 Athens study of children with diarrhoea [6], the overall incidence of viral gastroenteritis was significantly lower (12.5%). This could be attributed to the fact that different viral detection methods had been used and fewer viral pathogens had been investigated.

By contrast, the percentage of virus-positive cases was similar [7,8] or lower [9–12] to that reported in other studies. Many of these studies [10–12] were performed during the winter season, which is the peak season of viral diarrhoea. Limiting our study to this period, the percentage of

viral enteric pathogens would rise to 60% (746 virus-positive samples out of 1243 admissions). Moreover, investigation of other enteric viruses, such as enteroviruses, sapoviruses, toroviruses, picornaviruses and coronaviruses would probably increase the overall proportion of viral gastroenteritis.

As reported worldwide [9,12–14], group A rotavirus was the most frequent viral agent detected in children from north-west Greece. Infection severity (dehydration, fever >38 °C, diarrhoea, vomiting and duration of symptoms) was greater in rotavirus cases than in those associated with other viruses [3,4,9]. As previously suggested, our results confirm norovirus as the second commonest viral agent among hospitalized children with acute gastroenteritis [10–12]. Astrovirus and adenovirus infection rates were also comparable [9,13,15]. Although enteric adenovirus types 40 and 41 predominated, non-enteric subgenera (A and C) were also causally implicated [8]. As the detection of norovirus and astrovirus infections was performed only by EIA, one can speculate that their incidence may have been underestimated.

The most important observation, as with other reports, remains that, in the majority of cases, despite the use of molecular techniques, no pathogen was isolated: this implies that hitherto unknown enteropathogenic viruses/bacteria

may exist, or that our current diagnostic efficacy in acute gastroenteritis may actually still be low. The addition of molecular techniques resulted in an increase in detection of rotavirus and adenovirus cases by c. 10%, increasing the overall diagnostic efficacy by 2.5%.

There are limited data available on co-infections in gastroenteritis cases, and the rate of such infections varies widely in the literature. In our study, the rate of co-infections (4.4%) was lower than reported in other European studies [7,12–14]. Rotavirus plus astrovirus (55.5%) represented the most frequent co-infection [7,8,12,14].

In conclusion, although it has been demonstrated that rotavirus plays a significant role in hospitalized children with diarrhoea in Greece, the potential cost-effectiveness of routine vaccination cannot be speculated upon. Molecular techniques for detecting viral enteropathogens marginally enhanced diagnostic sensitivity in this patient series, and the majority of cases remained of unknown cause. One can suggest, however, that routine testing for norovirus may be warranted, it being the second most frequent viral causative agent, and that the presence of dual infections also warrants further epidemiological and pathogenetic discussion. Although implementation of routine testing for viral agents causing acute diarrhoea may have an impact on the public health burden of viral gastroenteritis, there is still a long way to go to achieve a satisfactory diagnostic yield.

## Transparency Declaration

The authors declare that they have no competing interests.

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