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A large infantile gastroenteritis outbreak in Albania caused by multiple emerging rotavirus genotypes

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SUMMARY

By the end of December 2000, the epidemiological system 'Alert' of the Public Health Institute in Tirane reported an outbreak of acute gastroenteritis. The outbreak involved children in Tirane and in the rural area. In total, 2722 children were seen in Tirane Hospital and 982 (56·4%) were treated for acute gastroenteritis. The age group with the highest morbidity was 0–5 years (89·7%), followed by the 6–9 (6·2%) and 10–15 years age groups (4·1%). The distribution of acute gastroenteritis cases, which occurred along the same water distribution system, suggests a waterborne origin. The nucleic acid amplification confirmed the co-circulation of different genotypes of rotavirus, mainly P[8]G9 and P[8]G3, responsible for the outbreak. Other enteric viruses such as astrovirus serotype 1, adenovirus and Norovirus, genogroups I and II were detected. Co-infections with different rotavirus genotypes and even with different enteric viruses were detected in several samples.

INTRODUCTION

Each year viral infections of the intestinal tract represent the major cause of morbidity in young children in industrialized countries, whereas in developing countries they are responsible for an estimated annual death rate of 800 000 persons [1], the causative agents include rotaviruses, enteric adenoviruses, astroviruses and caliciviruses, which include Norovirus (NV) and Sappovirus (SV).

Group A rotaviruses are the leading cause of infantile diarrhoea worldwide [2], and are associated with over 600 000 annual deaths, mainly in developing countries [3]. Rotavirus strains may be serotyped on the basis of two outer capsid proteins that are the targets of neutralizing antibodies produced following natural infection; the glycoprotein VP7, which determines G serotypes, and the protease-sensitive protein VP4, which determines P-types [2]. Fourteen rotavirus G serotypes, including 10 which infect humans, and 21 P-types, including 9 that infect humans have been identified [4]. Serotyping and genotyping studies indicate that four G–P combinations: P[8]G1, P[4]G2, P[8]G3, P[8]G4 are common worldwide. An emerging fifth combination, P[8]G9, is becoming more common [3]. The incidence of acute gastroenteritis, especially in developing countries, is largely underestimated because of the lack of surveillance systems. Ford [5], in a study conducted in India, calculated that the incidence of acute gastrointestinal disease was underestimated by a factor of 200, whereas Mead [6] estimated this value to be from 20 to 38 in the United States.

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Albania is a Balkan country, with heavy socio-economic problems that affect the public health. The country suffered the re-emergence of diseases such as cholera in 1994 and poliomyelitis in 1996–1997.

Tirane, the capital, is the most overcrowded city, housing approximately one sixth of the entire population of the country, however the population is in continuous transition. The poor infrastructure of the country causes a low quality of drinking water, with interrupted chlorine treatments. These factors may explain the high frequency of outbreaks of acute gastroenteritis which mostly affect children [7, 8].

The actual incidence of the different enteric viruses in Albania is completely unknown, and they are not included in the list of notifiable diseases set by the Ministry of Health.

During the period December 2000–January 2001, an increase in the incidence of acute gastroenteritis was observed in the Paediatric Unit of Tirane Hospital. Stool samples were collected from patients and analysed by molecular methods for the presence of enteric viruses.

METHODS

Twenty-eight stool samples from children with gastroenteritis were collected during the outbreak period: 19 males and 9 females. The samples were from children from the urban areas of central Tirane (10), Bathore (3) and Lac (1). Additionally, stools from children inhabiting the rural areas around Tirane (13) and Durrës (1) were also collected. The rural areas include immigrants from different districts of the country, whereas Bathore, although located near the city, is a crowded area, due to people arriving from the Northeast of Albania, with poor hygienic conditions and a low economic level. All the information about the outbreak has been collected by the Public Health Institute in Tirane using two national surveillance systems for infectious diseases. The first is the 'Weekly Surveillance Syndrome System' or 'Alert system' which collects information on all common syndromes, such as acute diarrhoea, without, however, discriminating between the different aetiologies. The second system is the 'Monthly Diagnosed Database Surveillance System' which actually gathers information only on bacterial gastroenterical diseases, mainly because of the limitations in the technology and equipment available to approach virus diagnosis.

Stools were suspended (10%, w/v) in phosphate-buffered saline (PBS) and, after vigorously mixing, the faecal sample was clarified by centrifugation at 2500 ***g*** for 15 min at 4 °C. The resulting supernatant was stored at −20 °C and shipped frozen to Italian and Spanish laboratories to be analysed for the presence of enteric viruses.

Nucleic acids were extracted, following the manufacturer's specifications, using the Qiagen Viral RNA kit (Qiagen, Milan, Italy) for RNA viruses and Seek Viral DNA (Talent, Trieste, Italy) for adenovirus assays respectively.

The primers, probes and procedures employed for the detection and typing of NLV have been described elsewhere [9], the hybridization was performed in liquid phase using the DiaSorin kit (DiaSorin, Saluggia, Italy). A previously described two-step PCR was used for the generic diagnosis of adenovirus [10]. Adenovirus sequences from the general hexon region were determined for typification. For astrovirus detection, an RT–PCR–Southern blot system was carried out with published primers and probe [11]. Genotyping of the astrovirus-positive samples was performed by a previously described method [12] based on sequence analysis of a 348-bp amplimer from ORF2. All sequences were determined after purification of the amplified products with QIAgen PCR purification kit, and sequenced with the Big Dye Terminator Cycle Sequencing Ready Reaction version 2.0 (PerkinElmer, Rome, Italy). The reading was performed using an ABI Prism 377 automated DNA sequencer (PerkinElmer).

For the generic detection of rotaviruses, a new RT–PCR hybridization method based on the amplification of a VP6 fragment and confirmed by Southern blot hybridization with a digoxigenin-labelled internal probe was used. Primers VP6-3 (5′-GCTTTAAAAC-GAAGTCTTCAAC-3′) and VP6-4 (5′-GGTAAAT-TACCAATTCCTCCAG-3′) at a 1 μM concentration were used in an RT reaction of 10 μl final volume containing 4 U of M-MLV enzyme (Roche, Monza, Italy), 0·2 mM of each nucleotide and 5 μl of denatured (5 min at 99 °C) double-stranded RNA sample. The reaction was run for 60 min at 50 °C. A total of 5 μl of the RT product was further processed by a PCR method using 3·5 U of the Expand enzyme (Roche) in a final volume of 50 μl, again supplemented with 1 μM of each primer and 2 mM of each nucleotide. The PCR programme included a 9 min denaturation step at 95 °C and 40 cycles of amplification with 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C each and a final elongation step of 7 min at 72 °C. In order to confirm the rotaviral nature

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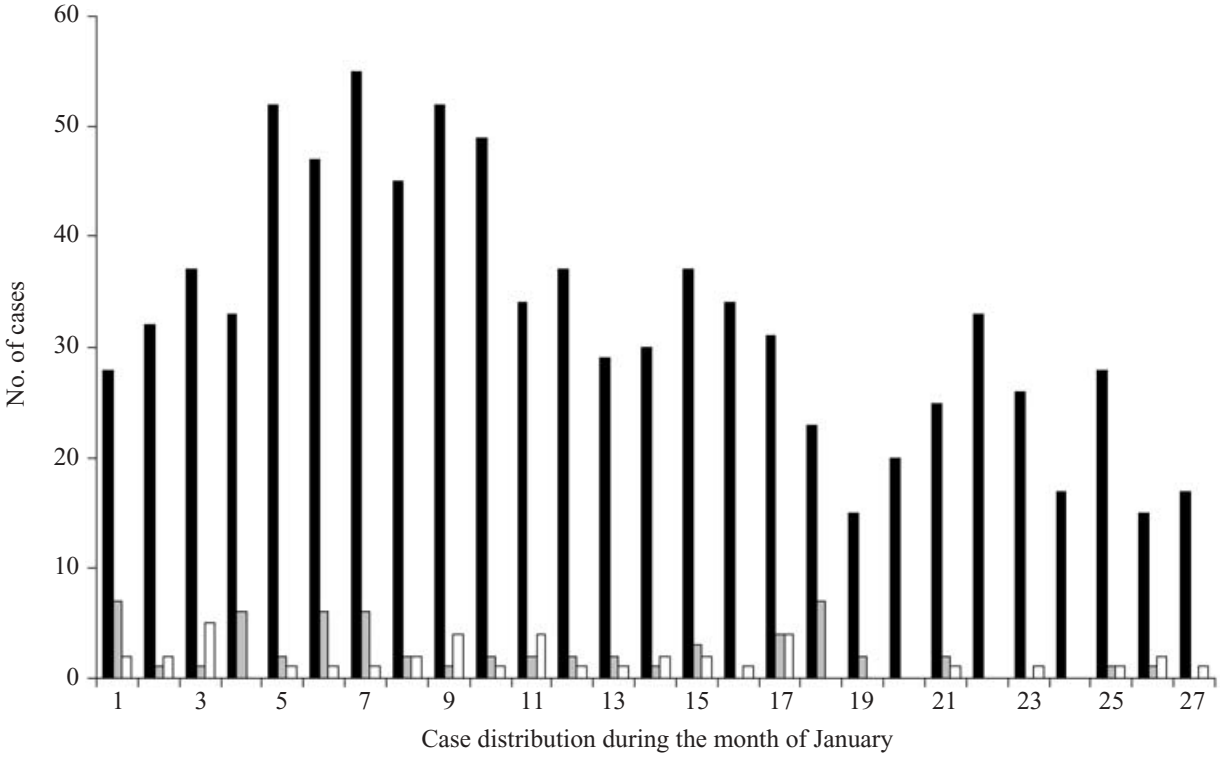

Fig. 1. Acute gastroenteritis cases by age group during the month of January in the Emergency Paediatric Unit. ■, 0–5 years; ▨, 6–9 years; □, 10–15 years.

of the 186-bp amplimer a Southern blot hybridization with the digoxigenin-labelled probe 5′-CAAATGATAGTTACTATGAATGG-3′ was performed.

VP6-positive samples were further analysed, being the G- and P-types determined following the methods described by Gouvea [13] and Gentsch [14] respectively. The cocktail of primers used in the present study allows the determination of the G1, G2, G3, G4, G5, G8 and G9 VP7-types and P[4], P[6], P[8] and P[9] VP4-types.

RESULTS

In December 2000, the Weekly Surveillance Syndrome System for acute gastroenteritis (Alert system) in the Public Health Institute in Tirane observed an increase in the number of cases of acute gastroenteritis and the number of children requiring medical treatment in the Paediatric Unit of Tirane Hospital. Overall, 2722 children were seen, 982 for gastroenteritis (36%) and 1740 for other illnesses. The maximum number of cases were observed during the first week of January, but cases were reported throughout the entire month. The age distribution of acute gastroenteritis showed a preponderance of cases in the 0–5 years age group (89·7%), whereas in the 6–9 and 10–15 years age groups it was 6·2 and 4·1% respectively (Fig. 1). No deaths were recorded during the outbreak.

Twenty-five out of the 28 samples were positive for the presence of rotavirus. One of these samples was co-infected with astroviruses, three with adenoviruses and six with NV. Co-infections with more than two viral agents were not detected. Altogether, 28% had co-infections with different agents.

The sequence analysis of the adenovirus amplimers, employing primers from the hexon region for generic amplification, showed the maximum homology with adenovirus type 6. Seven samples were identified positive for NV, six were confirmed by liquid hybridization, one was unspecific. Two other samples, negative in RT–PCR were scored as positive after the hybridization test. In particular, all 8 NV-positive samples contained GGII strains, two of them also contained a GGIA strain and a third one contained a GGIB strain. The positive astrovirus sample was typed by sequencing of a fragment of the capsid region and corresponded to an astrovirus serotype 1.

Twenty-one rotavirus-positive samples could be G-typed (84%) while 22 (92%) could be P-typed. Seventy-one per cent of the samples contained a

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Table 1. *Number of patients infected with one or more G-types*

	G1	G2	G3	G9
G1	2	1	0	2
G2	—	2	0	0
G3	—	—	1	7
G9	—	—	—	4

G9-type, 47% a G3-type, 33% a G1-type and 14% a G2-type. Co-infections with different G-types were commonly detected (Table 1) but were rather uncommon among the P-types. Eighty-two per cent of the samples contained only P[8] strains, 14% only P[6] and 4% contained both strains. The combination of P/G-types revealed the P[8]G9 as the most frequent, accounting for 44% of all the cases, followed by the P[8]G3 in 33% of the cases, and by P[8]G1, P[6]G2, P[8]G2 and P[6]G1, which accounted for 10, 7, 3 and 3% of the cases respectively.

DISCUSSION

Among the different countries in the Balkan area, Albania has several socio-economic and environmental problems. In the early 1990s, the capital city sustained a rapid and intensive urbanization due to large shifts of the population, which caused severe public health problems, such as high environmental microbiological pollution and major congestion in the medical system. The increasing environmental degradation led to continuous problems in drinking water quality and waterborne transmission of enteric pathogens, among them the cholera [15] (1994) and poliomyelitis [16] (1996–1997) outbreaks have been reported.

In a study of waterborne disease outbreaks reported between 1946 and 1980 [17], water system deficiencies that caused or contributed to these outbreaks were categorized under five major headings: (*a*) the use of contaminated, untreated surface water; (*b*) the use of contaminated, untreated groundwater; (*c*) inadequate or interrupted treatment; (*d*) distribution network problems and (*e*) miscellaneous. More than 80% of the outbreaks were associated with deficiencies in the treatment and distribution of water. The magnitude of this outbreak, with cases concentrated all along a particular potable water piping system serving the affected areas, pointed to drinking water as the likely source of the infection (M. Dhimolea, personal communication). Several breakdowns were detected in the water distribution system after environmental examination [8]. However, although a definitive case-control study could not be performed, due to problems related with the local socio-economic situation, a questionnaire, answered by the patients, pointed to drinking water as the sole common risk factor among other selected exposures, which included different kinds of food. High-risk factors such as shellfish or vegetable consumption was not acknowledged by the affected population.

One of the few studies conducted in Albania [8] reported that a high proportion of the drinking water samples lacked chlorine residuals; statistical analysis indicated a high significant risk of coliform presence and of diarrhoeal disease.

The huge public health burden of infectious diarrhoea in childhood in Albania is evidenced by the extremely high number of clinically recognized cases: 11 206 episodes in 2000 and a clearly underestimated official figure of 7712 in 2001, which represents 1% of the total infantile population [18]. The gastroenteritis attack rate in Albania is 2·5 times that reported for the United States and Spain [1].

In a previous 1-year study conducted in 1996 by the Laboratory of Enterobacterial Pathogens of the High Institute of Health in Rome (I. Luzzi, personal communication), the aetiology of 367 diarrhoea cases in Albania was 28·3% bacteria, 10·4% protozoa, 1·1% worms and 20·1% rotaviruses, which were the only assayed viruses, using a commercial immunoenzymic test.

Since it is well documented that once in the environment human enteric viruses are much more persistent than bacteria [19], it can be assumed that the risk of waterborne virus infection is extremely high. Moreover, in another study Divizia and co-workers [16] confirmed the evident circulation of enteric viruses in Albania during the outbreak of poliomyelitis declared in 1996–1997. Our previous data confirm the large circulation of the enterically transmitted viruses in Albania because of the large population movement from rural to urban areas and the absence of any wastewater treatment plant.

Our data generated by using molecular detection procedures confirm the wide circulation of rotaviruses as well as of the other three assayed viruses.

The most frequent rotavirus strain isolated in stool samples from this very large outbreak was P[8]G9, followed by P[8]G3. The latter, which was detected in 33% of patients' stools, is considered a common strain worldwide, although with much less prevalence

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than the P[8]G1, which was found in only 10% of these cases. P[8]G9, detected in 44% of the outbreak samples, should be regarded as an emerging strain whose prevalence is continuously increasing [20]. The P[6]G2, P[8]G2 and P[6]G1 strains, which were also isolated from these stools, have only occasionally been reported previously [20, 21].

The distribution of the different enteric virus types may vary between distinct geographic and socio-economic regions of the world, or even between years in a given community. Although serotyping is a classification based on neutralization of virus infectivity, the available information on gene sequences of rotavirus strains allows for the prediction of the serotype of a given strain by PCR, using type-specific primers.

The first live attenuated rotavirus vaccine, licensed in 1998, was withdrawn from the market in 1999, due to its possible association with intussusception [22]. When future vaccines are designed, antigens from the prevalent genogroups across the world should be included to ensure protection against all circulating strains. Since rotavirus serotypes (genotypes) circulating in a given region have a direct influence on the predicted efficiency of a potential vaccine for that region, the search for its G- and P-type distribution becomes necessary.

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