

Waterborne gastroenteritis outbreak in Albania

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Abstract Three different studies are reported concerning the environmental pollution caused by viruses in Albania. The first study describes an outbreak of gastroenteritis in the capital city, involving 2,722 children attending the Paediatric Unit of Tirana Hospital. The age group with the highest morbidity was 0–5 years, with 89.5%; no fatalities were recorded during the outbreak. Rotavirus was detected in 26/28 faecal samples by RT–PCR, although astrovirus, adenovirus and calicivirus were also present. The second study describes an outbreak of hepatitis A virus involving the city of Lac. Two hundred cases were recorded, with the highest incidence in the age-group 5–9 years. Phylogenetic analysis of the VP1/2A region showed the presence of a unique sequence: genotype IA. Rotavirus was identified in drinking-water samples collected during the outbreak. The third study describes the prevalence of HAV and HEV in 202 sera randomly collected from 12 different cities in Albania. HAV showed a high incidence (66.2%), whereas none was positive for HEV. The genomic analysis of the VP1/2A junction revealed the presence of only one genotype (IA) with few point mutations and just two amino acid substitutions at codons 22 and 34. Additionally, two potential antigenic variants were detected, the first at position 46 of VP3 and the second at position 23 of VP1.

Keywords Gastroenteritis; hepatitis A virus; rotavirus

Introduction

The cause of many gastroenteritis cases remains frequently unknown because of (a) the absence of specific and sensitive tests or (b) the absence of facilities, particularly in developing countries (CDC, 1990). Untreated sewage discharges can cause pollution of different surface waters and, in particular, of drinking waters. The microbiological quality of drinking water in Tirana is poor, especially in the suburban areas, where faecal coliforms are usually present. The possibility of isolating enteric viruses in cell culture is limited by the absence of susceptible cell lines for all the enteric viruses and by the high cost of virus isolation on monolayers. The development of molecular tests, in particular the polymerase chain reaction (PCR), has improved diagnosis, and nucleotide analysis has enabled better characterisation of different genotypes.

Materials and methods

Serum samples and serological tests

All sera were collected at the Public Health Institute, Tirana, and kept frozen (–20°C) with limited freeze–thaw for serological tests and for transport to Italy and Spain for molecular analysis. IgM anti-HAV tests were performed in Albania, and antibody anti-HEV in Italy, both with and according to Abbott kits.

Water samples

Drinking-water (1,500 L) and sewage (1.5 L) samples were collected and filtered in

Albania using electropositive filters (Cuno 1MDS). The filters were eluted with 1.3 L 0.05 M glycine (pH 9.5), with the eluate being neutralised and frozen to be sent to Italy. Each sample was re-concentrated by PEG precipitation: the pellet was resuspended in 5–6 mL of sterile PBS and further concentrated by ultracentrifugation (Beckman Optima rotor TLA100, 60,000×g, 4°C, 60 min). The final pellet was resuspended in 500 µL sterile PBS.

RNA extraction and RT-PCR test

All the RNA extractions for molecular biological assays were performed using the commercial TRIzol kit (Life Technology). The protocols for RT and PCR have been previously described for all the viruses (Villena *et al.*, 2003).

Sequence analysis of VP1/2A, VP1 and VP3 HAV regions

The amplified products were purified (QIAgen PCR purification kit) and sequenced using 0.8 ng/bp with the Big Dye Terminator Cycle Sequencing Ready Reaction v2.0, and the reading was performed using an ABI prism DNA sequencer (Perkin Elmer).

Results

Study 1: Waterborne gastroenteritis outbreak in Tirana

The epidemiological system “Alert” of the Public Health Institute in Tirana reported an outbreak of gastroenteritis involving the capital city and the rural area. In total, 2,722 children attended Tirana Hospital, and 982 (56.4%) were treated for acute gastroenteritis (Figure 1). The age group with the highest morbidity was 0–5 years (89.7%), followed by the age-groups 6–9 (6.2%) and 10–15 years (4.1%). No fatalities were recorded during the outbreak.

Stool samples (28) were collected during the outbreak of gastroenteritis and tested by RT-PCR for rotavirus, astrovirus, adenovirus and norovirus. Rotavirus was present in 25/28 samples with 28% of co-infections with the other enteric viruses – however, no more than two viral agents were ever detected in the same sample. Genomic analysis showed astrovirus type I and three adenovirus type 6. Norovirus PCR-positive samples (6) were confirmed by liquid hybridisation including GG1a, GG1b and GG2 strains. Rotavirus positive samples (21) could be G- and P-genotyped using a cocktail of primers showing 71% of G9 type, 47% G3 type, 33% G1 type and 14% G2 type. P[8] strains were found in 82%, 14% were P[6] and 4% showed both. The combination of P/G types revealed 44% P[8]G9, followed by P[8]G3 in 33% of the cases, and more frequently P[8]G1 (10%), P[6]G2 (7%), P[8]G2 (3%) and P[6]G1 (3%).

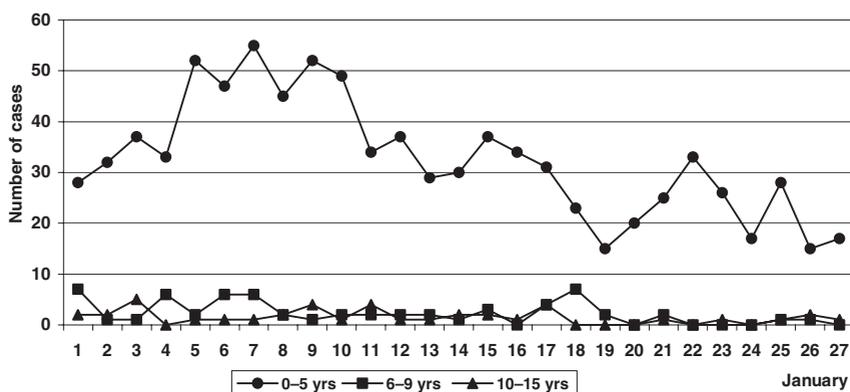


Figure 1 Acute diarrhoea cases by age group in Tirana Emergency Paediatric Unit

Study 2: Waterborne hepatitis A outbreak in Lac

During the period November 2002–January 2003, HAV cases (210) were recorded, with a peak at the beginning of January. Age distribution of HAV cases showed a peak in the age group 5–10 (43.5%). Wastewater and drinking-water samples were collected during January, the first tested for HAV and the second for rotavirus and HAV. Wastewaters were HAV positive, whereas the drinking-water samples were only rotavirus positive. The genomic analysis of all the sera and environmental samples of the HAV isolates showed a unique genotype: IA. Two isolates presented numerous point mutations in the third position determining only one amino acid substitution in the position 25 (Arg→Lys) (Table 1); two other isolates had the same sequence of the GBM strain (acc. n. X75215). All the other isolated HAV strains showed only two point mutations present both in human and in environmental samples.

Study 3: Hepatitis in Albania

Sera (202) were tested serologically for hepatitis A and E markers, with the HAV positive samples being characterised by sequence analysis. No sera presented specific antibodies against HEV, whereas 66.2% were positive for HAV. The genomic analysis of 50 HAV positive sera showed, after sequence alignment, only 11 point mutations (4%), with all being genotype IA. Only two amino acid substitutions were present, one in the codon 34 (Arg→Lys) and the other in the codon 22 (Glu→Asp). Analysis of VP3 and VP1 identified two amino acid substitutions at positions 46 of VP3 (Ile→Val) and 23 of VP1 (Ile→Val) (Table 1).

Discussion

In the past few years Albania has suffered from various socio-economic problems resulting from a large movement of the population, leading to a collapse of the medical system and serious environmental microbiological pollution: both responsible for outbreaks of cholera (1994) and poliomyelitis (1996–1997). In a previous study, Palombi *et al.* (2001) showed the low quality of the drinking water in Tirana and the suburban area, where several breakdowns occurred in the water system distribution; microbiological parameters clearly indicated faecal contamination of the drinking water associated with a complete absence of chlorine residuals. Luzzi (pers. comm.), evaluating the aetiology of 367 diarrhoea cases, reported 28.3% due to bacteria and 20.1% due to rotavirus. In our data, the most frequently isolated virus was rotavirus (study 1), in particular the strain P[8]G9, followed by P[8]G3. While the latter is a common strain worldwide, the former is regarded as an emerging strain. This outbreak showed a concentration of the cases along the potable water distribution system (pers. comm. Epidemiology Unit, Public Health Authority), whereas

Table 1 Nucleotide mutations and amino acid substitutions in the VP1/2A junction region in the Albanian HAV isolates

Nt	Study 2			Study 3			
	Substitution	Nt	Substitution	Nt	Substitution	Nt	Substitution
3029	T → C	3080	T → C	3029	T → C	3089 ²²	G → T
3041	A → G	3095 ²⁵	G → A	3041	A → G	3124 ³⁴	G → A
3056	C → T	3135	C → T	3056	C → T	3134	C → T
3062	G → A	3159	G → C	3062	G → A	3158	G → C
3068	A → G	3167	A → C	3068	A → G	3173	A → G
				3080	T → C		

* The nucleotide substitutions in **bold** were responsible for three amino acid substitutions in the codons 22 (Glu → Asp), 25 (Arg → Lys) and 34 (Arg → Lys)

the environmental examination detected several breakdowns along the water system (Palombi *et al.*, 2001). However, since a case-control study could not be performed, we can only suggest a waterborne origin of the infection (Figure 1).

Considering the relevance of enteric viruses in the gastroenteritis incidence, a surveillance of the hospitalised gastroenteritis cases was developed. The analysis of almost 140 stool samples, collected in Tirana Hospital, showed 19.7% negative for enteric virus (by RT-PCR), 37.6% showed specific amplified bands for rotavirus, 27.1% for astrovirus, 54.8% for enterovirus, 1.0% for norovirus type I and 6.3% for norovirus type II. The analysis of only virus positive samples showed 62.3% of samples with only one virus, 26.0% with two enteric viruses, and 10.4% and 1.3% with three and four enteric viruses respectively (unpublished data). The large presence of enterovirus in our data was dependent on the age group of the patients (0–3 years old) and the vaccination schedule against poliomyelitis performed in Albania: 2–4–6 months and two other boosters at 2–3 years.

Hepatitis A is a common infection in developing countries, largely acquired in childhood, whereas in industrialised countries the first contact tends to be in young adults. Albania recorded more than 3,200 cases in 1998, with the most affected being 0–5 years of age. The prevalence of the different hepatitis types has been confirmed (Santantonio *et al.*, 1993; Chironna *et al.*, 2000; Adhami and Angoni, 2001). Although genotype IA is largely endemic in the Mediterranean area, samples collected from different zones show different circulating genotypes. In study 2, analysis of the randomly collected samples from 12 cities and several rural areas showed a unique genotype IA with only few point mutations and only two amino acid substitutions in the codon 22 and 34 of the protein 2A (Table 1). One of these substitutions, codon 22, was identified by Chironna *et al.* (2003) in the Apulia region, whereas several other strains have been identified in both Albania and Italy. The presence of a unique genotype has been confirmed by the outbreak in Lac where, as in the previous study, the only serologically identified genotype was IA (third study) (Table 1).

Fewtrell and Bartram (2001), analysing waterborne outbreaks between 1946–1980, identified 80% of outbreaks as being associated with deficiencies in treatment and distribution of drinking waters. During the Lac outbreak, drinking-water samples were collected but the RT-PCR test for HAV was always negative using 400 L samples, despite the same samples being positive for rotavirus – clearly showing the low quality of the potable water.

The genomic analysis identified only two point mutations present in both human and environmental samples, although two other human isolates presented eight point mutations not present in the other human and environmental cases.

Conclusion

Overall, our data confirmed the low quality of drinking water and the large faecal pollution of the environment. The presence of a unique HAV genotype confirmed the isolation of the country until the beginning of the 1990s when a continuous migratory movement was established between Albania and Italy. The presence of several identical strains in the two countries could have been a result of this situation.

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