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Wading pool water contaminated with both noroviruses and astroviruses as the source of a gastroenteritis outbreak

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SUMMARY

In July 2001, an outbreak of gastroenteritis occurred in Helsinki among children and adults after bathing in an outdoor wading pool. The epidemiological survey revealed that at least 242 persons were affected. Microbiological testing of both patient stool samples and of the pool water revealed the presence of two different gastroenteritis viruses: a norovirus (NV) and an astrovirus. Amplicon sequencing of the NV samples showed nucleotide sequence identity between the virus from patients and the water. After changing the pool water and the sand at the bottom of the pool followed by shock chlorination, no virus could be detected in the water. However, NV was continuously detected in the water outlet well as much as 8 months after the incident. Here we show how molecular methods aided in tracing the source of the epidemic and in finding the causative pathogens both in patients and in the environment.

INTRODUCTION

Noroviruses (NVs), previously called Norwalk-like viruses, which belong to the family Caliciviridae, are known to be major causative agents of diarrhoea in developed and developing countries [1]. NVs have been shown to be transmitted most often by person-to-person contact or by contaminated food or water. Waterborne outbreaks have almost without exception been associated with consumption of contaminated drinking water [2–4]. Contamination has mainly occurred through leaks of sewage into wells [5], while infections caused by swimming water have rarely been described [6–9]. In addition, there is insufficient information on the occurrence of NVs in natural waters.

Since the identification of caliciviruses and astroviruses as the main constituents of the group of small round-structured viruses (SRSVs [10]) detected by electron microscopy (EM) in stools and subsequently the development of sensitive genome-based detection methods, it has become possible to monitor their role in waterborne outbreaks [11, 12]. These viruses, particularly NVs, possess properties that favour their efficient spread via the environment. Their abundant excretion (10^8–10^{10} virions/g of stool), their resistance to heat and disinfection [1, 13] and exceptional survival time in both dry and moist environments are factors critical to their circulation. The short duration of protective immunity caused by NVs together with the large number of immunologically distinct strains also explains the abundance of outbreaks and infections in the population both in developed and developing countries [1].

We report a waterborne outbreak associated with contamination of a children's wading pool, in which both an astrovirus and an NV could be demonstrated in the water and in patient stool samples.

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METHODS

Description of the pool

The children's outdoor pool in question was located in a recreational area of the city of Helsinki. The bottom of the pool is formed partly of natural rock and partly of concrete, covered with gravel and sand. It is filled with purified municipal water at the beginning of the bathing season, but also has some natural influx. The outlet pipes lead into the municipal sewage system via a well, but some water is absorbed into the surrounding soil.

The pool, set up in 1946, has a water volume of approximately 200 m^3 and depth of 30–100 cm. It is a popular recreation site, with approximately 20 000–34 000 bathers using it during the summer months or as many as 500 bathers per day. The water was manually chlorinated three times per week. Municipal health inspectors irregularly check the microbiological quality of the water, but no routine measurements for the concentration of free chlorine were undertaken. No previous reports on outbreaks associated with use of the pool have been recorded. During the 5 years prior to the outbreak, 10 water samples had been studied. They showed at most a concentration of 40 thermotolerant coliforms and six faecal streptococci per 100 ml of water. A level of less than 100 colony-forming units (c.f.u.)/100 ml has been used as an acceptable value to indicate adequate cleanliness.

Description of events

The first reported cases of gastroenteritis among those having bathed in the pool occurred on 7 and 8 July 2001. Following these announcements the Environmental Centre forbade bathing in the pool. A preliminary investigation undertaken by the Environmental Centre revealed that a rather extensive outbreak was occurring, and a more detailed epidemiological investigation was initiated. In included an announcement in the main newspaper directed towards possibly predisposed pool users, and a questionnaire was sent to all those reporting illness.

An inspection at the site revealed that the hygienic condition of the two toilets located quite close to the pool was unsatisfactory, with traces of excreta on the floor. Water samples for microbiological investigations were taken immediately and the pool was subsequently emptied. The surface sand was replaced to a 20-cm depth and the basin refilled with municipal water and shock-chlorinated (10 mg/l). The water was replaced and chlorinated twice, after which the basin was left empty over the winter. Rainwater and snowmelt passed through the outlet well, but no water was added to the pool. A water sample from the outlet well was taken in the spring for virus testing. Later, for the 2002 swimming season, the pool was equipped with continuous filtration and chlorination of the water.

Laboratory and environmental investigation

Stool samples were obtained from a total of six patients. A 10% suspension from faecal samples was prepared in a 50 mM Tris–HCl/0·1 M NaCl/1 mM $CaCl_2$ buffer (pH 7·4). For NVs, reverse transcription–polymerase chain reaction (RT–PCR) was performed as described with separate reactions for genogroups I and II [14]. Amplicons of lengths 117 and 150 bp respectively, were obtained from the polymerase region. The product was initially identified on agarose gel and confirmed in an enzyme immunoassay (EIA) type of hybridization reaction. Subsequently, the amplicons were sequenced as described in [14]. For astrovirus, the primers used were those described by Mitchell et al. [15].

Water samples (1 litre) were obtained from the pool and the outlet well. Initial concentration was achieved by binding the virus to positively charged filters according to Gilgen et al. [4, 16]. The RT–PCR was performed as described previously after a phenol-based RNA extraction and ethanol precipitation [14]. The sand sediment samples were treated similarly, with the exception that the RNA was extracted with the glass milk from the sand suspension [17].

The amplicons of the NV strains from both patients and water samples were sequenced using direct sequencing as previously described [14]. The sequences obtained were analysed in the PHYLIP program dnaml [18] and compared with corresponding sequences from reference strains of genogroup II NV and the Norwalk agent of genogroup I.

National standard methods were used in microbiological analyses of faecal pollution indicated by thermotolerant coliforms and faecal streptococci. The Colilert Quanti-Tray (IDEXX Laboratories Inc., Westbrook, ME, USA) test for *Escherichia coli* was used during the epidemiological survey. All the methods used are accredited.

Table 1. *Symptoms and signs among cases*

Symptoms	No. of patients with symptoms/ No. of persons that answered the questions	Patients with symptoms (%)
Nausea	216/224	96
Fatigue	196/205	96
Vomiting	199/224	89
Stomach pain	187/211	89
Fever	118/180	66
Headache	118/178	66
Diarrhoea	126/200	63

Table 2. *Virological findings in patient samples*

Age (years)	Sample date	Norovirus RT–PCR		Astrovirus RT–PCR	EM†
		GI*	GII		
9	9 July	Neg.	Pos.‡	Neg.	Neg.
10	11 July	Neg.	Pos.	Neg.	Neg.
9	11 July	Neg.	Pos.	Pos.	Neg.
19	17 July	Neg.	Neg.	Neg.	Neg.
8	20 July	Neg.	Pos.	Pos.	Neg.
4	20 July	Neg.	Pos.	Neg.	Neg.

* Genogroup I.
† Electron microscopy.
‡ The first three patients in the list developed the disease 9 July; the illness date for the last three patients is not known.

RESULTS

Epidemiological survey

Approximately 200 persons replied to the newspaper announcement and received, by mail, a questionnaire concerning possible disease and behaviour in the pool; in total, 242 questionnaires were returned. The main symptoms and signs of affected persons are presented in Table 1.

Most of the cases were children. The median age was 9 years (range 9 months to 73 years). The median incubation period was 31 h and the median duration of disease 32 h. Three persons were hospitalized. Of those contracting the disease 85% reported having swum in the pool and 79% having in some cases submerged their heads in the water, although others only waded. The median time spent at the pool was 90 min.

Microbiology

No pathogenic bacteria (salmonellas, shigellas, campylobacters, yersinias, aeromonads, *Plesiomonas shigelloides*, *Bacillus cereus*, *Clostridium perfringens* or *Staphylococcus aureus*) were detected in the stool samples of six patients. No signs of faecal contamination were detected in water samples taken less than 2 weeks before the outbreak, while 370 c.f.u./100 ml of *E. coli* were detected in the sample collected on 7 July and 24000 c.f.u./100 ml in the outlet well 2 weeks after the onset of the outbreak. After chlorination the amount of *E. coli* decreased within a few days to below 100 c.f.u./100 ml.

Five out of the six patient samples obtained were positive for genogroup II NV (Table 2). The amplicons from all patients reacted to a probe panel similarly in the microplate hybridization assay, which suggested that they all represented the same virus strain (results not shown). Amplicon sequencing revealed that the NV strain was most closely related to the Bristol-like NVs GII 4 (see Fig.), although the nucleotide identity was only 89·2% with the strain Grimsby (based on 65 nucleic acids). The simultaneous presence of astrovirus could also be demonstrated with RT–PCR (Table 2) in two of the NV-positive stools.

The findings of virus in the water samples are summarized in Table 3. The first water samples from the pool were taken on 9 July. The water was cloudy and only 250 ml could be passed through the filter in the concentration step; it resulted, however, in a strong reaction with RT–PCR for GII NV suggesting heavy contamination of the water. Subsequently, it could be shown that even a 100-μl sample of the unconcentrated water yielded a positive result by RT–PCR. Since the patient samples were shown to contain astroviruses, the water was tested for their presence. The concentrated sample was also positive for astroviruses.

All three sand samples taken (days 6, 16 and 18 after the first cases) from the bottom of the pool were found to be positive for GII NV by RT–PCR.

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Table 3. *Norovirus (NV) and astrovirus (AV) findings in water samples*

Date of sampling	Pool water filtered (ml)	RT–PCR NV/AV	Outlet well water filtered (ml)	NV
2001				
9 July	250	Pos.*/Pos.		n.d.†
23 July	350	Pos./Neg.	250	Pos.
4 Aug.	Chlorination			
6 Aug.	400	Neg./Neg.	400	Pos.
8 Aug.	450	Neg.		n.d.
10 Aug.	450	Neg.	600	Pos.
30 Aug.	Pool was emptied‡			
18 Oct.			350	Neg.
25 Oct.			500	Pos.
2002				
10 Apr.			1000	Pos.
16 Apr.			1000	Pos.

* All positive noroviruses were of genogroup GII.
† n.d., Not done.
‡ The pool was emptied and remained so until summer 2002.

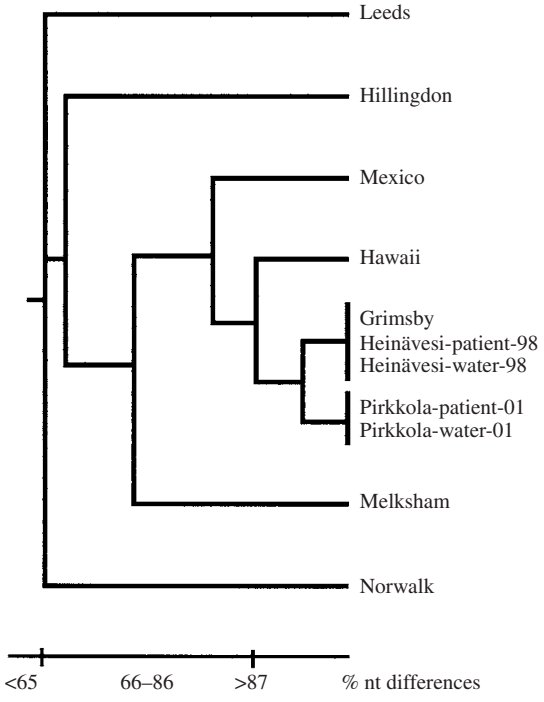

Fig. Phylogenetic tree derived with norovirus polymerase sequences (54 nucleic acids). Corresponding nucleotide sequences from a previous waterborne outbreak in Finland (Heinävesi-patient-98 and Heinävesi-water-98, AJ243788) and prototype noroviruses from GenBank/EMBL: Hawaii (U07611), Melksham (X81879), Mexico (U22498), Leeds (AJ313030), Norwalk (M87661), Grimsby (Bristol-like virus) and Hillingdon [37] were included, for comparison.

The pool water remained positive until chlorination was performed on 4 August. The pool was emptied, refilled and the water was chlorinated up to the calculated free chlorine concentration of 10 mg/l. After the chlorination no virus was detected in the water samples (Table 3). The samples taken simultaneously from the outlet well tested positive for GII NV. The outlet well remained continuously NV-positive by RT–PCR even during spring of the following year. No astroviruses were, however, found in subsequent water samples of the pool and the outlet well.

The NV strains obtained from the patient and water samples were compared using nucleotide sequencing. The NVs from both sources showed a nucleotide sequence identity of 100% for the amplicons (Fig.). For astrovirus, attempts to sequence the PCR-positive water sample were unsuccessful; thus, comparison of the strains was not possible.

DISCUSSION

Enteric viral infections are, next to respiratory diseases the most common ailments occurring in man [19–21]. Most of these infections are short-lived, mild and remain largely unnoticed. During an NV outbreak caused by tap water in a community of 5000 inhabitants, 2500 contracted the disease but only approximately 50 persons reported to the municipal health-care centre [4]. The ubiquity of NVs and infections caused by them makes proper identification of the causative virus strain crucial to source tracing. The RT–PCR methodology applied for viral gastroenteritis is expensive, but it offers an excellent opportunity for precise strain identification. Even a short

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diagnostic amplicon sequence is in most cases enough to link the patient and environmental isolates in a given outbreak [4], as has also been demonstrated in the present case.

Natural bathing waters have been recognized as sources of infections, especially enteric infections [22–27]. In case of sewage contamination, a multitude of agents can be expected to be found in the faeces of exposed people. Swimming pools have also been reported to have been associated with bacterial, protozoan and viral infections and outbreaks (e.g. NVs and adenovirus type 8) [28]. The present findings indicate that the wading pool had been heavily contaminated with human faecal material. The local conditions suggested that the contamination was apparently carried from the filthy public toilets, a view supported by the presence of both astrovirus and NV in the water. Since it can be estimated that the contamination corresponded to about 10^{10} PCR units of NV, even intentional contamination of the pool was suspected. The finding of four nappies at the bottom of the pool during the cleaning operation indicates that other potential ways of contamination also exist.

NVs of genogroup II were found in patient, pool water and outlet well samples. The amplicon nucleotide sequences were identical in the sequenced patient and water samples, which strongly suggests the presence of an infection route from water to humans in this epidemic. Identical capsid sequences would have given even stronger evidence, but capsid sequences were not obtained. Based on the polymerase sequence, the NV strain belongs to the Bristol-like virus group or similar, possibly as a new genotype. In recent years (1999–2002), short nucleotide sequences identical to ours have been found in Canada (af218038), Japan (ab089900, ab053210, ab0444366) and Germany (ay274326). Although the patient samples were clearly positive for NV in the PCR assay, viruses were not found by EM, probably because the timing may not have been optimal for EM analysis in most of the samples.

The outlet well remained positive for NV by PCR for a prolonged period, although NVs were not found in the pool water after shock chlorination. It is not known whether this positivity also represented an infectious virus. It was reported for polioviruses that positive results by PCR obtained after disinfections with chlorine may result in overestimations of infections titres [29].

Astroviruses, as well as caliciviruses, have only been accurately identified since specific diagnostic methods have become established [30]. Astroviruses apparently account for approximately 5–10% of gastroenteritis cases among children. Most of these cases remain unrecognized due to their relatively mild clinical symptoms [31]. Thus the astrovirus finding reported here in the children's pool correlates with their known epidemiology, taking into account the median age of the pool users. In a recent study, a role for waterborne astrovirus infection was suggested, since astroviruses were found in raw and some treated water samples [32]. In the present epidemic the amount of astroviruses in the water was not measured, but appeared to be remarkably lower than the amount of NVs, since the pool water was found to be positive only once for astrovirus.

Contamination of recreational waters and especially of children's pools with viruses is difficult to avoid. An underestimation of these incidents apparently exists, mostly due to the resulting mild disease. The present case shows that extensive outbreaks may result from contamination. A single patient infected with hepatitis A virus (HAV) could under these circumstances lead to a much more serious situation. HAV-infected children show mostly mild symptoms and could pass unrecognized. In a non-immune population, such as those found in most industrialized countries, a mild outbreak among children may – taking into account the long incubation time – lead to a more extensive outbreak before being recognized. It is therefore important to carefully monitor the hygiene of swimming facilities such as the one reported here.

Wading pools are usually equipped with chlorination and filtration. Despite this, it is often difficult to control the water quality and proper maintenance of a pool, even if its construction and management are better than in the pool reported here. A recent survey conducted in the United States, including data from 22 131 inspections of various types of swimming pools, showed that low, attributable free chlorine concentrations and pH occurred in 4·5–18·4% and 4·7–16·7% of investigations respectively. The highest percentages were observed in children's wading pools [33]. Young children, who often swallow the water indiscriminately and contaminate it faecally, are at increased risk for infections [33–35]. In addition, the shallow depth and relatively low water volume in the wading pools may lead to a more rapid depletion of disinfectant by ultraviolet light and thus a higher level of organic contamination by children.

Swimmer education is important in the prevention of swimming-related illnesses. Reports [33, 36] have advised swimmers not to swim when ill with diarrhoea, not to swallow pool water, and to practice good hygiene when using a pool. Parents should take children for toilet breaks regularly, use appropriate nappy-changing areas, wash hands after using the toilet or changing nappies, and shower before entering the pool [33, 36].

The present case also indicates the need for regular surveillance of swimming water for the presence of viruses. Since the amount of *E. coli* is not always indicative of virus-contaminated water, monitoring and using specific virus assays is necessary. Since methods for the detection of viruses in water samples are becoming established, viral testing is currently feasible and highly recommended.

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