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Detection of Human and Animal Rotavirus Sequences in Drinking Water

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Reverse transcription-PCR analysis of drinking water in the homes of 56 children suffering from rotaviral gastroenteritis has shown the presence of the rotavirus genome in four samples. These strains were different from human rotaviruses detected in the children's feces, as determined by sequencing of the VP7-amplified fragments—three of them of animal origin (porcine or bovine) and one of human origin.

Rotavirus is the most common diarrheal pathogen in children worldwide. Rotavirus infections occur in winter epidemics, with a high level of interhuman transmission, which is mainly of fecal-oral origin. There are a wide variety of rotaviruses. The majority of human strains belong to G serotypes 1 to 4, as determined by seroneutralization of the VP7 glycoprotein. Rotaviruses are also widespread throughout the animal kingdom, and natural reassortants, arising from different species, may appear; the latter could have a role in human pathology (16).

Rotaviruses are excreted in very large quantities in the feces of infected subjects, at a rate of up to 10^{11} virus particles per gram. Very resistant in the environment, they are therefore present in large amounts in wastewater (3, 5) and generally in environmental water (2). Their physicochemical characteristics lead us to suspect that certain treatments of water for human consumption may not be completely effective (1).

Several studies have mentioned the presence of rotaviruses in drinking water (4, 12) or the occurrence of epidemics originating from contaminated water (10, 11). In parallel with interhuman contamination, drinking water might thus play a role in the occurrence of sporadic cases (3).

The purpose of this study was to look for the presence of rotaviruses in drinking water distributed in the homes of children with acute gastroenteritis caused by rotavirus; when a rotavirus was detected by reverse transcription-PCR (RT-PCR) analysis, we compared the child's virus with that found in the water by using nucleotide sequencing.

A prospective study of the circulation of rotaviruses in water was carried out during the usual epidemic period, from 3 January to 7 March 1994. Samples were collected from three towns in the Isère department of southeast France (Grenoble, Voiron, and Saint-Marcellin) because of differences in the natural filtration of the water coming from three different geological grounds. Fifty-six samples of drinking water were taken from the homes of 56 children who had been hospitalized for acute gastroenteritis of rotaviral origin. Two liters of water was collected within 24 h after the hospitalization; this volume represents the average drinking consumption for an individual. Over the same period, in each of the three towns (24 control samples), a 2-liter sample was taken systematically each week in a public area chosen for its easy access (school or city hall).

For the 56 children, the rotavirus infection was diagnosed on the basis of detection of an antigen in the feces by agglutination (Slide Rota-kit2; bioMérieux, Lyon, France). The water samples were concentrated 4,000 times by tangential ultrafiltration (Minitan device, followed by Centriprep 100; Millipore Corporation, Bedford, Mass.) before analysis. For the fecal and water samples, total RNA was extracted and purified on silica (Sigma, Saint Louis, Mo.) (14). An RT-PCR analysis was carried out with the Beg9 and RTB primers, as previously described by Gouvea et al. (7) and Flores et al. (6), respectively, and then a seminested PCR was carried out with the RTA-RTB primer pair (6), which allows amplification of a 341-bp fragment of the gene coding for the VP7 capsid protein. The sensitivity of the whole procedure, including ultrafiltration, extraction, and RT-PCR, was previously determined with the bovine strain RFC 67 (i.e., 1 50% tissue culture infective dose [$TCID_{50}$] in 1 liter) (14). The amplified fragments were sequenced after molecular cloning (Sequenase; Amersham-Pharmacia-Biotech, Freiburg, Germany). Sequencing concerned one of the three hypervariable regions of VP7, region A (45 bases), which determines the serotype (9). This allows us both to correlate the serotypes of the viral strains present in the drinking water and in the feces of the corresponding child and to compare them with sequences listed in the National Center for Biotechnology Information (Bethesda, Md.) database.

Four of the 56 water samples taken at the homes of the infected children were found to be positive by RT-PCR (7%), while none of the 24 control samples was. The four positive water samples complied with bacterial standards (absence of total coliform, absence of thermotolerant coliform, and absence of enterococci). The analyzed sequences showed a single strain of human origin but three viruses of animal origin, while the children were all infected by human strains. The strains found among the children and in their homes were not similar (see Table 1 for details of the sequencing results).

The aim of this study was to underline the possible part played by the drinking water in the occurrence of rotavirus gastroenteritis.

Of the samples taken from the homes of the infected children, 7% (4 of 56) were positive, whereas no positive result (0 of 24) was obtained from the control samples; this difference is not statistically significant (the Fisher exact test, $P = 0.31$). In fact, the probability of detecting no virus in control samples

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TABLE 1. Amino acid sequences of region A of VP7 protein (underlined) of rotaviruses detected in water at four children's homes and found in the children's feces

Sample origin	Sample type	Sequence	Characteristics
Voiron area			
Nantouin	Water	LCLYYP SEAP TQIS DNEW KDT LS	Human strain, G serotype 4
	Feces	LCLYYP TEAS TQIN DGEW KDS LS	Human strain, G serotype 1
La Côte Saint-André	Water	LCLYYP NEAA TEIA DDKW TDT LS	Porcine strain
	Feces	LCLYYP SEAP TQIS DNEW KDT LS	Human strain, G serotype 4
Rives	Water	LCLYYP VEAS NEIA DTEW KDT LS	Bovine strain
	Feces	LCLYYP TEAS TQIN DGEW KDS LS	Human strain, G serotype 1
Saint-Marcellin	Water	LCLYYP VAAS NKYA DTEW KDT LS	Bovine strain
	Feces	LCLYYP TEAI TQIN DGEW KDS LS	Human strain, G serotype 1

was high (assuming a Poisson distribution, the 95% confidence interval included 0 value). The presence of rotavirus RNA in drinking water does not prove that the water is infectious. Only a positive result in cell culture would prove the integrity of the virus in the original sample; in fact, growth of rotavirus in cell culture is fastidious, and no such attempt has been made. Nevertheless, the relative fragility of an RNA molecule leads us to expect that a nonencapsided viral RNA would have been altered during the concentration and purification procedures. This makes it reasonable to assume that an RNA detected by RT-PCR probably corresponds to the presence of complete viral particles in the original sample.

Furthermore, could the viral load present in the positive samples be sufficient to cause an infection? The volume of water tested was 2 liters, chosen because this quantity could easily be absorbed in 1 day by an individual (15). Since the minimum infectious dose is low for rotaviruses (i.e., close to 1 $TCID_{50}$) (17) and the sensitivity of our technique is approximately 1 $TCID_{50}$ (14), human contamination from such samples would be likely.

The study of the amplified fragment sequences shows no correlation between the serotypes present in the water and those of the corresponding feces. This does not, therefore, allow us to highlight the water as the source of contamination for the four children concerned. Nevertheless, the water samples taken 24 h after hospitalization did not correspond to the water actually consumed by the children. More generally, our results show that the homes of these children are served by water supplies which might have been contaminated by the rotavirus at a given moment in time.

Sequencing results of rotavirus strains detected in the feces show human serotypes, with the G serotype 1 being predominant. This corresponds to the usual distribution described in the literature (18). In contrast, the majority of animal strains were detected in the water samples. The positive samples came from an area where several crops and livestock were grown. The presence of bovine and porcine strains probably indicates contamination by fecal matter from cattle, perhaps due to the practice of muck spreading in areas of intensive agriculture. Can the presence of animal viruses in water destined for human consumption have any consequences? In the case of a real viral infectivity, it could favor the appearance of reassortant viruses. In fact, recent studies have suggested the importance of an interspecies genetic reassortment phenomenon in rotaviruses, although the epidemiological phenomena involved are not understood yet (8, 13). At this level, water may act as a medium for the spread of animal strains to the human population, and thus lead to the appearance of hybrid viruses.

The results obtained in this study also question the lack of efficiency of some drinking water treatments as to eradication of viruses. The presence of viruses in water that complies with bacterial standards has already been reported (12). It should be noted that in the present study, three of the four water samples containing rotavirus RNA were from sources of mediocre quality, requiring purification treatment to make them potable (two treated with chlorine and one with UV radiation). In addition, the samples were taken after heavy rainfall. Three of the water sources came from the Chartreuse range of mountains (Voiron), and one came from the Vercors range (Saint-Marcellin), with karstic rock formations which do not filter the water. We did not detect any rotavirus in the water originating from the Belledonne range (Grenoble), with a crystalline rock formation, which provides a good filtration of water.

In conclusion, this study shows the presence of rotaviral RNA of human or animal origin in water samples collected from the homes of children with rotaviral acute gastroenteritis. The sequences found in the water were different from those found in the feces of the corresponding children, and it is not possible, from this study, either to confirm or to negate the role of drinking water in the occurrence of rotaviral infections. Nevertheless, the presence of viral sequences of animal origin in water intended for human consumption leads to the assumption that water can convey animal rotaviruses and therefore could participate in the occurrence of reassortant viruses.

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