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Molecular Epidemiology of Group A Rotaviruses in Water Sources and Selected Raw Vegetables in Southern Africa

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Group A rotaviruses (RVs) are the most important cause of acute viral gastroenteritis in infants and young children. In this study raw and treated drinking water supplies at plants in two geographic areas, as well as selected irrigation water and corresponding raw vegetables in three regions of southern Africa, were screened for the presence of RVs using molecular techniques. Group A RVs were detected in 11.8% of partially treated and 1.7% of finally treated drinking water samples and in 14% of irrigation water samples and 1.7% of corresponding raw vegetable samples. Type-specific reverse transcriptase-PCR and sequence analysis revealed the presence of multiple types (G1, G2, G8, and G9) in irrigation water and single types (G1 or G3) in raw and treated drinking water. Group A RVs detected in all samples consisted of mixed P types (P[4], P[6], P[8], and P[9]), with P[6] predominating. The detection of types G8, G9, and P[6] reflects the emergence of these types in clinical infections. The similarity of environmental types to those in patients with clinical RV infections confirms the value of wastewater screening as a tool for assessing RVs circulating in communities, with the benefit of detecting types that cause both clinical and subclinical infections. The results provide new information on RV types in water and related environments and identify the potential risk of waterborne transmission. In addition, the presence of RVs in drinking water underlines shortcomings in quality specifications. These data provide valuable information regarding the prevalence of RVs in environmental sources, with important implications for vaccine development.

Group A rotaviruses (RVs) are a major cause of acute viral gastroenteritis in infants and children but can cause gastroenteritis in all age groups (26, 41). An estimated 110,000 to 150,000 children younger than 5 years of age die annually on the African continent due to RV infection (33, 37). The high burden of rotaviral disease worldwide (37) has promoted the development of an effective vaccine for the control of severe RV-associated disease (6, 19). As demonstrated in the rest of Africa (48), RVs have been shown to be a significant cause of sporadic (62) and epidemic (43, 46, 52) pediatric gastroenteritis in South Africa.

Based on the diversity of the VP4 and VP7 outer capsid proteins group A RVs are classified into P (protease sensitive) and G (glycoprotein) types, respectively (27). To date, 15 G genotypes, 14 P serotypes, and 24 P genotypes have been described (10, 54). A possible 25th P genotype from a Bangladeshi patient has recently been described (40). The G serotypes correlate fully with the G genotypes, whereas only 12 P serotypes correlate with the genotypes (25). Ten G and eleven P genotypes are associated with human RVs and, as the genes segregate separately, different G and P combinations coexist (1). Worldwide, the most common serotypes associated with human infection are G1 to G4, with G5, G8, and G12 less frequently described (10, 49). With its global emergence G9 is currently regarded as the fifth most important G type (60). The most common P types in human infection are P[4] and P[8] (25), with different P and G combinations occurring in natural infections (12). G1P[8], G2P[4], G3P[8], and G4P[8] are the most common combinations associated with human rotaviral disease worldwide (1), with unusual genotypes and P and G combinations emerging, for example, G9P[11] in India (9), G8P[?] in South Africa and United Kingdom (49), G3P[14] in Belgium (10) and G11P[25] in Bangladesh (40). Between 1996 and 1999, G1 and G3 were the predominant serotypes detected in southern Africa, while G9 emerged in both southern Africa and West Africa (48). P[8] was the predominant P type detected, while P[6] was the emerging P type detected in all regions of Africa investigated (48). The types of RVs circulating in a specific region will have a direct effect on the efficacy of the vaccine in use (19). Surveillance and assessment of circulating G and P types is therefore essential to monitor the efficacy of vaccines and to provide epidemiological information important for future vaccine development (12).

RVs are predominantly transmitted via the fecal-oral route (26). After replication in the gastrointestinal tract, RVs are shed in high numbers and may contaminate surface water (42), groundwater, drinking water, and food (5, 29). Group A RVs have been implicated in waterborne and food-borne outbreaks worldwide (14, 24, 30), and sensitive molecular assays and group- and type-specific reverse transcriptase PCR (RT-PCR) methods have been used to detect and genotype RVs from contaminated water sources (3, 15, 57). Group A RVs have been detected in untreated and treated drinking water samples in southern Africa (56), but there are no data regarding the molecular epidemiology of RVs in contaminated water and

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food sources in this region. The aim of the present study was to optimize and apply type-specific RT-PCR methods, developed for the typing of clinical isolates of group A RVs by Gouvea et al. (20), Das et al. (9), and Gentsch et al. (17), to characterize the VP7 gene and the VP4 gene, respectively, of the RV strains detected in water and raw vegetable sources in southern Africa. The types of RVs identified in the environmental sources could then be compared to published data on the types associated with clinical infections from the same regions to establish whether contaminated water and raw vegetables were a possible source or reservoir of RV infection.

MATERIALS AND METHODS

Sampling strategy. (i) Water purification and treatment plant. From January 2003 to February 2005, samples of untreated source water (borehole, empoundments, and wastewater effluent), partially treated (samples collected either after ozonation or dissolved air flotation or ultrafiltration in a multistage purification process), and "final treated" (i.e., after chlorine disinfection and stabilization) drinking water were collected at selected processing points from a water purification and treatment plant. Ten liters of each of the above-mentioned samples were drawn on a weekly basis. Samples were kept at 4°C and analyzed within 48 h of collection.

(ii) Water treatment plant. From January 2004 to February 2005, untreated river and dam water samples and treated drinking water samples were collected from a water treatment plant. The water treatment process included coagulation with slaked lime, flocculation, sedimentation of the flocs, carbonation, filtration, and chlorination. Ten liters of the source water and 200 liters of the chlorinated drinking water were collected on a weekly basis. Samples were kept at 4°C and analyzed within 48 h of collection.

(iii) Irrigation water and associated raw vegetables. From April 2002 to February 2004, irrigation water (10 liters) and corresponding raw vegetable samples were collected in the Western Cape, Gauteng, and Limpopo regions. These regions represent three geographically distinct regions in the far south, the central region, and the far north of South Africa, respectively. The irrigation water was collected from the rivers and boreholes, and the raw vegetables were sampled directly from the fields where they were irrigated. The raw vegetables included cabbage, lettuce, onion, spinach, and tomato and are known to be eaten uncooked by the local rural communities. Outer leaves (100 g) of the cabbage and lettuce were removed and used, while individual onions, spinach, and tomatoes (100 g) were used whole and unprocessed.

(iv) Microbiological analysis. Concurrent water samples were subjected to routine microbiological analysis for indicators of microbial water quality by independent collaborating investigators.

Virus recovery. Rotaviruses were recovered from the raw, partially treated, and final drinking water by means of a glass wool adsorption-elution technique, with an estimated efficiency of recovery of 40% for enteric viruses (59), as described previously (61). The raw vegetable samples (100 g) were vigorously shaken in 100 ml of phosphate-buffered saline (pH 7.4; Sigma Chemical Co., St. Louis, MO) for 2 min and were gently stirred in the same solution overnight at 4°C. RVs were recovered from 100 ml of raw vegetable wash suspensions by using the PEG/NaCl precipitation technique (32).

Cell culture amplification. Propagation in cell culture was used to improve the molecular detection of RVs from the water and raw vegetable samples and to ascertain whether they were viable or not. The human colonic carcinoma cell line, CaCo-2 (ATCC HBT 37), and the primary hepatocellular carcinoma cell line, PLC/PRF/5 (ATCC CRL 8024), both proven to be susceptible to infection with fastidious enteric viruses (21, 39, 51), were used between passages 50 and 100. Using standard cell culture procedures, the cells were grown to confluent monolayers in 25-cm^2 cell culture flasks as described previously (51, 56). The suspensions of recovered viruses were treated with an antibiotic-antimycotic cocktail mix and 10 μg of trypsin/ml (Trypsin 250; Difco, Detroit, MI) as previously described (56). Two sets of cell cultures were infected per sample: one maintained with trypsin (Difco) and one without. Prior to infection the cells were washed with 5 ml of 0.01 M phosphate-buffered saline (Sigma) and starved for 60 min in 1 ml of serum-free Eagle minimum essential medium (Highveld Biological [Pty], Ltd., Kelvin, South Africa). After withdrawal of the starvation medium, 1 ml of the treated virus suspension was inoculated onto the cells. The virus was allowed to adsorb for 90 min at 37°C with gentle rotation every 15 min. Thereafter, the inoculum was removed, and 4 ml of serum-free EMEM, containing a penicillin-streptomycin-amphotericin B (Fungizone) mix (BioWhittaker, Walkersville, MD), with or without 10 μg of trypsin (Difco)/ml added to each flask. The infected cell cultures were incubated at 37°C in the presence of 5% CO_2 and blind-passaged 3 days postinfection by scraping cells off the surface of the flask and inoculating them onto a freshly prepared monolayer of cells in a 25-cm^2 cell culture flask to promote cell-to-cell transfer and enhance the propagation of any virus that may have be present. Four days after passage (7 days postinfection), the cells were scraped off the surface of the flask into the surrounding medium and mixed thoroughly to form a uniform suspension of which 1.5 ml was harvested for molecular analysis. The trypsin was found to affect the integrity of the monolayers; consequently, no virus-induced cytopathic effect was discernible, and viral growth was monitored by RT-PCR.

Molecular detection of group A RVs. To avoid the possibility of contamination, the water and raw vegetable samples were processed and analyzed in a facility separate from that where stool samples were tested for RVs. The cell culture, RNA extraction, preparation of RT-PCRs, amplification, and gel electrophoresis procedures were performed in separate rooms and by different analysts. Nuclease-free water was used for RT-PCRs, and all RT-PCRs were combined together in a UV-irradiated hood. Filter tips were used exclusively during all extraction, amplification, typing, and gel electrophoresis procedures. Unless stated otherwise, the RT-PCR reagents and enzymes for the molecular detection of RVs were from Promega Corp., Madison, WI. Primers were manufactured by Sigma-Genosys, Ltd. (Pampisford, United Kingdom).

Viral RNA was extracted from both the infected cell cultures (1.5 ml) and the recovered virus suspensions (1 ml), using either TRIzol reagent (Invitrogen Life Technologies, Paisley, Scotland) or the QIAamp Ultrasens virus kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The RT-PCR and nested PCR for the detection of group A RVs was performed by using the method of Baggi and Peduzzi (3) with minor modifications as described previously (56). A total of 20 μl of the nested-PCR products was separated on a 2% agarose gel using a 100-bp MW marker (Promega Corp.) and visualized by ethidium bromide staining.

A negative control, consisting of 1 ml of nuclease-free water (Promega Corp.) was included in each set of RNA extractions. Negative controls, consisting of 10 and 2 μl of nuclease-free water were included in each run of RT-PCR and nested PCR, respectively, and simian rotavirus SA11 RNA was included as a positive control.

Molecular characterization of group A RVs by RT-PCR. Unless stated otherwise, the reagents and enzymes used for the typing reactions were from QIAGEN. Primers were manufactured by Sigma-Genosys, Ltd.

(i) G genotyping. Molecular methods initially developed for the typing of RVs from clinical samples were applied for the characterization of the VP7 gene of the environmental RV strains. Briefly, the RT-PCR products from the initial detection step using the primers R1 and End9 (3) were used as a template for a seminested PCR using the primers 9con1 (9) and EndA (16). An additional seminested multiplex PCR was subsequently applied by using primers specific for genotype regions of the VP7 gene (20). The G1 to G4, G8, and G9 genotypes were deduced from the resultant amplicon sizes. All G type results were confirmed with a seminested multiplex PCR system as described by Das et al. (9). Seminested and typing multiplex reactions were done using the following PCR mix: 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (Promega Corp.), 20 pmol of each of primer, and 1.5 U of *Taq* DNA polymerase. Initial denaturation for 2 min at 94°C was followed by 30 cycles of 1 min at 94°C, 1 min at 42°C, and 1 min at 72°C, with a final extension step of 7 min at 72°C. Then, 20 μl of the seminested multiplex PCR products was separated on a 2% agarose gel using a 100-bp MW marker (Promega Corp.) and visualized by ethidium bromide staining.

(ii) P genotyping. The P genotype of the environmental strains was determined according to the RT-PCR method developed by Gentsch et al. (17) for the typing of clinical isolates. Briefly, primers con3 and con2 were used in a RT-PCR, and the resultant products were used as templates for a seminested multiplex PCR. All seminested multiplex reactions were done using the following PCR mix: 10 mM Tris-HCl (pH 9), 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (Promega Corp.), 20 pmol of each of primer, and 1.5 U of *Taq* DNA polymerase. Initial denaturation for 2 min at 94°C was followed by 30 cycles of 1 min at 94°C, 2 min at 50°C, and 2 min at 72°C, with a final extension step of 7 min at 72°C. A total of 20 μl of the seminested multiplex PCR products was separated on a 2% agarose gel using a 100-bp MW marker (Promega Corp.) and visualized by ethidium bromide staining. The P genotypes could be determined from the resultant multiplex PCR product sizes.

Characterization of VP4 gene by nucleotide sequence analysis. RV RNA that was unreactive using the seminested multiplex for P genotyping was amplified in a nested PCR with the primers con1 and con4 (13). The resultant amplicons, ranging from 150 to 180 bp depending on the type, were subjected to nucleotide

TABLE 1. Water samples analyzed for the presence of RV RNA

Source of samples	Sampling period (mo/yr)	Type of sample (region)	Total no. of samples	No. of samples in which RV RNA was detected
Water purification and treatment plant	1/2003 to 2/2005	Raw water	63	0
		Partially treated water	17	2
		Treated drinking water	216	3
Water treatment plant	1/2004 to 2/2005	Raw water	100	0
		Treated drinking water	200	4
Irrigation water and raw vegetables	4/2002 to 2/2004	Irrigation water (Western Cape)	12	3
		Irrigation water (Gauteng)	39	5
		Corresponding raw vegetables (Gauteng)	39	0
		Irrigation water (Limpopo)	12	1
		Corresponding raw vegetable (Limpopo)	22	1

sequencing on an ABI 3130 Automated Analyzer. Both strands of the amplified fragments were sequenced to verify the nature of the PCR amplicons. Nucleotide sequences were compared to sequences in the NCBI database by using the Basic Local Alignment Search Tool (BLAST) program (www.ncbi.nlm.gov/BLAST/).

Electropherotyping. One milliliter of recovered viral suspension and infected cell culture suspensions, where sufficient sample and cell culture suspensions were still available, was further concentrated to 200 μl using Centricon YM-100 spin columns (Amicon Bioseparations; Millipore Corp., Beverly, MA). Genomic RNA was subsequently extracted by using TRIzol reagent (Invitrogen/Life Technologies) according to the manufacturer's instructions. The method of Steele and Alexander (46) was applied for the electropherotyping of the environmental RV strains.

Nucleotide sequence accession numbers. The nucleotide sequence data for RV isolates have been deposited in the EMBL GenBank database under the accession numbers DQ641037 (IW TKR) and DQ641038 (WPP 17b).

RESULTS

Prevalence of RVs. RV RNA was detected in 5 of 296 (1.7%) samples of source, partially treated, and final treated drinking water drawn from the water purification and treatment plant (Table 1). Two (11.8%) of the RV strains were detected in partially treated water samples, while three (1.4%) were detected in final treated drinking water samples. No RV RNA was detected in the raw water samples. Rotavirus RNA was detected in 4 of 200 (2%) treated drinking water samples from the water treatment plant from a geographically different region (Table 1). No RVs were detected in the river and dam water samples serving this treatment plant. The seven final treated drinking water samples in which RVs were detected met the World Health Organization (63) and the South African Bureau of Standards (45) microbial specifications for drinking water quality (results not shown).

The water and raw vegetable samples included 12 river water samples from the Western Cape, 39 river water and 39 raw vegetable samples from the Gauteng, and 12 water (3 river and 9 borehole) and 22 raw vegetable samples from the Limpopo regions. Group A RVs were detected in 9 (16.7%) of the river water samples used for irrigation and domestic purposes, and in 1 (1.7%) of the associated raw vegetable samples, namely, a tomato sample (results not shown). None of the borehole samples yielded RVs. Group A RVs were only detected in three water samples (WTP 4, IW G, and IW AK) after amplification in the CaCo-2 or PLC/PRF/5 cell cultures, with the remaining strains detected in the recovered virus suspensions (Tables 2 and 3). Apart from four strains, all of the RVs were detected in samples collected between May and October of each year (Tables 2 and 3).

Rotavirus G typing. The G genotypes of 18 of the 19 (94.7%) environmental RV strains could only be determined after the application of a nested PCR using the primers 9con1 and EndA, followed by a seminested multiplex PCR of the VP7 gene. With the exception of one strain, a G1 genotype, all nine of the RVs detected in the water samples from both treatment plants were G3 genotype (Table 2). Mixed G types,

TABLE 2. Detection and characterization of RVs from raw and treated water samples from water treatment plants in southern Africa between April 2002 and February 2005[a]

Source of samples	Sampling date (yr/mo/day)	Type of sample	Derivation of RV	Genotype[b]
WPP	2003/06/17	Final treated drinking water (WPP 29)	Recovered viral suspension	G3P[?]
	2003/07/01	Partially treated water (WPP 17a)	Recovered viral suspension	G3P[?]
	2004/06/28	Partially treated water (WPP 17b)	Recovered viral suspension	G1P[8]
	2004/09/06	Final treated drinking water (WPP 26)	Recovered viral suspension	G3P[?]
	2005/01/25	Final treated drinking water (WPP 16)	Recovered viral suspension	G3P[?]
WTP	2003/03/03	Final treated drinking water (WTP 4)	CaCo-2 cells plus trypsin	G3P[?]
	2004/10/11	Final treated drinking water (WTP 6)	Recovered viral suspension	G3P[?]
	2005/01/24	Final treated drinking water (WTP 12)	Recovered viral suspension	G3P[?]
	2005/02/01	Final treated drinking water (WTP 2)	Recovered viral suspension	G3P[?]

[a] WPP, water purification and treatment plant; WTP, water treatment plant.
[b] ?, no result due to insufficient sample or unsuccessful typing.

TABLE 3. Detection and characterization of RVs in irrigation water and selected raw vegetables in southern Africa between April 2002 and February 2005

Sampling area	Sampling date (yr/mo/day)	Type of sample[a]	Derivation of RV	Genotype[b]
Western Cape	2002/04/15	River water (IW GS)	Recovered viral suspension	G1, G8, G9P[6]
Western Cape	2002/04/15	River water (IW G)	Recovered viral suspension	G1, G2, G9P[4], P[6], P[8]
			PLC/PRF/5 cells	G1, G9P[?]
			PLC/PRF/5 cells plus trypsin	G1, G9P[4]P[6]P[9]
Western Cape	2002/06/24	River water (IW AK)	Recovered viral suspension	G1, G8, G9P[6]
			PLC/PRF/5 cells plus trypsin	G1, G8, G9P[6]
Limpopo	2002/06/26	Tomato (TTo)	Recovered viral suspension	Untypeable
Limpopo	2002/07/09	River water (IW TKR)	Recovered viral suspension	G1, G9P[8]
Gauteng	2003/05/26	River water (IW TMa)	Recovered viral suspension	G1, G2, G9P[?]
Gauteng	2003/05/26	River water (IW TXa)	Recovered viral suspension	G1P[?]
Gauteng	2003/06/23	River water (IW TMb)	Recovered viral suspension	G1, G2, G9P[?]
Gauteng	2003/06/23	River water (IW TXb)	Recovered viral suspension	G1P[?]
Gauteng	2003/07/21	River water (IW TMc)	Recovered viral suspension	G1, G2, G9P[8]

[a] IW, irrigation water.
[b] ?, no result due to insufficient sample or unsuccessful typing.

namely, G1, G2, G8, and G9, were detected in the irrigation water samples, with G1 (100%) and G9 (77.8%) predominating (Table 3). Notably, G1, G2, and G9 types were identified in the recovered viral suspension from water sample IW G, but only types G1 and G9 were identified in the infected cell culture suspensions from the same sample. The types identified in the recovered viral suspension of IW AK, namely, G1, G8, and G9, corresponded to the types identified in the infected cell culture suspensions (Table 3). Despite several attempts the RV strain detected on the raw vegetable sample (tomato) could not be G typed.

Rotavirus P typing. P types could only be determined for six of a total of 19 (31.6%) RV-containing samples. P[8] was identified in a partially treated drinking water sample (WPP 17b) (Table 2), whereas mixed P genotypes, namely, P[4], P[6], P[8], and P[9] were detected in the irrigation water samples, with P[6] predominating (Table 3). Two strains which were unreactive by the seminested multiplex typing PCR were identified by a different nested PCR, using the primers con1 and con4, followed by nucleotide sequence analysis (results not shown). The two strains, identified as P[8], were detected in samples WPP 17b (partially treated water) and IW TKR (irrigation water). The P genotypes of the remaining RV strains could not be determined due to insufficient sample material (Tables 2 and 3).

Electropherotyping. Due to insufficient RNA, none of the RV strains detected in the water and associated raw vegetable samples could be characterized by electropherotyping (results not shown).

DISCUSSION

In this study molecular methods were applied to the detection and characterization of RVs detected in selected water and associated raw vegetable samples in southern Africa. In all instances, RV RNA could only be detected after application of the nested-PCR step. The single-step RT-PCR, which readily detects RV RNA in stool samples, was thus not sensitive enough to detect the low titers of RV RNA in the water and raw vegetable samples. This finding confirms the results of previous studies (3, 56). The presence of RVs in seven of the treated drinking water samples is, however, not unexpected since human enteric viruses have previously been detected in treated drinking water in southern Africa (55, 58) and other regions of the world (23). The majority of the RVs were detected in river water sources used for domestic and irrigation purposes, which again was not unexpected since high levels of fecal pollution, namely, *Escherichia coli* counts of 493,000 CFU 100 ml^{-1} were reported for the river in the Western Cape (4) and >1,000 CFU 100 ml^{-1} for the river in Gauteng (53), indicating that the river water did not meet the microbial specifications for drinking water (45, 63) or agricultural use (11). The failure to detect RVs in the raw water samples could possibly be ascribed to the small volumes (10 liters) used for viral recovery, the decreased survival rate of RVs under adverse environmental conditions (M. van der Linde and W. B. van Zyl, unpublished data), and the lower efficiency of the recovery technique for turbid water (22), as well as the possible presence of viral and PCR-inhibitory agents in these samples. Whether the lower efficiency of recovery of the turbid water was due to association of viruses with the suspended material or the physical quality of the water was not further investigated. Although the extraction methods used in the present study reportedly remove PCR inhibitors, no method has been proven to remove all inhibitors (38). Therefore, the possibility of PCR-inhibitory agents in these samples cannot be excluded, suggesting that an internal control should be included in future studies. Group A RVs could only be detected after amplification in the CaCo-2 or PLC/PRF/5 cell cultures from three samples (WTP 4, IW G, and IW AK). The inability to isolate more RVs in the cell culture systems used could possibly be due to the fastidious nature of wild-type RVs (26) or inactivation of the viruses by environmental elements (raw water) and disinfection processes (treated drinking water). Since the majority of the RV strains could not be amplified in cell culture, the potential infectiousness of these viruses detected by molecular methods is unknown.

The G typing methods, as described by Gouvea et al. (20) and Das et al. (9), could, with minor adaptations (namely, the introduction of an additional seminested PCR using primers 9con1 and EndA), be successfully applied for the typing of

RVs detected in environmental water samples. The G types were identified in 94.7% of RV-containing samples compared to the 70% typing rate for samples from Egypt and 43.6% for samples from Spain (57). In addition, the G2 types in the Gauteng irrigation water samples (IW TMa, -b, and -c) could only be detected when the primers specific for G2 in a monoplex nested typing-PCR were used. Similar G2 typing problems were encountered by Villena et al. (57) when typing RVs detected in sewage samples. However, the method for the P typing, as described by Gentsch et al. (17), was less successful, identifying the P types in only 31.6% of RV-containing samples compared to the >73.3% typing rate for samples from Egypt and Spain (57). This low rate of P typing could possibly be due to differences in virus titers and sample types analyzed in the two studies (57).

The RVs detected in the water samples from the water purification and treatment plants were predominantly G3 RVs with only single types (G1 or G3) detected in these water samples (Table 2). Both G1 and G3 are common types associated with diarrhea worldwide (18) and were reported to be the most common G types detected in southern Africa between 1996 and 1999 (48). The RVs detected in the irrigation water from the Limpopo province were types G1 or G9, while the samples from the Western Cape and Gauteng provinces yielded types G2 and G8, highlighting the distribution of RV types between geographical areas as described in Denmark (12). This cocirculation of mixed RV populations could favor reassortment and contribute to strain diversity (60). The high frequency at which the G9 genotype was detected in the irrigation water sample supports epidemiological data documenting the emergence of this type in clinical specimens from other African countries such as Malawi (8), Kenya (35), and Ghana (2). The global emergence of G9 strains has been demonstrated in India, Bangladesh, Argentina, Brazil, the United Kingdom, and the United States (41). The P genotypes in the irrigation water samples were predominantly P[6], whereas P[4], P[8], and P[9] were also detected (Table 3). The occurrence of types G8, G9, and P[6] in the water sources is reflected in the emergence of these types in clinical infections in the same geographical regions (36, 47, 50), suggesting that water serves as a potential reservoir and vehicle of infection. The emergence of unusual RV types (G9 and P[6]) in sewage samples in a different part of Africa, namely, Cairo, has also recently been documented (57). Surveillance of water and wastewater may thus be an important means to assess the circulation of RVs in the community, and additional analysis for animal RVs would have provided a complete picture of the RVs circulating in the environment. This method of surveillance has been applied for the assessment of circulating astrovirus (34), enterovirus (44), norovirus (31), and rotavirus (28) in communities.

Group A RV was detected on only one raw vegetable sample (tomato, raw vegetable TTo), but despite several attempts the virus appeared to be untypeable. Since the molecular detection method of Baggi and Peduzzi (3) reportedly detects only human group A RVs, the question arises as to whether the RV detected on the raw vegetable samples is an as-yet-undescribed strain. The low number of raw vegetable samples from which the virus could be detected may be due to inadequate sampling and recovery techniques of the viruses from the food samples or that the titers of the viruses were below the detection limit of the molecular methods applied. Although it appears that the raw vegetables did not pose a health risk in terms of RV infection, in a concurrent study untyped enteroviruses were detected on 10% of the raw vegetable samples from the Gauteng region (53). The irrigation water sample IW AK from the Western Cape, however, yielded RV, human adenovirus, and human astrovirus type 1 (4), thus highlighting the potential health risk associated with the use of this water source for irrigation and domestic purposes.

With the exception of four samples (WPP 16, WTP 4, WTP 12, and WTP 2), all samples in which RV RNA was detected were collected between April and October of each year. This corresponds with the reported occurrence of RV infection during the fall and winter in temperate regions (26). However, since the seasonality of RV infection is less marked in developing countries (7), the detection of RV RNA in some water sources during the summer months (January to March) in the present study was not unexpected. However, due to the small sample size further surveillance is required to accurately establish the seasonality of RV in water sources in the regions studied.

The data obtained in the present study represent the first typing information on RVs circulating in the environment in southern Africa. This approach provides valuable information regarding the source and extent of fecal waste contamination of untreated and treated water sources (28). Surveillance of the RV serotypes detected in the environment in terms of geographic and temporal distribution provides information regarding the serotypes circulating in the community, which may have important implications for RV vaccine efficacy. This preliminary study has highlighted the need for further in-depth surveillance of water environments in southern Africa to identify the RV strains circulating in the community.

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