JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2006, p. 395–399 Vol. 44, No. 2
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.2.395–399.2006

Environmental Monitoring for Gastroenteric Viruses in a Pediatric Primary Immunodeficiency Unit

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Received 8 August 2005/Returned for modification 14 November 2005/Accepted 1 December 2005

The aim of this study was to determine if gastroenteric viruses were present on surfaces and equipment in a pediatric primary immunodeficiency unit (PPIU) by environmental sampling using swabs and subsequent nucleic acid extraction and reverse transcriptase PCR assays. A PPIU was chosen, and 11 swabs were taken at the same sites every 2 weeks for 6 months. Nested/heminested PCR assays were used to screen for astroviruses (AsV), noroviruses (NoV), and rotaviruses (RV). AsV, NoV, and RV were detected at multiple swab sites during the study period. NoV was the most frequently detected virus on environmental surfaces; however, RV was detected on 79% and NoV on 50% of swabbing dates during the study period. Toilet taps were the most contaminated sites. Fecal samples from selected patients in the unit were also screened during the study period, and patients excreted AsV, NoV, and RV at times during the study. New cleaning schedules and changes in some of the PPIU sanitary furniture have been suggested as a means of reducing environmental contamination.

The viruses that commonly cause gastroenteritis in children and adults are rotaviruses (RV), noroviruses (NoV), sapoviruses, astroviruses (AsV), and enteric adenoviruses. These viruses are frequently associated with diarrhea and vomiting in children under 5 years of age, and sporadic cases have been described for hospitalized children (9, 10, 22, 25). NoV in particular are a major cause of outbreaks of gastroenteritis in adults in semiclosed institutions, including hospitals (11, 24), nursing/retirement homes (11, 17), cruise ships (13, 30), and other settings (2, 3, 12).

The usual transmission route for NoV is person to person spread by the fecal-oral route. However, other transmission routes have been implicated and include environmental routes (6, 16), which in hospitals can include work surfaces, floors, medical equipment, light switches, taps, door handles, and television/game consoles. Several groups have monitored environmental contamination by viruses in various settings, including pediatric units (29), hospitals (5, 16), day care facilities (4), and hotels (6).

RV has been detected on environmental surfaces by several groups. Studies which examined the prevalence of RV on high-risk fomites in day care facilities and RV environmental contamination in a pediatric unit have been reported (29).

There have been few reports of environmental contamination with AsV (14), although several groups have examined the prevalence of AsV in hospitalized children with gastroenteritis (18, 27, 28). Cubitt et al. monitored an outbreak of AsV gastroenteritis in a pediatric bone marrow transplant unit (7), using three different assays, including reverse transcriptase PCR, to examine fecal samples from patients.

There is little information on the role of environmental contamination with multiple gastroenteric viruses in hospital wards and what effect the environmental contamination has on patient morbidity.

This study was undertaken following an outbreak of AsV gastroenteritis that occurred in a pediatric primary immunodeficiency unit (PPIU) in March 2004 and in which environmental contamination with AsV was detected (14).

In this study, we examined a PPIU over a 6-month period for environmental contamination with NoV genogroup II (GII) strains (representative of the most predominant NoV genotypes circulating), AsV, and group A RV as representative enteric viruses.

MATERIALS AND METHODS

Environmental swabs and patient fecal specimens. Eleven swab sites were chosen to represent areas commonly in contact with hands (Table 1). The environmental swabs were taken approximately every 2 weeks for 6 months. Environmental swabs were collected from sites by methods previously described (14). The first batch of swabs was collected at the time of an AsV outbreak (14). Supplementary cleaning was performed through the use of alcohol wipes in addition to the normal protocol (14), and swabs were collected during the study period in which the normal cleaning protocol was used (14). The swabs were sent to the Enteric Virus Unit (EVU), Virus Reference Department (VRD), Centre for Infections (CFI), Health Protection Agency (HPA), after each swabbing. Multiple fecal specimens were collected from three patients (patients 1, 3, and 5) who were in the unit during the study period.

Clinical details. Clinical details for patients 1 and 3, both less than 12 months of age, have been described in a previous study (14). Briefly, patient 1 had a diagnosis of immunodeficiency, polyendocrinopathy, enteropathy, and X-linked syndrome, had an umbilical cord hemopoietic stem cell transplant on 16 January 2004, and engrafted 31 weeks later. Patient 3 had T-lymphocyte-negative, B-lymphocyte-negative severe combined immune deficiency, underwent a pheno-

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TABLE 1. Combined enteric PCR results on environmental swabs for AsV, NoV, and RV

Swab site	Result for swab batch and date (mo/day)[a]													
	Pr 3/24	Po 4/18	A 5/6	B 5/21	C 9/6	D 6/23	E 7/8	F 7/22	G 8/13	H 8/27	I 9/8	J 9/23	K 10/7	L 10/21
Staff toilet door handle	+	−	−	+	−	−	$+^{1}$	−	−	+	−	$+^{3}$*	−	−
Staff toilet taps	−	$+^{1}$*	−	−	+	+	$+^{2}$, +	$+^{1}$	$+^{1}$	−	−	+	−	−
Telephone outside rooms 3 and 4	−	−	−	−	−	−	−	−	−	$+^{1}$	−	−	−	−
Microwave oven	−	+	−	+	−	−	−	−	$+^{1}$	$+^{1}$	−	−	$+^{2}$	−
Room 4 outside flow syringe pump	$+^{3}$	−	−	−	−	−	+	$+^{2}$	$+^{2}$	−	−	$+^{3}$*	$+^{1}$	−
Room 3 outside flow syringe pump	$+^{3}$	−	+	−	−	+	$+^{2}$	−	$+^{1}$	−	−	−	−	$+^{1}$
Parents' phone	−	−	−	−	−	−	$+^{1}$	$+^{1}$	$+^{1}$	$+^{2}$	−	−	$+^{1}$, +	−
Parents' room handle	−	−	+	−	+	−	$+^{1}$	−	$+^{1}$	−	−	−	−	−
Game console	$+^{3}$	−	+	+	−	+	$+^{2}$	−	−	−	−	−	−	−
Parents' toilet door handle	−	−	−	−	−	−	+	−	−	−	−	−	−	$+^{2}$
Parents' toilet taps	$+^{4}$	+	−	+	−	−	$+^{1}$	$+^{1}$	$+^{1}$	−	+	−	$+^{2}$*	−

[a] Pr, swabs pre-deep clean (14); Po, swabs post-deep clean (14); A–L, swabs (this study). Positive results without superscript numbers are RV. Superscript numbers: 1, NoV GII-4; 2, NoV rGII-3a; 3, AsV-3; 4, AsV-1. Symbol: *, single-round PCR positive.

typically identical whole-marrow bone marrow transplant on 29 January, and engrafted 6 weeks later.

Patient 5 was referred to the PPIU at 4 years of age with a diagnosis of CD40 ligand deficiency. He was described as having lactose intolerance from 3 months of age. There were concerns about malabsorption, as he often passed semi-formed mucous stool, between two and three times per day. There was no vomiting. Examination of a stool sample showed no evidence of viral or cryptosporidial infection. Large-bowel biopsy was negative for viral or cryptosporidial infection by PCR. At the age of 4.5 years, the patient received a replete bone marrow transplant on 27 May from an unrelated donor, following myeloablative chemotherapy conditioning with busulfan and cyclophosphamide. He engrafted by day 39 posttransplant. He had no gastrointestinal symptoms subsequent to bone marrow transplant.

RNA extraction of swab and fecal specimens and reverse transcription assay. Swab specimens were prepared as previously described (14). Briefly, swab specimens were prepared by addition of 1 ml of guanidinium thiocyanate solution (L6 buffer; Severn Biotech Ltd., Kidderminster, United Kingdom) to each swab in a 2-ml screw-cap tube and incubation at room temperature for 1 h. The supernatant was transferred to a new tube to which 20 μl of silica matrix (Severn Biotech Ltd., United Kingdom) was added. This was followed by washing with L2 buffer (Severn Biotech Ltd., United Kingdom). Thereafter, the guanidinium thiocyanate-silica method was followed (15). RNA was eluted in 40 μl of nuclease-free water (Promega, Southampton, United Kingdom). Fecal specimens were prepared as previously described (14).

cDNA was prepared as previously described (10). cDNA from swabs (pre-deep clean and post-deep clean) (Table 1) from the outbreak investigation (14) were retrospectively tested for NoV and RV as part of this study.

PCR primers for enteric viruses. Single-round and nested or heminested primers were used for the detection of AsV, GII NoV, and group A RV. Standard control measures and unidirectional workflow were used for all nested PCRs, with the second-round PCR setup performed with a PCR workstation with UV decontamination. Water controls were used in each assay as negative controls. All primers were synthesized by Invitrogen, Paisley, United Kingdom.

(i) Astrovirus. AsV was amplified using a single-round and a heminested PCR using Mon269/Mon270 (26) primers in the first round of PCR. The primers are located between nucleotides (nt) 4574 and 5022 in the open reading frame 2 (ORF2) capsid gene of AsV type 1 (AsV-1). A heminested PCR used a forward primer internal to Mon269 and designated Mon269N (5′-GAC CAA AAC CTG CAA TAT GTC A-3′), between nt 4599 to 4620 (5 nt upstream from the end of Mon269), which was designed (14) from a conserved region by means of sequence analysis of all eight genotypes of AsV. PCR mix and cycling conditions were carried out as previously described (14).

(ii) Norovirus. NoV (GII strains only) were amplified using a single-round and a heminested PCR using GIIFB-1/GIISKR, GIIFB-2/GIISKR, and GIIFB-3/GIISKR primers in the first round of PCR (21).

The heminested PCR used primers GIIFBN/GIISKR. Primer GIIFBN (5′-TGG GAG GGC GAT CGC AAT CT-3′) (8) was internal to primers GIIFB-1, -2, and -3 at nt 5048 to 5067 of GII-4 strain Lordsdale/1995/United Kingdom (X86557). PCR mix and cycling conditions were as previously described (8).

(iii) Rotavirus. RV was amplified using single-round primers VP6-F/VP6-R (20), which amplify a region of the VP6 gene, and in order to improve sensitivity of detection for RV, a nested PCR using primers VP6NF (5′-GCW AGA AAT TTT GAT ACA-3′) and VP6NR (5′-GAT TCA CAA ACT GCA GA-3′) was developed for this study. The nested PCR involved adding 1 μl of first-round PCR product to a 49-μl PCR mix containing 10 mM Tris (pH 8.0), 50 mM HCl, 2.5 mM $MgCl_2$, 1 mM of each deoxynucleoside triphosphate (Invitrogen), 20 pmol of VP6NF primer, 20 pmol of VP6NR primer, and 1 U of *Taq* DNA polymerase (Invitrogen). Cycling conditions for VP6NF/VP6NR were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 42°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 5 min.

Detection and sequencing. PCR amplicons were examined by gel electrophoresis in 2% agarose gels (MP agarose; Roche Molecular Biochemicals, United Kingdom), stained with ethidium bromide (0.5 μg/ml), and photographed using a Bio-Rad GelDoc system (Bio-Rad, Hemel Hempstead, United Kingdom).

Purified DNA was sequenced in both directions using PTAG5′ and PTAG3′ primers (cloned amplicons) (23) or virus-specific primers (direct sequencing), a Beckman Coulter CEQ2000 dye terminator cycle sequencing quick start kit according to the manufacturer's instructions (Beckman Coulter, High Wycombe, United Kingdom), and a Beckman Coulter CEQ2000 capillary sequencer (Beckman Coulter, United Kingdom).

Generation of contiguous sequences and pairwise alignments of the 429-bp interprimer region (Mon269/Mon270) and 402-bp interprimer region (Mon269N/Mon270) of the AsV ORF2 sequences, of the 425-bp interprimer region (GIIFB-1/GIISKR, GIIFB-2/GIISKR, and GIIFB-3/GIISKR) and the 300-bp interprimer region (GIIFBN/GIISKR) of the NoV GII ORF1 and ORF2 sequences, and the 155-bp interprimer region (VP6NF/VP6NR) of the RV VP6 gene were performed using Genebuilder and Clustal in Bionumerics v. 3.5 (Applied Maths, Kortrijk, Belgium).

RESULTS

Screening reverse transcriptase PCR for enteric viruses. Combined PCR results are shown in Table 1 for AsV, NoV, and RV from the environmental swabs taken from 11 swab sites from 24 March to 21 October 2004 in a PPIU. PCR results for AsV, NoV, and RV from patient 1, 3, and 5 fecal specimens covering the study period are shown in Tables 2, 3, and 4, respectively.

Typing of enteric viruses. Of the AsVs detected, AsV-3 was detected in the majority of environmental swabs and patient fecal specimens and AsV-1 was detected in one environmental swab on 24 March (14) (Tables 1 to 4).

Of the NoV genotypes detected in swabs, 68% were GII-4 (Grimsby virus) and 32% were a recombinant NoV, designated Harrow/Mexico virus (rGII-3a) (8). Both were detected in mul-

TABLE 2. Enteric PCR results for fecal samples collected from patient 1 over study period[a]

Date of specimen (mo/day)	Result for: AsV		RV		NoV GII	
	Single-round PCR	Heminested PCR	Single-round PCR	Nested PCR	Single-round PCR	Heminested PCR
3/24	+AsV-3	+	−	−	−	−
6/15	+AsV-3	+	−	−	−	−
7/7	+AsV-3	+	−	−	−	−
7/12	+AsV-3	+	−	+	−	−
7/19	+AsV-3	+	−	−	−	+rGII-3a
7/27	−	+AsV-3	−	−	−	−
8/2	−	+AsV-3	−	+	−	+GII-4
8/16	−	−	−	−	−	−
8/25	−	−	−	−	−	+rGII-3a
9/6	−	−	−	−	−	−
9/22	−	−	−	−	−	−

[a] Stem cell transplant date was 16 January, reconstituted at 31 weeks.

tiple environmental swabs during the study period (Table 1) and were excreted in the feces of patient 1 (Table 2).

Group A RV strains were detected in multiple environmental swabs during the study period (Table 1) and in all three patients but were not further typed. NoV was not detected in the fecal samples collected from patients 3 and 5.

Mixed infections. Mixed enteric viruses were detected in two swabs, with swab 2 containing a NoV (rGII-3a) and an RV and swab 7 a NoV (GII-4) and an RV, from 8 July and 7 October, respectively (Table 1).

Patient 1 was excreting AsV-3 and RV on 12 July, AsV-3 and NoV rGII-3a on 19 July, and AsV-3, RV, and NoV GII-4 on 2 August. Patient 3 was excreting AsV-3 and RV from 15 June to 7 July. Patient 5 was found to be excreting RV on 17 June and 19 July and AsV on 6 July (Tables 2 to 4). Symptomatic infection was associated only with astrovirus, and these infections cleared following immunological reconstitution.

TABLE 3. Enteric PCR results for fecal samples collected from patient 3 over study period[a]

Date of specimen (mo/day)	Result for: AsV		RV		NoV GII	
	Single-round PCR	Heminested PCR	Single-round PCR	Nested PCR	Single-round PCR	Heminested PCR
3/25	+AsV-3	+	−	−	−	−
4/5	+AsV-3	+	−	−	−	−
4/13	+AsV-3	+	−	−	−	−
5/17	+AsV-3	+	−	−	−	−
6/7	+AsV-3	+	−	−	−	−
6/15	+AsV-3	+	−	+	−	−
6/21	+AsV-3	+	−	+	−	−
7/7	−	+AsV-3	−	+	−	−
7/13	−	+AsV-3	−	−	−	−
8/2	−	−	−	−	−	−

[a] Bone marrow transplant date was 29 January, reconstituted at 6 weeks.

TABLE 4. Enteric PCR results for fecal samples collected from patient 5 over study period[a]

Date of specimen (mo/day)	Result for: AsV		RV		NoV GII	
	Single-round PCR	Heminested PCR	Single-round PCR	Nested PCR	Single-round PCR	Heminested PCR
6/8	−	−	−	−	−	−
6/14	−	−	−	−	−	−
6/17	−	−	−	+	−	−
7/6	+AsV-3	+	−	−	−	−
7/19	−	−	−	+	−	−
8/3	−	−	−	−	−	−
8/19	−	−	−	−	−	−

[a] Bone marrow transplant date was 27 May, reconstituted at 39 days.

DISCUSSION

This study was initiated to determine the extent of environmental contamination within a PPIU following an AsV outbreak which demonstrated the likely role of environmental contamination as a source of infection (14) and to determine the possibility of transmission to patients undergoing treatment within the unit during the study period through regular monitoring of the patients.

Environmental contamination with NoV, AsV, and RV was demonstrated. An enteric virus was detected in at least one swab site on every occasion that swabbing was performed over a 6-month period. On one swab date, 9 of 11 (82%) swabs were positive for NoV and/or RV, whereas on other swabbing occasions only 1 swab site had detectable enteric viruses (Table 1).

The most likely sites for environmental contamination were those which hands had contacted postdefecation. Light switches and taps in both the staff (swab 2) and parent (swab 11) toilets were found to be contaminated on seven and eight occasions, respectively (Table 1). However, more unusual sites, such as the microwave oven and the television game console (situated within the parents' restroom), were found to be contaminated on five occasions. On only two occasions was more than one enteric virus detected at an individual swab site (Table 1).

AsV was first detected in environmental swabs during the astrovirus outbreak (14); however, two swabs were positive for AsV on 23 September and both were positive by single-round PCR, indicating a high level of contamination. Interestingly, one of the two swab sites (syringe pump) with AsV contamination was identical to one of the swab sites during the AsV outbreak (14).

RV was detected in 11 of 14 (79%) of the swabbing occasions and more commonly between April and July, whereas NoV was detected in only 7 of 14 (50%) of the swabbing occasions and was more common between July and August. No NoV was detected in the months of May and June (Table 1).

The detection of RV more often than other viruses in environmental sites during the whole study period may be related to their greater ability to survive for longer periods on environmental surfaces. The detection of NoV and AsV RNA in environmental sites would indicate that the viral RNA is encapsidated within virus particles, as naked RNA would be extremely vulnerable to

degradation; however, this does not prove it is infectious, only that it is likely to be infectious (6, 16).

Overall, NoV RNA was detected in 18% of swabs, RV RNA in 14%, and AsV RNA in 4%, with 36% of swabs positive for one or more gastroenteric viruses. This would indicate that NoV is a more common enteric virus in environmental swabs than the other viruses. Sixty-four percent of the NoV-positive swabs were collected on three dates between 8 July and 13 August, and during this period 55% of the swabs were positive for NoV, indicating a possible increase in contamination through repeated introductions within the PPIU.

On two occasions, each AsV and NoV was detected in swab sites by use of a single-round PCR, which would indicate a higher level of contamination. Sites included toilet taps, a toilet light switch, a toilet door handle, and a syringe pump. AsV, NoV, and RV were detected in the remaining swabs by use of nested- or heminested-PCR assays, which would indicate a lower level of surface contamination or degradation of viral nucleic acid in the environment. Although single-stranded RNA is prone to degradation, the absence of high concentrations of RV would suggest that the contamination was derived from asymptomatic individuals shedding virus at low levels.

As indicated in the AsV outbreak in March, transmission from one patient to a second patient may have occurred via environmental contamination (14). In this study, all three patients acquired rotavirus infection; however, these were asymptomatic infections. NoV was detected in only one patient, although the patient did acquire two different strains between 19 July and 25 August, which was the period when these viruses were more commonly detected at the swab sites. RV was detected in patients between June and August; however, fecal specimens were not available from two of the patients in April and May, and patient 3 did not appear to acquire RV during these months when it was more commonly detected in environmental swabs.

This study demonstrates contamination of a variety of surfaces and equipment in a PPIU with a range of enteric viruses. The unit has a good infection control procedure, and both staff and parents are educated and informed of the correct procedures while working in or visiting the unit. It is not known if the infections acquired by these patients were a result of transmission by environmental contamination or by person-to-person contact, following continual introductions of enteric viruses into the PPIU; however, gastroenteric symptoms associated with NoV and RV were not observed with the patients.

Although the lack of symptomatic infection may be associated with contamination with nonviable virus particles, a low infecting dose or the presence of preexisting immunity (although unlikely in profoundly immunocompromised children) is also a possible cause of asymptomatic infection.

It was noted that the toilet taps in both staff and parent toilets were of a traditional hand-operating type and that using taps with elbow handles or noncontact triggers, which are more commonly seen in operating theaters, would be an effective way of reducing contamination of the hands following defecation.

Clearly, the cleaning/decontamination procedures used were ineffective against the enteric viruses sought in this study. The use of a chlorine-based disinfectant should be considered for cleaning of hard surfaces.

Future studies may include the introduction of a more rigorous cleaning protocol (1, 19) in order to eliminate or reduce the level of contamination on surfaces and equipment and to determine the effect of any new protocol on the incidence of viral gastroenteritis within the unit.

ACKNOWLEDGMENTS

We thank Lesley Wilson and Angela Reed, infection control nurses in Newcastle upon Tyne Hospitals NHS Trust, for carrying out the environmental swabbing.

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