Polyomavirus Shedding in the Stool of Healthy Adults

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We recently reported the frequent detection of polyomaviruses (BK virus [BKV] or simian virus 40 [SV40]) in 46% of stool samples from hospitalized children. In order to determine if adults exhibit fecal shedding of polyomavirus, single stool specimens from healthy adults were evaluated by PCR. Overall, 20 (18.2%) of 110 specimens were positive for human polyomaviruses: 9 with BKV, 9 with JC virus (JCV), 1 with SV40, and 1 with both JCV and SV40. Among the 94 subjects without immune compromise, 17 (18.1%) were excreting polyomaviruses. This shedding frequency in adults was significantly lower than that observed in children (P < 0.001). These findings support the hypothesis that the gastrointestinal tract may be a site of polyomavirus persistence, and they suggest a fecal-oral route of viral transmission.

Seroprevalence studies from various parts of the world have documented the ubiquity of early childhood infection with BK virus (BKV), a polyomavirus whose host-range is limited to humans (15, 35). Infection with BKV has not been associated with any known clinical syndrome in healthy individuals, and BKV persistence is believed to be lifelong, primarily in the kidney and in leukocytes (32). BKV is occasionally excreted in the urine of healthy adults and children (20). BKV viruria may be common among pregnant women, perhaps reflecting the mild immune compromise of pregnancy (2, 10). Among kidney transplant recipients, BKV reactivation can lead to high titers of virus in the urine and the development of polyomavirus-associated nephropathy, which has emerged as a significant cause of renal allograft loss (13, 17, 18). After hematopoietic stem cell transplantation, BKV reactivation can cause hemorrhagic cystitis, a painful syndrome that usually resolves following engraftment of T-cell lineages (24, 25).

JC virus (JCV) infection occurs somewhat later in life, more commonly in the second and third decades (22). As with BKV, there is no recognized clinical syndrome associated with primary JCV infection, and it has emerged as an important cause of disease among severely immunocompromised patients (4, 31, 41). JCV reactivation in patients with AIDS and other T-lymphocyte deficiencies can result in progressive multifocal leukoencephalopathy, which is uniformly fatal in the absence of restored T-lymphocyte function. JCV has been detected in both colon and gastric cancers and adjacent normal tissues, suggesting that the gastrointestinal tract is a site of JCV persistence, but it has not previously been detected in feces (23, 30, 34, 39). SV40 has been detected in urine and feces from humans and in mixed fecal and urine specimens from cynomolgus monkeys (3, 39, 40).

Despite the ubiquity of BKV infection, its route of transmission has not been clearly determined. In addition to urinary excretion, BKV has been detected rarely in respiratory secretions and tonsil tissue (16, 37). It has also been detected in placental tissue, but whether it is transmitted in utero has not been determined (5, 28). Recent studies by our laboratory have detected polyomaviruses in stool specimens from a large proportion of hospitalized children (46%), independent of immune status and age (39). Thirty-eight percent were positive for BKV and 8% for simian virus 40 (SV40); JCV shedding was not detected. This observation raises the possibility of a fecal-oral route of spread for BKV and perhaps for other polyomaviruses. In the present study, we analyzed stool specimens from healthy adults in order to determine the presence and prevalence of polyomavirus fecal shedding by older individuals.

MATERIALS AND METHODS

After approval by the Institutional Review Board for Studies of Human Subject Research at Baylor College of Medicine (Houston, TX), subjects were recruited from the General Medicine and Gastroenterology clinics at Ben Taub General Hospital (Houston, TX) and the Michael E. DeBakey Veterans Affairs Medical Center (Houston, TX). Informed consent was obtained from each subject prior to study participation. Demographic and medical information was collected from each subject using a standard data collection form. Stool specimens were obtained from the glove of the examining physician after routine digital rectal examination. Stool specimens (~0.5 to 2 g) were placed in viral transport medium (39). Stool specimens were stored at −20°C prior to analysis.

DNA templates for qualitative and quantitative PCR were prepared from stool specimens by 1:10 dilution of samples in sterile phosphate-buffered saline and boiling for 10 min, followed by clarification by low-speed centrifugation and further 10-fold serial dilution, as previously described (39). Qualitative PCR was performed using universal primers (PYV-for and PYV-rev) that detect a conserved segment of the T-antigen genes of BKV, JCV, and SV40 (1, 40). The limit of detection of this assay is approximately 200 genome copies per reaction for each virus. PCR products of the appropriate size were analyzed by direct DNA sequencing to confirm the identity of the virus detected. Virus-specific quantitative PCR was performed as previously described (26).

The prevalence of fecal shedding of polyomaviruses was compared among subgroups of study subjects using 2×2 tables to determine P values in StatCalc (Epi Info, version 6; U.S. Centers for Disease Control and Prevention, Atlanta, GA).

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TABLE 1. Demographic characteristics and polyomavirus fecal shedding by study subjects

<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>Polyomavirus negative (n = 90)</th>
<th>Any polyomavirus positive (n = 20$^b$)</th>
<th>Positive for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any polyomavirus (n = 20$^b$)</td>
<td>BKV (n = 9)</td>
<td>SV40 (n = 2)</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>56.4</td>
<td>58.5</td>
<td>51.1</td>
</tr>
<tr>
<td>Age range (yr)</td>
<td>26.0–85.6</td>
<td>22.9–69.8</td>
<td>22.9–68.0</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (n = 38)</td>
<td>28</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>African-American (n = 29)</td>
<td>27</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hispanic (n = 38)</td>
<td>31</td>
<td>7$^b$</td>
<td>4</td>
</tr>
<tr>
<td>Asian/mixed/other (n = 5)</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n = 24)</td>
<td>21</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Male (n = 86)</td>
<td>69</td>
<td>17$^b$</td>
<td>7</td>
</tr>
<tr>
<td>Immune compromise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 16)</td>
<td>13</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>No (n = 94)</td>
<td>77</td>
<td>17$^b$</td>
<td>7</td>
</tr>
</tbody>
</table>

$^a$ Data are numbers of patients, unless otherwise indicated.

$^b$ One subject was excreting both JCV and SV40. For statistical analyses, polyomavirus-positive subjects were compared with polyomavirus-negative subjects, BKV-positive with BKV-negative subjects, SV40-positive with SV40-negative subjects, and JCV-positive with JCV-negative subjects. For all characteristics, the results were not statistically significant (P > 0.05) by $\chi^2$ analyses.

RESULTS

Clinical and demographic characteristics of study participants. One hundred ten subjects were enrolled between 3 November 2004 and 7 June 2006; 24 (21.8%) of them were women (Table 1). The mean age of subjects was 56.8 years, with a range of 22.9 to 85.6 years. The race/ethnicity of subjects was reflective of the local community (i.e., 34.5% white, 26.4% black, 34.5% white Hispanic, and 4.5% Asian, mixed, or of other descent). Sixteen (14.5%) subjects were considered immunocompromised by virtue of human immunodeficiency virus/AIDS, a history of colon or rectal cancer, or a history of cancer chemotherapy. Fourteen subjects reported a history of colon polyps, including one with a history of colon cancer.

Prevalence of polyomavirus shedding in stool. Qualitative PCR using primers PYV-for and PYV-rev detected 16 polyomavirus-positive specimens. The specific polyomaviruses present were identified by sequence analysis; nine samples were confirmed with JCV, five with BKV, one with SV40, and one with a coinfection of JCV and SV40. The nucleotide sequences of amplicons produced from BKV- and JCV-positive specimens were distinct from those of polyomavirus strains used in the laboratory (data not shown). Single-nucleotide polymorphisms at several positions within the BKV and JCV amplicons provided conclusive evidence that these results were not due to laboratory contamination, since none of the DNA sequences from PCR amplicons was identical to that of BKV strain Dunlop or JCV strain Mad-1, which were used as positive controls in our laboratory. Quantitative PCR using BKV-specific primers detected four additional BKV-positive specimens.

Overall, 20 (18.2%) fecal specimens contained detectable polyomavirus DNA, based either on the confirmation of qualitative PCR amplicons by DNA sequencing or on the detection of >10 genomes per reaction by quantitative PCR (Table 1). Nine subjects were shedding BKV; nine were shedding JCV; one was shedding SV40; and one was shedding both SV40 and JCV. Excluding those with human immunodeficiency virus/AIDS (n = 7), a history of colon cancer (n = 6), or a history of cancer chemotherapy (n = 7), 17 (18.1%) immunocompetent individuals were shedding polyomaviruses in stool. There was no significant difference in polyomavirus shedding between those who were considered immunocompromised and those who were not (3 of 16 versus 17 of 94 [P = 1.0]).

There was no discernible difference in the prevalence of polyomavirus fecal shedding among the different racial/ethnic groups represented in the study population (Table 1). Similarly, there was no association between gender and polyomavirus fecal shedding (3 of 24 females versus 17 of 86 males [P = 0.55]). Fourteen subjects had a history of colon polyps; of these, two were excreting BKV, one was excreting JCV, and one was excreting SV40, proportions not significantly different from those of subjects without polyps (4 of 14 versus 16 of 96 [P = 0.28 by Fisher’s exact test]). When results were stratified by age, the prevalence of polyomavirus shedding was highest for subjects 60 to 69 years old, among whom 7 (22.6%) of 31 were shedding polyomaviruses (Fig. 1). However, a trend toward increased fecal shedding of polyomaviruses with age could not be demonstrated statistically.

DISCUSSION

This study provides evidence that polyomaviruses are shed in stool specimens from healthy adults. BKV was excreted by 8.2% of subjects, JCV by 9.1%, and SV40 by 1.8%. These findings extend our previous observation of frequent fecal shedding of polyomaviruses in children and support the hypothesis that the gastrointestinal tract is a site of polyomavirus
previously detected polyomavirus shedding in 46% of children, appears to be less than that of children (39). Whereas we reports of an association of JCV with the gastrointestinal tract infection. The presence of JCV is not surprising, given the suggesting that JCV infection occurs later in life than BKV stool specimens (39) but is consistent with seroprevalence data persistence in humans. The detection of JCV in specimens number of subjects in each age group is given inside the bar.

The prevalence of polyomavirus fecal shedding by adults appears to be less than that of children (39). Whereas we previously detected polyomavirus shedding in 46% of children, here we observed shedding in only 18.2% of adults (P < 0.001). BKV was excreted by 38.4% of children versus 8.2% of adults, and SV40 was shed by 8.1% of children and 1.8% of adults (i.e., the prevalence of fecal shedding by adults was about 20% that of children). This observation could reflect the increased likelihood of recent infections among pediatric subjects with higher concomitant levels of virus replication and excretion. The duration of fecal polyomavirus shedding after primary infection has not yet been determined. Comparison of this study with published findings of urinary BKV shedding in adulthood suggests that fecal shedding occurs with comparable frequency (21, 38), although direct comparisons involving specimens from the same subjects are needed. Larger studies of fecal polyomavirus shedding that include serial sample collections to control for intermittent excretion will be necessary in order to clarify whether there is a relationship between the frequency of polyomavirus shedding and aging.

Although humans were exposed to polyomavirus SV40 through contaminated poliovirus vaccines used decades ago (9, 11, 36), it is evident that SV40 is being transmitted among humans today. For example, SV40 has recently been detected in tissues and excreta of healthy humans, including blood, tonsils, urine, and stool, as well as in diseased kidneys and certain malignancies (6, 7, 9, 19, 27, 39, 40). The prevalence of infection in different populations is not known but appears to be low (9, 33). In our previous studies of infants and children, the rate of SV40 seropositivity was 5.9% (20/337) (8) and excretion rates were 5.5% (4/72) in urine and 8.1% (8/99) in stool (39, 40). This report extends the observation of the presence of SV40 in stool from childhood to adulthood. SV40 excretion rates in other populations remain to be determined.

Although this study provides important new insights into the natural history of human polyomavirus infection, it does have several limitations. The true prevalence of fecal polyomavirus shedding may be underestimated by this analysis, because only a single stool specimen was obtained from each subject, and the amount of stool placed in each specimen vial was not controlled.Specimens that contained low titers of polyomaviruses would have been missed if the viral content was below the limit of detection in the aliquots tested. The sample-processing methodology used (boiling and dilution) was not optimal for the quantitative PCR assay, limiting its applicability in this study. The predominantly male subject population limited the ability of this study to detect possible gender-specific associations with fecal polyomavirus shedding. As noted, testing of concurrently obtained urine specimens was not done; this would be an important addition to future studies. It is also possible that immune compromise might lead to increased replication and fecal shedding of polyomaviruses, similar to the increased polyomavirus viruria that occurs in immunosuppressed transplant patients (13, 17). Subsequent studies should include increased numbers of patients with possible immune compromise as well as a robust sampling of the elderly.

In conclusion, we have observed shedding of polyomaviruses BKV, JCV, and SV40 in the stool of healthy adults. These data provide additional evidence that the human polyomaviruses JCV and BKV persist in the gastrointestinal tract and that fecal-oral transmission could be an important route of polyomavirus dissemination in the human population.

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