

## Human Polyomavirus 6 and 7 Are Associated with a Pruritic and Dyskeratotic Dermatitis

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1    **CAPSULE SUMMARY**

- 2        •    The contribution of Human Polyomavirus 6 and 7 to skin diseases remains unclear.
- 3        •    Novel strains of Human Polyomavirus 6 and 7 are associated with pruritic dermatoses
- 4            showing dyskeratosis and irregular columns of parakeratosis on histology.
- 5        •    Human polyomavirus 6 and 7 associated pruritic and dyskeratotic dermatoses should be
- 6            considered in immunosuppressed patients. Their identification could facilitate the
- 7            characterization and treatment of these diseases.

8 **ABSTRACT**

9 **Background:** Human Polyomavirus 6 (HPyV6) and Human Polyomavirus 7 (HPyV7) are shed  
10 chronically from human skin. HPyV7, but not HPyV6, has been linked to a pruritic skin eruption  
11 of immunosuppression.

12 **Objective:** We determined whether biopsies showing a characteristic pattern of dyskeratosis and  
13 parakeratosis might be associated with polyomavirus infection.

14 **Methods:** We screened biopsies showing “peacock plumage” histology by PCR for human  
15 polyomaviruses. Cases positive for HPyV 6 or 7 were then analyzed by immunohistochemistry,  
16 electron microscopy (EM), immunofluorescence, quantitative PCR, and complete sequencing,  
17 including unbiased, next generation sequencing (NGS).

18 **Results:** We identified three additional cases of HPyV6 or 7 skin infections. Expression of T  
19 antigen and viral capsid was abundant in lesional skin. Dual immunofluorescence staining  
20 experiments confirmed that HPyV7 primarily infects keratinocytes. High viral loads in lesional  
21 skin compared to normal skin and the identification of intact virions by both EM and NGS  
22 support a role for active viral infections in these skin diseases.

23 **Limitation:** This was a small case-series of archived materials.

24 **Conclusion:** We have found that HPyV6 and HPyV7 are associated with rare, pruritic skin  
25 eruptions with a unique histologic pattern and describe this entity as “HPyV6- and HPyV7-  
26 associated pruritic and dyskeratotic dermatosis (H6PD and H7PD).”

## 27 INTRODUCTION

28 Human polyomaviruses (HPyVs) were first described in 1971, when JC polyomavirus  
29 and BK polyomavirus were identified in immunosuppressed individuals with progressive  
30 multifocal leukoencephalopathy and nephropathy, respectively <sup>1,2</sup>. In the past decade, an  
31 additional 11 human polyomaviruses have been described <sup>3</sup>. Of these, several appear to reside  
32 chronically in human skin—Merkel cell polyomavirus (MCV), trichodysplasia spinulosa  
33 polyomavirus (TSPyV), HPyV6, and HPyV7. MCV was discovered within, and has been  
34 strongly linked to the pathogenesis of, a rare but deadly skin malignancy, Merkel cell carcinoma  
35 (MCC) <sup>4</sup>. Trichodysplasia spinulosa polyomavirus has been linked to a folliculocentric eruption  
36 first described in an immunosuppressed individual <sup>5,6</sup>.

37 HPyVs 6 and 7 are closely related polyomavirus species first identified through rolling  
38 circle amplification (RCA) of DNA isolated from swabs of healthy human skin <sup>7</sup>. They are  
39 thought to infect the skin in a latent or subclinical manner in the majority of people. In healthy  
40 individuals with clinically normal skin, previous studies have detected HPyV6 and HPyV7  
41 sequences from skin swabs in 14-28% and 11-13% of samples, respectively <sup>7,8</sup>. Less is known  
42 about skin diseases associated with HPyV6 and 7. Recent studies have revealed that HPyV7  
43 could infect and actively replicate in biopsies taken from immunosuppressed, lung transplant  
44 recipients. In these patients, the skin infection presented as pruritic, scaly, brown plaques.  
45 Biopsies from lesional skin showed a characteristic pattern of parakeratosis described as  
46 “peacock plumage” <sup>9</sup>. In contrast to HPyV7, HPyV6 has not yet been linked with specific skin  
47 disease. Low levels of HPyV6 DNA have been detected in several types of epithelial neoplasms  
48 and a contribution of HPyV6 to specific epithelial neoplasms has not been excluded<sup>10,11</sup>.

49           We identified biopsies showing a characteristic pattern of dyskeratosis and parakeratosis,  
50 previously described as “peacock plumage” for HPyV7 skin infections and investigated  
51 whether polyomavirus infections might be associated with these eruptions. We identify HPyV6  
52 and 7 infection in three additional patients with pruritic dermatoses and provide extensive  
53 evidence for the involvement of these viruses in the pathogenesis of the eruptions<sup>12,13</sup>. Our work  
54 expands the spectrum of skin diseases associated with HPyV6 and 7 and yields novel insights  
55 into the biology of these ubiquitous skin polyomaviruses.

56 **METHODS**

57 This was a retrospective case series of archived skin biopsies. Histologically normal skin and  
58 archived biopsy samples were obtained through an IRB-exempt protocol. For Patient B, written,  
59 informed consent was obtained for collecting skin swabs for diagnostic and research purposes.

60 **Human polyomavirus PCR**

61 Formalin-fixed, paraffin-embedded (FFPE) sections were deparaffinized with xylene (Sigma, St.  
62 Louis, MO, USA) and DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen,  
63 Hilden, Germany). Typically, PCR was performed on 20ng of genomic DNA with polyomavirus  
64 screening primers (Table S3). Quantitative PCR (qPCR) was used to determine the copy number  
65 of HPyV6, HPyV7, and MCV using SYBR green and primers targeting the small T antigen  
66 region. LINE1 primers were used as a normalization reference. Anonymized skin biopsies from  
67 8 patients with histologically normal skin were assessed by qPCR as a control.

68 **Histology, immunohistochemistry, and immunofluorescence studies**

69 5 µm FFPE sections underwent xylene deparaffinization, rehydration, antigen retrieval, and  
70 blocking. These slides were stained overnight at 4°C with 6V32 antibody (Buck lab, 1:100) to  
71 detect HPyV6 and 7 viral capsid protein, 2t10t (Buck lab, 1:100) for HPyV7 small T antigen, or  
72 1t1 (Buck lab, 1:200) for HPyV6 small T antigen. Slides were then stained with appropriate  
73 secondary antibody conjugated to HRP (Santa Cruz Biotechnology, Dallas, TX). Lastly, the  
74 slides were developed with the VECTOR VIP Peroxidase Substrate Kit (Vector Laboratories,  
75 Burlingame, CA). For co-staining experiments, slides were incubated with HPyV7 antibodies  
76 and rabbit anti-cytokeratin 10 (clone EP1607IHCY, 1:10,000, Abcam), rabbit anti-cytokeratin 14  
77 (catlog#PA5-28002, 1:5,000, Thermo Scientific), or rabbit anti-vimentin (clone D21H3, 1:400,

78 Cell Signaling Technology). Goat anti-mouse Alexa Fluor 546 and goat anti-rabbit Alexa Fluor  
79 647 were used as secondary antibodies. Confocal images were taken using a Zeiss LSM 880.

### 80 **Electron microscopy**

81 Biopsies were deparaffinized in xylene, dehydrated, and placed in Trump's fixative. The tissue  
82 was treated with osmium tetroxide, 2% uranyl acetate, rinsed, and dehydrated in alcohol. The  
83 tissue was embedded in epoxy resin, and thick and thin sections were generated and stained with  
84 toluidine blue (with sodium borate).

### 85 **HPyV6 and HPyV7 Genome Sequencing**

86 PCR was performed in a total volume of 50 $\mu$ l with 20ng of DNA using Sapphire Amp Fast PCR  
87 Master Mix (Takara Bio Inc, Shiga, Japan) and 0.3 $\mu$ M of sequence-specific primers (Table S3).  
88 Additional HPyV6 sequencing primers were designed with Primer3 to ensure overlapping  
89 coverage of the genome. Reactions were denatured at 95°C for 5 m, followed by 35 cycles with  
90 denaturation at 95°C for 1 m, annealing at 56°C for 1 m, and extension at 72°C for 2 m.  
91 Amplification products were agarose gel-purified with the NucleoSpin Gel and PCR Clean-up  
92 kits (Machery-Nagel, Düren, Germany) and sent for bidirectional Sanger (GeneWiz, South  
93 Plainfield, NJ, USA). Methods for unbiased sequencing of circular DNA viruses were previously  
94 described<sup>7, 14</sup>. Briefly, skin swabs were layered onto an Optiprep (Sigma) gradient, centrifuged  
95 to purify encapsidated virions, Benzonase Nuclease (Sigma) digested, and amplified by RCA  
96 prior to whole genome sequencing (Illumina MiSeq).

97 **RESULTS**

98           The clinical characteristics of the patients, which have been previously reported, are  
99 summarized in Table I<sup>12, 13</sup>. All three patients presented with generalized, scaly, hyperpigmented  
100 papules coalescing into plaques, which had all been present for at least 12 months. All  
101 dermatoses were associated with some degree of pruritus (Fig 1A, Table I). While Patient A was  
102 immunosuppressed from HIV that had progressed to AIDS, Patient C was immunosuppressed  
103 from a kidney/pancreas transplant. Curiously, Patient B did not report any known  
104 immunosuppression at the time of initial diagnosis and biopsy in 2008. Importantly, all cases  
105 were first identified based through biopsies that showed a characteristic histologic pattern—  
106 scattered dyskeratotic cells throughout the epidermis along with irregular columns of  
107 parakeratosis in the stratum corneum (Fig 1B). This pattern has been described in the literature as  
108 “columnar dyskeratosis,” “tiered parakeratosis with dyskeratosis,” or “peacock plumage”<sup>9, 12, 13</sup>.  
109 FFPE lesional skin biopsy samples from these patients were screened for known human  
110 polyomaviruses by endpoint PCR. Sample A amplified HPyV7, while samples B and C  
111 amplified HPyV6 (Fig 1C). Weaker bands consistent with MCV were detected in samples A and  
112 C in replicate experiments; however, MCV was not detected by quantitative PCR (data not  
113 shown). We did identify and screen three additional cases that showed “peacock plumage” on  
114 histology. However, none of those cases showed evidence of HPyV6 or 7 by PCR (data not  
115 shown) suggesting that this histologic pattern is not pathognomonic for polyomavirus infection.  
116 Classically, electron microscopy (EM) has been used to identify virions in tissues with suspected  
117 polyomavirus infections. EM sections were prepared from samples A and B; insufficient tissue  
118 remained from sample C for processing. While no viral particles could be identified from sample  
119 A, the biopsy from patient B revealed cytoplasmic arrays of icosahedral structures approximately



120 40nm in size, consistent with the reported size of polyomavirus virions (Fig 1D). The viral  
121 particles in sample B were identified in cells without recognizable features.

122 To confirm the expression of viral proteins in lesional skin, sections were stained with  
123 antibodies recognizing HPyV6 and HPyV7 capsid and small T antigen proteins. T antigen  
124 expression was detected in scattered cells throughout all levels of the epidermis. In contrast,  
125 capsid protein expression appeared to be enriched in the upper layers of the epidermis including  
126 within parakeratotic cells in the stratum corneum (Fig 2A). Viral capsid could be detected in both  
127 the nucleus and cytoplasm of infected cells (Fig 2C), while T antigen expression was largely  
128 limited to the nucleus of infected cells (Fig 2D). At higher magnification, the clear presence of  
129 desmosomes between infected cells provided histologic confirmation that keratinocytes could  
130 support active HPyV7 replication (Fig 2D).

131 To better characterize the skin infections caused by HPyV6 and 7, sections from patient  
132 A were co-stained with antibodies against viral antigens—capsid or small T—and cell-type  
133 specific proteins—cytokeratin 10 (CK10, a marker of more differentiated keratinocytes),  
134 cytokeratin 14 (CK14, a marker of basal keratinocytes), or vimentin (a mesenchymal/fibroblast  
135 marker). The majority of viral antigen positive cells co-stained for CK10 (66.7%) (Fig 3A) or  
136 CK14 (91.6%) (Fig 3B), while a very small number of cells also appeared to stain positively for  
137 vimentin (6.4%) (Fig 3C). These findings confirm that HPyV7 productively infects  
138 keratinocytes. Interestingly, we frequently noted the presence of CK10 negative, capsid positive  
139 cells in the upper layers of the epidermis (Fig 3A, arrowheads).

140 Because HPyV6 and 7 can be detected at low levels in clinically normal skin from  
141 healthy individuals<sup>8</sup>, we performed quantitative PCR (qPCR) and sequencing to better  
142 characterize the HPyV6 and 7 infections. DNA extracted from two distinct lesional biopsies from

143 Patient A revealed an average HPyV7 load of ~2904 copies/LINE repeat (a normalization  
144 control). In contrast, a collection of skin biopsies from asymptomatic skin donors (n=8) showed  
145 an average of ~14.3 copies/LINE repeat. Patient B was calculated to have a mean HPyV6 load of  
146  $\sim 1.44 \times 10^6$  copies/LINE repeat, and Patient C,  $\sim 2.37 \times 10^6$  copies/LINE repeat. In contrast, control  
147 samples showed an average of ~31.1 copies/cell (Fig 4A). Thus, the levels of HPyV6 and 7  
148 detected in multiple independent lesional biopsies were found to be several orders of magnitude  
149 more abundant than normal skin controls, suggesting a possible pathogenic role for the virus in  
150 the described rashes. The complete genomes of HPyV7 and 6 (GenBank KX771234-5),  
151 respectively, were successfully sequenced through primer walking. These sequences clearly  
152 diverged from published genomes (Fig 4B, Table S1). Specifically, the HPyV6 strain present in  
153 patient B demonstrated a complex deletion/insertion in the non-coding control region (NCCR)  
154 along with multiple nucleotide substitutions in the NCCR, major capsid protein VP1, and large T  
155 (LT) antigen regions. The HPyV7 strain from patient A had a deletion in the NCCR and  
156 nucleotide substitutions in major capsid proteins VP1/2/3 and LT antigen regions. The possible  
157 effects of the mutations in the non-coding control region, capsid proteins, and large T antigens  
158 on viral replication and virulence are yet to be determined. Moreover, swabs collected from  
159 Patient B in July 2016, after the dyskeratotic dermatosis had resolved, were analyzed through  
160 unbiased deep sequencing of encapsidated, circular DNA viruses<sup>7, 14</sup>. In addition to abundant  
161 reads representing the complete genomes of nine human papillomaviruses and a MCV strain  
162 (GenBank KX781279-88), 121 reads matching HPyV6 isolate UTSW6.1 were observed (Fig  
163 4C). The results show that patient B continued to shed HPyV6 (alongside other typical members  
164 of the human skin virome) after resolution of symptoms.

165 **DISCUSSION**

166 Ho et al<sup>9</sup> first associated HPyV7 infection with pruritic rashes in immunosuppressed transplant  
167 patients with a characteristic “peacock plumage” histology. Our study confirms and extends  
168 these seminal findings by linking HPyV6 with similar rashes. This study also broadens the range  
169 of immunosuppressed states that may allow for HPyV7 infection from iatrogenic  
170 immunosuppression from organ transplantation to the immunocompromised state of HIV  
171 infection. Interestingly, the HIV/AIDS patient (Patient A) was initially diagnosed with a variant  
172 of epidermodysplasia verruciformis<sup>13</sup>. While we can not exclude that papillomavirus co-infection  
173 contributed to some of the patient’s findings, our analyses indicate that HPyV7 infection alone  
174 could be sufficient to explain the observed clinical and histologic changes. It is also worth noting  
175 that Patient B in this study was not reported to be immunosuppressed at the time of the original  
176 biopsies. However, we speculate that the patient may have an uncharacterized, acquired  
177 immunosuppression based on his recent, frequent hospitalizations for severe infections.

178         These studies also expand our understanding of the biology of HPyV7. First, we  
179 definitively identify keratinocytes as the primary targets of HPyV7 infection in the skin. While  
180 MCV, TSV, HPyV9, and HPyV10 DNA can be detected on the skin<sup>8, 15</sup>, no other  
181 polyomaviruses have been demonstrated to infect non-follicular keratinocytes. Given the  
182 detection of HPyV6 and 7 in other tissues<sup>16, 17</sup>, it is likely that HPyV6 and 7 can infect or remain  
183 latent in non-epidermal tissues, as is the case for other polyomaviruses. Our studies also suggest  
184 that like other human polyomaviruses, HPyV6 may persist as a latent infection in patients  
185 without clinical evidence of disease. Specifically, Patient B possessed detectable levels of  
186 HPyV6 on his skin despite the fact that his pruritic dermatosis had resolved. How  
187 polyomaviruses, including HPyV6 and 7, maintain latency remains unclear. The decreased

188 expression of CK10 in some HPyV7-infected keratinocytes, in concert with the notable  
189 dyskeratosis in these infections, suggests that latent infections might be maintained, in part,  
190 through the inhibition of normal keratinocyte differentiation. Moreover, the numerous sequence  
191 changes in the strains identified in our work could also contribute to the virulence and/or  
192 persistence of these strains and deserve further investigation.

193         Because our case series is very small and largely retrospective in nature, additional cases  
194 showing HPyV6 and 7 infection are necessary to confirm our findings. Moreover, the limited  
195 number of cases prevents us from definitively assigning causation to HPyV6 and 7. We speculate  
196 that additional cases of H6PD and H7PD will be identified based on the characteristic “peacock  
197 plumage” histologic pattern, in which nucleated, eosinophilic keratinocytes are present within  
198 the stratum corneum. In future studies, it may be worthwhile investigating whether patients  
199 previously diagnosed with epidermodysplasia verruciformis show clinical or histologic changes  
200 consistent with the polyomavirus infections described here. Cases showing the characteristic  
201 histology could then be further screened for the presence of human polyomaviruses, especially  
202 HPyV6 and 7.

203         In summary, we provide multiple lines of evidence suggest that highly active HPyV6 and  
204 7 infections are associated with skin disease, including high viral copy number in lesional skin,  
205 the expression of viral proteins by IHC in dyskeratotic keratinocytes, and the detection of  
206 encapsidated virions by both electron microscopy and unbiased sequencing. Given the  
207 characteristic clinical and histologic presentation of this rash, we propose that this skin disease  
208 be described as HPyV6- and HPyV7-associated pruritic and dyskeratotic dermatosis (H6PD and  
209 H7PD). This rare condition could be included in the differential diagnosis of immunosuppressed  
210 patients with recalcitrant, pruritic rashes. The identification of additional cases would be an

211 important step toward improving our understanding of human polyomavirus biology and  
212 ultimately developing better treatments for their associated diseases.

213

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217 **REFERENCES**

- 218 1. Gardner SD, Field AM, Coleman DV , Hulme B. New human papovavirus (B.K.) isolated  
219 from urine after renal transplantation. *Lancet* 1971;1:1253-7.
- 220 2. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ , Dessel BH. Cultivation of papova-like  
221 virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* 1971;1:1257-  
222 60.
- 223 3. DeCaprio JA , Garcea RL. A cornucopia of human polyomaviruses. *Nature reviews*  
224 *Microbiology* 2013;11:264-76.
- 225 4. Feng H, Shuda M, Chang Y , Moore PS. Clonal integration of a polyomavirus in human  
226 Merkel cell carcinoma. *Science* 2008;319:1096-100.
- 227 5. van der Meijden E, Janssens RW, Lauber C, Bouwes Bavinck JN, Gorbalenya AE , Feltkamp  
228 MC. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an  
229 immunocompromized patient. *PLoS Pathog* 2010;6:e1001024.
- 230 6. Matthews MR, Wang RC, Reddick RL, Saldivar VA , Browning JC. Viral-associated  
231 trichodysplasia spinulosa: a case with electron microscopic and molecular detection of the  
232 trichodysplasia spinulosa-associated human polyomavirus. *J Cutan Pathol* 2011.
- 233 7. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL , Buck CB. Merkel cell  
234 polyomavirus and two previously unknown polyomaviruses are chronically shed from human  
235 skin. *Cell Host Microbe* 2010;7:509-15.
- 236 8. Wieland U, Silling S, Hellmich M, Potthoff A, Pfister H , Kreuter A. Human polyomaviruses  
237 6, 7, 9, 10 and Trichodysplasia spinulosa-associated polyomavirus in HIV-infected men. *J Gen*  
238 *Virol* 2014;95:928-32.

239 9. Ho J, Jedrych JJ, Feng H, Natalie AA, Grandinetti L, Mirvish E et al. Human Polyomavirus 7-  
240 Associated Pruritic Rash and Viremia in Transplant Recipients. *The Journal of infectious*  
241 *diseases* 2014.

242 10. Schrama D, Groesser L, Ugurel S, Hafner C, Pastrana DV, Buck CB et al. Presence of human  
243 polyomavirus 6 in mutation-specific BRAF inhibitor-induced epithelial proliferations. *JAMA*  
244 *dermatology* 2014;150:1180-6.

245 11. Beckervordersandforth J, Pujari S, Rennspiess D, Speel EJ, Winnepeninckx V, Diaz C et al.  
246 Frequent detection of human polyomavirus 6 in keratoacanthomas. *Diagn Pathol* 2016;11:58.

247 12. Pock L , Stork J. Two case reports of columnar dyskeratosis, an unusual keratinisation  
248 disorder. *Dermatology* 2010;220:274-9.

249 13. Champagne C, Moore L, Reule R, Dyer JA, Rady P, Tying SK et al. Cornoid Lamella-Like  
250 Structures in HIV-Associated Epidermodysplasia Verruciformis: A Unique Histopathologic  
251 Finding. *The American Journal of dermatopathology* 2015;37:929-32.

252 14. Peretti A, FitzGerald PC, Bliskovsky V, Buck CB , Pastrana DV. Hamburger  
253 polyomaviruses. *J Gen Virol* 2015;96:833-9.

254 15. Sauvage V, Foulongne V, Cheval J, Ar Gouilh M, Pariente K, Dereure O et al. Human  
255 polyomavirus related to African green monkey lymphotropic polyomavirus. *Emerging infectious*  
256 *diseases* 2011;17:1364-70.

257 16. Salakova M, Koslabova E, Vojtechova Z, Tachezy R , Sroller V. Detection of human  
258 polyomaviruses MCPyV, HPyV6, and HPyV7 in malignant and non-malignant tonsillar tissues. *J*  
259 *Med Virol* 2016;88:695-702.

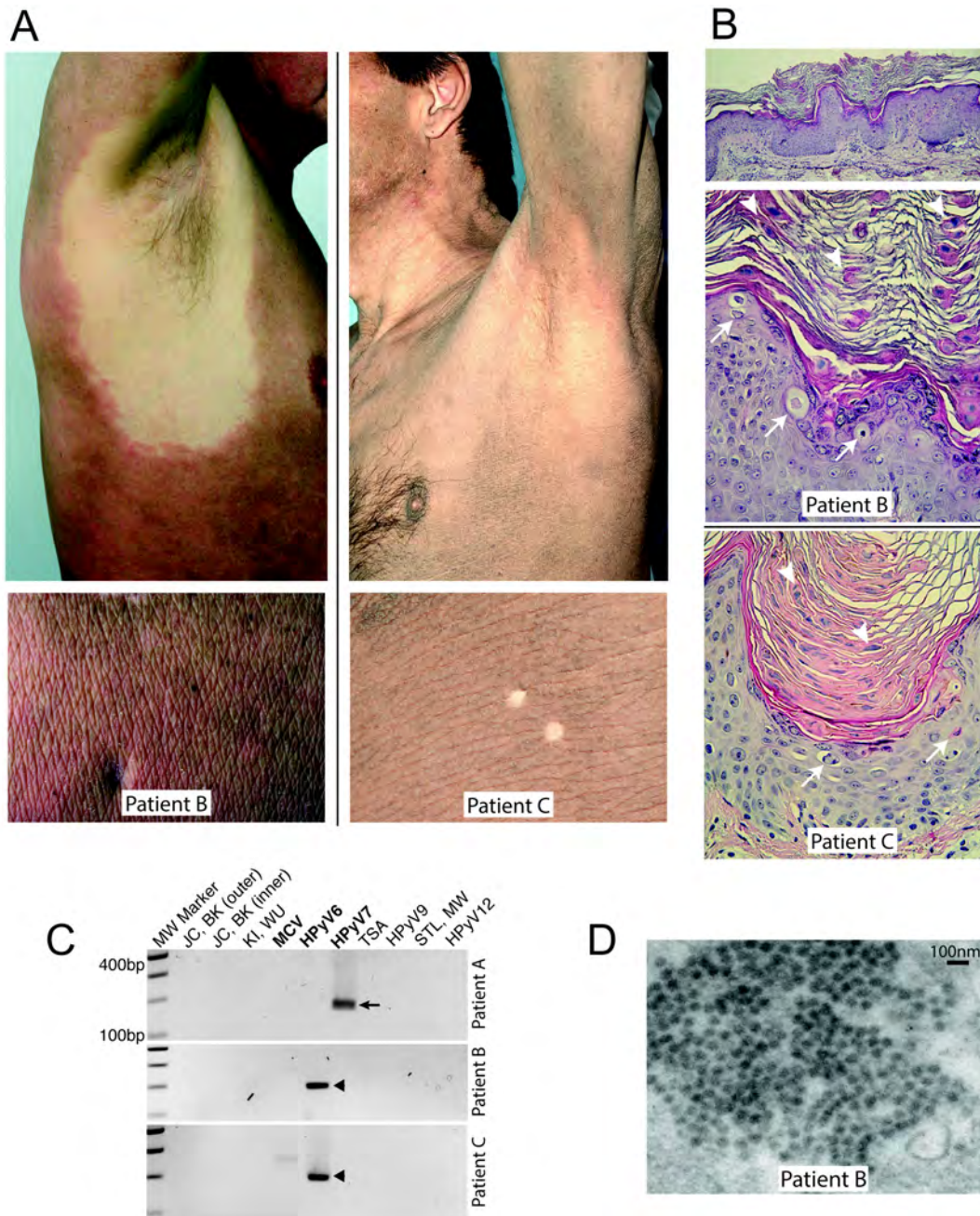
260 17. Rockett RJ, Sloots TP, Bowes S, O'Neill N, Ye S, Robson J et al. Detection of novel  
261 polyomaviruses, TSPyV, HPyV6, HPyV7, HPyV9 and MWPyV in feces, urine, blood,  
262 respiratory swabs and cerebrospinal fluid. PLoS One 2013;8:e62764.  
263



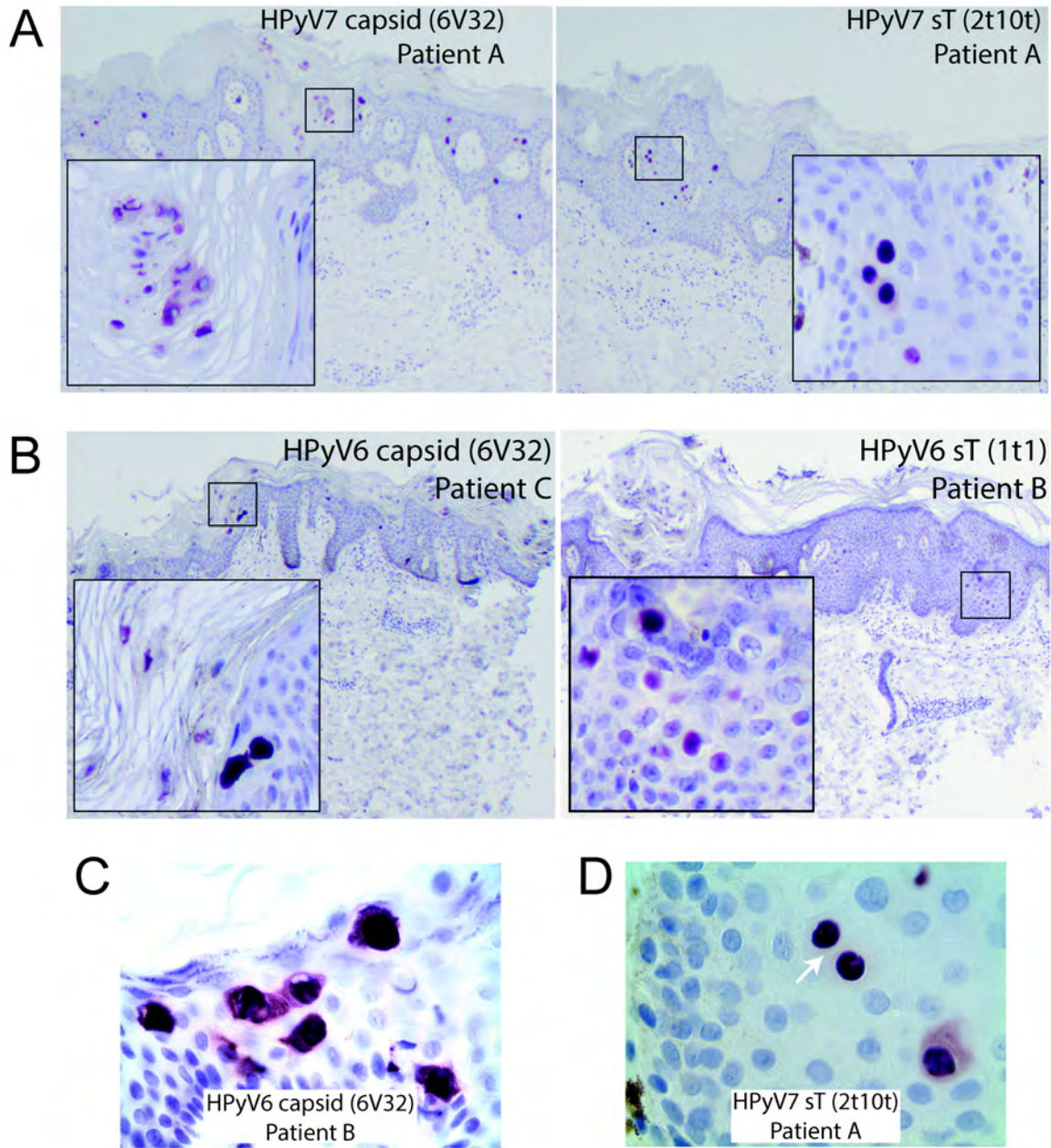
264 **Table I. Clinical characteristics of patients with HPyV6 and 7 associated dermatoses.**

<b>Patient</b>	<b>Age/Sex</b>	<b>Immunosuppression</b>	<b>Pruritus</b>	<b>Clinical History</b>
A	36/F	HIV/AIDS	severe	Worsening pruritic rash over 12 months; HPV-5, HPV-111, HPV-120, HPV-124, HPV isolate FA-88 positive; patient deceased
B	54/M	None at the time of diagnosis	present	Initial worsening over 2yrs followed by resolution over several years; HPV- by PCR; HIV negative; prominent palmar involvement; recent hospitalizations for sepsis, parapharyngeal abscess, pneumonia
C	52/M	Kidney/pancreas transplant (tacrolimus/rapamycin)	present	Pruritic rash over >2yrs; HPV-; prominent palmar involvement; patient deceased

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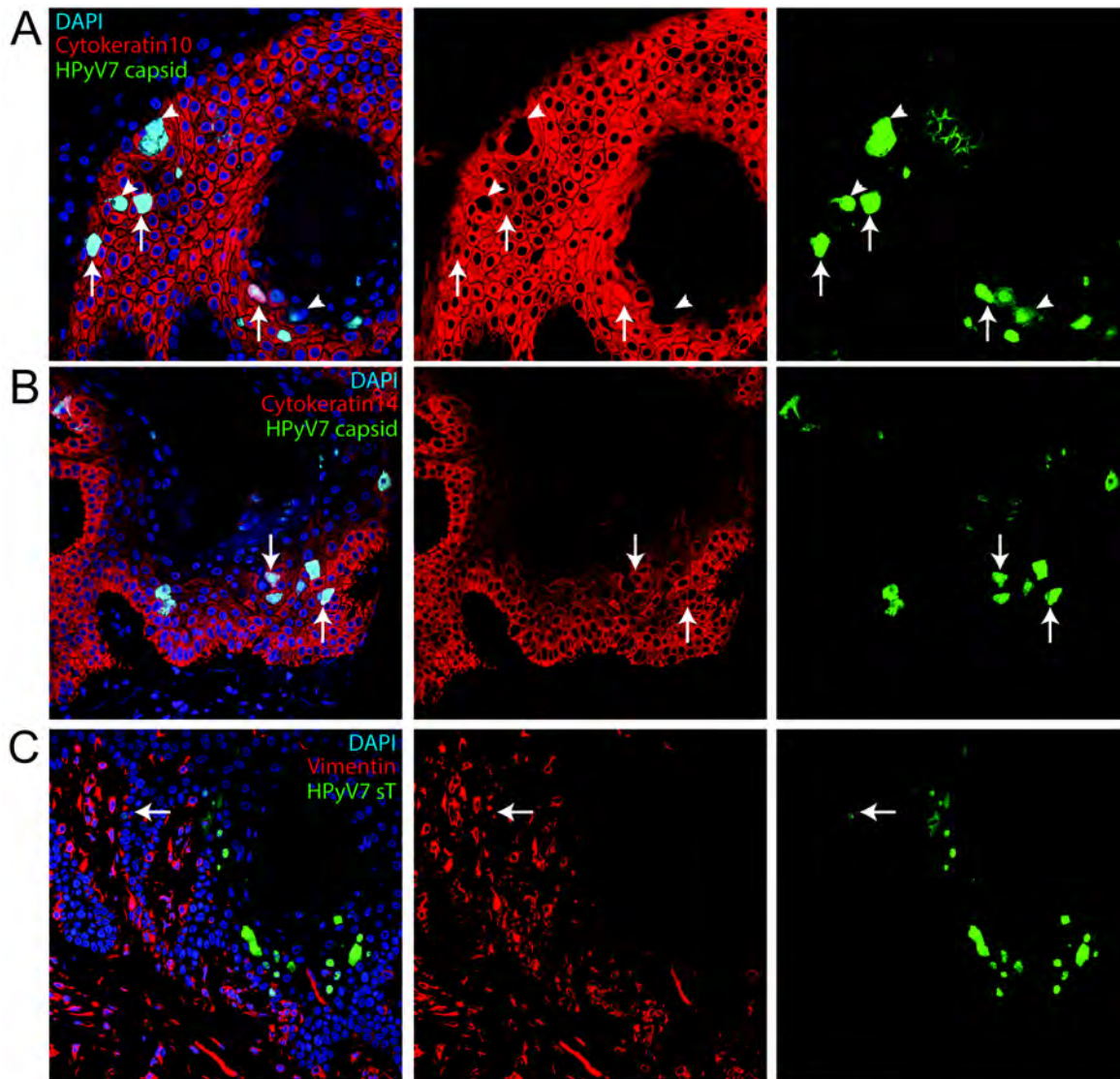
267 **Figure 1. HPyV6 and 7 are associated with a pruritic and dyskeratotic dermatosis.** (A) Generalized,  
 268 hyperpigmented, scaly eruptions in Patient B, with no known immunosuppression, and Patient C, a  
 269 kidney/pancreas transplant patient, (Pock and Stork 2010). Images from an HIV/AIDS patient (Patient A)  
 270 have previously been published (Champagne 2015); (B) From Patient B, on routine H&E sections, there  
 271 is mild acanthosis and papillomatosis (top, H&E, 40X total magnification); several dyskeratotic cells are  
 272 noted in the superficial epidermis (arrows), with eosinophilic, nucleated keratinocytes forming irregular  
 273 columns of parakeratosis (arrowheads) in the stratum corneum (middle, H&E, 200X). From Patient C, an  
 274 area with prominent columnar dyskeratosis showing dyskeratotic cells (arrows) and nucleated  
 275 keratinocytes in the stratum corneum (arrowheads) (bottom, H&E, 200X) (C) PCR for human  
 276 polyomaviruses yielded specific bands for HPyV7 (arrow) and HPyV6 (black arrowhead). (D) Electron  
 277 microscopy of affected cells revealed numerous cytoplasmic ~40-nm icosahedral virions from Patient B  
 278 (18000X, original direct magnification).



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**Figure 2. Immunohistochemistry against HPyV6 and 7 viral proteins.** (A) Sample A shows capsid (6V32) expression in the nucleus and cytoplasm of affected cells. HPyV7 small T antigen (2t10t) is detected more strongly in the nuclei of affected cells. Samples B and C show capsid and HPyV6 small T antigen (1t1) in affected cells (insets, 200X). (B) High power images of the immunohistochemical stains show capsid protein expression in the nucleus and cytoplasm of the keratinocytes with viral cytopathic changes (left, oil immersion, 1000X). (C) Small T antigen is detectable in adjacent cells attached by desmosomes (right, arrow, oil immersion, 1000X) confirming their identity as keratinocytes.

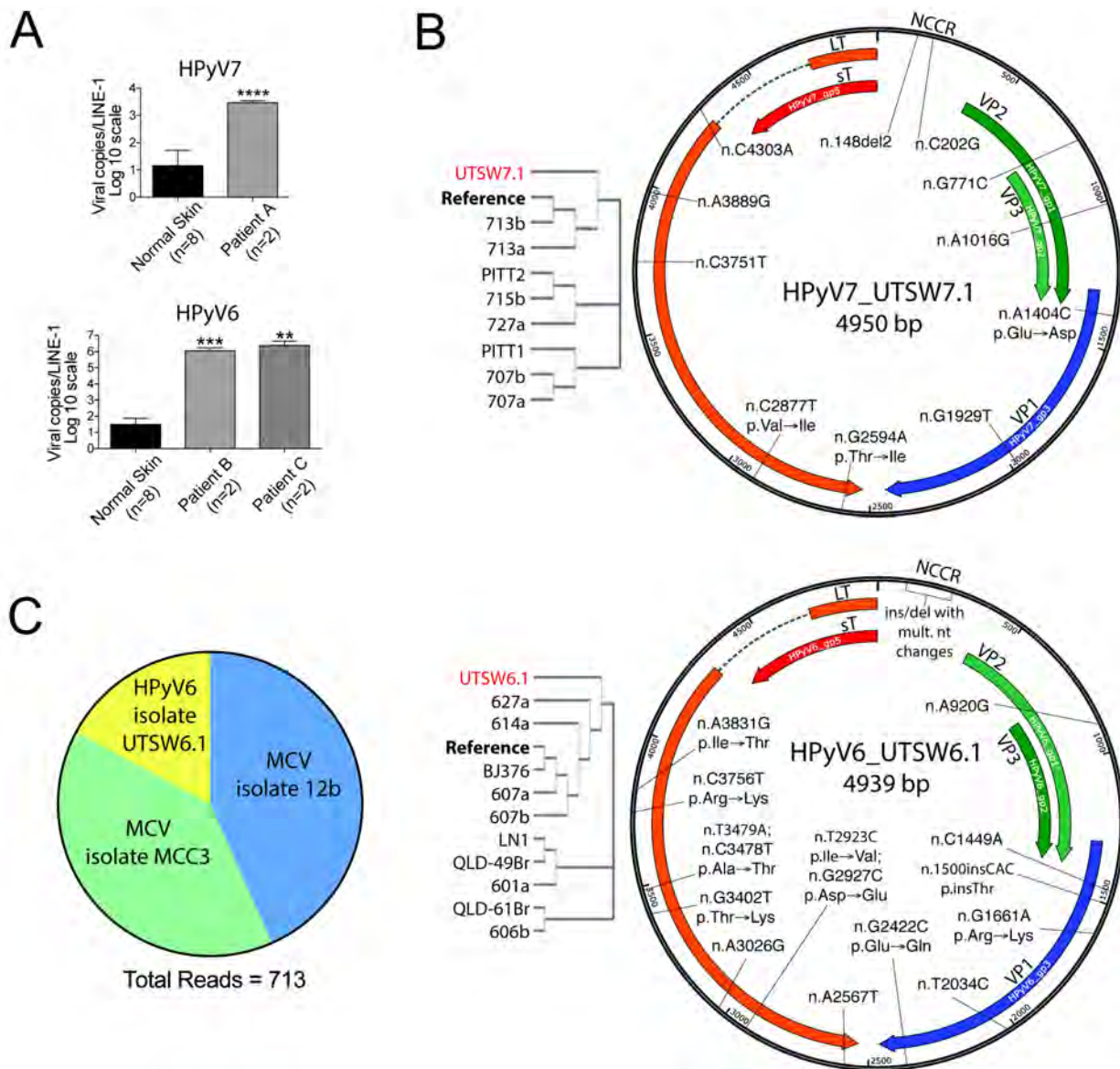




D	Markers	Cell Marker + Viral Protein+	Viral Protein+
	CK14; capsid	44	48
	CK10; capsid	32	48
	vimentin; small T	3	47

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290 **Figure 3. HPyV7 infects keratinocytes.** (A) Dual immunofluorescence (IF) staining for Cytokeratin 10  
 291 (CK10) and capsid (6V32) confirms that most capsid expressing cells also express CK10 (arrows, 400X).  
 292 However, there are several cells in which the expression of CK10 is undetectable (arrowheads). (B) Dual  
 293 IF staining for Cytokeratin 14 (CK14) and capsid (6V32) confirms that almost all capsid expressing cells  
 294 also express CK14 (arrows, 400X). (C) Dual IF staining for Vimentin and small T antigen (2t10t)  
 295 identifies rare T antigen expressing cells that also express vimentin (arrow, 400X). (D) Quantitation of  
 296 dual IF staining images.



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 299 **Figure 4. Novel strains of HPyV6 and 7 are abundant in lesional skin but can still be detected in**  
 300 **asymptomatic patients.** (A) Quantitative PCR comparing levels of HPyV6 and 7 detected in normal skin  
 301 compared to lesional skin normalized to LINE-1 repeats. (n=independent biopsy; error bars=SD; t-test;  
 302 \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001). (B) Schematic of HPyV7 isolate from Patient A  
 303 (HPyV7\_UTSW7.1) and HPyV6 isolate from Patient B (HPyV6\_UTSW6.1) with labels indicating the  
 304 approximate position of nucleotide and AA changes. Cladograms (left) indicate the phylogenetic  
 305 relationship of the current strains with previously sequenced strains. (C) Patient B possessed subclinical  
 306 infections of HPyV6 isolate UTSW6.1 and MCV isolates after clinical symptoms had resolved in 2016.  
 307 Pie chart indicates the proportion of reads (300bp or longer) corresponding to the indicated virus.

308 SUPPLEMENTAL MATERIALS

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310 Table S1. Viral Genomic Changes

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Location	HPyV7_UTSW7.1	HPyV6_UTSW6.1
NCCR	n.148del2	Ins/del with mult. nt changes
	n.C202G	
Early (sT, LT)	n.G2594A (p.Thr→Ile)	n.A2567T
	n.C2877T (p.Val→Ile)	n.T2923C (p.Ile→Val)
	n.C3751T	n.G2927C (p.Asp→Glu)
	n.A3889G	n.A3026G
		n.G3402T (p.Thr→Lys)
		n.T3479A; n.C3478T (p.Ala→Thr)
		n.C3756T (p.Arg→Lys)
		n.A3831G (p.Ile→Thr)
Late (VP1/2/3)	n.G771C	n.A920G
	n.A1016G	n.C1449A
	n.A1404C (p.Glut→Asp)	n.1500insCAC (p.insThr)
	n.G1929T	n.G1661A (p.Arg→Lys)
		n.T2034C
Other	n.C4303A	n.G2422C (p.Glu→Gln)

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314 **Table S2. Primers Used in the Study**

<b>Screening Primers</b>					
<b>Polyomavirus</b>	<b>Primer Name</b>	<b>Sequence (5' to 3')</b>	<b>Reference</b>		
JC, BK (Outer)	P-3	GTATACACAGCAAAGGAAGC	Giraud et al., 2008		
	P-4	GCTCATCAGCCTGATTTTGG			
JC, BK (Inner)	P-1	AGTCTTTAGGGTCTTCTACC	Giraud et al., 2008		
	P-2	GGTGCCAACCTATGGAACAG			
KI, WU	KIPyV2263.F	TTGGATGAAAATGGCATTGG	Giraud et al., 2008		
	KIPyV2404.R	TAACCCCTTCTTTGTCTAAAATGTAGCC			
MCV	MCV.F	GGAATTGAACACCCTTTGGA	Ho et al., 2014		
	MCV.R	CTACAATGCTGGCGAGACAA			
HPyV6	HPyV6.F	GGCATTTCAGGAACTGATT	Ho et al., 2014		
	HPyV6.R	AAGTTGAATGCCACCCTGAG			
HPyV7	HPyV7.F	AAACTCTGGCCCAAAAACCT	Ho et al., 2014		
	HPyV7.R	GAGATCCCCAGAAAATGCAA			
TSPyV	TSA.F	TGAAGGCCATTTTGCAGGT	Ho et al., 2014		
	TSA.R	AGCCAGGTAGGCTCATGGTA			
HPyV9	HPyV9.F	AGAGGGCGCAGCAAACCTAAA	Ho et al., 2014		
	HPyV9.R	TGGTGGATATCCTGATTGCAT			
STL, MW	STLMWScreen.F	GRATGAAAYRCWWTTACAGGTTGCCACC	Lim et al., 2013		
	STLMWScreen.R	GTGGWAAAACAACCTGTAGCWGCTGC			
HPyV12	HPyV12.VP1731.F	GTGGGAAGCTGTCAAGTGTGA	Korup et al., 2013		
	HPyV12.VP1868.R	CCACCTACTGCAACATGTG			
<b>Sequencing Primers</b>					
<b>Polyomavirus</b>	<b>Primer Name</b>	<b>Sequence (5' to 3')</b>	<b>Reference</b>		
HPyV6	H6_4628R	TGAAGACATTTGGTCCCTC	Schowalter et al., 2010		
	H6_395L	AAAGCAGCAGCAGACTGTAGAGCC			
	H6_160R	ACTGAGGTTGACCACCCTTG			
	H6_992L	ACTCCATTGCCCTCAAAC			
	H6_679R	AAGTTTGGTCTTGGGGAGG			
	H6_1465L	GGCTTCCCAAAACCAAAAGTATC			
	H6_2032L	CTCTCCTCTGTCTGAAGTGAAC			
	H6_3110R	CAGCAGCCAATGTTGTTTTG			
	H6_4117L	AATAGAGGCTGGGACGATG			
	H6_3774R	GCATTTAGCATAGCAGACACTC			
	H6_4539L	TTGCCCTTCAAAAAGGAGC			
	H6_1068F	GGGCCCTGTTAGAAGGAAGG		this study	
	H6_1710F	AGCCCCTTGGATGTCATTGG			
	H6_1710R	GAGGGGTTTACAGCTCACCC			
	H6_2384F	GCAAATTTGTTGGCCCTCCA			
	H6_2384R	CAAGGTGTCCAGCTGTTT			
	H6_2872F	CCTCTGGCAATTCTGGCCTT			
	H6_2872R	AGCAGCACAAAGAAGGGTGT			
	H6_3587F	CAGCTTGCTGGATCATTGGC			
	H6_3857R	GGTTTGGCCTACCTGTGGAA			
	H6_4143F	GAGAGTTGCCTTACCAGGG			
	HPyV7	H6_4143R		ATGGATCGCCTTTTAGCCAGAG	Ho et al., 2014
		H6_4716F		ACATTTGGTCCCTCAGGGTG	
		H6_4716R		TGGATCGCCTTTTAGCCAGAG	
		contig1.F		TAGAAGGGTGGAGGCAAATG	
		contig1.R		CTTCATTGGACACTGCCAGA	
		contig2.F		AGCCCCTCAAAGTGCTGCT	
		contig2.R		CTACTCAACAGGCTTGCCAAT	
contig3.F		TTCTACTACAGAACAGGAAGAGAG			
contig3.R		TGTGTCTCCGTGAGTGGTA			
contig4.F		GGAAATGGACCCACACAAAA			
contig4.R		ACTTGCTTTCTGAGGGCTTG			
contig5.F		TGAAGCCCTAGAAGGTCCAG			
contig5.R		GCTGCATTGCTCCATTGTA			
contig6.F		CAGAAGCCACAGGTGCAG			
contig6.R		GAACCAGTGTCTGACTTTGTTGA			
contig7.F		CCATATGACACCCACTGTTCA			
contig7.R		CAGCTGCCATTCTGGATTTA			
contig8.F		CAATAGCACATCCCAGTTCAAA			
contig8.R	TTTGCTACAGAGCCATCACTG				
contig9.F	TGCTTCTCATGGTGTGGTG				
contig9.R	GCCATGCCACTTTAGGTAAACA				
contig10.F	TTCTCATAAAGCAACATACTTTCTCA				
contig10.R	CACTTTTTGGCGGGCTAAT				
<b>qPCR primer</b>					
<b>Polyomavirus</b>	<b>Primer Name</b>	<b>Sequence (5' to 3')</b>	<b>Reference</b>		
HPyV6	HPyV6qPCR.F	TAGCACTTGTAGCACCAG	Schowalter et al., 2010		
	HPyV6qPCR.R	ATGCCCTCATTGCCCTTCT			
HPyV7	HPyV7qPCR.F	TCTAACCTTATGCTGTATG	Yu et al., 2008		
	HPyV7qPCR.R	GGTAGAGATGAAGTCAAG			
MCV	MCVqPCR.F	GGTGCAGATGCAGTAAGCAG	Yu et al., 2008		
	MCVqPCR.R	TTGTCTCGCCAGCATTGTAG			
LINE1	LINE1qPCR.F	TTGCGGCATTATTCACAATAGC	Yu et al., 2008		
	LINE1qPCR.R	GTGCCACATTTCTTAATCCAGTCT			