Antibodies response to Polyomaviruses primary infection: high seroprevalence of Merkel Cell Polyomavirus and lymphoid tissues involvement.

Carolina Cason¹, Lorenzo Monasta², Nunzia Zanotta², Giuseppina Campisciano², Iva Maestri³, Massimo Tommasino⁴, Michael Pawlita⁵, Sonia Villani⁶, Manola Comar¹², Serena Delbue⁶*.

Authors affiliations:

¹Department of Medical Sciences, University of Trieste, Piazzale Europa 1, 34127 Trieste, Italy.
²Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Via dell' Istria 65/1, 34137 Trieste, Italy.
³Department of Experimental and Diagnostic Medicine, Pathology Unit of Pathologic Anatomy, Histology and Cytology University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy.
⁴Infections and Cancer Biology Group, International Agency for Research on Cancer, Cours Albert Thomas 150, 69372 Lyon, France.
⁵German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany.
⁶Department of Biomedical, Surgical & Dental Sciences, University of Milano, Via Pascal 36, 20100 Milano, Italy.

*Corresponding author: serena.delbue@unimi.it, +390250315070
ABSTRACT

Human polyomaviruses (HPyVs) asymptotically infect the human population establishing latency in the host and their seroprevalence can reach 90% in healthy adults. Few studies have focused on the pediatric population and there are no reports regarding the seroprevalence of all the newly isolated HPyVs among Italian children. Therefore, we investigated the frequency of serum antibodies against 12 PyVs in 182 immunocompetent children from Northeast Italy, by means of a multiplex antibody detection system. Additionally, secondary lymphoid tissues were collected to analyze the presence of HPyVs DNA sequences using a specific Real Time PCRs or PCRs. Almost 100% of subjects were seropositive for at least one PyV. Seropositivity ranged from 3% for antibodies against Simian virus 40 (SV40) in children from 0 to 3 years, to 91% for antibodies against WU polyomavirus (WUPyV) and HPyV10 in children from 8 to 17 years. The mean number of PyV for which children were seropositive increased with the increasing of age: 4 standard deviations (SD) 1.8 in the 0-3 years group, 5 (SD 1.9) in the 4-7 years group, and 6 (SD 2.2) in the 8-17 years group. JC polyomavirus (JCPyV) DNA was detected in 1% of the adenoids, WUPyV in 12% of the tonsils and 28% of the adenoids and Merkel cell polyomavirus (MCPyV) was present in 6% and 2% of the tonsils and adenoids, respectively. Our study gives new insights on the serological evidence of exposure to PyVs during childhood, and on their possible respiratory route of transmission.

Keywords: Polyomaviruses; MCPyV; seroprevalence; lymphoid tissues.
INTRODUCTION

Polyomaviruses (PyVs) are non-enveloped viruses with a small, closed, circular, double-strand DNA genome. The PyVs genome is divided into three main regions: (i) the early region encoding for the large (TAg) and the small t antigens (tAg), (ii) the late region, encoding for the viral capsid proteins VP1, VP2 and VP3, and (iii) the non-coding control region (NCCR), containing the bidirectional origin of replication.

The late region of Human PyVs (HPyVs), JC virus (JCPyV), BK virus (BKPyV) and that of the monkey PyV Simian virus 40 (SV40) also encodes the agnoprotein (DeCaprio and Garcea, 2013; Garren et al, 2015).

The first Human PyVs (HPyVs) to be isolated from clinical specimens were JCPyV and BKPyV, in 1971 (Gardner et al. 1971; Padgett et al. 1971). After the primary asymptomatic infection, they remain latent in the cells of the urinary tract (Chesters et al. 1983), the central nervous system (CNS) and the hematopoietic system (Doerries, 2006), and are causative agents of progressive multifocal leukoencephalopathy (PML) and of hemorrhagic cystitis and nephropathy, respectively (Doerries, 2006; Imperiale, 2000).

Merkel cell polyomavirus (MCPyV) has subsequently been isolated in Merkel cell carcinoma (MCC), a rare and aggressive skin cancer (Feng et al. 2008). The trichodysplasia spinulosa-associated polyomavirus (TSPyV), discovered in 2010, can cause a rare skin disease in immunosuppressed patients (van der Meijden et al. 2010).

In the same year as TSPyV, HPyV6 and HPyV7 have been detected in human skin, and HPyV7 has been associated with pruritic rush (Schowalter et al. 2010).

The other newly discovered HPyV, namely KI polyomavirus (KIPyV), WU polyomavirus (WUPyV), Lyon IARC polyomavirus (LIPyV), HPyV12, STL polyomavirus (STLPyV), New Jersey polyomavirus (NJPyV), HPyV9 and MW polyomavirus (MWPyV), have been isolated from various specimens, but they have not been associated with any pathology and their pathogenic role is still

The HPyVs infect in a transversal way the adult population, and their worldwide seroprevalence ranges from 20% to 90%, depending on the virus, and on the geographical area (DeCaprio and Garcea, 2013), implying that healthy individuals are co-infected with several HPyVs.

Under particular conditions, such as impaired immune functions of the host, HPyV infection can reactivate, leading to the development of serious diseases, although the pathogenesis is not fully understood (Bhattacharjee and Chattaraj, 2017).

Currently, few studies have focused on the pediatric population and there is no report regarding the seroprevalence of all the newly isolated HPyVs among Italian children.

In this study we analyze the frequency of serum antibodies against 10 HPyVs, SV40 and the Limphotropic PyV (LPyV), as well as the presence of viral DNA in tonsil and adenoid samples, in immunocompetent children from Northeast Italy, employing a multiplex technique.

**METHODS**

**Subjects and samples**

Between May 2012 and January 2013, 182 samples of peripheral blood, were retrospectively analyzed for PyVs seroprevalence, while the presence of the viral genomes was assessed in 82 tonsils and 181 adenoids samples.

Clinical specimens were obtained from a population employed for a previous study (Nicol et al. 2014) and consisted of 182 immunocompetent pediatric subjects, who had undergone adeno-tonsillar surgery for hypertrophy of adenoids, defined, according to Brodsky’s classification (Brodsky, 1989), as adenoids occupying over 80% of the choanal space, and tonsils graded 3 (30/100) or 4 (70/100), at the Otolaryngology Department of the Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" of Trieste, Italy (Table 1).
**PyVs serology**

Sera were obtained by centrifuging venous blood samples at 1500 rpm for 10 minutes, and were then stored at -80 °C until subsequent assessment and analyzed for the presence of antibodies against VP1 and/or TAg of 12 PyVs: BKPyV (BKVP1, BKTag), JCPyV (JCVP1, JCTAg), LPyV (LPVP1, LPTAg), TSPyV (TSVP1, TSTAg), MCPyV (MCPyV344VP1, MCPyV344tAg, MCPyV344TAg), KIPyV (KIVP1), WUPyV (WUVP1), HPyV6 (HPyV6VP1, HPyV6TAg), HPyV7 (HPyV7VP1, HPyV7Tag), HPyV9 (HPyV9VP1), HPyV10 (HPyV10VP1, HPyV10TAg) and SV40 (SV40VP1, SV40Tag).

Serological analyses were performed, as previously described, using a multiplex antibody detection system based on the fluorescent beads technology, in combination with a glutathione S-transferase (GST) capture enzyme-linked immunosorbent assay method (Luminex corporation Austin, Texas) (Kjaerheim et al. 2007; Malhotra et al. 2016). Beads were examined on a Luminex 200 analyzer (xMAP, Luminex Corp.), that identifies the internal bead color and thus the antigen carried by the bead, quantifying the antibodies bound via the median R-phycoerythrin fluorescence intensity (MFI) of at least 100 beads of the same bead type.

Seropositivity for each PyV was defined as virus-specific VP1 antibody >250 MFI and virus-specific Tag antibody >400 MFI for Tag, except for seropositivity for MCPyV tag (MFI >200) and SV40 VP1 (MFI >300)(Waterboer et al. 2006).

Cut-offs were defined arbitrarily from visual inspection of frequency distribution curves (percentile plots) of all sera tested. Stringent criteria were chosen to increase specificity, as suggested by Karagas and colleagues, and Michael and colleagues (Karagas et al. 2006; Michael et al. 2008).

**DNA isolation from tonsils/adenoids**

Upon arrival at the laboratory, tonsil and adenoid tissue specimens were processed for DNA automated extraction, as previously described (Papa et al. 2016).

**Quantitative Real Time PCR (qPCR) and PCR for the detection of HPyVs genome**
QPCRs specific for the detection of JCPyV, BKPyV, SV40, MCPyV, WUPyV and KIPyV genomes were employed. The choice of these HPyVs was due to their confirmed or possible adenotropism. To measure the viral loads of JCPyV, BKPyV and SV40, the commercial kit Multiplex TaqMan qPCR, that simultaneously amplifies the viral genome and the reference human cellular β-globin gene, was used, following the manufacturer’s instructions (Euro-RT Polyoma Panel kit; Eurospital Spa, Trieste, Italy) (Comar et al. 2014a).

To assess the presence of MCPyV DNA, samples were analyzed using a quantitative real-time qPCR targeting the Tag sequence, as previously described (Maximova et al. 2011). WUPyV and KIPyV genomes were searched by conventional PCRs, targeting the WUPyV VP2 and KIPyV VP1 sequences, as previously described (Allander et al. 2007; Gaynor et al. 2007).

As control, the human lymphotropic Epstein-Barr Virus (EBV) was assayed using commercially available qPCR kit (Nanogen, Turin, Italy), according to manufacturer’s recommendations (Comar et al. 2014b).

**Sequencing of MCPyV PCR amplification products**

Samples resulted positive for MCPyV qPCR, were subjected to conventional PCR using the same primers. Amplification products were sequenced to confirm the identity of the virus with an ABI PRISM 310 Genetic Analyzer (Applied Biosystem, Foster City, USA), as previously described (Cason et al. 2016). Sequence homology search was performed using the Basic Local Alignment Search Tool (BLAST) program via the website of the National Center for Biotechnology Information (NCBI).

**Statistical analyses**

Statistical analyses were conducted using Stata/IC 14.2 (StataCorp LP, College Station, USA). The two-tailed Fisher Exact Test was used to determine whether there were significant differences in the distribution of age classes by sex, and to determine the possible association between seroprevalence and sex. The same test was used to evaluate the significant association between positivity to TAg and VP1, within the same infection. The two-tailed Fisher Exact Test was finally
used to evaluate the co-infections among age classes. The Bonferroni correction was applied to the level of significance to allow for simultaneous comparisons. A Cuzick’s test for trend was used when appropriate. A $p$ value <0.05 was considered as statistically significant, and as the base for the Bonferroni correction.

RESULTS

Seropositivity to HPyVs

The main demographic characteristics of the enrolled 182 children are described in Table 1. Results of HPyVs seroprevalence by age group are summarized in Table 2 and Fig. 1. Almost 100% of subjects were seropositive for at least one HPyV. Seropositivity ranged from 3% for antibodies against simian virus 40 (SV40) in children from 0 to 3 years, to 91% for antibodies against WUPyV and HPyV10 in children from 8 to 17 years.

In general, the prevalence of seropositive subjects was higher among children within 8 and 17 years, than among those within 0 and 3 years. In particular, among children aged 8-17 years, BKPyV, WUPyV and HPyV10 were the most seroprevalent, whereas lymphotropic polyomavirus (LPyV), HPyV9 and SV40 were the less seroprevalent. BKPyV ($p<0.0001$), TSPyV ($p<0.01$) and HPyV10 ($p<0.05$) showed an increasing significant trend of seropositivity among age classes as shown in Fig. 2, WUPyV showed an increasing trend although not significant.

The number of different HPyVs against which the subjects were seropositive is shown in Fig. 3, panels a-c. The mean number of HPyV for which children were seropositive increased with age: 4 (SD 1.8) in the 0-3 years group, 5 (SD 1.9) in the 4-7 years group, and 6 (SD 2.2) in the 8-17 years group.

Additionally, the co-presence of antibodies against single HPyV was evaluated and six associations were significant ($p<0.001$): BKPyV-SV40, BKPyV-WUPyV, JCPyV-SV40, LPV-HPyV9, HPyV6-HPyV7, SV40-HPyV9.

No significant distribution was observed between sex of subjects and any of the HPyVs.

HPyVs genomes in tonsil/adenoid tissues
JCPyV DNA was detected in 1% of adenoids, WUPyV in 12% of tonsils and in 28% of adenoids. MCPyV was present in 6% and 2% of tonsils and adenoids, respectively (Table 3). The lymphotropic Epstein-Barr virus (EBV), used as a control, showed the highest percentage of prevalence, 29% in tonsils and 42% in adenoids.

As regard the viral loads, JCPyV in adenoids showed a mean of $1 \times 10^3$ viral copies/5.5 $\times 10^4$ cells (cells range $4.5 \times 10^3$-1.6 $\times 10^6$); MCPyV showed a mean of $1.0 \times 10^1$ viral copies/5.5 $\times 10^4$ cells in adenoids, and of 19.8 viral copies/5.5 $\times 10^4$ cells in tonsils; EBV in tonsils and adenoids showed a range of 4.4 to 33 viral copies/5.5 $\times 10^4$ cells.

The sequencing results of the PCR products identified all the amplified strains as belonging to the MCPyV genome. Sequences were very similar to the MKL-1 strain isolated from France and Sweden confirming the circulation of this strain as previously published by our group (Data not shown) (Maximova et al. 2011).

**DISCUSSION**

In this retrospective study, for the first time we described the seroprevalence of a panel of 12 PyVs in a population of immunocompetent children, living in an area of Northeast Italy. Seroprevalence was determined using a multiplex system, by searching antibodies against the structural protein VP1 and against the viral functional protein Tag, in the serum of the patients.

Almost all tested subjects were seropositive for at least one virus, confirming that the components of the *Polyomaviridae* family are widespread in the human population and that the primary infection occurs early during childhood.

The most circulating viruses were HPyV10, WUPyV and BKPyV, followed by HPyV6, TSPyV and MCPyV. While this is not surprising for WUPyV and BKPyV, whose seropositivity usually ranges between 11% and 75% in children less than three years old (Kean et al. 2009; Martel-Jantin et al. 2014; Miller et al. 2012; Nicol et al. 2013; Sroller et al. 2016; Stolt et al. 2003; Taronna et al. 2013), HPyV10 was previously found at lower prevalence in Italy (Nicol et al. 2014).
The JCPyV seroprevalence reached 30% in the 8-17 years group, which was similar to the results of several previous studies (Chang et al. 2002; Hennes et al. 2016; Kean et al. 2009; Sroller et al. 2014), but far from what reported by Elia and colleagues in an Italian recent survey (Elia et al. 2017). These differences may be due to the use of an ELISA assay based on multiplex antigens detection, that eliminates the issues related to the possible cross-reactivity, or may also be due to the different geographical areas (Gossai et al. 2016).

MCPyV, instead, showed a seroprevalence similar to that previously found in children from different areas of Italy (Nicol et al. 2013; Viscidi et al. 2011), and also from the United States (Kean et al., 2009).

As expected, since not strictly human, SV40 and LPyV showed the lowest values of seropositivity, with peaks of prevalence comprised between 10 and 20% (Kean et al. 2009). HPyV9 also showed lower prevalence compared to the other HPyVs and the seroprevalence reported in previous Italian and German studies, but it cannot be excluded that its infection might occur later in life (Nicol et al. 2013; Nicol et al. 2012; Trusch et al. 2012). Recent studies described a cross-reaction between HPyV9 and LPyV VP1 antibodies, (Trusch et al. 2012; Nicol et al. 2012) indicating that LPyV infection for humans needs to be further studied. By only considering the VP1 gene, the seroprevalence results of our study show that a cross reaction between HPV9 and LPyV was observed for all positive patients. When the Tag antibodies were considered, the serum of only one child showed positivity, confirming the existence of a real infection with LPyV, as hypothesized by Delbue and colleagues (2010).

Interestingly, BKPyV and MCPyV showed the higher seroprevalence in the 0-3 years group, compared to the other HPyVs, confirming that MCPyV primary infection could occur earlier during childhood than primary infection with the other HPyVs (Martel-Jantin et al. 2013; Signorini et al. 2014). The trends observed in seroprevalence by age were significant for BKPyV, TSPyV, and HPyV10 and may be explained by a continuous viral transmission throughout life (Gossai et al. 2016). The mean number of viruses against whom there was seroreactivity increased with age, ranging from 4 in the 0-3 age group, to 6 in the 8-17 age group, confirming previously published results related to
a large general US population (Gossai et al. 2016). Unexpectedly, the MCPyV seroprevalence was much higher in the 4-7 years old group of age, than in the 8-17 years old group of age: this result could be related to a diminished antibody reactivity against MCPyV over time or, more probably, to cohort effects in infection rates.

Regarding the analysis of co-infections, the significant association between some viruses opens up new hypotheses on the role of some PyVs in favoring coinfections by viruses of the same family. Our results confirm that the seropositivity against the HPyVs VP1 protein is much more frequent than that against the TAg (Malhotra et al., 2016) and that the presence of antibodies against JCPyV and BKPyV VP1 remains stable over time, identifying the seroprevalence of VP1 as a reasonable indicator of long-term antibody status in epidemiological studies (Antonsson et al. 2010).

Studying a cohort of young patients undergoing surgery for the ablation of tonsils and/or adenoids, we had the opportunity to collect part of the removed tissues and analyze them for the presence of some viral sequences. Most of the analyzed viruses resulted negative, whereas JCPyV, WUPyV and MCPyV genomes were amplified, with a prevalence comprised between 28% (WUPyV in the adenoids) and 1% (JCPyV in the adenoids). These findings confirm that HPyVs can infect the lymphoid tissue, especially during the primary infection, which might occur via the respiratory route (Kantola et al. 2009; Sadeghi et al. 2014). Thus, we hypothesize that the viruses might initially hit tonsils and adenoids, located in the first respiratory tract, and then reach the well-defined latency sites, such as kidney or B lymphocytes (Khalili et al., 2003). As expected, viral loads were very low: high copy number is usually and significantly associated with disease (Herberhold et al. 2017; Sadeghi et al. 2014; Salakova et al. 2016).

Very recently, Sadeghi and colleagues, using a luminex-based HPyV multiplex assay, with high sensitivity and specificity, were able to detect JCPyV, WUPyV, MCPyV, HPyV6, and TSPyV DNA altogether in 18% of tonsillar tissues collected from either adults or children. They concluded that tonsils might serve as shedding site in HPyV reactivation and thus contribute to HPyV transmission.
(Sadeghi et al., 2017). Additionally, the detection of other HPyVs, such as KIPyV, BKPyV and also SV40 has et al. been reported, confirming these findings (Astegiano et al. 2010; Patel et al., 2008).

In particular, MCPyV gained more interest as it is the certain etiologic agent of MCC, a rare but aggressive tumor affecting predominantly the elderly. Interestingly, this is the first study reporting the presence of MCPyV in tonsil and adenoids tissues in pediatric subjects, whereas its presence was already demonstrated in the secondary lymphoid tissues of healthy and tumor adult patients (Goh et al. 2009; Salakova et al. 2016). As our seroepidemiological data showed, the first contact with MCPyV probably occurs in early childhood; subsequently, this virus remains latent without any clinical manifestation, but may reactivate in specific conditions, such as immunosuppression, even in young subjects (Sourvinos et al. 2015). Shedding of MCPyV from this site of primary diffusion during replication might play a role also in MCPyV pathogenesis.

Although this study gave new insights on the serological evidence of exposure to PyVs during childhood, a topic very rarely studied, we are aware of the limitations that affect the survey. Firstly, the low homogeneity of the number of samples available among the different age classes could have added a bias in the analysis of the results. Furthermore, due to lack of sample, the active viral replication was not studied in any other clinical specimen (urine and/or blood) than adenoid/tonsil tissues.

However, seroepidemiological surveys are of great interest in the scientific community working on HPyVs, because they are frequently and asymptotically affecting the healthy human population, but they are also associated with important medical conditions, such as nephropathy, demyelinating diseases and tumors, and effective therapies are still lacking.

ETHICAL STATEMENTS

The study was approved by the Institutional Scientific Board of the Institute for Maternal and Child Health – IRCCS “Burlo Garofolo” (Trieste) and by the local Independent Ethics Committee. Parental
written informed consent was obtained for each participant in accordance with the principles outlined in the Declaration of Helsinki.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**ACKNOWLEDGMENTS**

The study was supported by Italian Ministry of Health, contract grant number: R.C. 11/2011.

**REFERENCE LIST**


Table 1. Main demographic characteristics of the children cohort.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Subjects</td>
<td>182</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian (100%)</td>
</tr>
<tr>
<td>Age in years, median (IQR)*</td>
<td>5.8 (4.4–6.3)</td>
</tr>
<tr>
<td>Age categories, number (percentage)</td>
<td></td>
</tr>
<tr>
<td>0-3 years</td>
<td>35 (19 %)</td>
</tr>
<tr>
<td>4-7 years</td>
<td>125 (69 %)</td>
</tr>
<tr>
<td>8-17 years</td>
<td>22 (12 %)</td>
</tr>
<tr>
<td>Female in age classes, number (percentage)</td>
<td></td>
</tr>
<tr>
<td>0-3 years</td>
<td>16 (46%)</td>
</tr>
<tr>
<td>4-7 years</td>
<td>59 (47%)</td>
</tr>
<tr>
<td>8-17 years</td>
<td>11 (50%)</td>
</tr>
</tbody>
</table>

*IQR: interquartile range*
Table 2. Seropositivity of HPyVs among Italian children aged 0-17 years.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Antigens</th>
<th>0-3 years</th>
<th>4-7 years</th>
<th>8-17 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n°</td>
<td>%</td>
<td>n°</td>
<td>%</td>
</tr>
<tr>
<td>BKPyV</td>
<td>VP1</td>
<td>17</td>
<td>49%</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>17</td>
<td>49%</td>
<td>102</td>
</tr>
<tr>
<td>JCPyV</td>
<td>VP1</td>
<td>3</td>
<td>9%</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>5</td>
<td>14%</td>
<td>42</td>
</tr>
<tr>
<td>TSPyV</td>
<td>VP1</td>
<td>7</td>
<td>20%</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>7</td>
<td>20%</td>
<td>56</td>
</tr>
<tr>
<td>MCPyV</td>
<td>VP1</td>
<td>12</td>
<td>34%</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>VP1 ± sTAg ± TAg</td>
<td>12</td>
<td>34%</td>
<td>77</td>
</tr>
<tr>
<td>KIPyV</td>
<td>VP1</td>
<td>12</td>
<td>34%</td>
<td>41</td>
</tr>
<tr>
<td>WUPyV</td>
<td>VP1</td>
<td>25</td>
<td>71%</td>
<td>99</td>
</tr>
<tr>
<td>HPyV6</td>
<td>VP1</td>
<td>17</td>
<td>49%</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>17</td>
<td>49%</td>
<td>60</td>
</tr>
<tr>
<td>HPyV7</td>
<td>VP1</td>
<td>7</td>
<td>20%</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>8</td>
<td>23%</td>
<td>25</td>
</tr>
<tr>
<td>HPyV9</td>
<td>VP1</td>
<td>3</td>
<td>9%</td>
<td>10</td>
</tr>
<tr>
<td>HPyV10</td>
<td>VP1</td>
<td>25</td>
<td>71%</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>25</td>
<td>71%</td>
<td>109</td>
</tr>
<tr>
<td>SV40</td>
<td>VP1</td>
<td>1</td>
<td>3%</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>1</td>
<td>3%</td>
<td>25</td>
</tr>
<tr>
<td>LPyV</td>
<td>VP1</td>
<td>3</td>
<td>9%</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>VP1 ± TAg</td>
<td>3</td>
<td>9%</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 3. Detection of HPyVs genomic sequences in tonsil/adenoid tissues.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Specimens</th>
<th>Subjects no°</th>
<th>Positive no° (%)</th>
<th>Mean viral load (virus copies/5.5x10^4 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCPyV</td>
<td>Tonsils</td>
<td>182</td>
<td>0</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>Adenoids</td>
<td>181</td>
<td>2 (1%)</td>
<td>1x10^3</td>
</tr>
<tr>
<td>WUPyV</td>
<td>Tonsils</td>
<td>50</td>
<td>6 (12%)</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Adenoids</td>
<td>83</td>
<td>23 (28%)</td>
<td>n.a.</td>
</tr>
<tr>
<td>MCPyV</td>
<td>Tonsils</td>
<td>150</td>
<td>8 (6%)</td>
<td>1x10^1</td>
</tr>
<tr>
<td></td>
<td>Adenoids</td>
<td>179</td>
<td>3 (2%)</td>
<td>2x10^1</td>
</tr>
</tbody>
</table>
Figure captions

**Fig. 1** Seroprevalence of 12 PyVs among the 182 study participants, by age group

**Fig. 2** Trend of seropositivity among age classes. Percentage of children positive to antibodies against **a)** BKPyV (VP1 ± TAg); **b)** TSPyV (VP1 ± TAg); **c)** HPyV10 (VP1 ± TAg), and **d)** WUPyV (VP1)

**Fig. 3** Number of different PyVs against which a subject tested positive, by age: panel **a)** 0-3 years; panel **b)** 4-7 years; panel **c)** 8-17 years.