Seroprevalence of fourteen human polyomaviruses determined in blood donors

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Abstract

The polyomavirus family currently includes thirteen human polyomavirus (HPyV) species. In immunocompromised and elderly persons HPyVs are known to cause disease, such as progressive multifocal leukoencephalopathy (JCPyV), haemorrhagic cystitis and nephropathy (BKPyV), Merkel cell carcinoma (MCPyV), and trichodysplasia spinulosa (TSPyV). Some recently discovered polyomaviruses are of still unknown prevalence and pathogenic potential. Because HPyVs infections persist and might be transferred by blood components to immunocompromised patients, we studied the seroprevalence of fourteen polyomaviruses in adult Dutch blood donors. For most polyomaviruses the observed seroprevalence was high (60-100%), sometimes slightly increasing or decreasing with age. Seroreactivity increased with age for JCPyV, HPyV6 and HPyV7 and decreased for BKPyV and TSPyV. The most recently identified polyomaviruses HPyV12, NJPyV and LIPyV showed low overall seroprevalence (~5%) and low seroreactivity, questioning their human tropism. Altogether, HPyV infections are common in Dutch blood donors, with an average of nine polyomaviruses per subject.

Introduction

The *Polyomaviridae* family comprises non-enveloped double-stranded DNA viruses that infect a broad spectrum of hosts. After primary infection, usually in childhood, they cause asymptomatic persistent infection accompanied by low-level replication and shedding, for instance in urine [1,2]. Since 2007 the number of identified human polyomaviruses (HPyV) has greatly increased. They are currently grouped in thirteen species, including the 'classic' BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV) [2,3]. A novel

polyomavirus called the Lyon IARC polyomavirus (LIPyV) that was identified in 2017 has not been assigned to a polyomavirus species yet [4].

BKPyV is the main cause of polyomavirus-associated nephropathy (PVAN) that occurs in up to 10% of kidney transplant patients [5]. Haemorrhagic cystitis, also caused by BKPyV, complicates between 6-30% of hematopoietic stem cell transplantations [6]. JCPyV causes progressive multifocal leukoencephalopathy (PML), a potentially lethal, demyelinating brain disease, which is found in HIV-infected AIDS patients, immunosuppressed transplantation patients, and nowadays especially in multiple sclerosis (MS) patients treated with immunomodulatory drugs, such as natalizumab [7]. The incidence of PML in natalizumab-treated MS patients can be as high as 20 per 1000 patients [8,9]. Merkel cell polyomavirus (MCPyV) is an important cause of Merkel cell carcinoma (MCC). The incidence of MCC is low, approximately 0.4 per 100.000 person-years, though this appears to increase [10]. Less is known about the incidence of diseases caused by other polyomaviruses, for example *trichodysplasia spinulosa* caused by the trichodysplasia spinulosa polyomavirus (TSPyV) [2,11]. Karolinska Institute polyomavirus (KIPyV) and Washington University polyomavirus (WUPyV) have been implicated in respiratory disease [12–14], HPyV6 and HPyV7 in dyskeratotic dermatosis, and HPyV7 in thymomagenesis [15–18]. Furthermore, the New Jersey polyomavirus likely caused a unique but severe case of vasculitis resulting in blindness, dermatitis and myositis. Altogether, the polyomaviruses are a significant cause of disease in the immunocompromised population.

Blood components (red blood cells, platelets and fresh frozen plasma) are administered to haematological, transplant, and other immunocompromised patients in huge numbers. Therefore, it is important to understand the epidemiology of polyomaviruses among healthy adults and potential blood donors, including HPyVs that have been recognized just recently and of which still very little is known. In this study the seroprevalence and

seroreactivity were determined of fourteen polyomaviruses identified thus far in humans, in 1050 Dutch blood donors subdivided into age categories. HPyV serology was performed using a custom bead-based immunoassay which was recently validated for this purpose [19].

Materials and methods

Study population

The study population consisted of serum samples from 1050 Dutch blood donors. Donors were included using weighted random selection from Dutch blood donations to obtain groups of equal size in terms of age and sex. Serum samples from eighty blood donation centres were collected over a period of two weeks to ensure an even geographic distribution over the Netherlands (Fig 1). Every blood donation in the Netherlands is routinely screened for presence of human immunodeficiency virus, hepatitis B and C virus and syphilis, only samples with a negative result were included in this study. Sex and age characteristics are summarized in Table 1. The donors were divided in five age categories: 18 – 29, 30 – 39, 40 – 49, 50 – 59 and 60 – 69 years of age. In total, 529 males and 521 females were included.

The study involves anonymous 'left over' samples from blood donors who gave permission to use this material for studies into blood-borne agents. Hence Sanquin's scientific board, and the secretary of Sanquin's Ethical Advisory Board, decided that for this study permission from the Ethical Advisory Board is not applicable.

Human polyomavirus multiplex immunoassay

A customized, recently described Luminex xMAP assay was used to measure IgG seroreactivity against the VP1 major capsid protein of BKPyV, JCPyV, KIPyV, WUPyV, MCPyV, HPyV6, HPyV7, TSPyV, HPyV9, Malawi polyomavirus (MWPyV), Saint Louis polyomavirus (STLPyV), HPyV12, NJPyV and LIPyV [19–21]. As described, each GST-VP1 fusion protein was expressed in BL21 Rosetta bacteria and coupled to uniquely colored, glutathione-casein cross-linked magnetic fluorescent polystyrene beads.

In the multiplex immunoassay the 1:100 diluted serum samples were incubated for one hour in blocking buffer to suppress non-specific binding [21,22]. Biotinylated goat-α-human IgG (H+L) (1:1000 Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA, catalogue number: 109-065-088, antibody registry number: AB_2337628) followed by streptavidin-R-phycoerythrin (SAPE) (1:1000 Invitrogen, Waltham, MA, USA, catalogue number: S866) were used to detect IgG responses against the individual VP1 antigens. A serially diluted mix of four serum samples with known seroreactivity against various polyomaviruses was included in each plate to measure intertest variability [19,20], which was low. The intraclass correlation coefficient for the 1:100 diluted controls was 0.91 (95% confidence interval: 0.81-0.97; $P<0.001$). Specific seroreactivity was calculated by subtracting the median fluorescence intensity (MFI) values of both a blank sample and of beads coupled to an irrelevant GST fusion protein, in this case GST-SV40 small T-antigen. Serum samples with a high response against GST-SV40 small T-antigen (resulting in specific seroreactivity below or equal to minus 1000 MFI), were excluded for further analysis (n=6).

Determination of the cut-off value

For each HPyV a cut-off value for seropositivity was determined based on seroresponses of Dutch children (n=36) between 10 and 15 months old, as previously described [20]. To determine a seronegative population, a frequency distribution analysis

with a bin width of 250 MFI was performed and samples in bins with a frequency percentage above 10% were used in the calculation of the cut-off score. The cut-off value is calculated by the mean seroresponse of the seronegative population and adding three times the standard deviation. This resulted in the following cut-off values, expressed as MFI, for BKPyV 391, JCPyV 349, KIPyV 341, WUPyV 403, MCPyV 509, HPyV6 322, HPyV7 1069, TSPyV 346, HPyV9 446, MWPyV 325, STLPyV 357, HPyV12 326, NJPyV 994, and LIPyV 438.

Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 23. Intraclass correlation coefficient was calculated based on a single measures form, absolute-agreement and 2-way mixed-effects model. Associations between categorical variables (e.g. sex, age categories) and seropositivity were analysed by χ^2 test (for trend) or Fisher's exact test where appropriate. Mann-Whitney U test was used to analyse differences in seropositivity numbers between the different age categories per donor. Seroreactivity was not normally distributed and was therefore analysed by a non-parametric test, Jonckheere's trend test for ordinal variables (in this case the association between age and seroreactivity in seropositive samples).

Results

Human polyomavirus seroprevalence

In 1050 Dutch blood donors, the seroprevalence of each polyomavirus was determined by calculating the proportion of serum samples with seroreactivity above the established MFI cut-off points. For the majority of polyomaviruses, the overall seropositivity was high, at least 60% (Fig 2 and Table 2). However, for HPyV9 and especially for HPyV12,

NJPyV and LIPyV the overall seropositivity was low, 19.2%, 4.0%, 5.2% and 5.9% respectively. When the seroprevalences were analysed in relation to age, a significant positive association was observed for KIPyV (P<0.001), HPyV6 (P<0.001), HPyV7 (P<0.001) and TSPyV (P= 0.04). For MCPyV, a negative association between seroprevalence and age was observed (P= 0.013). Due to low numbers of seropositives, age comparisons were not performed for HPyV12, NJPyV and LIPyV. For all HPyVs no significant differences in seropositivity were observed related to sex.

All blood donors were seropositive for at least four polyomaviruses. The mean number of infections per donor (± SD), based on seropositivity, was 8.7 ± 1.6 per subject (Fig 3). Participants in the lowest age category (18-29) had a mean of 8.2 ± 1.6 infections, which was significantly lower ($P \leq 0.001$) than the other age categories, which showed a mean number of infections as follows: 30-39 years: 8.8 ± 1.7, 40-49 years: 8.7 ± 1.5, 50-59 years: 8.7 ± 1.5, and 60-69 years: 8.9 ± 1.6. No differences regarding the mean number of infections per donor were observed between the sexes.

Human polyomavirus seroreactivity

Seroreactivity detected in seropositive donors differed between the analysed HPyVs. The highest median MFI-values were measured for BKPyV (Fig 4). Intermediate values were measured for KIPyV, WUPyV, MCPyV, HPyV6, HPyV7, TSPyV and MWPyV. Low to intermediate median MFI values were measured for JCPyV, HPyV9 and STLPyV, although some highly reactive serum samples were noted for HPyV9. The seroresponses against HPyV12, NJPyV and LIPyV were generally very low.

Seroreactivity was further analysed in relation to age by investigating potential trends within the five age-categories shown in Fig 4. A significant age-dependent increase of seroreactivity was observed for JCPyV, HPyV6 and HPyV7 (P<0.001, P<0.001 and P=0.047,

150 respectively). For HPyV9 a substantial increase was seen for the 60-69 age category. In
151 contrast, for BKPyV and TSPyV a significant decrease of seroreactivity was observed in the
152 higher age categories ($P<0.001$ for both viruses). No significant trends were observed for the
153 other HPyVs. The analyses were not performed for HPyV12, NJPyV and LIPyV, due to low
154 numbers of seropositives.

155 In general, no differences in seroreactivity between the sexes were observed, but for
156 HPyV7 the overall median seroreactivity among seropositives was higher in men (9250 MFI
157 in men vs. 6464 MFI in women, $P=0.018$).

158

159 Discussion

160 In this study we determined the seroprevalence and seroreactivity of all currently
161 known human polyomaviruses in a large Dutch blood donor cohort. Our findings indicate that
162 seropositivity for a large number of polyomaviruses is common in this healthy population. At
163 the same time, for some recently identified HPyVs the seroprevalence was low.

164 With regard to most HPyVs that have been serologically analysed before, the
165 seroprevalences reported here are in line with previous seroepidemiological studies in
166 immunocompetent populations from different continents [11,19,20,23–28], therefore we
167 assume our findings to be representative for most other immunocompetent populations. For
168 KIPyV, HPyV6, HPyV7 and TSPyV an increase in seroprevalence with higher age was
169 noticed, which has also been reported previously [20,23–26]. This could reflect continuous
170 viral exposure throughout life or frequent reactivation of persistent infection, which can boost
171 HPyV seroresponses as well [29]. Furthermore, we observed a decrease in seroprevalence

with age for MCPyV, which was not published before to our knowledge, [20,23,24], and could represent a cohort effect. HPyV infections with a stable seroprevalence in adult life are probably acquired during childhood, as previously indicated by a rapid increase in seropositivity during the first years of life [20,24,30]. Due to the age restrictions on becoming a blood donor in the Netherlands, we did not investigate the HPyV seroreactivity patterns in individuals under 18 years of age or older than 69.

The seroreactivity of seropositive individuals differed with age between the HPyVs, with decreasing intensity for BKPyV and TSPyV, increasing intensity for JCPyV, HPyV6 and HPyV7, and stable intensity for KIPyV, WUPyV, MWPyV and STLPyV. Comparable trends were obtained in healthy Australian, Czech and Italian populations [20,25,26,31,32], though MCPyV seroreactivity did not increase with age in our cohort. The decrease in seroreactivity for BKPyV and TSPyV suggests gradually less immunological boosting, possibly related to a decrease in environmental exposure or diminished reactivation of these HPyVs, while the increase in seroreactivity seen for JCPyV, HPyV6 and HPyV7 might reflect continuous exposure or reactivation [29].

The serological profile of HPyV9 is unique compared to other polyomaviruses, with a small subset of seropositive individuals that display very high seroreactivity in a background of weak seroresponders. It was previously shown that HPyV9 has unique receptor binding properties, and preferentially binds to a ligand which cannot be synthesized by humans, but can be acquired through diet (red meat and milk) [33]. The necessity for a dietary ligand might explain why this virus is less prevalent among humans than most other HPyVs. Whether highly HPyV9-seroreactive subjects indeed ingest more dairy and meat-containing products could be the subject of further study.

For HPyV12, NJPyV and LIPyV we detected a very low seroprevalence, approximately 5%, with low seroreactivity for all three. In a pilot study, we obtained similar

197 results and confirmed the antigenicity of the used HPyV12- and NJPyV-VP1 antigens by
198 specific polyclonal antibody recognition [19]. Therefore, we believe the observed low
199 seroprevalence of these polyomaviruses to be genuine, and we consider the possibility that
200 these polyomaviruses do not frequently circulate in humans, and perhaps do not represent
201 human polyomaviruses at all. For HPyV12, this would fit with recent observations suggesting
202 that HPyV12 represents a shrew rather than a human polyomavirus [34]. For NJPyV it could
203 very well be that the only published patient was infected from an animal reservoir under
204 exceptional circumstances, when fleeing from flooding during hurricane Sandy [35]. LIPyV
205 was identified in a skin swab sample and subsequently detected in a small subset of oral
206 fluids (2%), skin swabs (2%) and eyebrow hair follicles (0.2%) [4]. In this case, the measured
207 low seroprevalence might reflect the LIPyV detection rate, though more studies are needed to
208 further clarify the epidemiology of LIPyV.

209 In contrast to our data, a very recent study reported 90% seroprevalence for HPyV12,
210 in an Italian adult population [36]. This percentage is considerably higher than our finding
211 and the 20% seroprevalence obtained previously for HPyV12 by Ehlers and co-workers using
212 recombinant VP1 and VP1-based VLP ELISA [34,37]. Since HPyV VP1-based and VLP
213 based assays generally obtain comparable results, as we have recently demonstrated for
214 BKPyV [19], we have no explanation thus far for this large discrepancy except differences in
215 cut-off value determination and striking geographic differences in virus exposure. Suboptimal
216 HPyV12 antigen recognition, resulting from the use of a premature translation initiation site
217 in HPyV12 VP1, causing a 16 amino acids longer version of VP1 [38], was experimentally
218 ruled out (S1 Fig). Also for NJPyV a much higher seroprevalence was found in the Italian
219 population (50%) than in our population (5%) [36]. Overall, more (sero)epidemiological
220 studies are needed to solve these discrepancies and to define the natural host(s) of these

221 viruses, for example by studying seroprevalence in different geographic regions while using
222 comparable serological methods.

223 In conclusion, by analysing a large group of Dutch blood donors we showed that most
224 HPyV infections are common, although we found little indication of HPyV12, NJPyV and
225 LIPyV circulation in humans. Considering that blood donors are persistently infected with, on
226 average, nine different polyomaviruses and assuming that episodes of viremia sometimes
227 occur, the consequences for the safety of blood transfusion, especially for
228 immunocompromised recipients, remains to be established.

229

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233

References

1. Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, et al. Prevalence of Polyomavirus BK and JC Infection and Replication in 400 Healthy Blood Donors. J Infect Dis. 2009;199: 837–846. doi:10.1086/597126

2. Feltkamp MCW, Kazem S, van der Meijden E, Lauber C, Gorbalenya AE. From Stockholm to Malawi: recent developments in studying human polyomaviruses. J Gen Virol. 2013;94: 482–496. doi:10.1099/vir.0.048462-0

3. Moens U, Krumbholz A, Ehlers B, Zell R, Johne R, Calvignac-Spencer S, et al. Biology, evolution, and medical importance of polyomaviruses: An update. Infect Genet Evol. 2017;54: 18–38. doi:10.1016/j.meegid.2017.06.011

4. Gheit T, Dutta S, Oliver J, Robitaille A, Hampras S, Combes J-D, et al. Isolation and characterization of a novel putative human polyomavirus. Virology. 2017;506: 45–54. doi:10.1016/j.virol.2017.03.007

5. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. Transplantation. 2005;79: 1277–1286.

6. Foster JH, Cheng WS, Nguyen N-Y, Krance R, Martinez C. Intravesicular cidofovir for BK hemorrhagic cystitis in pediatric patients after hematopoietic stem cell transplant. Pediatr Transplant. 2018; doi:10.1111/petr.13141

7. Reuwer AQ, Heron M, van der Dussen D, Schneider-Hohendorf T, Murk J l. The clinical utility of JC virus antibody index measurements in the context of progressive multifocal leukoencephalopathy. Acta Neurol Scand. 2017;136: 37–44. doi:10.1111/ane.12840

8. Vennegoor A, van Rossum JA, Polman CH, Wattjes MP, Killestein J. Longitudinal JCV serology in multiple sclerosis patients preceding natalizumab-associated progressive multifocal leukoencephalopathy. Mult Scler J. 2015;21: 1600–1603. doi:10.1177/1352458514567728

9. Schwab N, Schneider-Hohendorf T, Melzer N, Cutter G, Wiendl H. Natalizumab-associated PML: Challenges with incidence, resulting risk, and risk stratification. Neurology. 2017;88: 1197–1205. doi:10.1212/WNL.0000000000003739

10. Fondain M, Dereure O, Uhry Z, Guizard AV, Woronoff AS, Colonna M, et al. Merkel cell carcinoma in France: A registries-based, comprehensive epidemiological survey. J Eur Acad Dermatol Venereol JEADV. 2018; doi:10.1111/jdv.14798

11. DeCaprio JA, Garcea RL. A cornucopia of human polyomaviruses. Nat Rev Microbiol. 2013;11: 264–276. doi:10.1038/nrmicro2992

12. Dehority WN, Eickman MM, Schwalm KC, Gross SM, Schroth GP, Young SA, et al. Complete genome sequence of a KI polyomavirus isolated from an otherwise healthy child with severe lower respiratory tract infection. J Med Virol. 2017;89: 926–930. doi:10.1002/jmv.24706

13. Teramoto S, Koseki N, Yoshioka M, Matsunami Y, Yanazume N, Nawate M, et al. WU Polyomavirus Infection Confirmed by Genetic and Serologic Tests in an Infant With Bronchitis. Pediatr Infect Dis J. 2011;30: 918. doi:10.1097/INF.0b013e3182252148

14. Siebrasse EA, Nguyen NL, Willby MJ, Erdman DD, Menegus MA, Wang D. Multiorgan WU Polyomavirus Infection in Bone Marrow Transplant Recipient. Emerg Infect Dis. 2016;22: 24–31. doi:10.3201/eid2201.151384

15. Nguyen KD, Lee EE, Yue Y, Stork J, Pock L, North JP, et al. Human polyomavirus 6 and 7 are associated with pruritic and dyskeratotic dermatoses. J Am Acad Dermatol. 2017;76: 932-940.e3. doi:10.1016/j.jaad.2016.11.035

16. Ho J, Jedrych JJ, Feng H, Natalie AA, Grandinetti L, Mirvish E, et al. Human Polyomavirus 7–Associated Pruritic Rash and Viremia in Transplant Recipients. J Infect Dis. 2015;211: 1560–1565. doi:10.1093/infdis/jiu524

17. Rennspiess D, Pujari S, Keijzers M, Abdul-Hamid MA, Hochstenbag M, Dingemans A-M, et al. Detection of Human Polyomavirus 7 in human thymic epithelial tumors. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer. 2015;10: 360–366. doi:10.1097/JTO.0000000000000390

18. Keijzers M, Rensspiess D, Pujari S, Abdul-Hamid MA, Hochstenbag M, Dingemans A-M, et al. Expression of pRb and p16INK4 in human thymic epithelial tumors in relation to the presence of human polyomavirus 7. Diagn Pathol. 2015;10. doi:10.1186/s13000-015-0418-6

19. Kamminga S, van der Meijden E, Wunderink HF, Touzé A, Zaaijer HL, Feltkamp MCW. Development and evaluation of a broad bead-based multiplex immunoassay to measure IgG seroreactivity against human polyomaviruses. J Clin Microbiol. 2018; doi:10.1128/JCM.01566-17

20. van der Meijden E, Bialasiewicz S, Rockett RJ, Tozer SJ, Sloots TP, Feltkamp MCW. Different Serologic Behavior of MCPyV, TSPyV, HPyV6, HPyV7 and HPyV9 Polyomaviruses Found on the Skin. PLoS ONE. 2013;8. doi:10.1371/journal.pone.0081078

21. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex Human Papillomavirus Serology Based on In Situ–Purified Glutathione S-Transferase Fusion Proteins. Clin Chem. 2005;51: 1845–1853. doi:10.1373/clinchem.2005.052381

22. Waterboer T, Sehr P, Pawlita M. Suppression of non-specific binding in serological Luminex assays. J Immunol Methods. 2006;309: 200–204. doi:10.1016/j.jim.2005.11.008

23. Gossai A, Waterboer T, Nelson HH, Michel A, Willhauck-Fleckenstein M, Farzan SF, et al. Seroepidemiology of Human Polyomaviruses in a US Population. Am J Epidemiol. 2016;183: 61–69. doi:10.1093/aje/kwv155

24. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. PLoS Pathog. 2009;5: e1000363. doi:10.1371/journal.ppat.1000363

25. Šroller V, Hamšíková E, Ludvíková V, Musil J, Němečková Š, Saláková M. Seroprevalence rates of HPyV6, HPyV7, TSPyV, HPyV9, MWPyV and KIPyV polyomaviruses among the healthy blood donors. J Med Virol. 2016;88: 1254–1261. doi:10.1002/jmv.24440

26. Nicol JTJ, Robinot R, Carpentier A, Carandina G, Mazzoni E, Tognon M, et al. Age-Specific Seroprevalences of Merkel Cell Polyomavirus, Human Polyomaviruses 6, 7, and 9, and Trichodysplasia Spinulosa-Associated Polyomavirus. Clin Vaccine Immunol CVI. 2013;20: 363–368. doi:10.1128/CVI.00438-12

27. Antonsson A, Neale RE, O'Rourke P, Wockner L, Michel A, Pawlita M, et al. Prevalence and stability of antibodies to thirteen polyomaviruses and association with cutaneous squamous cell carcinoma: A population-based study. J Clin Virol. 2018;101: 34–37. doi:10.1016/j.jcv.2018.01.013

28. Lim ES, Meinerz NM, Primi B, Wang D, Garcea RL. Common Exposure to STL Polyomavirus During Childhood. Emerg Infect Dis. 2014;20: 1559–1561. doi:10.3201/eid2009.140561

29. Wunderink HF, van der Meijden E, van der Blij-de Brouwer CS, Zaaijer HL, Kroes ACM, van Zwet EW, et al. Stability of BK polyomavirus IgG seroreactivity and its correlation with preceding viremia. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2017;90: 46–51. doi:10.1016/j.jcv.2017.03.015

30. Cason C, Monasta L, Zanotta N, Campisciano G, Maestri I, Tommasino M, et al. Antibody response to polyomavirus primary infection: high seroprevalence of Merkel cell polyomavirus and lymphoid tissue involvement. J Neurovirol. 2018; 1–9. doi:10.1007/s13365-017-0612-2

31. Šroller V, Hamšíková E, Ludvíková V, Vochozková P, Kojzarová M, Fraiberk M, et al. Seroprevalence rates of BKV, JCV, and MCPyV polyomaviruses in the general Czech Republic population. J Med Virol. 2014;86: 1560–1568. doi:10.1002/jmv.23841

32. van der Meijden E, Kazem S, Burgers MM, Janssens R, Bavinck JNB, de Melker H, et al. Seroprevalence of Trichodysplasia Spinulosa–associated Polyomavirus. Emerg Infect Dis. 2011;17: 1355–1363. doi:10.3201/eid1708.110114

33. Khan ZM, Liu Y, Neu U, Gilbert M, Ehlers B, Feizi T, et al. Crystallographic and glycan microarray analysis of human polyomavirus 9 VP1 identifies N-glycolyl neuraminic acid as a receptor candidate. J Virol. 2014;88: 6100–6111. doi:10.1128/JVI.03455-13

34. Gedvilaite A, Tryland M, Ulrich RG, Schneider J, Kurmauskaite V, Moens U, et al. Novel polyomaviruses in shrews (Soricidae) with close similarity to human polyomavirus 12. J Gen Virol. 2017; doi:10.1099/jgv.0.000948

35. Mishra N, Pereira M, Rhodes RH, An P, Pipas JM, Jain K, et al. Identification of a novel polyomavirus in a pancreatic transplant recipient with retinal blindness and vasculitic myopathy. J Infect Dis. 2014;210: 1595–1599. doi:10.1093/infdis/jiu250

36. Gaboriaud P, Ferté M, Arnold F, Leblond V, Nicol J, Debare H, et al. Age-specific seroprevalence of human polyomavirus 12 and Saint Louis and New Jersey polyomaviruses. Emerg Microbes Infect. 2018;7: 22. doi:10.1038/s41426-018-0026-0

37. Korup S, Rietscher J, Calvignac-Spencer S, Trusch F, Hofmann J, Moens U, et al. Identification of a novel human polyomavirus in organs of the gastrointestinal tract. PloS One. 2013;8: e58021. doi:10.1371/journal.pone.0058021

38. Norkiene M, Stonyte J, Ziogiene D, Mazeike E, Sasnauskas K, Gedvilaite A. Production of recombinant VP1-derived virus-like particles from novel human polyomaviruses in yeast. BMC Biotechnol. 2015;15. doi:10.1186/s12896-015-0187-z

Fig 1. Geographic distribution of blood donors. The geographic origin of 1050 collected serum samples in the Netherlands is shown in a map by the location of the collection centres involved. Samples were collected over a period of two weeks to ensure the inclusion of blood donation centres from all regions of the Netherlands. The number of samples from each location is visualized by increasing circle size parallel to the number of samples from that location, with a minimum of 1 and a maximum of 51 samples from individual centres.

Fig 2. Seroprevalence of indicated polyomaviruses in Dutch blood donors. The percentage seropositivity of each polyomavirus is shown for the donor age categories 18-29 (checkers pattern, N=206), 30-39 (solid white bars, N=207), 40-49 (dots pattern, N=207), 50-59 (light grey bars, N=215) and 60-69 (diagonally striped pattern, N=209).

Fig 3. Distribution of the number of polyomavirus infections per donor. The distribution of the number of infecting polyomaviruses is shown among the tested blood donors, as indicated by seropositivity.

Fig 4. The level of polyomavirus seroreactivity in seropositive donors categorized by age. Box plots with whiskers represent 1-99th percentiles. Outliers are indicated by triangles. Age categories shown are 18-29 (checkers pattern), 30-39 (solid white bars), 40-49 (dots pattern), 50-59 (light grey bars) and 60-69 (diagonally striped pattern). Only MFI values from seropositive donors are shown. The total number of seropositives was for BKPyV: 1033; JCPyV: 660; KIPyV: 957; WUPyV: 1033; MCPyV: 855; HPyV6: 875; HPyV7: 749; TSPyV: 831; HPyV9: 200; MWPyV: 1039; STLPyV: 677; HPyV12: 42; NJPyV: 54; LIPyV: 62.

372 **Table 1. Demographics of study population**

		Sex		Total
		Male	Female	
	18-29	103	105	208
	30-39	106	103	209
Age category	40-49	106	102	208
	50-59	110	105	215
	60-69	104	106	210
Total		529	521	1050

373

374 **Table 2. Seropositivity numbers and seroprevalence**

	Total	Sex		Age category				
		Male	Female	18-29	30-39	40-49	50-59	60-69
Polyomavirus	(N=1044[a])	(N=527)	(N=517)	(N=206)	(N=207)	(N=207)	(N=215)	(N=209)
type	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
BKPyV	1033 (98.9)	523 (99.2)	510 (98.6)	204 (99.0)	205 (99.0)	204 (98.6)	213 (99.1)	207 (99.0)
JCPyV	660 (63.2)	336 (63.8)	324 (62.7)	120 (58.3)	138 (66.7)	128 (61.8)	133 (61.9)	141 (67.5)
KIPyV**	957 (91.6)	483 (91.7)	474 (91.7)	164 (79.6)	191 (92.3)	196 (94.7)	202 (94.0)	204 (97.6)
WUPyV	1033 (98.9)	521 (98.9)	512 (99.0)	204 (99.0)	204 (98.6)	205 (99.0)	213 (99.1)	207 (99.0)
MCPyV*	855 (81.9)	429 (81.4)	426 (82.4)	178 (86.4)	168 (81.2)	178 (86.0)	170 (79.1)	161 (77.0)
HPyV6**	875 (83.8)	444 (84.3)	431 (83.4)	156 (75.7)	175 (84.5)	173 (83.6)	184 (85.6)	187 (89.5)
HPyV7**	749 (71.7)	391 (74.2)	358 (69.2)	117 (56.8)	154 (74.4)	155 (74.9)	154 (71.6)	169 (80.9)
TSPyV*	831 (79.6)	431 (81.8)	400 (77.4)	156 (75.7)	160 (77.3)	163 (78.7)	183 (85.1)	169 (80.9)
HPyV9	200 (19.2)	91 (17.3)	109 (21.1)	44 (21.4)	43 (20.8)	40 (19.3)	36 (16.7)	37 (17.7)
MWPyV	1039 (99.5)	523 (99.2)	516 (99.8)	205 (99.5)	205 (99.0)	207 (100.0)	215 (100.0)	207 (99.0)
STLPyV	677 (64.8)	342 (64.9)	335(64.8)	118 (57.3)	145 (70.0)	139 (67.1)	143 (66.5)	132 (63.2)
HPyV12	42 (4.0)	15 (2.8)	27 (5.2)	11 (5.3)	10 (4.8)	5 (2.4)	4 (1.9)	12 (5.7)
NJPyV	54 (5.2)	22 (4.2)	32 (6.2)	8 (3.9)	4 (1.9)	4 (1.9)	16 (7.4)	22 (10.5)
LIPyV	62 (5.9)	33 (6.3)	29 (5.6)	10 (4.9)	14 (6.8)	10 (4.8)	14 (6.5)	14 (6.7)

375 a. 6 samples were excluded due to GST-SV40 small T reactivity.

376 * $P < 0.05$ ** $P<0.001$ $\chi 2$ test for trend for differences between age categories.

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Supporting information

S1 Fig. HPyV12 seroreactivity. Based on VP1 sequence alignment, the translation initiation site of the HPyV12-VP1 sequence may be located 48 nucleotides (16 amino acids) downstream of the 5' end of the VP1 open reading frame. In order to compare the antigenicity, both the 380 amino acids long version and the 364 amino acids long version of the HPyV12-VP1 protein were expressed and used to analyse a cohort of kidney transplant recipients (n=65). The seroreactivity measured with each protein was similar (Pearson R^2 = 0.99).

S2 File. Dataset. Dataset containing population characteristics and immunoassay results.

388 **Fig 1**

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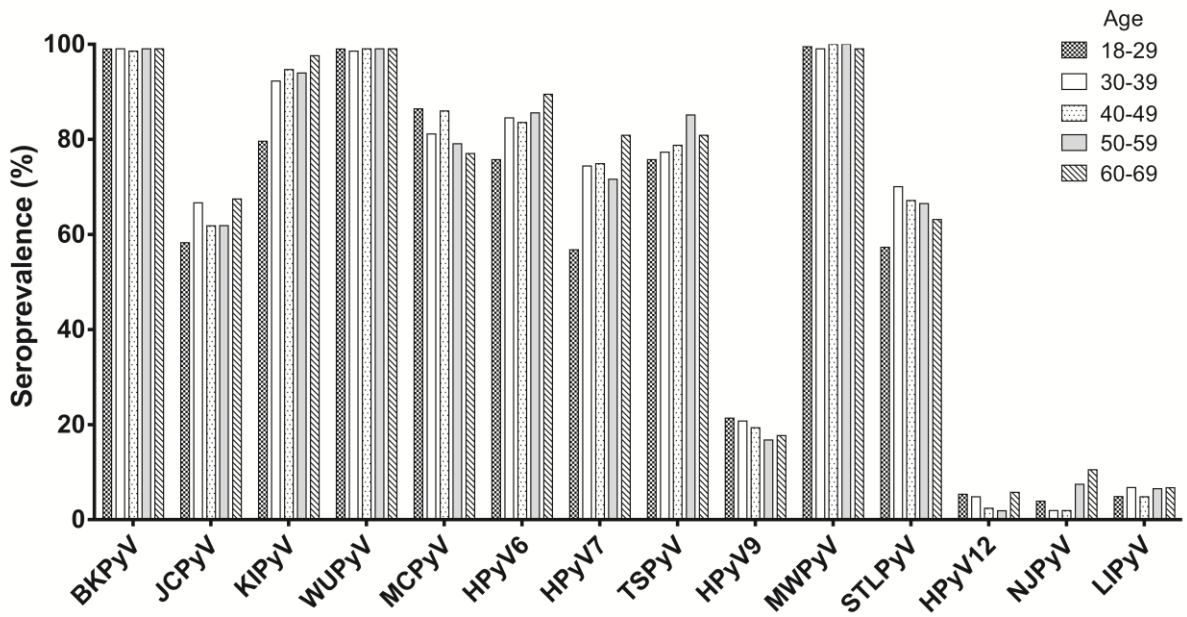
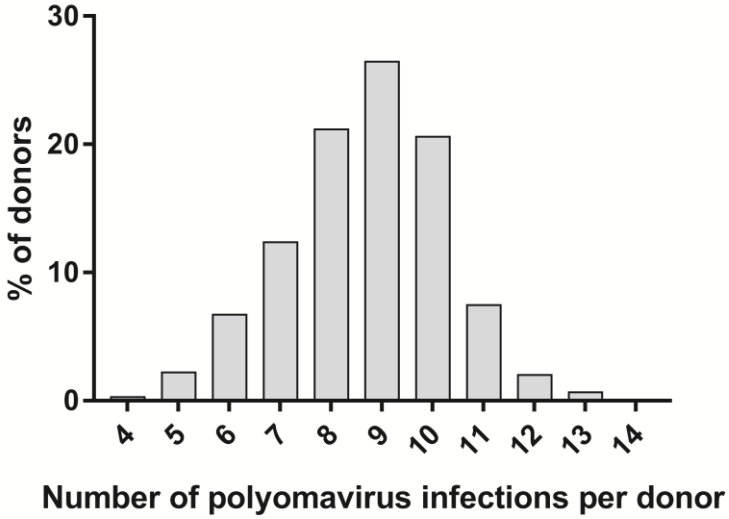
390

391 **Fig 2**



392

393 **Fig 3**



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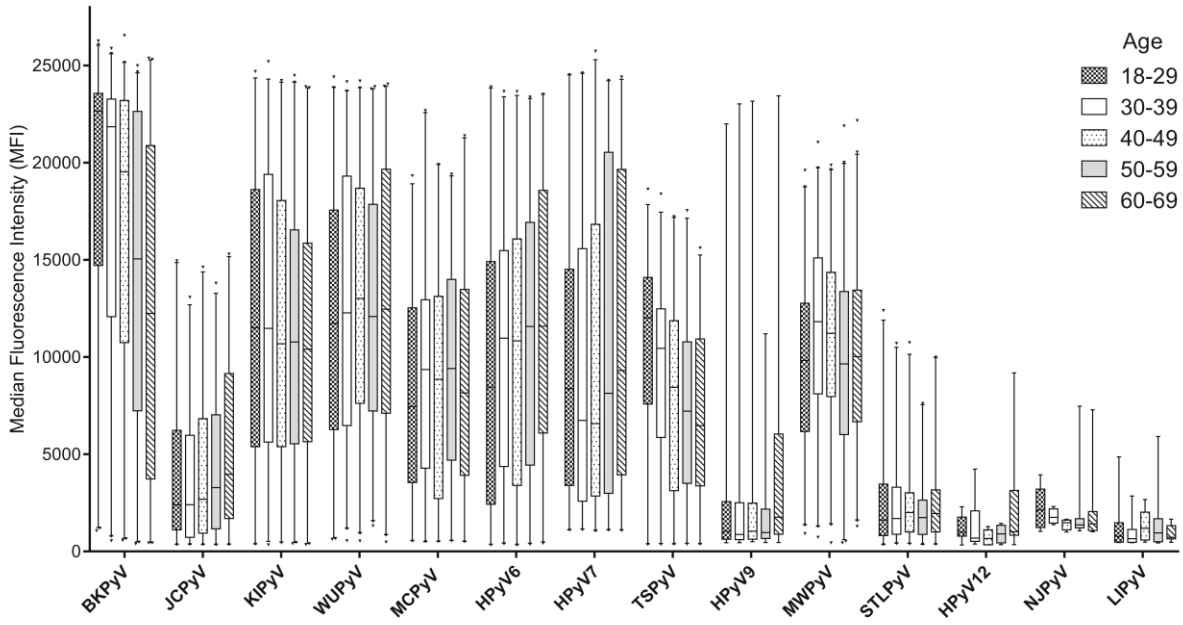
396 **Fig 4**



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