

Seroprevalence and stability of IgG antibodies to WU polyomavirus and human polyomavirus 6 among Finnish couples during a prospective follow-up

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ABSTRACT

Although WU polyomavirus (WUPyV) and human polyomavirus 6 (HPyV6) are widespread in adults, the long-term serological responses to these infections have not been systematically characterized. To address this gap, we assessed seroprevalence and IgG antibody stability of WUPyV and HPyV6 using data from the Finnish Family HPV (FFHPV) study, initiated in 1998. The cohort included 318 women in their third trimester of pregnancy and 133 male spouses, with follow-up samples available from 281 women and 119 men at 12, 24 and 36 months. IgG antibodies against the VP1 capsid proteins of both viruses were measured using Multiplex Serology. Persistent seropositivity was high for both WUPyV (97.5% in both sexes) and HPyV6 (66.9% in women; 72.3% in men), with antibody levels remaining stable over time. Men consistently exhibited significantly higher WUPyV and HPyV6 IgG antibody levels compared to women ($p < 0.033$ and 0.003 , respectively). A history of miscarriage was significantly associated with reduced odds of persistent HPyV6 seropositivity (OR 0.45 (95% CI: 0.21–0.97)), while other examined co-factors showed no significant associations. Antibody levels between spouses were not correlated, and pregnancy had no effect on maternal antibody levels. These findings demonstrate that WUPyV and HPyV6 infections are common, with long-lasting IgG responses, and suggest a potential link between miscarriage and HPyV6 serology that warrants further investigation.

1. Background

The Polyomaviridae family consists of small, non-enveloped double-stranded DNA (dsDNA) viruses with an average genome size of 5000 base pairs that can infect humans and a wide range of other species. The first human polyomavirus JCPyV, was discovered in 1965 after which rapid advances in sequencing technology led to the identification of several new polyomaviruses. (Moens et al., 2017a, 2017b; White et al., 2013). Currently, 13 human polyomaviruses have been identified in various locations of the body, including skin, respiratory and gastrointestinal tracts, liver, kidney, bladder, vascular endothelium and stool

(Cook, 2016). While some human polyomaviruses, such as BK polyomavirus (BKPyV), JC polyomavirus (JCPyV), Trichodysplasia spinulosa polyomavirus (TSPyV) and Merkel cell polyomavirus (MCV) are associated with disease, especially in immunocompromised individuals (Cook, 2016), the pathogenic potential of the others is not fully understood.

Among polyomaviruses, WU polyomavirus (WUPyV) and human polyomavirus 6 (HPyV6) are relatively recent discoveries. WUPyV was first identified in 2007 in respiratory secretions of pediatric patients with acute respiratory symptoms (Gaynor et al., 2007). Although this finding initially raised questions about its potential role in respiratory

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diseases, there is little supporting evidence that WUPyV is a direct causative factor. HPyV6, discovered in 2010, was originally identified in skin swabs from healthy individuals (Schowalter et al., 2010). HPyV6 infections are common and asymptomatic in normal skin but have also been linked to skin lesions such as pruritic and dyskeratotic dermatosis and several types of epithelial neoplasms (Nguyen et al., 2017; Schrama et al., 2014; Beckervordersandforth et al., 2016). Furthermore, both WUPyV and HPyV6 have also been detected in lymphoid tissue (Sharp et al., 2009; Rascovan et al., 2016), and various body fluid specimens (Rockett et al., 2013; Bialasiewicz et al., 2009).

Seroprevalence studies have consistently found high seroprevalence rates for both WUPyV and HPyV6. Age stratification data has indicated that primary infection for both viruses occurs in early childhood. WUPyV seroprevalence among healthy adult population is ranging from 69% to 98% being stable throughout adulthood (Kean et al., 2009; Carter et al., 2009; Gossai et al., 2016). Similarly, to WUPyV, HPyV6 seroprevalence is lower in infants but increases during childhood and then remains high in adulthood at around 70% to 80% (Gossai et al., 2016; Nicol Jérôme et al., 2013).

Despite WUPyV and HPyV6 infections being widespread, their transmission dynamics are not well-documented. The transmission modes of BKPyV and JCpV, are more widely studied and they are transmitted both vertically (mother-to-child) (Laine et al., 2023a), and horizontally, primarily through respiratory, urino-oral and feco-oral routes (Cook, 2016). Similarly, maternal antibodies to WUPyV and HPyV6 are found in neonates within the first six months, followed by a waning of the antibodies similarly as found for many other viruses as well (van der et al., 2013; Nguyen et al., 2009). WUPyV seroprevalence has been found to increase in children aged 4–6 years, raising a question if school attendance plays a role in viral transmission (Nguyen et al., 2009).

Several studies on WUPyV and HPyV6 infections, their epidemiology and clinical significance have been performed but there are still significant gaps in understanding the transmission, viral persistence and diseases if any caused by these two viruses in immunocompetent subjects. While both viruses are generally considered to cause asymptomatic infections, their high seroprevalence and ability to persist in the human body underscore the need for further investigation to fully determine their epidemiological and clinical impact.

To the best of our knowledge, this is the first study to examine WUPyV and HPyV6 antibodies in spouses longitudinally. In addition, the effect of pregnancy on these antibody levels has not been studied previously. Building upon our previous characterization of JCpV and BKPyV serological profiles within this same prospective cohort (Laine et al., 2023b), the current study expands our understanding of the cohort's overall polyomavirus status by focusing on the less-characterized WUPyV and HPyV6. Investigating pregnant women and their spouses in a longitudinal setting could provide valuable insights into potential immunological interferences of WUPyV and HPyV6 during pregnancy, transmission dynamics within couples and this way contribute to broader understanding of polyomavirus infections.

2. Methods

2.1. Study population

This research utilizes data from the Finnish Family HPV (FFHPV) Study, which is a prospective cohort study that was conducted at University of Turku and Turku University Hospital, Turku, Finland (Rintala et al., 2005). A total of 329 pregnant women in the third trimester, along with 133 fathers-to-be and their newborn offspring were recruited between 1998 and 2001 as described earlier (Louvanto et al., 2013). The mean age of women was 25.48 years (range, 18 to 46 years), and men 28.77 years (range, 19 to 46 years). The Research Ethics Committee of Turku University Hospital has approved the FFHPV study protocol and its amendments (3/1998, #2/2006, 45/180/2010 and 329/2018).

Written informed consent was obtained from all participants.

2.2. Data collection

Blood samples were collected from the spouses at baseline when the woman was pregnant at her 3rd trimester and at the 12-, 24- and 36-month follow-up visits (Syrjänen et al., 2009). For the present study, blood samples from 318 women and their 133 male spouses were available at baseline. At the 12-, 24- and 36-month follow-up visits, samples were available from 269, 256 and 241 women and 111, 101 and 97 men, respectively. The samples were centrifuged at 3000 g for 10 min (Sorvall GLC-2; DuPont Instrument), and each serum sample was divided into three 1 ml aliquots and stored first at −20 degrees Celsius for no longer than one week and then at −70 degrees Celsius until shipped on dry ice to German Cancer Research Center (DKFZ), Heidelberg, Germany, for serological analysis.

The participants were all asked to complete a structured questionnaire at baseline to provide information on various individual characteristics, background factors, general health, and reproductive health. The spouses were not allowed to see each other's completed questionnaires (Louvanto et al., 2013).

2.3. Serological analysis

Each participant's blood sample was analyzed for IgG antibodies to VP1 of WUPyV and HPyV6 with Multiplex Serology, a high-throughput assay that uses Glutathione S-Transferase (GST) fusion proteins combined with fluorescent bead technology for antibody detection (Waterboer et al., 2005). The WUPyV and HPyV6 antibodies were considered positive if they exceeded a cut-off level of 400 median fluorescence intensity (MFI) as pre-defined cut-off by The German Cancer Research Center (DKFZ). While inter-assay coefficients of variation have not been published separately for WUPyV and HPyV6, validation studies of this same Multiplex Serology platform for related human polyomaviruses (BKPyV, JCpV, and MCV) have demonstrated excellent reproducibility, with reported CVs of 13.7–20.8% across all samples and 2.5–7.6% among seropositive samples (Waterboer et al., 2005).

2.4. Statistical analysis

The scope of the current investigation was strictly limited to evaluating WUPyV and HPyV6 serological outcomes and transmission in adult couples. Participants were eligible in the analysis of longitudinal serological outcomes if they had at least two blood samples collected at different time points during follow-up. Those with no or only one sample analyzed for WUPyV and HPyV6 IgG antibodies were excluded, leaving 281 women and 119 men in the final FU analysis.

Serology outcomes were defined based on serum IgG antibody levels measured in different follow-up points. Persistence was defined as antibody levels that consistently remained above the MFI cut-off value throughout the follow-up period. Seroconversion was defined as a minimum of twofold increase in the previous serum antibody MFI value, with the new value exceeding the cut-off value of 400 MFI. Decay was defined as a reduction of at least twofold from the previous serum antibody MFI value, with the new antibody levels falling below the cut-off of 400 MFI. Fluctuation was defined as antibody levels increasing above and falling below the cut-off value during the follow-up period.

Because our cut-off of 400 MFI was considerably lower than the mean antibody level and the number of negative samples was low, a second MFI cut-off value was determined for both women and men. For each participant, their mean antibody levels at each follow-up point were assessed and used to calculate the median, lower and upper quartiles for these mean values. For WUPyV in women, the lower quartile (25% percentile) was 4.164 rounding to a 4200 MFI, to be used as the secondary cut-off. In men, the lower quartile was 5.190 rounding to 5200 MFI that was used as the secondary cut-off.

Logistic regression was used to determine crude and adjusted odds ratios (OR) with 95% confidence intervals (95% CI) for co-factors with binary outcomes. A Wilcoxon Rank-Sum test was used to compare MFI levels between women and men at different follow-up points. A Wilcoxon Sign-Rank Test (for paired samples) was used to compare women's antibody levels at baseline and at 12-month follow-up point to determine the potential impact of pregnancy on these antibody levels. Correlations of WUPyV and HPyV6 IgG antibody levels between spouses (in different follow-up points) were analyzed using Spearman's correlation. All statistical analyses were performed using Stata/MP 18.0 (StataCorp LLC, 4905 Lakeway Drive, College Station, TX 77845, USA). The significance level of two-sided p-value <0.05 was considered statistically significant for all tests.

2.5. Data availability statement

The data generated in this study are available upon request [KL].

3. Results

3.1. Population characteristics

Among the participants, the median IgG antibody levels of WUPyV and HPyV6 remained relatively stable over the 36-month follow-up period (Fig. 1). There was a slight variation in WUPyV IgG antibody levels, both in women (Fig. 1a) and their spouses (Fig. 1b), however, without any clear upward or downward trend during the follow-up. Outliers, defined as values exceeding 1.5 times the interquartile range (IQR), were observed in the WUPyV data at 12-month and 36-month follow-up visits in both spouses. For women, a total of two outliers at 12 months and eight outliers at 36 months. For men, a total of one outlier at 12 months and one outlier at 36 months. All outliers were manually verified for accuracy.

Similarly to WUPyV, median MFI values of HPyV6 IgG antibodies, in women (Fig. 1c) and men (Fig. 1d) remained stable during the 36-month follow-up. In women the mean HPyV6 IgG antibodies levels were lower than in their spouses, however. No outliers were observed for HPyV6.

MFI levels were compared between women and men at different

follow-up points (Table 1). For WUPyV, men had significantly higher MFI levels than women at all visits ($p < 0.033$). Similarly, for HPyV6, MFI levels were significantly higher for men in all visits ($p < 0.003$).

3.2. Serological outcomes

During the 36-month follow-up, IgG seropersistence to WUPyV was high, with 97.5% of both women (274/281) and men (116/119) showing persistent seropositivity. While HPyV6 seropersistence was lower, observed in 66.9% of women (188/281) and 72.3% of men (86/119) (Table 2). A substantial proportion of participants remained consistently seronegative for HPyV6, including 25.3% of women (71/281) and 16.8% of men (20/119). For WUPyV, only 1.4% of women (4/281) and 0.8% of men (1/119) were persistently seronegative. Seroconversion to HPyV6 occurred in 5.3% of women (15/281) and 4.2% of men (5/119), with median times to seroconversion of 14.8 months in women and 17.4 months in men. Seroconversion to WUPyV was rare, seen in only 0.7% of women (2/281) and 0.8% of men (1/119). Other serological patterns, including antibody decay and fluctuation, were uncommon. Fluctuating HPyV6 antibody levels were noted in 1.4% of women (4/281) and 5.9% of men (7/119), while decay was seen in 1.1% of women (3/281) and 0.8% of men (1/119). For WUPyV, decay occurred in 0.4% of women (1/281) and 0.8% of men (1/119), and fluctuation in 0.8% of men (1/119) and none of the women.

Table 1

Median fluorescence intensity (MFI) level comparisons^a between women and men for WUPyV and HPyV6 across time points in the 36-months follow-up.

	WUPyV		HPyV6	
	z	p-value	z	p-value
Baseline	-2.44	0.015	-4.01	<0.001
12 months	-2.14	0.033	-2.95	0.003
24 months	-4.48	0.015	-3.41	<0.001
36 months	-5.30	<0.001	-3.72	<0.001

z = standardized test statistic value.

p = probability value.

^a Two-sample Wilcoxon Rank-Sum (Mann-Whitney U) test.

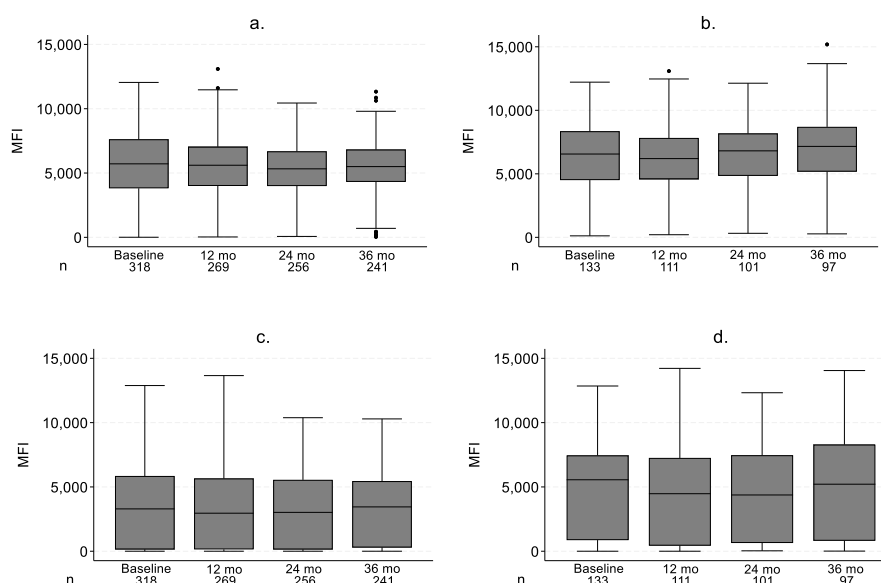


Fig. 1. Serum IgG antibody levels for WU polyomavirus in a) women and b) men, and for Human polyomavirus 6 c) women and d) men, at baseline and at 12-, 24- and 36-month follow-up visits.

Box representing the interquartile range (IQR), spanning from the 25th to the 75th percentile, with the horizontal line inside each box indicating the median MFI. The whiskers extend to the minimum and maximum values within 1.5 x IQR from the quartiles. Values beyond this range (>1.5 x IQR) are shown as outliers. Abbreviations: IQR = Interquartile range, MFI = Median Fluorescence Intensity, n = number of samples for each time point.

Table 2

Serological outcomes^a of WU polyomavirus (WUPyV) and human polyomavirus 6 (HPyV6) among the 281 women and their 119 male spouses during 36-months of follow-up.

	WUPyV		HPyV6	
	Women (n = 281)	Men (n = 119)	Women (n = 281)	Men (n = 119)
	n (%)	n (%)	n (%)	n (%)
Always negative	4 (1.4)	1 (0.8)	71 (25.3)	20 (16.8)
Persistence	274 (97.5)	116 (97.5)	188 (66.9)	86 (72.3)
Seroconversion	2 (0.7)	0 (0.0)	15 (5.3)	5 (4.2)
Decay	1 (0.4)	1 (0.8)	3 (1.1)	1 (0.8)
Fluctuation	0 (0.0)	1 (0.8)	4 (1.4)	7 (5.9)

n = number of samples in each group.

^a Outcome definitions (see Methods section).

3.3. Co-factor associations with seropositivity

Using the predefined 400 MFI cut-off, 97.8% of women and 98.5% of men were IgG seropositive to WUPyV. Among the 280 women who completed the questionnaire, 97.9% (274/280) were seropositive at the baseline (third trimester) visit. Similarly, 98.4% (123/125) of men were seropositive. This high prevalence limited our ability to analyze associations between patient characteristics and WUPyV seropositivity (Supplementary Tables 1 and 2). Using the lower 25% percentile cut-off of 4200 MFI for women and 5200 MFI for men, we were however able to evaluate the possible co-factors associated with higher IgG antibody levels of WUPyV (Table 3). Nevertheless, no statistically significant association between the co-factors and baseline WUPyV seropositivity was found. Using the pre-defined 400 MFI cut-off of HPyV6 seropositivity, 191 (68.2%) of the women were seropositive at the first baseline visit and 77.6% (97/125) of the were also HPyV6-seropositive. Similar to WUPyV, no statistically significant associations between the co-factors and baseline HPyV6 seropositivity was found.

3.4. Predictors of HPyV6 persistence

Further analysis of potential predictors of HPyV6 persistence over the three-year follow-up showed that women with a history of miscarriages were significantly less likely to have persistent IgG antibodies to HPyV6, compared to those who remained seronegative throughout the study, OR 0.45 (95%CI 0.21-0.97) (Table 4). Association remained statistically significant after adjusting for aged (OR 0.42 (95%CI 0.29-0.92)). No other significant associations were observed between HPyV6 seropersistence and evaluated co-factors. In men, none of the analyzed co-factors was a statistically significantly associated with HPyV6 seropersistence.

3.5. Horizontal transmission assessment and impact of pregnancy

To explore potential horizontal transmission, we conducted Spearman's correlation analysis on the IgG antibody levels of WUPyV and HPyV6 between spouses (Supplementary Table 3). No correlation was found between the WUPyV and HPyV6 IgG levels of the spouses at any follow-up points. In addition, we explored the role of pregnancy as all women were pregnant in enrolment of the study. No significant difference in the women's baseline WUPyV antibody level ranks and 12-month antibody level ranks were seen ($z = 1.21$, $p = 0.2273$). Similarly, no significant difference was found in the ranks for women's baseline HPyV6 antibody levels and 12-month antibody levels ($z = 0.18$, $p = 0.857$) (Data not shown).

4. Discussion

This study examined the serological outcomes of WUPyV and HPyV6 over a 36-month follow-up period in both women and men. We found no statistically significant associations between co-factors and baseline seropositivity for either WUPyV or HPyV6. However, women with a history of miscarriages were significantly less likely to exhibit persistent HPyV6 seropositivity. IgG antibody levels for both viruses remained relatively stable throughout the follow-up period for both sexes, with no significant fluctuations observed. WUPyV showed high rates of antibody persistence in both sexes (97.5%), with minimal seroconversion and antibody decay. In contrast, HPyV6 showed lower rates of persistent seropositivity (66.9% women, 72.3% men) and a higher proportion of participants who remained seronegative across the follow-up (25.3% women, 16.8% men). Despite exploring the potential for transmission between spouses, we found no significant association in antibody levels for either WUPyV or HPyV6 at any follow-up point.

It is important to note that our cohort consisted exclusively of adults – pregnant women and their partners. Because primary infection with many human polyomaviruses, including WUPyV and HPyV6, typically occurs in early childhood, the age distribution of our cohort limits our ability to investigate childhood transmission or the timing of seroconversion. Therefore, the high seropositivity observed in our cohort most likely reflects long-standing, persistent infections acquired earlier in life rather than recent viral exposure. Future studies incorporating infants, young children or birth cohorts are needed to more accurately characterize primary infection dynamics.

Our findings on the seroprevalence rates of both viruses are consistent with previous research. Variations in reported seroprevalence rates may be partially attributed to the use of different serological assays. Schowalter et al. and Nicol et al. used virus-like particle (VLP)-based ELISA and observed HPyV6 seroprevalence rates of 69% and 83%, respectively (Schowalter et al., 2010; Nicol Jérôme et al., 2013). In contrast, Kean et al. and Carter et al. used polyomavirus VP1-GST fusion proteins for antibody detection among general adult population, reporting WUPyV seroprevalence rates of 69% and 98%, respectively (Kean et al., 2009; Carter et al., 2009). In our study, antibody responses were measured using Multiplex Serology, a high-throughput assay platform that combines GST fusion proteins with fluorescent bead technology to simultaneously detect antibodies against multiple viral antigens. The assay has been shown to provide reproducible measurements of pathogen-specific antibody responses (Waterboer et al., 2005). Previous studies using Multiplex Serology have demonstrated generally high specificity for human polyomavirus VP1 capsid proteins. Gossai et al. reported little evidence of VP1 cross-reactivity among most polyomavirus types, with only potential cross-reactivity detected between the closely related HPyV6 and HPyV7 as well as MCV and HPyV9. Notably, these correlations were no longer apparent when analyses were restricted to seropositive individuals (Gossai et al., 2016). In contrast, WUPyV and HPyV6 are relatively distantly related polyomaviruses. Given that VP1 capsid protein is the target antigen in the serological assay, this low degree of genetic similarity suggests a low likelihood of cross-reactivity between WUPyV and HPyV6 and supports the specificity of the measured antibody responses.

The determination of cut-off values for seropositivity represents a critical factor influencing variability in reported seroprevalence rates. Previous studies on WUPyV and HPyV6 have all determined the cut-off values differently, which could affect the comparability of findings. Ideally, standardizing definitions for seropositivity and methods in determining MFI cut-offs could improve the comparability of serological studies. The German Cancer Research Center in Heidelberg has extensive experience with Multiplex Serology and has validated virus-specific cut-offs, ensuring reliable results across studies (Waterboer et al., 2005). In our study, we used a primary cut-off of 400 MFI, which is validated for determining seropositivity by the DKFZ. We also introduced a secondary cut-off for WUPyV (lower quartile of mean MFI levels) to enable

Table 3

Association of potential co-variables with baseline WUPyV and HPyV6 seropositivity (compared to seronegativity) in women and men, based on logistic regression analysis.

	Women's WUPyV seropositivity (MFI cut-off 4200 ^a)	Men's WUPyV seropositivity (MFI cut-off 5200 ^b)	Women's HPyV6 seropositivity (MFI cut-off 400)	Men's HPyV6 seropositivity (MFI cut-off 400)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Marital status				
Married or cohabiting (ref)	1.0	1.0	1.0	1.0
Single ^c	1.13 (0.43-3.01)	0.48 (0.03-7.90)	0.65 (0.27-1.58)	0.28 (0.02-4.65)
Level of education				
Upper secondary or higher (ref)	1.0	1.0	1.0	1.0
Primary + lower secondary	1.09 (0.44-2.72)	0.59 (0.15-2.34)	0.99 (0.41-2.39)	0.33 (0.08-1.32)
Smoking status				
Non-smoking (ref)	1.0	1.0	1.0	1.0
Smoking	1.22 (0.73-2.05)	1.20 (0.54-2.62)	1.30 (0.79-2.15)	0.49 (0.21-1.16)
Alcohol use				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.78 (0.81-3.92)	4.15 (0.37-47.21)	0.79 (0.34-1.87)	NA
Allergies				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.34 (0.79-2.26)	1.19 (0.55-2.60)	0.93 (0.56-1.54)	1.55 (0.63-3.80)
Atopy				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.40 (0.65-3.01)	1.54 (0.30-8.00)	0.96 (0.48-1.92)	2.12 (0.25-18.00)
History of miscarriages				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.26 (0.58-2.73)	2.02 (0.62-6.55)	0.55 (0.28-1.10)	1.68 (0.45-6.28)
History of infertility				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.22 (0.46-3.21)	2.02 (0.53-7.69)	1.34 (0.51-3.51)	0.68 (0.20-2.38)
Age at first intercourse				
Over 16 years (ref)	1.0	1.0	1.0	1.0
16 years or under	1.18 (0.70-1.97)	0.84 (0.40-1.79)	1.21 (0.73-2.01)	0.59 (0.25-1.38)
Number of lifetime sexual partners				
Under 10 (ref)	1.0	1.0	1.0	1.0
Over 10	1.60 (0.81-3.16)	1.26 (0.59-2.69)	0.84 (0.46-1.56)	0.81 (0.30-2.17)
Number of sexual partners by the age of 20				
Under 10 (ref)	1.0	1.0	1.0	1.0
Over 10	1.75 (0.57-5.39)	0.38 (0.12-1.23)	1.09 (0.41-2.95)	0.98 (0.25-3.84)
Practise of oral sex				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.20 (0.65-2.24)	2.21 (0.72-6.78)	1.06 (0.57-1.96)	2.20 (0.67-7.21)
Practise of anal sex				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.60 (0.80-3.23)	0.81 (0.33-1.98)	0.92 (0.49-1.72)	1.81 (0.57-5.78)
History of sexually transmitted diseases (STDs)				
No (ref)	1.0	1.0	1.0	1.0
Yes	1.35 (0.70-2.59)	1.03 (0.28-3.73)	1.24 (0.66-2.32)	1.58 (0.44-5.76)

Abbreviations.

MFI = Median Fluorescence Intensity.

OR = Odds Ratio.

95% CI = 95% Confidence Interval.

Ref = Reference Group.

NA = Not Applicable.

^a Lower quartile (25% percentile) cut-off of 4200 MFI.

^b Lower quartile (25% percentile) cut-off of 5200 MFI.

^c Including those that are divorced.

exploratory analyses between the lower and higher antibody levels due to nearly all participants being seropositive at baseline. This secondary cut-off should not be interpreted as validated diagnostic threshold for seropositivity but rather as an analytical tool to allow stratification within a highly seropositive study population. We defined seroconversion as a minimum of twofold increase in MFI value, with the new value exceeding the cut-off value of 400 MFI, and antibody decay as a minimum of twofold decrease in MFI value, with the new antibody levels falling below the cut-off of 400 MFI. Although virus-specific inter-assay coefficients of variations for WUPyV and HPyV6 are not yet available, the baseline technical variation of the platform, established via other polyomaviruses at roughly 14-21% globally and under 8% for

seropositive samples (Mentzer et al., 2022), is substantially lower than the twofold threshold used in the present study, supporting the interpretation that observed serological changes are unlikely to be explained by assay variability alone.

To our knowledge, no longitudinal studies have specifically examined WUPyV and HPyV6 serology over time. Our data demonstrates that IgG antibody levels for both viruses remain stable throughout the 36-month follow-up period, with median antibody MFI values showing no clear upward or downward trend for either virus or with pregnancy having no significant impact on antibody levels. These findings suggest a sustained immune response for both viruses in both sexes. This stability mirrors the serological patterns observed for well-characterized

Table 4

Association of patient characteristics with HPyV6 persistence (compared to always negative) in women and men, based on logistic regression analysis.

	Women's HPyV6 persistence	Men's HPyV6 persistence
	OR (95% CI)	OR (95% CI)
Marital status		
Married or cohabiting (ref)	1.0	1.0
Single	0.59 (0.23-1.50)	0.39 (0.02-6.39)
Level of education		
Upper secondary or higher (ref)	1.0	1.0
Primary + lower secondary	0.80 (0.31-2.06)	0.37 (0.09-1.57)
Smoking status		
Non-smoking (ref)	1.0	1.0
Smoking	1.31 (0.75-2.29)	0.53 (0.23-1.24)
Alcohol use		
No (ref)	1.0	1.0
Yes	1.11 (0.46-2.67)	NA
Allergies		
No (ref)	1.0	1.0
Yes	0.73 (0.42-1.28)	1.96 (0.67-5.67)
Atopy		
No (ref)	1.0	1.0
Yes	0.81 (0.38-1.72)	1.77 (0.21-15.36)
History of miscarriages		
No (ref)	1.0	1.0
Yes	0.45 (0.21-0.97)	1.81 (0.48-6.85)
History of infertility		
No (ref)	1.0	1.0
Yes	1.22 (0.43-3.49)	0.77 (0.15-4.04)
Age at first intercourse		
Over 16 years (ref)	1.0	1.0
16 years or under	1.62 (0.92-2.84)	0.52 (0.23-1.19)
Number of lifetime sexual partners		
Under 10 (ref)	1.0	1.0
Over 10	1.00 (0.49-2.03)	0.70 (0.26-1.92)
Number of sexual partners by the age of 20		
Under 10 (ref)	1.0	1.0
Over 10	1.16 (0.36-3.72)	2.03 (0.24-17.28)
Practise of oral sex		
No (ref)	1.0	1.0
Yes	0.79 (0.38-1.62)	2.25 (0.61-8.35)
Practise of anal sex		
No (ref)	1.0	1.0
Yes	0.74 (0.38-1.45)	2.49 (0.52-11.8)
History of sexually transmitted diseases (STDs)		
No (ref)	1.0	1.0
Yes	1.27 (0.63-2.54)	1.73 (0.39-7.72)

Abbreviations.

OR = Odds Ratio.

95% CI = 95% Confidence Interval.

Ref = Reference Group.

polyomaviruses, such as BKPyV and JCpV. For example, one study investigated the stability of antibodies to these viruses over an 11-year period and found that 96% of individuals remained seropositive for BKPyV, while 57% remained seropositive for JCpV (Antonsson et al., 2010). This supports the possibility that WUPyV and HPyV6 might also induce prolonged, stable immune responses. Because our study included only serology and not viral DNA detection, we were unable to assess viral reactivation or active replication as a sign of active viral infection. Future studies incorporating DNA-based detection could help clarify whether stable antibody levels correspond to persistent latent infection, intermittent viral shedding or periodic reactivation.

Previous studies have very limitedly examined possible risk factors influencing WUPyV and HPyV6 serology. Certain risk factors, including age and compromised immune systems due to organ transplantation or HIV, have been explored. However, limited data exists on individual characteristics and lifestyle factors that could relate to serological outcomes. Our findings indicate that sociodemographic factors (marital status and educational attainment), lifestyle factors (tobacco use and

alcohol consumption), immunological/allergic conditions and sexual behavior are not significantly correlated to baseline seropositivity for either virus in our study.

Previous research has indicated that viral infections are associated with miscarriage, although the mechanisms behind this are not yet fully understood (Giakoumelou et al., 2016). While no prior studies have specifically evaluated HPyV6 in this context, insights from other polyomaviruses could offer some insights. Zhou et al. reported a causal relationship between JCpV and MCPyV IgG seropositivity and miscarriage, suggesting that chronic polyomavirus infections may impact pregnancy outcomes through immune-mediated mechanisms (Zhou et al., 2024). Interestingly, these findings diverged from earlier work by Tagliapietra et al., who found no such association for JCpV based on viral DNA detection (Tagliapietra et al., 2019). In our study, women with a history of miscarriage were significantly less likely to maintain HPyV6 antibodies over time. Similarly to our findings, Mazziotta et al. also reported that the optical density of MCPyV IgG antibodies in serum samples of women with spontaneous miscarriage was lower compared to those with elective termination of pregnancy (Mazziotta et al., 2021). The underlying mechanism for this inverse relationship could involve several immunological and hormonal factors that regulate immune regulation, potentially impacting antibody production and maintenance. However, our findings are observational and do not allow conclusions about causality or directionality. It is possible that immunological alterations associated with prior miscarriage, rather than HPyV6 infection itself, influence antibody persistence. Therefore, the observed relationship should be considered hypothesis-generating and warrants further investigation. Future research should include larger cohorts and broader serological analyses of additional viruses to confirm this finding.

During pregnancy, the immune system undergoes modulation to tolerate paternal antigens expressed by the fetus, while still maintaining its ability to defend against pathogens. It has been suggested that pregnancy might lead to higher susceptibility to some infections and reactivation of certain latent infections. In our study, we were able to examine the impact of pregnancy on antibody levels by comparing baseline levels (when all women were pregnant) with those at the 12-month follow-up (when the women were no longer pregnant). We found no statistically significant differences in antibody levels for WUPyV and HPyV6 between baseline and 12 months. These findings suggest that pregnancy does not significantly affect antibody levels for either virus, further supporting the perceived overall stability of WUPyV- and HPyV6-specific antibody levels over time. One study aimed to assess whether pregnancy results in higher prevalence of WUPyV, but found no WUPyV DNA in plasma, urine, or respiratory samples from either pregnant or non-pregnant women (Csoma et al., 2012). As far as we know, no studies have yet explored the impact of pregnancy on HPyV6 prevalence.

Interestingly, men exhibited consistently higher MFI levels than women for both WUPyV and HPyV6 across all follow-up points, suggesting a possible difference in antibody response, although the significance of this observation remains unclear. Previous studies have demonstrated lower overall IgG antibody levels in men compared to women (Harkness et al., 2019; Gonzalez-Quintela et al., 2008). In addition, a review by Klein and Flanagan highlights that females generally mount stronger antibody responses than males, likely due to a combination of genetic, hormonal, and immunological factors (Klein and Flanagan, 2016). This phenomenon was not seen in our study, which might be due to several factors, including differences in exposure history, infection dynamics, and immune system regulation specific to WUPyV and HPyV6. Further research is needed to determine whether this finding reflects a true sex-based difference in immune responses to these polyomaviruses or is influenced by other confounding factors.

Our investigation into the potential relationship between spouses' antibody levels showed no significant associations for either WUPyV or HPyV6, suggesting that transmission within couples is unlikely to be the

primary mode of viral acquisition and instead transmission dynamics may be more strongly influenced by early-life exposure. Seroprevalence studies indicate that infants under six months of age exhibit high seropositivity rates, likely due to maternal antibody transfer, followed by a decline in seropositivity as maternal antibodies wane, and subsequent rapid increase during childhood (van der et al., 2013; Nguyen et al., 2009). Longitudinal studies with long-term follow-up are needed to determine how immune responses to these viruses fluctuate over the lifespan.

Several limitations should be considered when interpreting our findings. First, the cohort consisted exclusively of adults, which limits the ability to investigate initial seroconversion. As both WUPyV and HPyV6 are believed to be primarily acquired in early childhood, our findings most likely reflect long-standing immunity rather than recent or primary seroconversion event. Additionally, serological data for closely related polyomaviruses, such as KIPyV and HPyV7, were not available for this study cohort, which limits our ability to make direct comparative assessments regarding whether the observed serological patterns are virus-specific or broader generic characteristics. The relatively small sample sizes for certain subgroup analyses, particularly for WUPyV, may have limited the detection of more subtle associations. Furthermore, the study relied solely on serological data, and the absence of concurrent viral DNA detection restricted our ability to investigate active infection, such as viral reactivation or active replication, which could provide further insights into transmission dynamics. Finally, the use of a secondary data-driven cut-off value to stratify participants by high versus low antibody levels was introduced due to the high baseline seropositivity observed for WUPyV. This threshold has not been independently validated and should be interpreted with caution as an exploratory tool.

Importantly, while this investigation was restricted to WUPyV and HPyV6, the broader polyomavirus landscape of this specific cohort – specifically regarding JCPyV and BKPyV – has been fully characterized in our previous work (Laine et al., 2023b). Because those classic polyomaviruses belong to distinct phylogenetic groups and their epidemiological profiles in this population are already published, the current study was designed strictly to focus on these two less-characterized virus types.

Despite these limitations, our study has several strengths. To our knowledge, this is the first study to assess WUPyV and HPyV6 serology over a prolonged period, offering valuable insights into the stability of antibody responses. This is also the first study to analyze correlations in antibody levels between spouses, offering new information on how these viruses may be transmitted. Additionally, our study included the investigation of key demographic, immunological and lifestyle factors, expanding knowledge of whether these factors influence WUPyV and HPyV6 seropositivity.

Our findings highlight important research gaps, including the need for birth-cohort studies, mother-infant serological assessment and the inclusion of viral DNA detection to better understand the transmission routes, age of initial infection, viral reactivation and transmission dynamics across the lifespan.

To conclude, our study demonstrates high seroprevalence rates and long-term antibody persistence for both WUPyV and HPyV6. While our study provides valuable longitudinal data and our findings contribute to a deeper understanding of these viruses, further research with larger cohorts and longer follow-up duration is needed to better understand the long-term implications of WUPyV and HPyV6 infections.

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CRedit authorship contribution statement

Henni Huhtamo: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. **Hanna K. Laine:** Investigation, Methodology, Writing – review & editing. **Birgitta Michels:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. **Julia Butt:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. **Kari Syrjänen:** Data curation, Investigation, Software, Validation, Writing – review & editing. **Seija Grenman:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Tim Waterboer:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – review & editing. **Stina Syrjänen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – review & editing. **Karolina Louvanto:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: TW serves on advisory boards for Merck (MSD) Sharp & Dohme. The other authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virol.2026.111027>.

Data availability

Data will be made available on request.

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