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The association between viral load and concurrent human papillomavirus infection at the genital and anal sites of young women and the impact of vaccination

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ABSTRACT

Concurrent genital-anal human papillomavirus (HPV) infections may impose an increased anal cancer risk in women with HPV-related genital lesions. High viral load may facilitate genital-anal HPV concurrence. Genital and anal HPV is reduced by a bivalent HPV16/18 vaccine, yet the effect on concurrent genital-anal HPV remains unclear.

This study analyzed viral load in concurrent genital-anal HPV infections, relative to genital-only and anal-only HPV infections and the impact of vaccination in young women. We included 1074 women, who provided both genital and anal swabs. HPV detection and genotyping was performed using the SPF10-DEIA-LiPA25. HPV copy numbers were measured with type-specific qPCRs and corrected for cellular content to obtain the viral load.

Concurrent genital-anal HPV often had significantly higher genital viral load (0.09–371 c/cell) than genital-only HPV (3.17E-04-15.9 c/cell, $p < 0.0001$ to $p < 0.05$). Moreover, nearly all concurrent genital-anal HPV types had higher genital copy numbers per PCR reaction (157-416E04 c/rxn) than anal copy numbers (0.90–884E01 c/rxn, $p < 0.0001$ to $p < 0.001$). Vaccinated women had significantly less infections with HPV16/18 vaccine-types (2.8% vs 13.7%, $p < 0.0001$) and HPV31/35/45 cross-protective types (7.4% vs 21.1%, $p < 0.0001$) than unvaccinated women.

In conclusion, particularly high genital viral load is found in concurrent genital-anal HPV infections, which are effectively reduced by vaccination.

1. Introduction

While cervical cancer incidence has declined over the years, a rise in anal cancer incidence has been reported in developed countries [1,2]. Particularly women with a history of cervical, vulvar or vaginal intra-epithelial neoplasia (CIN/VIN/VAIN) or invasive cancer are at increased risk of anal cancer development [3]. Population-based cervical screening by the Papanicolaou (Pap) smear has become common

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practice. Anal cancer screening is only advised for high-risk groups, such as men who have sex with men [4]. As a consequence, women with a history of (pre)cancerous genital lesions are not screened for possible anal malignancies.

The vast majority of the cervical and anal cancer cases are attributed to a persistent infection with high-risk human papillomavirus (hrHPV) [5]. Concurrent HPV infections are regularly found in the cervical and anal canal of women. In women with high-grade CIN (≥CIN2) a higher frequency of concurrent cervical-anal HPV infections was observed compared to women with low-grade CIN (≤CIN1). Furthermore, the frequency of concurrent cervical-anal HPV infections, often with identical HPV types, increased with CIN lesion severity [6]. Therefore, women with a history of genital lesions who experience concurrent genital-anal HPV infections may be deficient in the immunological response controlling these infections and thus more prone to anal malignancies. In the current study, concurrent genital-anal HPV infections refers to identical HPV types that are concurrently present in the genital and anal sites of women.

Certain virological factors may be associated with the spread of HPV types throughout the anogenital site, one of which is HPV viral load. Previous research showed that HPV transmission within heterosexual couples is facilitated by increased HPV viral load [7]. Moreover, a dose-dependent relationship was found between viral load in men and type-specific HPV concordance in their female partner and vice versa [8, 9]. Individuals with hampered immunological control on an HPV infection tend to have highly productive HPV infections in multiple anatomical sites. Thus, concurrent genital-anal HPV infections may have increased viral load compared to HPV infections present only in the genital or anal site.

Approximately 70% and 87% of cervical and anal cancer cases are attributed to hrHPV 16 and 18, respectively [10]. To prevent the majority of HPV-related cancer cases, the Netherlands implemented a bivalent vaccine against hrHPV 16 and 18 in 2009 for teenage girls. Over the years, vaccination proved highly effective against genital and anal hrHPV 16 and 18 infections. Additionally, a cross-protective effect was reported against genital and anal hrHPV 31, 35 and 45 [11–13]. Nonetheless, these studies only examined the impact on separate genital or anal HPV infections and did not take vaccine impact on concurrent genital-anal HPV infections into account.

In present study we examined 1) the HPV viral load in concurrent genital-anal HPV infections compared to HPV viral load in genital-only and anal-only HPV infections and 2) the potential varying impact of vaccination on concurrent genital-anal HPV infections compared to genital-only and anal-only HPV infections.

2. Materials and methods

2.1. Study population

For this study, samples obtained from female participants from the Papillomavirus Surveillance among STI Sexually transmitted infection clinic YOungsters in the Netherlands (PASSYON) study were used for retrospective analysis. A detailed description of the study has been published previously [14]. In brief, the PASSYON study was set-up in 2009 before the introduction of the HPV vaccination program. It is a biennial cross-sectional study, which includes male and female sexual health center visitors aged 16 to 24. All participants were asked to provide a self-collected vaginal swab (hereafter named a genital swab) and a random subset was also asked for a self-collected anal swab. Personal information, including vaccination status, was self-reported. All participants provided informed consent. This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Medical Ethical Committee of the University of Utrecht, the Netherlands (protocol number 08/397).

2.2. HPV DNA isolation, detection, and genotyping

Genital and anal swabs from women were collected in 1 ml UTM^R (MLS, Menen, Belgium) and kept at −20 °C until analysis. Total DNA isolation was performed with 200 µl liquid per swab with the MagNA PuRE 96 (Total Nucleic Acid Isolation Kit, Roche, Rotkreuz, Switzerland). Total DNA was eluted in 100 µl elution buffer of which 10 µl was used for HPV-DNA amplification. Phocine herpes virus-1 (PhHV-1) was added as an internal control for DNA isolation. HPV amplification was performed with the broad-range SPF$_{10}$-DEIA-LiPA$_{25}$ assay according to manufacturer's protocol (DDL Diagnostic Laboratory, the Netherlands) to simultaneously detect and genotype up to 25 HPV types, including the hr types, in each sample. A detailed description on the SPF10-DEIA-LiPA25 assay is given elsewhere [15].

2.3. Type-specific viral load assays

Type-specific viral load assays were developed in-house to quantify the amount of HPV genomic copies in the DNA isolates for 11 hrHPV types (16/18/33/35/39/45/51/52/56/58/59), potential hrHPV66, and low-risk (lr) HPV6 and HPV11 [16,17]. Due to technical difficulties, the HPV31 viral load assay was excluded from this research. The time between initial HPV genotyping and viral load quantification was approximately 2–9 years. Each viral load assay contains HPV type-specific primers and probes that bind a region of the major capsid protein encoding L1 gene. Briefly, 5 µl total DNA isolate was suspended in 15 µl mastermix containing LightCycler 480 Probes Master (Roche), 400 nM forward primer, 400 nM reverse primer, and 100 nM probe. The viral load assays were carried out on the Roche LightCycler 480 platform (Roche) with cycling conditions of 95 °C for 10 min, 50 cycli of 95 °C for 15 s, and 60 °C for 30 s. The amount of HPV genomic copies per reaction (c/rxn) was corrected for cellular content with a β-actin qPCR to obtain the viral load, which is defined as copies per cell (c/cell). Samples that initially tested positive for HPV, but which were negative in the viral load and/or β-actin tests were excluded from further analyses.

2.4. Statistical analysis

In this study, we define concurrent genital-anal HPV infections as infections with identical HPV types found in both the genital and anal sites. Overall genital and anal HPV infection frequency and concurrent genital-anal HPV infection frequency were calculated for any HPV type, any hrHPV type, any lrHPV type and the individual HPV types. Genital, anal and concurrent genital-anal HPV infection frequencies were calculated from the number of women who provided a genital and anal swab. The expected frequencies of concurrent genital-anal HPV infections by chance (i.e. the presence of an HPV infection was independent of the other anatomical site) were calculated by multiplying the HPV genotype frequency in the genital samples with the HPV genotype frequency in the anal samples [7]. Potential significant differences between the observed and expected frequency of concurrent genital-anal HPV infections were analyzed with the Fisher's exact test. The phi-correlation statistic was used to measure the degree of genital-anal concurrence, ranging from a strong negative correlation (−1) to a strong positive correlation (+1). A value of zero suggests no correlation [18]. The Mann-Whitney U statistic was used to 1) compare the median genital and anal viral load of concurrent genital-anal HPV infections to the respective median viral load of HPV only found in the genital or anal site, 2) examine potential differences in the β-actin content in genital and anal swabs, 3) compare the median genital and anal copy number of concurrent genital-anal HPV infections and 4) compare the median copy number of genital-only and anal-only HPV infections. All analyses were performed in R 4.0.2. A p-value <0.05 was considered significant, with **** as $p < 0.0001$, *** as $p < 0.001$, ** as $p < 0.01$, * as $p < 0.05$ and "ns" as non-significant.

3. Results

3.1. Population overview and the frequency of concurrent genital-anal HPV infections

From 2009 to 2017, a total of 9589 people were enrolled in the PASSYON study, including 6506 (68%) women and 3083 men (32%; Fig. 1). For the present analysis, we included all women who provided a genital and anal swab (n = 1074 (17%)). Vaccine-eligibility was reported for 584 (54%) women, of which 352 (33%) women were vaccinated, 190 (18%) women were unvaccinated and 42 (4%) had unknown vaccination-status. The frequencies of concurrent genital-anal HPV infections were calculated for the HPV types detected with the SPF_{10}-DEIA-$LiPA_{25}$ (Table 1). Overall, more women were HPV positive in the genital swab (77.5%, n = 832) than anal swab (46.3%, n = 497). Also, hrHPV (43.9%, n = 472) was more prevalent in both the genital and anal sites compared to lrHPV (20.5%, n = 220). The frequency of concurrent genital-anal infections was significantly higher than could be expected by chance for nearly all HPV types, with $p < 0.0001$). HPV34 and HPV42 were borderline significant ($p = 0.044$) and non-significant ($p = 0.069$), respectively. No clear difference in genital-anal correlation was observed for any lrHPV (phi = 0.45) and any hrHPV (phi = 0.46), ranging from 0.11 (HPV42) to 0.63 (HPV6) and from 0.40 (HPV59) to 0.63 (HPV58).

3.2. Type-specific viral load of HPV in concurrent genital-anal, genital-only and anal-only infections

For lrHPV6/11, hrHPV16/18/33/35/39/51/52/56/58/59 and potential hrHPV66, a comparison was made between the genital and anal viral load (copies/cell) of concurrent genital-anal infections and the viral load of genital-only and anal-only infections, respectively. In this sample set, initial HPV typing found a total of 1276 HPV types, of which 1202 (94%) were viral load positive and 74 (6%) were either β-actin and/or HPV viral load negative. HPV11, HPV16 HPV33, HPV39, HPV51, HPV56 and HPV66 types in concurrent genital-anal infections had significantly higher median viral loads than in genital-only infections, with 0.7 vs 0.000317 c/cell, 19.5 vs 1.78 c/cell, 371 vs 0.0043 c/cell, 1.68 vs 0.018 c/cell, 1.21 vs 0.078 c/cell, 11.9 vs 1.15 c/cell and 2.69 vs 0.21 c/cell, respectively. HPV18, HPV35, HPV58 and HPV59 in concurrent genital-anal infections also had higher genital viral loads compared to the viral load of genital-only infections, though not significantly (Fig. 2A, Table S1). Furthermore, a trend was observed in which the anal viral load of HPV16, HPV18, HPV56 and HPV59 in concurrent genital-anal infections was higher than the viral load of corresponding HPV types in anal-only infections. Significance may not have been reached due to relatively few anal-only infections. Exceptions were the significantly higher anal viral load of HPV66 in concurrent genital-anal infections than in anal-only infections (25.9 vs 0.041 c/cell) and vice versa for HPV35 (0.016 vs 62.1 c/cell) (Fig. 2B, Table S1). No anal-only infections with HPV11, HPV45 and HPV58 were detected. We were not able to compare genital and anal HPV viral load in women with concurrent genital-anal infections and women with genital-only and anal-only infections, due to a significant difference in cellular content in genital swabs and anal swabs ($p < 0.0001$, Fig. S1). Nevertheless, a comparison of absolute HPV copy numbers indicated that concurrent genital-anal HPV infections had significant higher genital copy numbers than anal copy numbers (Fig. S2, Table S2). Similarly, genital-only infections also had higher absolute HPV copy numbers than anal-only infections, ableit not significantly (Fig. S3, Table S2).

3.3. The effect of vaccination on concurrent genital-anal HPV infections with HPV16/18 vaccine types and HPV31/35/45 cross-protective types

The impact of vaccination with the bivalent vaccine has only been analyzed for genital and anal HPV infections separately. The effect on concurrent genital-anal HPV infections has not been investigated so far. For this analysis, 542 vaccine-eligible women were selected, comprising 352 vaccinated women and 190 unvaccinated women. No HPV was detected in both swabs of 78 (22%) vaccinated women and 48 (25%) unvaccinated women. HPV was detected in at least one swab of 274 (78%) vaccinated women and 142 (75%) unvaccinated women. Considering all eligible women, infections with the bivalent HPV16/18 vaccine types and HPV31/35/45 cross-protective types were detected in 10 (2.8%) and 26 (7.4%) vaccinated women and in 26 (13.7%) and 40 (21.1%) unvaccinated women, respectively (Fig. 3). Infection prevalence was significantly different in vaccinated and unvaccinated women, with $p < 0.0001$ for both groups. Concurrent genital-anal infections with HPV16/18 and HPV31/35/45 were detected in one (0.3%) and seven (2.0%) vaccinated women and in 12 (6.3%) and 17 (8.9%) unvaccinated women and differed significantly between vaccinated and unvaccinated women ($p < 0.0001$ and $p < 0.001$), respectively. HPV16/18 and

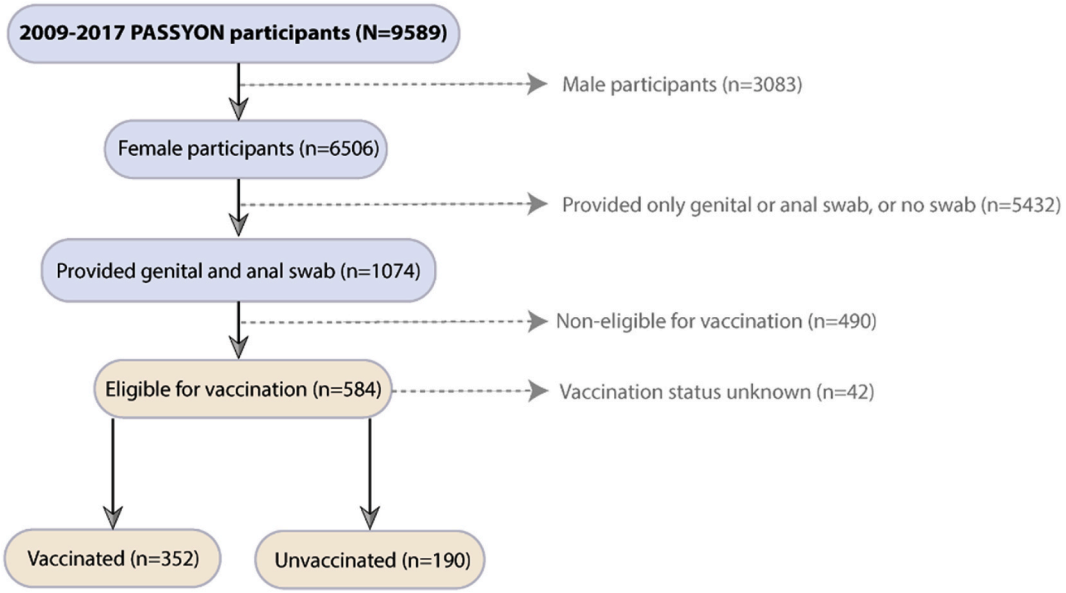

Fig. 1. Flowchart depicting the inclusion numbers from the PASSYON study.

Table 1
The frequencies (%) of any HPV, any hrHPV, any lrHPV and individual HPV types in the genital and anal sites. The observed and expected proportion of concurrent genital-anal infections are given. Phi-correlations indicate the degree of genital-anal concurrence.

HPV type	Prevalence of HPV infection		Genital-anal concurrence		Phi	Fisher's P
	Genital (n = 1074)	Anal (n = 1074)	Expected	Observed		
Any HPV	832 (77.5)	497 (46.3)	385 (35.8)	472 (43.9)	0.39	<0.0001
any hrHPV	729 (67.9)	414 (38.5)	281 (26.2)	394 (36.7)	0.46	<0.0001
HPV16	99 (9.2)	44 (4.1)	4.1 (0.4)	34 (3.2)	0.49	<0.0001
HPV18	53 (4.9)	27 (2.5)	1.3 (0.1)	17 (1.6)	0.43	<0.0001
HPV31	104 (9.7)	51 (4.7)	4.9 (0.5)	35 (3.3)	0.45	<0.0001
HPV33	45 (4.2)	21 (2)	0.9 (0.1)	15 (1.4)	0.47	<0.0001
HPV35	26 (2.4)	22 (2)	0.5 (0)	13 (1.2)	0.53	<0.0001
HPV39	83 (7.7)	38 (3.5)	2.9 (0.3)	27 (2.5)	0.45	<0.0001
HPV45	27 (2.5)	14 (1.3)	0.4 (0)	9 (0.8)	0.45	<0.0001
HPV51	249 (23.2)	148 (13.8)	34.3 (3.2)	117 (10.9)	0.53	<0.0001
HPV52	211 (19.6)	114 (10.6)	22.4 (2.1)	94 (8.8)	0.54	<0.0001
HPV56	116 (10.8)	67 (6.2)	7.2 (0.7)	49 (4.6)	0.52	<0.0001
HPV58	47 (4.4)	21 (2)	0.9 (0.1)	20 (1.9)	0.63	<0.0001
HPV59	47 (4.4)	21 (2)	0.9 (0.1)	13 (1.2)	0.40	<0.0001
HPV66 [a]	163 (15.2)	96 (8.9)	14.6 (1.4)	75 (7)	0.55	<0.0001
HPV68	53 (4.9)	26 (2.4)	1.3 (0.1)	19 (1.8)	0.50	<0.0001
any lrHPV	460 (42.8)	270 (25.1)	115.6 (10.8)	220 (20.5)	0.45	<0.0001
HPV06	132 (12.3)	107 (10)	13.2 (1.2)	80 (7.4)	0.63	<0.0001
HPV11	23 (2.1)	11 (1)	0.2 (0)	10 (0.9)	0.62	<0.0001
HPV34	12 (1.1)	4 (0.4)	0 (0)	1 (0.1)	0.14	0.04401
HPV40	24 (2.2)	16 (1.5)	0.4 (0)	8 (0.7)	0.40	<0.0001
HPV42	19 (1.8)	4 (0.4)	0.1 (0)	1 (0.1)	0.11	0.069
HPV43	42 (3.9)	30 (2.8)	1.2 (0.1)	20 (1.9)	0.55	<0.0001
HPV44	33 (3.1)	22 (2)	0.7 (0.1)	17 (1.6)	0.62	<0.0001
HPV53	187 (17.4)	102 (9.5)	17.8 (1.7)	77 (7.2)	0.50	<0.0001
HPV54	86 (8)	38 (3.5)	3 (0.3)	25 (2.3)	0.41	<0.0001
HPV70	19 (1.8)	7 (0.7)	0.1 (0)	5 (0.5)	0.43	<0.0001
HPV74	40 (3.7)	17 (1.6)	0.6 (0.1)	10 (0.9)	0.37	<0.0001

[a] HPV66 is a potential hrHPV type.

HPV31/35/45 genital-only infections were detected in seven (2.0%) and 15 (4.3%) vaccinated women and in 12 (6.3%) and 19 (10.0%) unvaccinated women, both with significantly different infection prevalence in vaccinated and unvaccinated women ($p < 0.05$).

4. Discussion

In this research, we showed that particularly genital HPV infections with high viral load are associated with a concurrent HPV infection in the anal site. Furthermore, we reported a specifically large impact of vaccination on the frequency of concurrent genital-anal HPV infections with the bivalent vaccine types (HPV16/18) and the cross-protective types (HPV31/35/45).

The prevalence of HPV in our study population was relatively high, which is likely due to the increased exposure to HPV in sexual health center visitors. Similar to previous studies, hrHPV types were more prevalent than lrHPV types [19,20]. We also found that the prevalence of concurrent genital-anal HPV infections with nearly all HPV types was higher than could be expected by chance. In contrast, Wei et al. (2018) reported an overall poor degree of HPV concurrence, especially between the vaginal or vulvar sites and the perianal site [21]. Nevertheless, their study population comprised women from the general population where lower exposure to HPV may influence HPV concurrence rates. We did not observe a clear difference in genital-anal correlation for lrHPV and hrHPV types. This is in line with previous research, which reported similar Phi correlations for cervical-anal concurrence with any lrHPVs (phi = 0.443) and any hrHPVs (phi = 0.402) [18].

Sexual intercourse is the main factor behind HPV transmission. Multiple studies have suggested alternative non-sexual routes of transmission, which include contact between the anogenital site and the fingers, mouth or other skin contact [22]. Self-inoculation or partner-assisted inoculation have also been described as a potential route of HPV transmission [23], in which the cervix is proposed as the main source for HPV infection in the anus [20]. Accordingly, a higher risk for cervical-to-anal HPV infection compared to anal-to-cervical HPV infection was reported with respective hazard ratios of 14.2 (95% CI = 9.86–20.05) and 7.08 (95% CI = 3.94–12.7). Moreover, 63% of incident anal HPV infections following a cervical HPV infection were detected in heterosexual women without a reported history of anal sex [24]. Therefore, genital intercourse appears to be a larger contributing factor in anal HPV positivity than anal intercourse. Wei et al., reported a hazard ratio of 51.5 (95% CI = 39.3–67.6) for acquiring an anal hrHPV infection with a prior genital hrHPV infection compared to no prior genital hrHPV infection, while the hazard ratio for acquiring a genital hrHPV infection with a prior anal hrHPV infection was 18.9 (95% CI = 11.3–31.5) compared with no prior anal hrHPV infection [25].

Previous research showed that type-specific HPV transmission in heterosexual couples is associated with a high viral load [7–9]. High viral load in general suggests a lack of the immune system to control the infection in an individual and increases transmission possibilities from the genital to anal site or vice versa. Unfortunately, no immunology data are available in this study. Nevertheless, we observed that concurrent genital-anal HPV infections also possess higher viral loads compared to genital-only and anal-only HPV infections. Particularly, the genital viral load of concurrent genital-anal HPV infections was increased compared to the viral load of genital-only HPV infections for the majority of HPV types. Therefore, our study suggests that the genital site seems to be the location where a HPV infection reaches high viral load, facilitating the spread to the anal site, creating a concurrent infection. In addition, nearly all HPV types in concurrent genital-anal infections had significantly higher genital copy numbers than anal copy numbers. Supporting this is the fact that many anal HPV infections in our populations concurrently existed in the genital site. Previous research reported significantly lower clearance rates of HPV in the genital site compared to the anal site of women [26,27]. Hence, prolonged HPV persistence, possibly due to lack of immunological control of the HPV infection, may contribute to higher viral loads in the genital site compared to the anal site. Alpha-papillomaviruses share a tropism for the mucosal epithelium

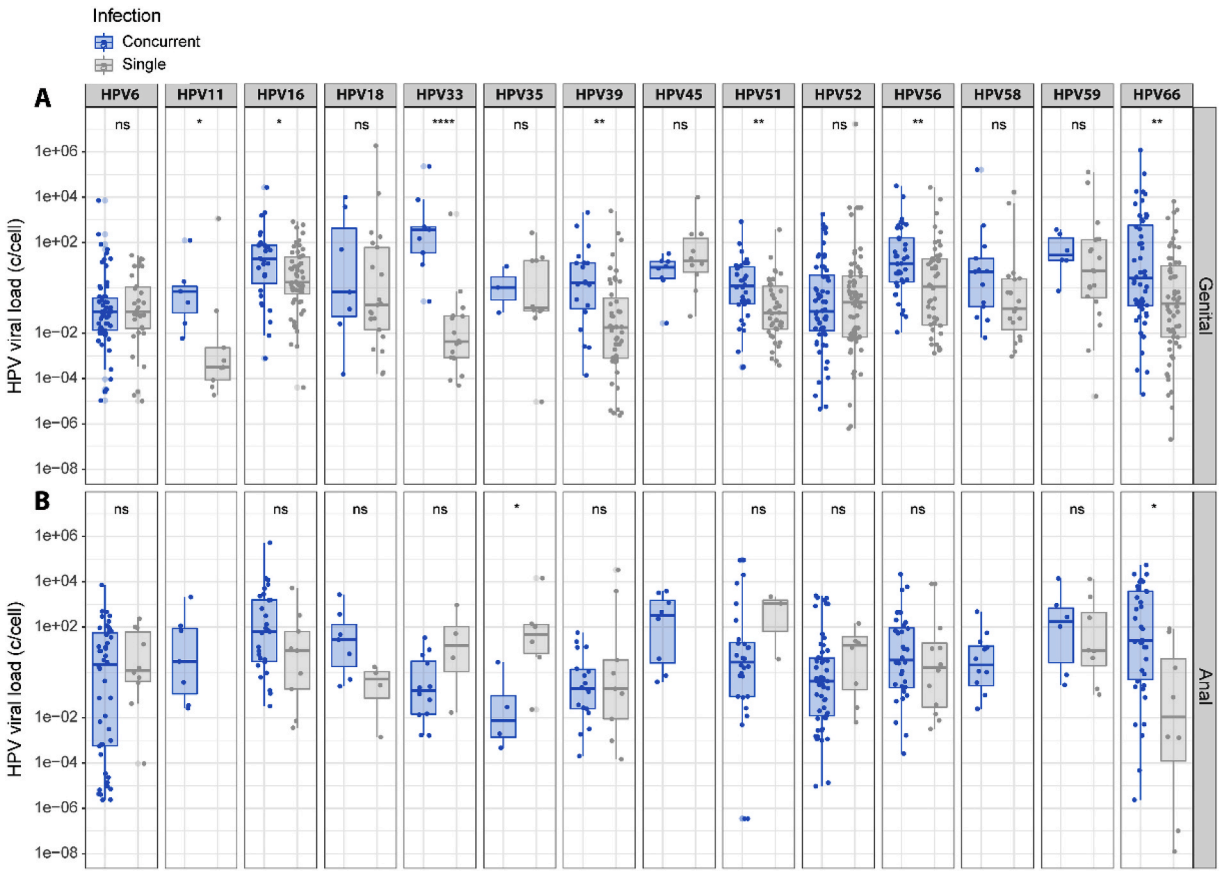

Fig. 2. Viral load of lrHPV6/11, hrHPV16/18/33/35/39/51/52/56/58/59 and potential hrHPV66. Panel A and B respectively visualize the comparisons of the genital and anal viral load of type-specific HPV concurrently found in the genital and anal sites (blue) to the viral load of HPV only found in the genital or anal site (grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of the genital site, yet the alpha-types have a varying predisposition for different anatomical sites, including the vagina, endocervix, ectocervix, or even the oral cavity [28–30]. This may be one explanation as to why there were no significant differences in viral load/copy number for alpha-7 members HPV18, HPV45, and HPV59. On the other hand, absence of significance may also be due to their relatively low prevalence.

Vaccination significantly reduced HPV infections with the bivalent vaccine types (HPV16/18) and cross-protective types (HPV31/35/45). Moreover, concurrent genital-anal HPV infections with these types were reduced the most, followed by genital-only infections. To our knowledge, this is the first study that shows the considerable impact of vaccination on concurrent genital-anal HPV infections. Previous research has shown that the bivalent vaccine reduced the prevalence of HPV16/18, but also cross-protects against HPV16-related HPV31/35 and HPV18-related HPV45, and potentially HPV51 [11]. Similarly, vaccination with the quadrivalent vaccine (against HPV6/11/16/18) reduced the prevalence of HPV types belonging to the alpha-7,9,10 species (alpha-7 and 9 include HPV18/45 and HPV16/31/35, respectively) in vaccinated women and their male partners compared to unvaccinated women and their male partners [31]. Interestingly, the genital viral load of HPV16/18 was reduced in women vaccinated with the bivalent vaccine, while this was not observed for the cross-protective HPV31/35/45 types [16]. The genital viral load of HPV6/11/16/18 was also reduced in women vaccinated with the quadrivalent vaccine compared to unvaccinated women [31]. Considering the above, it is likely that vaccination either completely eliminates or hampers the establishment of concurrent genital-anal HPV16 and 18 infections by reducing the viral load in the genital site. Little is known about the mechanism by which vaccination reduces the frequency of concurrent genital and anal infections with HPV31/35/45. It is has been suggested that cross-protection against these types is facilitated by a T-cell mediated immune response, which is responsible for the viral control of HPV16/18 breakthrough infections in vaccinated women [16]. Nonetheless, low numbers of infections with the vaccine types or cross-protective types may have affected our results.

Our study has several limitations. Vaccination status is self-reported and baseline HPV status prior to vaccination is unknown. Vaccine effectiveness is suggested to be impaired when the individual is already HPV-positive [32,33], although new evidence is emerging on potential benefits of vaccinating baseline positive individuals [34]. Also, previous research confirmed HPV16/18 seropositivity in 96% of the PASSYON women, minimizing recall bias [11]. Due to a difference in cellular content in the genital and anal swabs, we were limited to comparing the genital and anal HPV copy number. Differences in cellular content of swabs taken from different anatomical sites has been reported before. Specifically, appropriate swabbing of the anal cavity is challenging compared to the vaginal canal, because of the difference between the smooth mucosal uterine cervix membrane and the creased lining of the anus [35].

5. Conclusions

We report an increased genital viral load of HPV in concurrent genital-anal infections compared to the viral load of HPV in genital-only infections. We therefore suggest that the genital viral load facilitates the dispersal of HPV to the anal site. In addition, concurrent genital-anal HPV infections with the bivalent vaccine types (HPV16/18) and cross-protective types (HPV31/35/45) were most significantly reduced in vaccinated women compared to unvaccinated women. This study

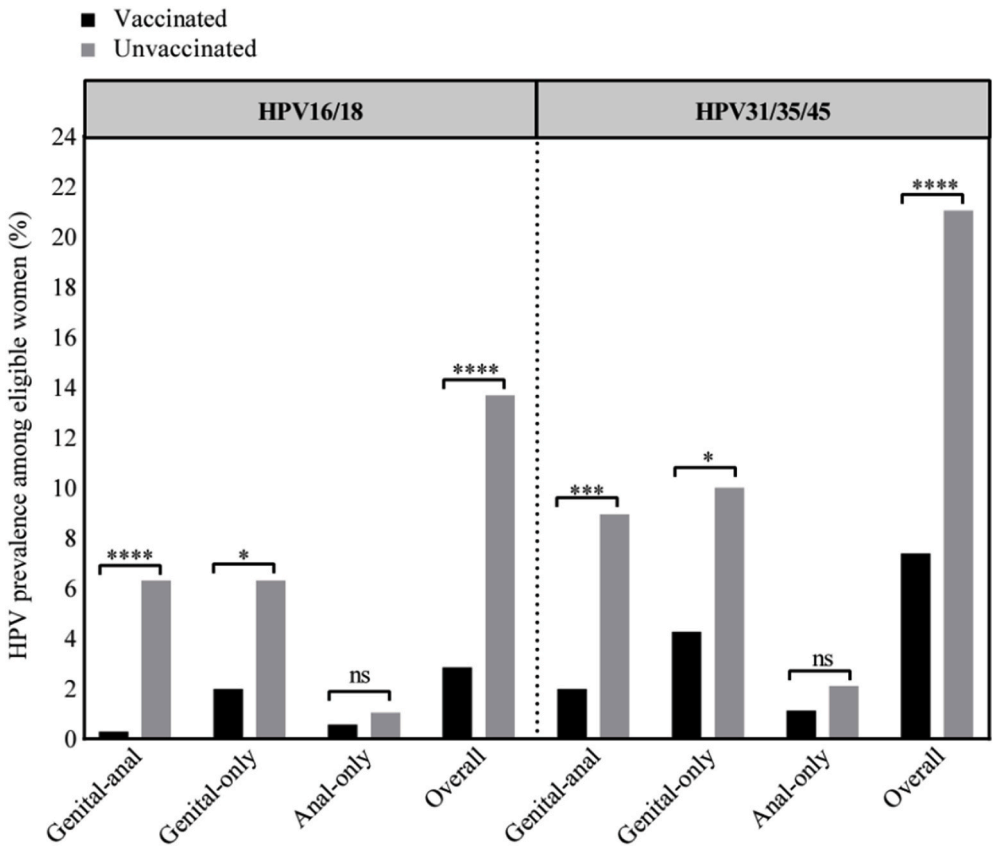

Fig. 3. The frequency of HPV infections with the bivalent vaccine types (HPV16/18) and bivalent cross-protective types (HPV31/35/45) in all eligible vaccinated and unvaccinated women. Infections were stratified into four categories: concurrent genital-anal, genital-only, anal-only, and overall.

provides new insights in the virological factors that contribute to the establishment of concurrent genital-anal HPV infections and highlights the importance of vaccination as a strategy against these infections.

Author contributions

Conceptualization, Kahren van Eer and Ihsane Laâbi; Data curation, Kahren van Eer; Formal analysis, Kahren van Eer; Funding acquisition, Audrey King; Investigation, Kahren van Eer, Ihsane Laâbi, Renske Steenbergen and Audrey King; Methodology, Kahren van Eer and Ihsane Laâbi; Project administration, Audrey King; Resources, Audrey King; Software, Kahren van Eer; Supervision, Renske Steenbergen and Audrey King; Validation, Kahren van Eer and Ihsane Laâbi; Visualization, Kahren van Eer; Writing – original draft, Kahren van Eer; Writing – review & editing, Kahren van Eer, Birgit van Benthem, Renske Steenbergen and Audrey King. All authors will be informed about each step of manuscript processing including submission, revision, revision reminder, etc. via emails from our system or assigned Assistant Editor.

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Institutional review board statement

This study was approved according to the guidelines of the Declaration of Helsinki and approved by the Medical Ethical Committee of the University of Utrecht, the Netherlands (protocol number 08/397).

Informed consent statement

Data was obtained anonymously. Informed consent was obtained from all subjects involved in the study.

Data availability statement

All relevant data are within the manuscript and its Supplementary Materials section.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: R. D.M.S. has a minority share in Self-screen BV, a university spin-off company. The other authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

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