

Betapapillomavirus natural history and co-detection with alphapapillomavirus in cervical samples of adult women

Talía Malagón¹, Aline Lopes Ribeiro², Emily Montosa Nunes², Tarik Gheit³, Mariam El-Zein¹, Luisa Villa², Eduardo Franco¹, and Laura Sichero²

¹McGill University Division of Cancer Epidemiology

²Universidade de Sao Paulo Instituto do Cancer do Estado de Sao Paulo

³International Agency for Research on Cancer

August 31, 2023

Abstract

Background: Human papillomaviruses (HPV) of the genus *Betapapillomavirus* can infect both cutaneous and mucosal sites, but research on its natural history at mucosal sites remains scarce. We examined the risk factors and co-detection patterns of HPVs of the *Betapapillomavirus* and *Alphapapillomavirus* genera in cervical samples of the Ludwig-McGill cohort study. **Methods:** We assessed a subset of 505 women from the Ludwig-McGill cohort study from São Paulo, Brazil. Cervical samples over the first year of follow-up were tested for DNA of over 40 alphapapillomavirus types and 43 betapapillomavirus types using a type-specific multiplex genotyping PCR assay. We assessed the risk factors for prevalent and incident betapapillomavirus type detection, and whether types were detected more frequently together than expected assuming independence using permutation tests, logistic regression, and Cox regression. **Results:** We observed significant within-genus clustering but not cross-genus clustering. Multiple betapapillomavirus types were co-detected in the same sample 2.24 (95%CI: 1.65-3.29) times more frequently than expected. Conversely, co-detections of alphapapillomavirus and betapapillomavirus types in the same sample occurred only 0.64 (95%CI: 0.51-0.83) times as often as expected under independence. In prospective analyses, positivity to one HPV genus was associated with a non-significant lower incidence of detection of types in the other genus. Lifetime number of sex partners and new sex partner acquisition were associated with lower risks of prevalent and incident betapapillomavirus detection. **Conclusion:** Betapapillomaviruses are commonly found in the cervicovaginal tract. Results suggest potentially different mechanisms of transmission for betapapillomavirus genital infections other than vaginal sex.

Introduction

Human papillomaviruses (HPV) constitute several genera. The vast majority of previous research on HPV has focused on the genus *Alphapapillomavirus*, whose types infect the mucosal epithelium and are an established cause of anogenital and some oropharyngeal cancers.¹ HPVs of the genus *Betapapillomavirus* normally infect the cutaneous epithelium.² Comparatively much less research has been done on the epidemiology of infections with betapapillomaviruses. However, growing evidence suggests that betapapillomaviruses may play a role in the development of skin cancers, and may be associated with the risk of a subset of head and neck cancers.^{1,3-5} More research on the incidence, transmission, and risk factors for betapapillomavirus infection is warranted.

While betapapillomaviruses were first isolated from the skin and are widely considered to prefer infecting the cutaneous epithelium, there is substantial evidence that they are also able to infect the mucosal epithelium.^{4,6} The detection of betapapillomaviruses in oral and genital samples suggests they are able to infect these sites along with alphapapillomaviruses.⁷⁻¹² There is therefore the potential for biological interactions between

HPV types of different genera. While co-detection patterns and the potential for interactions between HPV types within the alphapapillomavirus genus has been the topic of substantial previous research,¹³⁻¹⁶ there is scant research on cross-genus co-detection patterns and interactions. Biological interactions and co-detection patterns between different HPV types are important to study from a public health perspective due to the risk of genotype replacement following widespread implementation of HPV vaccination programs.¹⁷ Although there is currently little evidence that genotype replacement has occurred for alphapapillomaviruses,¹⁸ the potential for interactions with other HPV genera has not been studied.

We had previously found a fairly high prevalence of betapapillomaviruses in cervicovaginal samples of the Ludwig-McGill cohort study of HPV natural history.¹⁹ While we examined a number of putative risk factors, the only variable that was associated with betapapillomavirus prevalence in univariate analyses was lifetime number of sex partners; curiously, we found a lower betapapillomavirus prevalence among women with four or more lifetime sex partners. We also did not find that alphapapillomaviruses and betapapillomavirus co-detection patterns deviated significantly from expectations under independence. However, these analyses were underpowered as we only tested a restricted number of samples for betapapillomaviruses, and only looked at prevalent but not incident co-detections.

The objective of the current study was to assess a larger number of samples of the Ludwig-McGill cohort study than previously tested to i) examine whether there is evidence of biological interactions between alphapapillomaviruses and betapapillomaviruses, and ii) assess risk factors for betapapillomavirus genital prevalence and incidence.

Methods

Study population

The Ludwig-McGill study cohort study of HPV natural history recruited women at maternal health clinics in São Paulo, Brazil, between 1993 and 1997. Details of the study design have been published elsewhere.²⁰ Eligible women had to be between 18-60 years old, permanent residents of São Paulo, not currently pregnant, with an intact uterus, and with no history of treatment for cervical disease. During the first year, women were followed-up every 4 months; at each visit they provided cervical specimens for HPV genotyping and underwent an interview administered by study nurses collecting data on sociodemographic information, reproductive health, sexual behaviors, smoking, and diet. Women provided signed informed consent to study participation. The study was approved by the ethical review boards of all participating institutions: McGill University, Montreal, Canada; University of Toronto, Ontario, Canada; and the Ludwig Institute for Cancer Research and the Hospital Maternidade Vila Nova Cachoeirinha, both in São Paulo, Brazil.

The current analysis is restricted to a random subset of 505 women sampled from the full cohort who had a follow-up visit within 10 days of the 1-year follow-up date, had valid samples (either β -globin or HPV-positive samples) at the baseline and 1-year visits, and who had complete questionnaire and HPV genotyping data. Our previous publication details the selection process, baseline characteristics, and the betapapillomavirus prevalence at baseline (1st visit) and 1-year (4th visit) in this subset of women.¹⁹ For the current study, we extended genotyping to the 4 month (2nd visit) and 8-month (3rd visit) samples collected from these women in order to measure betapapillomavirus incidence rates over the first year of follow-up and increase the number of observations for analyses.

HPV genotyping

Exfoliated cervical cells were digested with 100 μ g/ml proteinase K for 3-18 hours at 55°C, and DNA was obtained by spin column chromatography. Samples were tested for alphapapillomaviruses by polymerase chain reaction (PCR) amplification using MY09/11 or PGMY09/11 generic primers, and genotyped using hybridization with HPV type-specific oligonucleotide probes or restriction fragment length polymorphism

analysis. This methodology allowed the identification of potentially more than 40 genital alphapapillomavirus genus types from the following species: α -1: HPVs 32, 42; α -3: HPVs 61, 62, 72, 81, 83, 84, 89; α -4: HPV57; α -5: HPVs 26, 51, 69, 82; α -6: HPVs 53, 56, 66; α -7: HPVs 18, 39, 45, 59, 68, 70; α -8: HPV40; α -9: HPVs 16, 31, 33, 35, 52, 58, 67; α -10: HPVs 6, 11, 44; α -11: HPVs 34, 73; α -13: HPV54; and α -14: HPV71.²¹

The presence of betapapillomaviruses was assessed by a type-specific, multiplex genotyping PCR assay followed by genotyping via a bead-based Luminex technology.²² This assay distinguishes 43 betapapillomavirus genus types from the following species: β -1: HPVs 5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47, 93, 98, 99, 105, 118, 124, 143; β -2: HPVs 9, 15, 17, 22, 23, 37, 38, 80, 100, 104, 107, 110, 111, 113, 120, 122, 145, 151; β -3: HPVs 49, 75, 76, 115; β -4: HPV92; and β -5: HPVs 96, 150. In this assay, results are expressed as the median fluorescence intensity (MFI) of at least 100 beads per bead set. For each probe, the MFI values obtained when no PCR product was added to the hybridization mixture were considered the background values. Different bead preparations impact MFI values; because the samples from visits 2 & 3 were tested using a different preparation of beads than the samples from visits 1 & 4, a different MFI cutoff value was used for these visits. Based on MFI values from positive and negative controls reads, cutoffs were computed by adding 20 MFI to 1.1X the median background values in samples obtained in visits 1 and 4;²⁰ and by adding 50 MFI to 1.1X the median background values for samples obtained in visits 2 and 3, with the exception of HPV100 for which the cutoff was computed by adding 100 MFI to 1.1X the median background values for visits 2 and 3.

Statistical analysis

All analyses were type-specific, with HPV type as the unit of observation. The prevalences of betapapillomaviruses were summed across the four visits to derive a time-averaged measure of prevalence over the first year of the study. The incidence rates of betapapillomaviruses were calculated in women who were negative for that specific betapapillomavirus type at the previous visit, with the denominator being the time between visits. We pooled results by summing observations over all HPV types.

To assess whether betapapillomaviruses and alphapapillomaviruses occur more frequently together than expected, we used permutation tests. Permutation tests work by performing rearrangements of the observed data without replacement (permutations).²³ The permutations allow deriving an expected distribution of betapapillomaviruses and alphapapillomaviruses under the assumption that infections are independently distributed across individuals. Because there was evidence of within-genus clustering, we implemented two versions of the permutation test (Figure 1). The first test assumed full independence of all HPV types; in this case, the results for each individual HPV type are permuted across participants, with each HPV type resampled separately. This first test will give the expected distribution of type-specific detections assuming all HPV types are fully independent, both across and within genera. The second test assumed independence between papillomavirus genera but not within genus; in this case, the set of results from all HPV types within a genus from the same person are permuted across participants, with each genus resampled separately. This second test will give the expected distribution of type-specific detections assuming that alphapapillomaviruses are independent from betapapillomaviruses and vice-versa, but accounting for the tendency of HPV types from the same genus to cluster together. We performed 2500 permutation resamples for both tests. We divided the observed number of co-detections by the mean number of co-detections in the permutation resamples to obtain observed over expected values. The 95% confidence intervals were obtained using the 2.5-97.5 percentiles of the permutation resample distribution.

To assess the cross-sectional association between alphapapillomavirus and betapapillomavirus prevalence, we calculated odds ratios (OR) using random effects logistic regression models, with a woman-level random intercept. To assess the prospective association between alphapapillomavirus and betapapillomavirus incidence, we calculated hazard ratios (HR) using Cox proportional hazard regression models, using the robust sandwich estimate of the covariance to account for multiple HPV types per woman.²⁴ All models were fitted separately for alphapapillomavirus and betapapillomavirus types and used the HPV type as the unit of analysis. To assess interactions between genera, the models included as predictors whether the woman was

positive for any HPV type of a different genus at either the same visit sample (logistic regressions) or the previous visit sample (Cox regressions). Multivariable models were adjusted for age and sexual behaviors reported since the previous visit. Sexual behavior variables were included as a priori predictors because our previous publication had found that lifetime number of sex partners might be inversely associated with betapapillomavirus prevalence.¹⁹

Results

All 505 women contributed four study visits each over the 1st year; however, there were missing samples for eight women (1.6%) at the 4-month visit and for twelve women (2.4%) at the 8-month visit, for a final total of 2000 study visits with samples. All available samples were valid (positive for either β -globin or HPV). Baseline characteristics of the subcohort have previously been reported;¹⁹ the mean age was 33.5 years (range: 18–57) and most women were married (49%) or living as married (33%). There were 281/2000 (14%) samples positive for any betapapillomavirus across all study visits. The prevalence and incidence of individual betapapillomaviruses are presented in Table 1. The most prevalent and incident types were HPV38, HPV21, HPV5, HPV22, and HPV8, respectively; these were the only types with a time-averaged prevalence above 1%.

We observed significant clustering within HPV genera (Table 2). Over the four visits, we observed 44 samples positive for two or more alphapapillomavirus types, and 56 samples positive for two or more betapapillomavirus types. This represents respectively 1.74 (95%CI 1.26-2.44) times more alphapapillomavirus type co-detections than expected and 2.24 (95%CI 1.65-3.29) times more betapapillomavirus type co-detections than expected when assuming independence of types. The betapapillomavirus types most often found in co-detection with others were also the most commonly detected types (HPV 38, HPV21, HPV5, HPV22, and HPV8).

Conversely, there were fewer than expected co-detections of alphapapillomaviruses and betapapillomaviruses (Table 2). Over the four visits, we observed 33 samples positive for both an alphapapillomavirus and a betapapillomavirus type together. This represents 0.64 (95%CI 0.51-0.83) times fewer cross-genus co-detections than expected when assuming independence of all types. After accounting for the within-genus expected clustering of both genera, this represented 0.80 (95%CI 0.62-1.06) times fewer cross-genus detections than expected when assuming cross-genus independence.

Cross-sectional and prospective associations between betapapillomavirus positivity, alphapapillomavirus positivity, and sexual risk factors are presented in Table 3 and Table 4. Samples were somewhat less likely to be positive for alphapapillomavirus types if a betapapillomavirus type was present (OR 0.73, 95%CI 0.50-1.07). They were also somewhat less likely to be positive for betapapillomavirus types if an alphapapillomavirus type was present (OR 0.79, 95%CI 0.56-1.12), but neither of these associations were significant. Women were also less likely to become newly positive for alphapapillomavirus types if a betapapillomavirus type was present at the previous visit (HR 0.84, 95%CI 0.49-1.43), and were less likely to become newly positive for betapapillomavirus types if an alphapapillomavirus type was present at the previous visit (HR 0.72, 95%CI 0.45-1.15), but these associations were also not significant. Alphapapillomavirus prevalence and incidence were strongly associated with age, increasing lifetime number of sex partners, and having new sex partners in the previous interval. Conversely, betapapillomavirus prevalence and incidence were not associated with age, and were inversely correlated with the number of lifetime sex partners. While having a new sex partner in the previous interval was not associated with prevalence or incidence, having sex with any partner in the previous interval was associated with a higher incidence of betapapillomavirus (HR 1.56, 95%CI 1.00-2.46). Multivariable adjustment did not change these associations.

Discussion

We found that alphapapillomavirus and betapapillomavirus types were detected together significantly less often than expected under independence of HPV types. Some of this was due to intra-genus clustering: both alphapapillomavirus and betapapillomavirus types are more likely to be detected with other types from the same genus. This clustering is likely due to shared common transmission routes with closely related types. There were still fewer than expected co-detections of alphapapillomavirus and betapapillomavirus types than expected after adjusting for intra-genus clustering, though confidence intervals could not exclude independence.

A substantial amount of previous research has shown that alphapapillomaviruses tend to cluster together; individuals who are positive for one alphapapillomavirus type are at a significantly higher risk of becoming positive for other alphapapillomavirus types.¹³⁻¹⁶ Presumably a large part of this association reflects shared risk factors across all alphapapillomavirus types, the most important being a common sexual transmission route as well as host-level susceptibility to infection. Therefore, it is unsurprising that we also found betapapillomaviruses tend to cluster together. This presumably also reflects a shared common transmission route across all betapapillomavirus types. However, what is much more surprising is the definite lack of clustering between alphapapillomaviruses and betapapillomaviruses. While the CIs of many of the measures of association include the null value expected under independence (1.0), all previous evidence¹³⁻¹⁶ supports that independence between HPV types is not the expectation and that the expected clustering of HPVs of the alphapapillomavirus genus is of the order of OR/HRs of at least 1.5-3.0. Therefore, while the CIs include ORs and HRs of 1.0, we consider that this is a highly unusual result. We examine below two potential hypotheses for why alphapapillomaviruses and betapapillomaviruses may be less likely to be found together than expected.

The first hypothesis relates to potential biological interactions between genera. This could arise, for example, if infection with a first genus induces cross-immunity to subsequent infection with the other genus, or if both genera occupy the same ecological niche and infection with one genus leads to competitive exclusion of the other genus.¹⁷ We believe this hypothesis to be implausible for several reasons. HPV antibodies induced by natural infection tend to be type-specific,²⁵ so it is unlikely that infection with types from one HPV genera would generate strong neutralizing cross-protection against infection with types from another HPV genera. Alphapapillomaviruses and betapapillomaviruses are also believed to have distinct tissue tropisms with preferred anatomical sites of infection.²⁶ While alphapapillomaviruses have evolved to infect the mucosal epithelium, betapapillomaviruses have evolved to infect the cutaneous epithelium.² Nonetheless, both genera are able to infect epithelial sites outside of their preferred trophic niche,^{6,8,27} so it is not impossible they could have overlapping niches leading to competitive exclusion. If this were the case, we would expect alphapapillomavirus types to be more likely to exclude cervicovaginal infections with betapapillomavirus types than the reverse due to their mucosal tropism. However, we did not find that alphapapillomavirus positivity reduced incident betapapillomavirus more than the reverse, so the hypothesis of competitive exclusion does not seem to be supported.

The second hypothesis is that the negative associations between genital alphapapillomaviruses and betapapillomaviruses could reflect independent or inversely correlated transmission mechanisms. Alphapapillomaviruses are sexually transmitted infections, with major risk factors including lifetime number of sex partners and new sex partners.²⁸ Betapapillomaviruses are believed to be transmitted through skin-to-skin contacts,² which include but are not restricted to sexual contacts. Interestingly, we found that lifetime and new sex partners were not risk factors for betapapillomavirus incidence, but having any sex during the previous interval was a risk factor. Most of the women who reported having sex in our study were having sex with a regular partner rather than a new one, and women who were married or living with their partner reported higher frequencies of sexual contacts than single or separated women. It could be that, in this study, having sex is an indicator for being in an ongoing intimate relationship with more skin-to-skin contacts, which include but may not be restricted to sex. It is also possible that betapapillomavirus detections may be detections from recent sex rather than true infections, as the penile epithelium has been found to have a high

betapapillomavirus prevalence.⁹ However, a previous study did not find that vaginal sex within the last 24 hours increased genital betapapillomavirus concordance between heterosexual partners.⁸ Nevertheless, that study and others have found that concordance was higher within than between couples, suggesting some form of transmission through intimate contact.^{8,29} Fingernails could be a potential reservoir for transmission and autoinoculation in the case of oral betapapillomavirus infections.¹¹ We believe it is plausible that the negative association observed between betapapillomavirus and alphapapillomavirus types could partly reflect confounding due to inversely correlated transmission mechanisms, as women in our study who were having more regular sex and intimate skin-to-skin contact were women with fewer lifetime sexual partners or new sex partners. However, our adjustment for these sexual behaviors in multivariable models did not completely attenuate the negative association between alphapapillomaviruses and betapapillomaviruses. There could therefore remain some unmeasured confounding transmission risk factors explaining the negative association between betapapillomaviruses and alphapapillomaviruses.

The most detected betapapillomavirus types in cervicovaginal samples were HPV38, HPV5, HPV21, HPV22, and HPV8, respectively. Moscicki *et al.* also found that HPV38 was the most commonly detected betapapillomavirus type in cervicovaginal samples.⁸ HPV38 is also one of the most commonly detected types in women's oral samples,¹¹ and among the most common betapapillomavirus types in men's genital samples.⁹ Genital HPV21 and HPV22 positivity were also common in male genital samples.⁹ HPV5 and HPV8 were conversely not among the most commonly reported detected types in male genital samples.⁹ HPV5 and HPV8 are the betapapillomavirus types which have been isolated from patients with *epidermodysplasia verruciformis* (EV) and are deemed to be possibly carcinogenic for individuals with EV by the International Agency for Research on Cancer.^{1,30} Oral HPV5 has also been associated with the risk of some head and neck cancers.³

By increasing the numbers of samples tested for betapapillomavirus, we were able to expand on our previous results which only included two observations per woman.¹⁹ The current study confirms our initial surprising findings that lifetime number of sex partners was inversely correlated with betapapillomavirus prevalence, and we propose above a potential explanation for this finding based on a multivariable analysis of sexual risk factors. However, this finding might reflect confounding from sexual behaviors that is specific to our study population, as studies of oral and penile betapapillomavirus infections have not found that number of lifetime sex partners was associated with betapapillomavirus prevalence at these sites.^{9,11,12,31} We are unaware of any other studies that have looked at risk factors for cervicovaginal betapapillomavirus prevalence. Due to the larger sample size, we were able to calculate incidence rates of betapapillomavirus positivity, and also detect lower than expected prevalent and incident co-detections of alphapapillomavirus and betapapillomavirus types which were not previously evident with fewer observations. However, despite the larger sample size, there were still large CIs for some associations; we therefore cannot exclude that our results could be due to chance. Our results would need to be confirmed in other studies of alphapapillomavirus and betapapillomavirus co-detection to verify whether they represent a more general trend across populations.

In conclusion, we found fewer prevalent and incident co-detections of alphapapillomaviruses and betapapillomaviruses than expected if HPV types belonging to these genera were transmitted independently. It is not clear whether these highly unusual findings reflect potential biological interactions between HPV genera, or are the result of inversely correlated transmission mechanisms, or simply represent a chance result. These findings need to be confirmed in other populations. This would help further elucidate the biology of betapapillomaviruses, a genus for which there is growing evidence of a role in carcinogenesis.

Acknowledgements

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

Availability of Data, Software, and Research Materials

Participants of the Ludwig-McGill cohort study did not consent to have their data made publicly available, and confidentiality precludes the publishing of their data. To access the data for research purposes, please contact Eduardo Franco (eduardo.franco@mcgill.ca) or Luisa Villa (l.villa@hc.fm.usp.br). Code for the permutation analysis is available at the Borealis repository: <https://doi.org/10.5683/SP3/MQR8H9>.

Funding

This work was supported by an intramural grant from the Ludwig Institute for Cancer Research (to LLV and ELF); the U.S. National Cancer Institute (CA70269 to ELF); the Canadian Institutes of Health Research (grants MA-13647, MOP-49396, CRN-83320 to ELF). The funders of the study had no involvement in study design; in the collection, analysis, and interpretation of data; neither in the writing of the report; and in the decision to submit the article. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflicts of Interest

ALR, EMN, TG, and LS have no conflicts of interest to declare. TM is a board member of the International Papillomavirus Society. LLV reports grants from Merck & Co. and personal fees from Merck & Co. outside the submitted work. ELF reports grants to his institution from CIHR and the National Institutes of Health during the conduct of the study; and personal fees from Merck. MZ and ELF hold a patent related to the discovery “DNA methylation markers for early detection of cervical cancer”, registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada.

Ethics statement

Study participants provided signed informed consent to study participation. The study was approved by the ethical review boards of all participating institutions: McGill University, Montreal, Canada; University of Toronto, Ontario, Canada; and the Ludwig Institute for Cancer Research and the Hospital Maternidade Vila Nova Cachoeirinha, both in São Paulo, Brazil. The study was performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

Figures & Tables

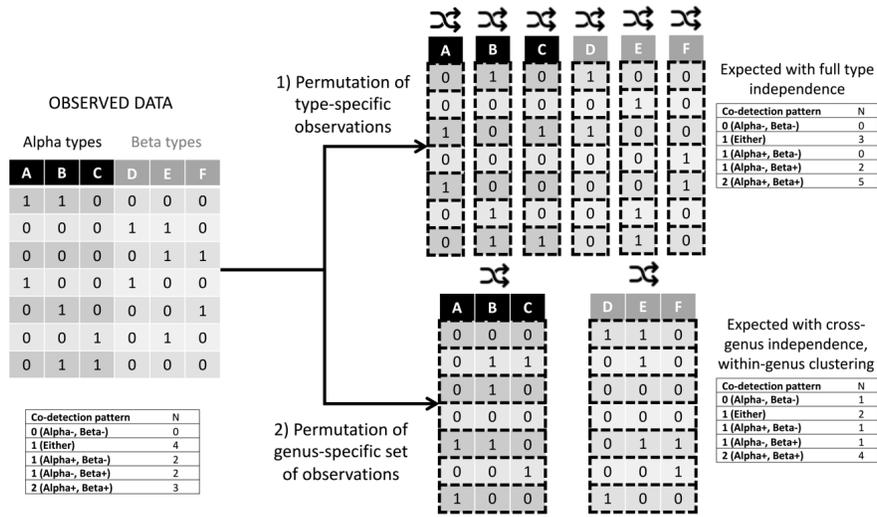


Figure I. Graphical representation of permutation tests. The first test permutes the data for each HPV type individually and derives the expected distribution of HPV positivity assuming full independence for all HPV types. The second test permutes the data for the set of HPV types within each genus and derives the expected distribution of HPV positivity assuming independence between genera while maintaining the clustering of types within a genus.

Table I. Betapapillomavirus time-averaged prevalences and incidence rates, overall and by type, age, and lifetime number of sex partners.

		Cross-sectional	Cross-sectional	Prospective	Prospective
Variables		n/N	Time-averaged prevalence (%) ^a	Events (n)	Incidence rate (
Total	All (summed across types)	352/86000	0.41	257	1.2
HPV type	HPV5	33/2000	1.65	28	5.6
	HPV8	25/2000	1.25	19	3.8
	HPV9	2/2000	0.10	1	0.2
	HPV12	11/2000	0.55	9	1.8
	HPV14	4/2000	0.20	4	0.8
	HPV15	5/2000	0.25	2	0.4
	HPV17	7/2000	0.35	5	1.0
	HPV19	4/2000	0.20	3	0.6
	HPV20	0/2000	0.00	0	0.0
	HPV21	40/2000	2.00	19	3.8
	HPV22	30/2000	1.50	22	4.4
	HPV23	8/2000	0.40	7	1.4
	HPV24	18/2000	0.90	15	3.0
	HPV25	0/2000	0.00	0	0.0
	HPV36	12/2000	0.60	12	2.4
	HPV37	0/2000	0.00	0	0.0
	HPV38	46/2000	2.30	31	6.3
	HPV47	6/2000	0.30	6	1.2
	HPV49	11/2000	0.55	10	2.0
	HPV75	0/2000	0.00	0	0.0

	Cross-sectional	Cross-sectional	Prospective	Prospective
HPV76	13/2000	0.65	10	2.0
HPV80	0/2000	0.00	0	0.0
HPV92	1/2000	0.05	1	0.2
HPV93	0/2000	0.00	0	0.0
HPV96	3/2000	0.15	2	0.4
HPV98	0/2000	0.00	0	0.0
HPV99	0/2000	0.00	0	0.0
HPV100	13/2000	0.65	10	2.0
HPV104	0/2000	0.00	0	0.0
HPV105	3/2000	0.15	2	0.4
HPV107	2/2000	0.10	2	0.4
HPV110	11/2000	0.55	8	1.6
HPV111	16/2000	0.80	6	1.2
HPV113	1/2000	0.05	0	0.0
HPV115	0/2000	0.00	0	0.0
HPV118	0/2000	0.00	0	0.0
HPV120	15/2000	0.75	13	2.6
HPV122	8/2000	0.40	8	1.6
HPV124	2/2000	0.10	1	0.2
HPV143	0/2000	0.00	0	0.0
HPV145	1/2000	0.05	1	0.2
HPV150	0/2000	0.00	0	0.0
HPV151	1/2000	0.05	0	0.0

HPV=human papillomavirus^a Time-averaged over four study visits.

Table II. Observed versus expected number of samples positive for alphapapillomavirus and betapapillomavirus types based on permutation tests.

	Observed (n)	Expected ^a (n)	Observed/ Expected ^a	95% CI
Within-genus clustering				
Alphapapillomavirus, number of types detected in sample		Expected with total type independence		
0	1718	1693	1.01	1.01-1.02
1	257	299	0.86	0.81-0.91
2+	44	26	1.74	1.26-2.44
Betapapillomavirus, number of types detected in sample		Expected with total type independence		
0	1719	1675	1.03	1.02-1.03
1	225	299	0.75	0.71-0.80
2+	56	26	2.24	1.65-3.29
Cross-genus clustering				

	Observed (n)	Expected ^a (n)	Observed/ Expected ^a	95% CI
Number of genera detected in sample		Expected with total type independence		
0 (Alpha-, Beta-)	1456	1405	1.04	1.02-1.05
1 (Either)	511	543	0.94	0.90-0.99
1 (Alpha+, Beta-)	263	270	0.97	0.93-1.03
1 (Alpha-, Beta+)	248	273	0.91	0.86-0.96
2 (Alpha+, Beta+)	33	52	0.64	0.51-0.83
Number of genera detected in sample		Expected with within-genus clustering		
0 (Alpha-, Beta-)	1456	1463	1.00	0.99-1.00
1 (Either)	511	495	1.03	0.99-1.08
1 (Alpha+, Beta-)	263	256	1.03	0.99-1.07
1 (Alpha-, Beta+)	248	239	1.04	0.99-1.09
2 (Alpha+, Beta+)	33	42	0.80	0.62-1.06

CI=confidence interval, based on 2.5th and 97.5th percentiles of 2500 permutations.^a Mean of 2500 permutations.

Table III. Cross-sectional association between alphapapillomavirus and betapapillomavirus type-specific positivity (summed over all types within genera), age, and lifetime number of sex partners.

	Alphapapillomavirus prevalence, summed over all types	Betapapillomavirus prevalence, summed over all types								
Risk factor	n/N	Time-averaged prevalence (%) ^a	OR	95%CI	ORadj ^b	95%CI	n/N	Time-averaged prevalence (%) ^a	OR	95%CI
Positivity to other HPV genus ^c										
Positive any	34/10678	0.32	0.73	0.50-1.07	0.75	0.51-1.11	42/12728	0.33	0.79	0.56-1.12
Negative any	314/65322	0.48	1.00	Ref	1.00	Ref	310/73272	0.42	1.00	Ref
Age years										
<25	105/13832	0.76	1.00	Ref	1.00	Ref	67/15566	0.43	1.00	Ref

	Alphapap prevalence, summed over all types	Alphapap prevalence, summed over all types	Alphapap prevalence, summed over all types	Alphapap prevalence, summed over all types	Alphapap prevalence, summed over all types	Alphapap prevalence, summed over all types	HPV positivity	Betapap prevalence, summed over all types	Betapap prevalence, summed over all types	Betapap prevalence, summed over all types	Betapap prevalence, summed over all types
25-34	123/ 28614	0.43	0.48	0.29- 0.80	0.49	0.30- 0.81		138/ 32121	0.43	0.96	0.68- 1.36
35-44	93/ 22838	0.41	0.44	0.25- 0.76	0.46	0.27- 0.78		91/ 25542	0.36	0.79	0.55- 1.15
[?]45	32/ 11438	0.28	0.32	0.16- 0.63	0.31	0.15- 0.61		56/ 12771	0.44	1.01	0.66- 1.55
Lifetime num- ber of sex partners											
0-1	102/ 35188	0.29	1.00	Ref	1.00	Ref		179/ 39388	0.45	1.00	Ref
2-3	149/ 27018	0.55	1.65	1.13- 2.42	1.62	1.10- 2.37		122/ 30401	0.40	0.89	0.68- 1.16
4+	102/ 14478	0.70	2.17	1.38- 3.42	2.13	1.36- 3.36		50/ 16168	0.31	0.68	0.47- 0.97
Any sex in last interval											
No	47/ 7600	0.62	1.00	Ref	1.00	Ref		30/ 8514	0.35	1.00	Ref
Yes	306/ 69122	0.44	0.65	0.40- 1.05	0.58	0.35- 0.94		322/ 77486	0.42	1.21	0.80- 1.84
New sex part- ner in last interval											
No	331/ 74480	0.44	1.00	Ref	1.00	Ref		343/ 83505	0.41	1.00	Ref
Yes	22/ 2242	0.98	1.10	0.62- 1.95	1.11	0.62- 1.98		9/ 2494	0.36	0.89	0.44- 1.80

CI=confidence interval; OR=odds ratio; ORadj=adjusted odds ratio; Ref=reference level.^a Time-averaged over four study visits.^b Adjusted for positivity to the other HPV genus, age, lifetime number of sex partners, any sex in last interval, and new sex in last interval.^c Positive for any type of the other HPV genus at same visit; corresponds to betapapillomavirus positivity for columns where alphapapillomavirus prevalence

is the outcome, and alphapapillomavirus positivity for columns where betapapillomavirus prevalence is the outcome.

Table IV. Prospective association between alphapapillomavirus and betapapillomavirus type-specific positivity (summed over all types within genera), age, and lifetime number of sex partners.

	Alphapapillomavirus incidence, summed over all types	Betapapillomavirus incidence, summed over all types									
Risk factor	Events (n)	Incidence rate (/100 person-years)	HR	95%CI	HRadj ^a	95%CI	Events (n)	Incidence rate (/100 person-years)	HR	95%CI	HR
Positivity to other HPV genus ^b											
Positive any	18	0.7	0.84	0.49-1.43	0.86	0.51-1.45	30	0.9	0.72	0.45-1.15	0.72
Negative any	131	0.8	1.00	Ref	1.00	Ref	227	1.2	1.00	Ref	1.00
Age years											
<25	46	1.4	1.00	Ref	1.00	Ref	42	1.1	1.00	Ref	1.00
25-34	56	0.8	0.58	0.36-0.92	0.58	0.37-0.92	104	1.3	1.16	0.81-1.66	1.16
35-44	35	0.6	0.46	0.27-0.79	0.48	0.29-0.81	66	1.0	0.96	0.65-1.41	0.96
[?]45	16	0.3	0.41	0.22-0.75	0.31	0.17-0.57	45	1.4	1.27	0.83-1.93	1.27
Lifetime number of sex partners											
0-1	43	0.5	1.00	Ref	1.00	Ref	135	1.4	1.00	Ref	1.00
2-3	66	1.0	2.02	1.27-3.23	1.89	0.19-3.00	80	1.0	0.77	0.58-1.01	0.77
4+	44	1.2	2.41	1.49-3.91	2.23	1.36-3.64	42	1.0	0.73	0.51-1.03	0.73
Any sex in last interval											

	Alphapap inci- dence, summed over all types	Alphapap inci- dence, summed over all types	Alphapap inci- dence, summed over all types	Alphapap inci- dence, summed over all types	Alphapap inci- dence, summed over all types	Alphapap inci- dence, summed over all types	Betapap inci- dence, summed over all types	Betapap inci- dence, summed over all types	Betapap inci- dence, summed over all types	Betapap inci- dence, summed over all types	Betapap inci- dence, summed over all types
No	30	1.6	1.00	Ref	1.00	Ref	18	0.8	1.00	Ref	1
Yes	123	0.7	0.48	0.31- 0.74	0.44	0.28- 0.70	239	1.2	1.57	1.00- 2.46	1
New sex part- ner in last interval											
No	144	0.8	1.00	Ref	1.00	Ref	250	1.2	1.00	Ref	1
Yes	9	1.7	2.04	0.96- 4.36	1.87	0.87- 4.01	7	1.2	0.95	0.38- 2.38	1

CI=confidence interval; HR=hazard ratio; HRadj=adjusted hazard ratio; Ref=reference level. ^a Adjusted for positivity to the other HPV genus, age, lifetime number of sex partners, any sex in last interval, and new sex in last interval. ^b Positive for any type of the other HPV genus at previous visit; corresponds to betapapillomavirus positivity for columns where alphapapillomavirus incidence is the outcome, and alphapapillomavirus positivity for columns where betapapillomavirus incidence is the outcome.

References

1. International Agency for Research on Cancer. Biological agents. Volume 100 B. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum* 2012; **100** (Pt B): 1-441.
2. Gheit T. Mucosal and Cutaneous Human Papillomavirus Infections and Cancer Biology. *Front Oncol* 2019; **9** : 355.
3. Agalliu I, Gapstur S, Chen Z, et al. Associations of Oral α -, β -, and γ -Human Papillomavirus Types With Risk of Incident Head and Neck Cancer. *JAMA Oncology* 2016; **2** (5): 599-606.
4. Tommasino M. The biology of beta human papillomaviruses. *Virus Research* 2017; **231** : 128-38.
5. Sabol I, Smahelova J, Klozar J, et al. Beta-HPV types in patients with head and neck pathology and in healthy subjects. *Journal of Clinical Virology* 2016; **82** : 159-65.
6. Altamura G, Tommasino M, Borzacchiello G. Cutaneous vs. Mucosal Tropism: The Papillomavirus Paradigm Comes to an "and". *Front Microbiol* 2020; **11** : 588663.
7. Bottalico D, Chen Z, Dunne A, et al. The oral cavity contains abundant known and novel human papillomaviruses from the Betapapillomavirus and Gammapapillomavirus genera. *J Infect Dis* 2011; **204** (5): 787-92.
8. Moscicki AB, Ma Y, Gheit T, et al. Prevalence and Transmission of Beta and Gamma Human Papillomavirus in Heterosexual Couples. *Open Forum Infect Dis* 2017; **4** (1): ofw216.

9. Nunes EM, Sudenga SL, Gheit T, et al. Diversity of beta-papillomavirus at anogenital and oral anatomic sites of men: The HIM Study. *Virology* 2016; **495** : 33-41.
10. Hampras SS, Rollison DE, Giuliano AR, et al. Prevalence and Concordance of Cutaneous Beta Human Papillomavirus Infection at Mucosal and Cutaneous Sites. *The Journal of Infectious Diseases* 2017;**216** (1): 92-6.
11. Winer RL, Gheit T, Feng Q, et al. Prevalence and Correlates of β - and γ -Human Papillomavirus Detection in Oral Samples From Mid-Adult Women. *The Journal of Infectious Diseases* 2019; **219** (7): 1067-75.
12. Lang Kuhs KA, Gonzalez P, Struijk L, et al. Prevalence of and risk factors for oral human papillomavirus among young women in Costa Rica. *J Infect Dis* 2013; **208** (10): 1643-52.
13. Carozzi F, Ronco G, Gillio-Tos A, et al. Concurrent infections with multiple human papillomavirus (HPV) types in the New Technologies for Cervical Cancer (NTCC) screening study. *Eur J Cancer* 2012;**48** (11): 1633-7.
14. Chaturvedi AK, Katki HA, Hildesheim A, et al. Human papillomavirus infection with multiple types: pattern of coinfection and risk of cervical disease. *J Infect Dis* 2011; **203** (7): 910-20.
15. Mendez F, Munoz N, Posso H, et al. Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. *J Infect Dis* 2005;**192** (7): 1158-65.
16. Rousseau MC, Pereira JS, Prado JC, Villa LL, Rohan TE, Franco EL. Cervical coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. *J Infect Dis* 2001; **184** (12): 1508-17.
17. Tota JE, Ramanakumar AV, Jiang M, et al. Epidemiologic approaches to evaluating the potential for human papillomavirus type replacement postvaccination. *Am J Epidemiol* 2013; **178** (4): 625-34.
18. Tota JE, Struyf F, Merikukka M, et al. Evaluation of Type Replacement Following HPV16/18 Vaccination: Pooled Analysis of Two Randomized Trials. *J Natl Cancer Inst* 2017; **109** (7).
19. Sichero L, El-Zein M, Nunes EM, Ferreira S, Franco EL, Villa LL. Cervical Infection with Cutaneous Beta and Mucosal Alpha Papillomaviruses. *Cancer Epidemiol Biomarkers Prev* 2017;**26** (8): 1312-20.
20. Franco E, Villa L, Rohan T, Ferenczy A, Petzl-Erler M, Matlashewski G. Design and methods of the Ludwig-McGill longitudinal study of the natural history of human papillomavirus infection and cervical neoplasia in Brazil. Ludwig-McGill Study Group. *Rev Panam Salud Publica* 1999; **6** (4): 223-33.
21. Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol* 2000;**38** (1): 357-61.
22. Gheit T, Billoud G, de Koning MNC, et al. Development of a sensitive and specific multiplex PCR method combined with DNA microarray primer extension to detect Betapapillomavirus types. *Journal of clinical microbiology* 2007; **45** (8): 2537-44.
23. Berry KJ, Johnston JE, Mielke Jr PW. Permutation methods. *WIREs Computational Statistics* 2011; **3** (6): 527-42.
24. Lin DY, Wei LJ. The Robust Inference for the Cox Proportional Hazards Model. *Journal of the American Statistical Association* 1989; **84** (408): 1074-8.
25. Scherpenisse M, Schepp RM, Mollers M, Meijer CJ, Berbers GA, van der Klis FR. Characteristics of HPV-Specific Antibody Responses Induced by Infection and Vaccination: Cross-Reactivity, Neutralizing Activity, Avidity and IgG Subclasses. *PLoS One* 2013; **8** (9): e74797.

26. Egawa N, Egawa K, Griffin H, Doorbar J. Human Papillomaviruses; Epithelial Tropisms, and the Development of Neoplasia. *Viruses*2015; **7** (7): 3863-90.

27. Soler C, Chardonnet Y, Allibert P, Euvrard S, Schmitt D, Mandrand B. Detection of Mucosal Human Papillomavirus Types 6/11 in Cutaneous Lesions from Transplant Recipients. *Journal of Investigative Dermatology* 1993; **101** (3): 286-91.

28. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. *Vaccine* 2006; **24**, **Supplement 3** : S52-S61.

29. Plasmeijer EI, Green AC, de Koning MNC, et al. Transmission of betapapillomaviruses between domestic partners in an Australian community. *Journal of Clinical Virology* 2010; **47** (3): 216-8.

30. Orth G, Jablonska S, Favre M, Croissant O, Jarzabek-Chorzelska M, Rzesza G. Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. *Proc Natl Acad Sci U S A* 1978; **75** (3): 1537-41.

31. Smelov V, Muwonge R, Sokolova O, et al. Beta and gamma human papillomaviruses in anal and genital sites among men: prevalence and determinants. *Scientific Reports* 2018; **8** (1): 8241.

