Sex-specific patterns of incidence, transmission, and concordance of human papillomavirus infections in newly formed heterosexual couples

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October 2024

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Epidemiology

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ABSTRACT

Background: Human papillomavirus (HPV) infections are easily transmissible through sexual contact. Most HPV infections resolve without sequelae, but persistent infections with high-risk types can lead to cervical, anogenital, and head and neck cancers. A nuanced understanding of HPV transmission within couples could help improve prevention and screening policies. Yet, few HPV studies focus on couples.

Objectives: The first objective (Manuscript 1) is to describe sex-specific genital incidence and transmission rates of type-specific HPV infections and the effect of recent vaccination on those rates. The second objective (Manuscript 2) is to describe type-specific HPV concordance at multiple anatomical sites (i.e., anal, genital, oral) within individuals and between partners.

Methods: Data come from the Transmission Reduction and Prevention with HPV Vaccination (TRAP-HPV) study, a randomized controlled trial conducted in Montreal, Canada (2014 -2022) with heterosexual couples aged 18+, formed within the previous 6 months. Individuals (n=372) were randomized to receive an HPV (intervention) or hepatitis A vaccine. This created 4 groups: 1) neither partner vaccinated; 2) male partner vaccinated; 3) female partner vaccinated; and 4) both partners vaccinated against HPV. Genital, oral, and anal samples from baseline and 5 follow-up visits over 12 months were genotyped for up to 36 HPV types. The analytical sample in Manuscript 1 included couples with at least one follow-up visit and valid or imputable baseline genital samples (n=308). We estimated sex-specific rates (in events/1000 infection months) in the 4 study arms. The analytical sample in Manuscript 2 included all participants (n=372). We calculated observed/expected (O/E) type-specific concordance (with self or partner)

by visit and overall. Additionally, we used mixed-effects logistic regression models to estimate odds ratios for type-specific concordance over all of follow-up and to evaluate predictors of concordance within couples.

Results: In Manuscript 1, in females, recent vaccination was not consistently associated with lower incidence of vaccine-targeted HPV; while the lowest rate was in the group with only female partners vaccinated, 1.05 (95% CI: 0.42, 3.45), the highest rates were in the groups with only male partners vaccinated and with both partners vaccinated, 1.58 (95% CI: 0.55, 6.17) and 1.58 (95% CI: 0.71, 4.25), respectively. In males, recent vaccination was weakly associated with lower incidence of vaccine-targeted HPV. Recent vaccination was not associated with reduced HPV transmission. The group with both partners vaccinated had the highest rate of vaccine-targeted HPV transmission to females, 29.83 (95 % CI: 7.26, 145.53). In Manuscript 2, self-concordance between genital and anal sites was high; O/E: 23.37 (95% CI: 15.55, 38.05) and 14.79 (95% CI: 9.20, 43.45) in females and males, respectively. Between partners, there was high concordance between genital sites, O/E: 14.99 (95% CI: 12.47, 18.41).

Conclusions: In Manuscript 1, we did not find conclusive indications of a protective effect from recent vaccination. Factors that could have contributed to this lack of an observed effect are the age of participants, time since couple formation, length of follow-up, and sample size. In Manuscript 2, findings demonstrated high HPV type-specific concordance within individuals and between partners. In particular, the high genital/anal concordance in females suggests that HPV infections are passed easily between these sites.

RÉSUMÉ

Contexte: Les infections par le virus du papillome humain (VPH) se transmettent facilement par voie sexuelle. La plupart des infections se résolvent sans séquelles, mais les infections persistantes par des souches de VPH à hauts risques peuvent entraîner des cancers du col de l'utérus, des cancers anogénitaux, et des cancers de la tête et du cou. Une compréhension nuancée de la transmission au sein des couples pourrait améliorer les politiques de prévention et de dépistage. Néanmoins, peu de recherches sur le VPH ciblent les couples.

Objectifs: Le premier objectif (manuscrit 1) est de décrire les taux d'incidence et de transmission en fonction de sexe (et de la souche de VPH), ainsi que l'influence de la vaccination récente sur ces taux. Le deuxième objectif (manuscrit 2) est de décrire la concordance des infections à plusieurs sites anatomiques (anal, génital, oral) au sein des individus et entre partenaires.

Méthodes: Les données viennent de l'étude TRAP-HPV (Transmission Reduction and Prevention with HPV Vaccination), un essai contrôlé randomisé mené à Montréal, Canada, (2014-2022) auprès de couples hétérosexuels formés dans les 6 mois précédents, âgés de 18 ans et plus. Des individus (n=372) ont été répartis de manière aléatoire pour recevoir le vaccin contre le VPH (traitement) ou un vaccin contre l'hépatite A. 4 groupes ont été créés : aucun des partenaires vaccinés; seul le partenaire masculin vacciné; seule la partenaire féminine vaccinée; les deux partenaires vaccinés contre le VPH. Des prélèvements génitaux, oraux, et anaux ont été effectués à l'inscription et lors de 5 visites de suivi sur 12 mois, et testés pour 36 génotypes de VPH. Le manuscrit 1 inclut les couples ayant au moins deux visites et des échantillons génitaux valides (ou imputables) à la visite de référence (n=308). Nous avons estimé les taux (en

événements par 1000 mois à risque), selon le sexe, dans les 4 branches de l'étude. Le manuscrit 2 inclut tous les participants (n=372). Nous avons calculé la concordance observée/attendue (O/A ; avec soi-même ou avec son partenaire), en fonction de la souche, par visite et au total. Nous avons utilisé la régression logistique à effets mixtes afin d'estimer les rapports de cotes pour concordance sur l'ensemble du suivi et d'évaluer les variables prédictives de la concordance au sein des couples.

Résultats: Dans le manuscrit 1, chez les femmes, la vaccination récente n'était pas systématiquement associée à un taux d'incidence plus bas des souches de VPH ciblées par le vaccin ; le taux le plus fiable était dans le groupe où la partenaire féminine était vaccinée, 1.05 (IC 95% : 0.42, 3.45), mais les taux les plus élevées étaient dans les groupes avec le partenaire masculin vacciné et les deux partenaires vaccinés : 1.58 (IC 95% : 0.55, 6.17) et 1.58 (IC 95% : 0.71, 4.25), respectivement. Chez les hommes, la vaccination récente était faiblement associée à un taux d'incidence plus bas des souches de VPH ciblées par le vaccin. En outre, le groupe avec les deux partenaires vaccinés présentait le taux le plus élevé de transmission aux femmes. Dans le manuscrit 2, la concordance entre les sites génitaux et anaux, chez le même individu, était élevée, O/A : 23.37 (IC 95% : 15.55, 38.05) et 14.79 (IC 95% : 9.20, 43.45) chez les femmes et les hommes, respectivement. Dans les couples, la concordance pour les sites génitaux était élevée, O/A : 14.99 (IC 95% : 12.47, 18.41).

Conclusions: Dans le manuscrit 1, nous n'avons pas trouvé d'indications concluantes d'un effet protecteur d'une vaccination récente. Des facteurs qui ont pu contribuer à cette absence d'effet incluent l'âge des participants, le temps déjà en couple, la période de suivi, et la taille de

l'échantillon. Dans le manuscrit 2, les résultats montrent de fortes concordances intra-individuelle et entres les partenaires. En particulier, la concordance génitale/anale chez les femmes indique que les infections au VPH se transmettent facilement entre ces sites.

ACKNOWLEDGEMENTS

It has truly been an honour to work on research from the Division of Cancer Epidemiology for my MSc thesis project. I am indescribably grateful to my thesis supervisor, Dr. Franco, for the opportunity to be part of such an amazing team and for his ongoing mentorship, guidance, insight, and inspiration. Dr. Franco has created a wonderful and enriching academic environment at the Division of Cancer Epidemiology, and it was a privilege to be able to work with and learn from such accomplished researchers.

Likewise, I am enormously grateful to Dr. Mariam El-Zein, Associate Director for Research at the Division of Cancer Epidemiology, for her guidance, encouragement, and training. Dr. El-Zein's detailed and nuanced feedback has been extraordinarily valuable to my progress and development as a student. I would also like to thank Dr. Paul Brassard for being part of my thesis committee and for providing valuable insights and guidance.

Also, thank you to Dr. Talía Malagón for feedback and guidance on various aspects of my thesis work (as well as for the opportunity to be involved in her fascinating research on cancer survival in Canada). Thank you to the staff and students, both past and present, at the Division of Cancer Epidemiology. Thank you to the staff and faculty at the McGill Department of Epidemiology, Biostatistics and Occupational Health.

For financial support, many thanks to: Canada Graduate Scholarships-Master's, CIHR; McGill Faculty of Medicine Internal Studentships, in part through the C. Epstein Fellowship in Women's Health); Dr. Franco and the Division of Cancer Epidemiology, in part through the Hirsh & Schulman Education Fund; and McGill University, Epidemiology, Biostatistics and Occupational Health GREAT travel award.

Thank you to my partner, Mickaël, who is wonderfully kind and supportive, and to my family and friends who convinced me to follow my academic dreams.

Most of all, thank you to the participants of the TRAP-HPV study, without whom this research would not be possible.

PREFACE AND CONTRIBUTION OF AUTHORS

This thesis comprises a review of pertinent literature, two original manuscripts (which have been formatted for submission to academic journals), and an overall discussion and conclusion based on the research findings from the two manuscripts. Below is information regarding the two manuscripts.

Manuscript 1:

Moore, A., El-Zein, M., Burchell, A. N., Tellier, P., Coutlée, F., & Franco, E. L. Human papillomavirus incidence and transmission by vaccination status among heterosexual couples. This manuscript was submitted to *The Journal of Infectious Diseases*. It is formatted accordingly.

Author contributions: ELF and ANB conceived the study design. ELF and MZ planned and supervised the study. MZ oversaw data collection and management. AM carried out analyses and drafted the manuscript with support from MZ and ELF. FC supervised HPV genotyping. PPT supervised the study nurses and advised on participant recruitment and sexual health. All authors reviewed, provided critical feedback, and approved the final versions of the manuscript.

Manuscript 2:

Moore, A., El-Zein, M., Burchell, A. N., Tellier, P., Coutlée, F., & Franco, E. L. Genital, oral, and anal type-specific human papillomavirus concordance within individuals and between partners. This manuscript will be submitted to the journal *Clinical Infectious Diseases* and is formatted accordingly.

Author contributions: ELF and ANB conceived the study design. ELF and MZ planned and supervised the study. MZ oversaw data collection and management. AM carried out analyses and drafted the manuscript with support and guidance from MZ and ELF. FC supervised HPV genotyping. PPT supervised the study nurses and advised on participant recruitment and sexual health. All authors reviewed the manuscript and provided critical feedback.

LIST OF TABLES

Regarding prefixes of table titles: M1 indicates that the table is part of Manuscript 1; M2 indicates that the table is part of Manuscript 2; T indicates that the table is part of the thesis but not part of a particular manuscript.

M2-Table S5. Anal HPV-level ever positivity [n (%)] during the study (N=43 couples).

LIST OF FIGURES

Regarding prefixes of figure titles: M1 indicates that the figure is part of Manuscript 1; M2 indicates that the figure is part of Manuscript 2; T indicates that the figure is part of the thesis but not part of a particular manuscript.

LIST OF ACRONYMS & ABBREVIATIONS

AP	Anyplex II HPV28 Detection Assay
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
DNA	Deoxyribonucleic acid
GEEs	Generalized estimating equations
HIM	HPV Infection in Men study
HITCH	HPV Infection and Transmission among Couples through Heterosexual Activity cohort study
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesions
IARC	International Agency for Research on Cancer
ICO	Catalan Institute of Oncology (*Institut Català d'Oncologia*)
LA	Linear Array HPV Genotyping Test
LSIL	Low-grade squamous intraepithelial lesions
M1-	Manuscript 1
M2-	Manuscript 2
MuFu	Male unvaccinated, female unvaccinated
MuFv	Male unvaccinated, female vaccinated
MvFu	Male vaccinated, female unvaccinated
MvFv	Male vaccinated, female vaccinated

NHANES	National Health and Nutrition Examination Survey
O/E	Observed/expected
OR	Odds ratio
p53	Tumour protein p53
Pap	Papanicolaou
Q1	First quartile
Q3	Third quartile
Rb	Retinoblastoma protein
SCJ	Squamocolumnar junction
SD	Standard deviation
STI	Sexually transmitted infection
T-	Thesis (table or figure found in thesis only, it is not part of one of the component manuscripts)
TRAP-HPV	Transmission Reduction and Prevention with HPV vaccination study
USA	United States of America

CHAPTER 1: INTRODUCTION

1.1 RATIONALE FOR CURRENT ANALYSES

In a 2020 review of couple-based studies on heterosexual human papillomavirus (HPV) transmission, Balaji and colleagues remarked that their meta-analysis was somewhat hindered by the low number of pertinent studies,[1] thereby highlighting the need for additional couple-based studies on HPV. They also commented on the need for future couple-based studies to assess the effect of HPV vaccination on transmission dynamics within couples.[1] Furthermore, in a 2015 review that compared sex-specific HPV infection and HPV-related cancer rates, Giuliano and colleagues remarked on a lack of studies reporting HPV prevalence at various anatomical sites and for both males and females from the same underlying population, which would enable more valid comparisons.[2] They explain that comparing HPV prevalences reported for females and males from different populations across different studies can be difficult because of the sizable regional differences in HPV prevalence and/or methodological variability that may affect results.[2] The manuscripts included in this thesis help address these lacunae in the literature.

Couple-based studies permit the comparison of HPV prevalence in males and females from the same population. The Transmission Reduction and Prevention with HPV Vaccination (TRAP-HPV) study, which is the source of the data analyzed in the following manuscripts, is one such study. Furthermore, the TRAP-HPV study included testing for HPV at multiple anatomical sites (i.e., genital, oral, anal).[3] Therefore, findings from this study can provide much-needed direct comparisons of sex-specific intra-individual concordance patterns within males and females from the same population, as well as concordance between partners. The TRAP-HPV study is a randomized, controlled trial designed to assess the efficacy of HPV vaccination in preventing the transmission of vaccine-targeted HPV infections to the partners of vaccinated

individuals.[3] The study population consists of adults in new heterosexual relationships.[3] Thus, data from the TRAP-HPV study can help elucidate the effect of vaccination on HPV transmission dynamics within sexual partnerships. Furthermore, data from the TRAP-HPV study can shed light on the potential benefits of vaccination of adult heterosexual males, a population for whom there is relatively little data on HPV vaccination.[4] Additionally, by detailing patterns of HPV type-specific concordance within individuals, Manuscript 2 contributes to our knowledge and understanding of HPV natural history and intra-individual transmissibility to both adjacent and non-adjacent anatomical sites. This also has great potential clinical utility. Anal cancer screening is recommended for certain high-risk populations.[5]A better understanding of the increased risk of type-specific HPV infection at one anatomical site, given infection at another anatomical site (e.g. increased risk of anal HPV infection given genital HPV infection) could be informative for such screening programs.

1.2 THESIS OVERVIEW AND OBJECTIVES

Overall, the aim of this thesis is to describe type-specific HPV incidence, transmission, and concordance among sexually active adults in relatively new ($\leq$ 6 months) heterosexual relationships. This thesis is manuscript-based and contains two manuscripts, each of which pertains to one of the following objectives:

1. Describe sex-specific genital incidence and transmission rates of type-specific HPV infections in newly formed couples and the effect of recent vaccination on those rates, considering three types of HPV: 1) vaccine-targeted HPV types, 2) HPV types which are phylogenetically related to vaccine-targeted types, and 3) HPV-types which are phylogenetically unrelated to vaccine-targeted types.
2. Describe sex-specific HPV type-specific concordance at multiple anatomical sites (i.e., anal, genital, oral) within individuals and between partners, as well as couple-level predictors of genital/genital HPV type-specific concordance.

1.3 LITERATURE REVIEW

1.3.1 Virology & Lifecycle of HPV

There are distinct papillomaviruses unique to many vertebrates,[6] and HPVs have co-evolved with humans for millennia.[7,8] HPVs are a large family of small, non-enveloped, double-stranded circular DNA viruses.[6] The HPV genome contains around 8,000 base pairs, which code for eight proteins and a long control region.[8] HPV virions have an icosahedral capsid made up of two capsid proteins coded by the late region; L1 is the major capsid protein, and L2 is the minor capsid protein.[7] The early region of the HPV genome contains six genes, E1, E2, E4, E5, E6, and E7, that function in various stages of viral genome replication and persistence.[6–9] E1 and E2 function in viral DNA replication, E4 is involved in the release of virions[8], and E5 is thought to promote proliferation.[10] E6 and E7, which are regulated by E2,[8] are the primary oncogenes in high-risk HPV types, and their increased expression is key to the progression of infections to precancerous lesions (discussed further below).[11] E6 and E7 are also involved in the downregulation of the host's immune response.[12]

Viral entry and initial infection occur in basal epithelial cells; minor skin trauma or abrasion is needed for the virions to gain access to these target cells.[8,9,13] The HPV lifecycle requires differentiation of basal squamous epithelial cells into mature keratinocytes[12] as the HPV genome does not contain the necessary genes for its own DNA replication, and thus, needs the host cell to be actively replicating DNA.[9]

After initial infection, viral DNA is replicated as an episome, and the copy number is maintained at around 50-100 per infected cell.[12] In productive infections, as the infected cell completes differentiation and becomes a mature keratinocyte, the viral genome copy number increases manyfold, and proteins L1 and L2 are expressed, enabling the formation of infectious

virions, which can then be shed.[12] The incubation period of HPV can be up to 8 months, but it is usually shorter.[14] It takes a minimum of 3 weeks from the initial infections to infectivity (i.e., from the infection of basal keratinocytes to viral shedding from the top level of skin cells).[12]

1.3.2 HPV Taxonomy

There are over 200 types of HPV,[15] 150 of which are fully sequenced.[7] On the basis of genetic sequence in the L1 gene, HPVs are divided into 5 genera: *Alphapapillomavirus*, *Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus and Nupappillomavirus.*[7,8,16] Overall, the sequence of L1 is highly conserved, but it also contains regions that vary substantially between HPV types.[8,17] By definition, the different genera of HPV are $<$ 60% identical to each other in the L1 region, and new HPV types must be $<$ 90% identical to already identified types in the L1 region.[16] While the other genera cause benign cutaneous infections, the *Alphapapillomavirus* genus is of most interest with regards to disease; it includes the HPV types which infect cutaneous epithelia and cause warts and the HPV types that infect the mucosal epithelium,[7] of which there are over 40.[18] Twelve types (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC), one type (HPV 68) is classified as "probably carcinogenic to humans" (Group 2A), and twelve types (HPVs 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97) are classified as "possibly carcinogenic to humans" (Group 2B).[19]

1.3.3 HPV Epidemiology

1.3.3.1 Cervical and genital HPV prevalence

HPV infections are highly prevalent,[20] easily transmissible,[21] and represent the most common sexually transmitted infection (STI) worldwide.[18] Most individuals will, over the course of their lives, contract one or more sexually transmitted HPV infections.[19,22]

According to a meta-analysis of data from 194 studies (mostly from the pre-vaccine era) pertaining to over a million participants, among females with normal cervical cytology, global cervical HPV prevalence was 11.7%; however, there was substantial variation in HPV prevalence across different regions of the world.[23] Cervical HPV prevalence was estimated to be 21.1% in Africa, 16.1% in Latin America and the Caribbean, 4.7% in North America, 9.4% in Asia, and 14.2% in Europe (with additional regional variation within these categories).[23] The highest prevalence estimate was for Eastern Africa (33.6%), and the lowest prevalence estimate was for Western Asia (1.7%).[23]

According to data compiled in the latest Human Papillomaviruses and Related Diseases Report from the Catalan Institute of Oncology/International Agency for Research on Cancer (ICO/IARC) Information Centre on HPV and Cancer, globally, in females with normal cervical cytology, type-specific cervical prevalence is highest for HPV 16 (i.e., 2.8% prevalence).[24] Among the high-risk HPV types, the second and third highest prevalences are seen for HPVs 52, and 31 (i.e., 1.5%, and 1.2%, respectively).[24] Cervical prevalence for HPV 16 and/or 18 is 3.9% globally, 3.8% in Africa, 4.5% in the Americas, 3.4% in Asia, 3.8% in Europe, and 8.3% in Oceania.[24]

In females, HPV prevalence is highest in young adults and peaks around age 25, with another smaller peak in later middle-age, around menopause.[25] The later peak in females may be due to reactivation of latent infections, new exposures from new sexual partners, or cohort

effects.[18,23,25,26] However, there are regional variations in these patterns; for example, the decline with age is less pronounced in Asia and Africa than in the Americas and Europe, and the later second peak is not observed in all populations (i.e., it is not seen in Asia).[20,25]

In contrast to the general pattern observed in females, genital HPV prevalence in males remains relatively stable in adulthood and does not decline with age.[2,27] A 2023 meta-analysis of males aged 15 and over from 35 countries calculated an overall global genital HPV prevalence of 31%.[28] Prevalence for high-risk HPV was estimated to be 21%, and similar to the results seen for females, HPV 16 was the most commonly detected type, with an estimated global prevalence of 5%.[28] In the USA, based on National Health and Nutrition Examination Survey (NHANES) data, genital prevalence for any HPV in males 14-59 is estimated to be 42.2%.[29]

HPV is a highly transmissible STI; a modeling study estimated the probability of heterosexual HPV transmission per instance of sex to be between 5-100%, with a median probability estimate of 40%.[21] Thus, sexual behaviours are predictive of HPV detection; lifetime number of sexual partners is major predictor of cervical and/or genital HPV detection in both females [19,30] and males.[19,27,29] In a cohort of young (18 -24 year old) heterosexual couples in new relationships, overall type-specific HPV concordance between couples was four times greater than would be expected by chance [31] and the strongest predictor of type-specific prevalent HPV detection in both males and females, was their partner testing positive for that HPV type.[32] In this same cohort, 67% of couples were positive for any HPV in one or both partners, and HPV positivity was highest in couples where both partners had concurrent partners.[33]

Gay, bisexual, and other men who have sex with men, as well as transgender women, generally face higher rates of HPV incidence and HPV-related disease compared with heterosexual males.[34] This is especially the case for those living with HIV.[34] There is relatively

little data on HPV in lesbian, bisexual, and other women who have sex with women, or individuals assigned female at birth who do not identify as women.[35] However, perceptions of HPV-related risk in these populations may be erroneously low, potentially leading to inadequate screening.[35]

1.3.3.2 Oral HPV prevalence

Oral HPV is relatively uncommon and there is considerable variation in estimates of its prevalence.[2,20] In the studies included in the most recent report from ICO/IARC Information Centre on HPV and Cancer, estimates of oral HPV prevalence in healthy populations range from 0-24.1%.[24] Estimates of oral HPV prevalence are higher for males than females.[2,20] For example, in a 2023 article looking at adults in the USA, Giuliano and colleagues reported that oral HPV prevalence for any type was 9.1% in males, and 4.6% in females.[36] Oral HPV prevalence was highest in older males, and predictors were lifetime number of male sex partners and lifetime number of female oral sex partners.[36] In a meta-analysis of 48 studies examining oral HPV prevalence, Mena and colleagues estimated an overall oral HPV prevalence of 4.9%.[37] This study did not report global prevalence by sex, but the global relative risk ratio for males vs. females was 1.2 (95% CI: 0.74- 1.9).[37]

Multiple hypotheses have been put forth to explain sex-specific differences in oral HPV prevalence, including more efficient genital-to-oral transmission from females to males than vice versa, and sex-specific differences in immune response to genital infections (i.e., higher rates of seroconversion leading to some protection in females).[2,38]

1.3.3.3 Anal HPV prevalence

Anal HPV is most common in men who have sex with men, but in heterosexual populations, anal HPV is more common in females than in males.[2,20] Based on previous studies in HIV-negative populations, Giuliano and colleagues estimated anal HPV prevalence to be 58.8%, 30.7%, and 14.2% in men who have sex with men, heterosexual females, and heterosexual males, respectively.[2]

However, anal HPV prevalence estimates vary. For example, in a 2008 study by Nyitray and colleagues, anal HPV was detected in 24.8% of 222 men who reported sex only with women.[39] Considering only high-risk HPV infections, according to a 2015 systematic review among HIV-negative females without apparent HPV-related diseases, the estimates anal HPV prevalence were between 5% and 22%.[40] There are similar age- and sex-related patterns in anal and genital HPV prevalence; specifically, anal HPV is less prevalent at older ages among females but does not decline with age in males.[2,41]

1.3.4 HPV-Related Disease Epidemiology

Even considering only high-risk HPVs, most infections do not lead to cancer.[7] The majority of HPV infections are asymptomatic and are resolved by the host's immune system within two years and without causing adverse health consequences.[12,19,42] However, a small fraction of infections persist much longer.[25,42] Persistent infection with a high-risk HPV type is a necessary cause of cervical cancer and also contributes to the burden of anal, penile, vaginal, vulvar, and head and neck (mostly oropharyngeal) cancers.[19,43] Other non-oncogenic or low-risk HPV types (mostly HPVs 6 and 11) cause anogenital warts and recurrent respiratory papillomatosis.[44]

Globally, an estimated 4.5% of cancer cases are attributable to HPV; more specifically, 8.6% of cancers in females and 0.8% of cancers in males.[45] Based on data from 2018, an estimated 690,000 new cancer cases were attributable to HPV.[46] Among females of all ages, cervical cancer is the 4th most common cancer, and among females 15-44 years of age, cervical cancer is the 2nd most common cancer.[24] Globally, in 2022 there were 662,301 new cases of cervical cancer, and 348,874 cervical cancer deaths;[47] this means that on average, cervical cancer kills one person every two minutes.[48] Globally, a majority (83%) of HPV-related cancers are cervical cancers [20] and the majority of cervical cancer incidence and mortality is in low- and middle-income countries.[24] Indeed, the burden of HPV-related cancer varies starkly by country income level, with low-income countries facing age-standardised incidence rates of HPV-attributable cancer of 16.1 cases/100,000 person-years compared to 6.9 cases/100,000 person-years in high-income countries.[46] In contrast, HPV-related head and neck cancers are most common in high-income counties and more common in males than in females.[20] In high-income countries, incidence rates of oropharyngeal cancer have been rising over the past 30 years, mostly in males aged 50-60.[38,49] Half of HPV-related cancers diagnosed in males are head and neck cancers.[20] In Canada, oropharyngeal cancers and cervical cancers represent about the same proportion of HPV related cancers (about a third each).[50] Combined with declining rates of cervical cancer in women, (due to the success screening of and vaccination programs) the increasing incidence HPV related head and neck cancers in males means that head and neck cancers in males is likely to become the most common HPV-related cancer in some high-income countries, such as the USA.[49,51] Possible explanations for the increase in HPV-related oropharyngeal cancer in males in high-income countries include decreasing tonsillectomies and changes in sexual norms and practices.[38]

Anal cancer incidence is highest in men who have sex with men.[52] However, the global burden of anal cancer (irrespective of sexual orientation) is slightly higher in females than in males; the standardized incidence rates per 100,000 population are 0.58 in females vs. 0.49 in males.[24] Anal cancer incidence in both males and females is also increasing in many countries, likely due to an increase in persistent anal HPV infections.[53]

While essentially all cervical cancers are attributable to HPV, approximately 88% of anal cancers and less than half of other genital cancers are attributable to HPV.[2] Globally, 30% of oropharyngeal cancers are attributable to HPV.[45] However, in countries in Northern Europe and North America, over 70% of oropharyngeal cancers are attributable to HPV.[38]

Considering all cancer sites together, HPVs 16 & 18 cause 73% of HPV-attributable cancer, while the seven vaccine-targeted oncogenic HPV types (i.e., HPVs 16, 18, 31, 33, 45, 52, and 58) cumulatively account for 90% of HPV-attributable cancers.[45] While different HPV types have different attributable fractions of HPV-related cancers at various sites, HPV16 is particularly carcinogenic at all sites. HPV 16 causes 50-55% of cervical cancers, 90% of HPV-related anal cancers,[54] and 80-90% of HPV-related head and neck cancers (mostly oropharyngeal squamous cell carcinoma).[49] HPV 18 is responsible for the 2nd largest number of cervical cancers, followed by HPV 45.[25] However, with respect to oropharyngeal cancer, HPV 33 is the 2nd most common type, HPV 35 is the 3rd most common type, and HPV 18 is the 4th most common.[38]

1.3.5 Progression from HPV Infection to Cancer

HPV is best characterized in the context of cervical infection and pathogenesis.[7,55] The majority of cervical cancers are squamous cell carcinomas.[56] The development of squamous cell

carcinomas is well characterized;[56] precursor lesions were previously referred to as cervical intraepithelial neoplasia (CIN) and categorized as CIN1, CIN2, or CIN3, based on the level of observed dysplasia (mild, moderate, or severe) and the proportion of the epithelium thickness affected (i.e., one-third, two-thirds, or the complete thickness, respectively.[24,57] CIN1 itself (while a necessary step in the progression to more pronounced dysplasia) is not considered precancer but rather "an insensitive histopathological sign of HPV infection" (p.893).[58] CIN2 is ambiguous as an indication of precancerous changes; it may represent precancer but may also result from HPV infection with non-high-risk types, while CIN3 is considered precancer.[58]

More recently, a two-level classification terminology has been adopted, wherein cervical precancerous lesions are classified as either low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL).[59] The change to the two-level classification system allows for standardization of terminology for cervical and other anogenital lesions caused by HPV.[59,60] A minority (10-25%) of cervical cancers are adenocarcinomas.[61] The histological progression of these cancers is not as well characterized,[56] and they are more heterogeneous than squamous cell carcinomas.[62]

The time from HPV infection to precancerous changes at the cervix can be only a few years.[58] However, most precancerous lesions will not become invasive cancer, and the time required for invasive cervical cancer to develop from a high-risk HPV infection is usually much longer, possibly decades.[58] Mechanisms by which HPV can lead to the development of cancer include immortalization and genome instability.[63,64]

Genome instability refers to a tendency towards a higher buildup of DNA mutations, which are passed on to daughter cells, and it is a defining feature of cancer.[65,66] High-risk HPV infection can lead to host genome instability via multiple mechanisms, for example, by co-opting

cellular DNA repair machinery,[67] by interfering with the cell cycle, or indirectly via the host's immune responses to infection.[9,64] Furthermore, the HPV genome, or a portion of it, may integrate into the host genome; this occurs in an estimated 80% of cervical cancers and 25-70% of head and neck cancers.[64] Integration of HPV DNA into the host genome results in increased expression of the oncoproteins E6 and E7 (because E2, which regulates these genes, is lost), and this causes dysregulation of the cell cycle.[9,64] E6 and E7 target the tumour suppressor genes p53 and Rb, respectively.[9,10,12,64] Rb proteins regulate the cell cycle by activating transcription factors that negatively regulate S-phase genes.[10] Thus, targeting of Rb by E7 leads to the over-expression of S-phase genes, evasion of cell cycle checkpoints, and increased DNA replication.[10] E6 prevents apoptosis;[58] its main target, p53, encodes a tumour suppressor protein which would normally inhibit the cell growth resulting from destruction of Rb proteins by E7.[10] Differences in the E6 and E7 genes are instrumental to the differences between high-risk and low-risk HPV, in terms of their propensity to cause disease.[7] In general, E6 and E7 overexpression in high-risk HPVs causes cell proliferation (in basal cells) and entry into the cell cycle (in upper skin layers).[8] HPV-induced immortalization occurs via telomerase activation by E6.[68] Epigenetic mechanisms, such as methylation, which influence gene expression, are also thought to play a role in the development of cervical cancer.[56]

HPV infection is at least as common in males as in females, with some research indicating higher prevalence in males.[2,20,24,69,70] However, globally, females bear the brunt of HPV-related cancer incidence. The reason for the considerably larger HPV-related cancer burden in females may be due in part to histological features of the cervix.[70] The cervical transformation zone or squamocolumnar junction (SCJ) is the region on the border of the endocervix and ectocervix where the columnar epithelium (endocervix) becomes stratified squamous epithelium

(ectocervix).[58,71] At the SCJ, HPV gene expression in infected cells is particularly likely to become disordered, leading to infections that do not go through the normal viral life cycle and do not produce infectious virions, but rather develop into precancerous lesions and possibly invasive cancer.[7,8,71] The anus also contains a squamocolumnar junction (i.e., between the anal squamous epithelium and the rectal columnar epithelium), and most anal cancers and precancers develop at this anorectal junction.[72–74] Thus, HPV-related cancer risks or attributable fractions at different sites do not depend solely on sex-specific HPV infection risk or rates. High-risk HPV infections at the cervical and anal transformation zones as well as the tonsillar reticulated epithelium, are more likely (due to histological characteristics at these sites) than infections at other sites to progress to cancer or precancer.[7,71]

Other risk factors that may increase the likelihood that a high-risk HPV infection develops into pre-cancer of cancer include immunosuppression, due to either HIV or anti-organ rejection medications,[13] as well as oral-contraceptive use, multiple pregnancies, smoking, and possibly, concurrent chlamydia infection and socio-economic status.[58] There are also believed to be host-specific immune response factors which influence whether infections persist and ultimately lead to carcinogenesis, however, these are not yet well elucidated.[19]

1.3.6 HPV Vaccination

HPV vaccines utilize virus-like particles formed from the L1 capsid protein generated via recombinant DNA technology.[75] The first HPV vaccine approved in Canada in 2006 was Gardasil® (Merck), a quadrivalent vaccine that protects against HPVs 6, 11, 16 & 18.[76] Gardasil® was discontinued in 2019.[76] Cervarix® (GlaxoSmithKline Biologicals) is a bivalent vaccine which protects against HPV 16 & 18,[76] it has been approved for use in females Canada

since 2010.[77] Gardasil®9 (a nine-valent vaccine), approved in Canada in 2015, protects against HPVs 6 & 11 and seven oncogenic types (HPVs 16,18, 31, 33, 45, 52, and 58).[77] It is estimated that Gardasil®9 could prevent nearly 90% of cervical cancer cases, 79% of anal cancer cases, 21.3% of oropharyngeal cancer cases, as well as 4% of oral cavity cancer cases, and 2.7% of laryngeal cancer cases, 24.5% of penile cancer cases, 60.7% of vaginal cancer cases and 22.8% of vulvar cancer cases.[43] In Canada, HPV vaccination is recommended routinely for individuals up to 26 years of age, and on a case-by-case basis thereafter.[76]

According to the World Health Organization, 141 countries (of 194 that reported) have fully implemented HPV vaccination as part of their national vaccination program, with vaccination of both males and females implemented in 75 countries.[78] The Canadian provinces began school-based vaccination of females in 2007/2008 or 2008/2009 (depending on the province), with the Yukon and Northwest Territories beginning in 2009/2010, and Nunavut in 2010/2011.[79] In Canada, gender-neutral vaccination was implemented first in PEI in 2013/2014, and in all jurisdictions by 2017/2018.[79] School-based HPV vaccination occurs in grades 4 - 7, depending on the province or territory.[80] Currently, in the province of Quebec, school-based HPV vaccination consists of one dose of Gardasil®9 in the fourth grade and one dose of Cervarix® five years later.[79,81] All other Canadian jurisdictions now administer a 2-dose schedule of Gardasil®9 within the same school year.[79,82] Between the introduction of vaccination programs and the 2018/2019 school year, the provinces of Newfoundland & Labrador and Prince Edward Island were the only Canadian jurisdictions to consistently achieve over 80% completion for the full course of vaccination.[79] For the whole of Canada, vaccination coverage of at least one dose by age 14 was 84% in 2021.[83] More recent data on HPV vaccine coverage in Canada is only

available for Alberta, Saskatchewan, Manitoba, New Brunswick, Nova Scotia, and the Yukon; according to this data, coverage in 2023 was 77.1% for females and 75.0% for males.[83]

HPV vaccination is highly effective at creating a stable long-term immune response,[75] preventing HPV infections and cervical and genital lesions,[75,84,85] and reducing rates of cervical cancer at the population level.[86] Vaccination also protects against HPV infection at oral, anal, and vulvar sites in females,[75] as well as oral and anal sites in males.[87] Vaccination programs are most effective when the vaccine is received before sexual debut and exposure to HPV.[85] HPV vaccination has not been shown to help clear pre-existing infections[88] or to inhibit the development of pre-existing infections to cancer or precancer.[75] Furthermore, the immune response to HPV vaccination is more robust at younger ages and weaker at older ages.[75]

1.3.7 Vaccine-Preventable and Phylogenetically Related HPV Types

The HPV types in the *Alphapapillomavirus* genus can be delineated into subgenera according to tissue tropism and oncogenicity, as well as phylogeny; HPV types in subgenus 1 have mucosal tissue tropism and low oncogenicity, HPV types in subgenus 2 have mucosal tissue tropism and high oncogenicity, and HPV types in subgenus 3 have mucocutaneous tissue tropism and result in commensal infections.[89–92] Of the HPV types assayed for the in TRAP-HPV study, HPVs 6, 11, 40, 42, 44, and 54 belong to subgenus 1; HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 and 82 belong to subgenus 2 and HPVs 61, 62, 71, 72, 81, 83, 84, and 89 belong to subgenus 3.[89–92] Subgenus 2 contains all the HPV types considered to be high-risk.[92]

Gardasil®9 protects against seven high-risk types (i.e. HPVs 16, 18, 31, 33, 45, 52, 58).[93] Within subgenus 2, HPV 18 and 45 are part of the α7 lineage (also known as species), while

HPV 16, 31, 33, 52, and 58 are part of the α9 lineage.[89] The α7 lineage also contains HPVs 39, 59, 68, and 70; while the α9 lineage also contains HPVs 35 and 67.[89] The low-risk HPVs (HPVs 6 and 11) that Gardasil®9 protects against are from the α10 lineage in subgenus 1.[89]

Previous research has found some level of cross-protection against HPV types that are phylogenetically related (i.e., within the same species or α lineage) to vaccine target types: for example, some protection against persistent infections with HPV 31, 33, and 45 has been reported in women vaccinated with the bivalent vaccine (which specifically targets HPV 16 & 18).[94] However, a systematic literature review looking at studies on the cross-protective effects of the bivalent and quadrivalent vaccines concluded that "cross-protective efficacy for non-vaccine HPV types appears to be partial, inconsistent across the non-vaccine HPV types assessed and to wane over time" (p. 2234).[95] The review also found that HPVs 31 and to a lesser extent 45 were the primary types against which cross-protection occurred.[95]

1.3.8 Screening

In Canada, cervical cancer screening is mostly via cytology, i.e., the Papanicolaou (Pap) test.[96] Guidelines vary slightly by jurisdiction, but generally, the recommendations are to start screening between 21 and 25 years of age and screen every 2-3 years until age 65-70.[96] Pap testing has a specificity of 96.8% and a sensitivity of 55.4% for detecting CIN2 or CIN3.[97] This means that a large proportion of women with potentially precancerous changes go undetected by Pap testing, so frequent screening is necessary.[98] HPV DNA testing has a specificity of 94.1% and a sensitivity of 94.6% for detecting CIN2 or CIN3[97] and, therefore, detects a higher percentage of precancerous changes and would allow for longer intervals between screenings.[13,98] The province of British Columbia recently implemented HPV DNA testing as the

primary screening modality,[99] and some other Canadian jurisdictions are also planning to implement HPV DNA testing.[100]

Whether progression from oral HPV infection to head and neck cancer occurs via detectable precancerous lesions that would allow for screening and intervention at the precancer stage is not clear.[101] There is currently no method of screening for oral precancer.[49] Some authors argue that implementing such screening programs could support early diagnosis and improve outcomes for HPV-related head and neck cancers,[55] while others argue that such screening would not be helpful.[101]

There are no screening programs for anal cancer, and studies have not indicated that anal cancer screening would be beneficial.[53] However, anal cancer is known to develop via precursor lesions analogous to cervical cancer development[102] and it has been argued that screening programs should be considered in areas with large populations at high risk for anal cancer.[103] Given the frequency of HPV type-specific concordance between cervical (or genital) and anal samples in females (discussed below), cervical cancer screening via HPV DNA testing may have the added benefit of helping to identify females at risk for anal cancer.[104]

1.3.9 Self-inoculation & Intra-individual Concordance Between Anatomical Sites

HPV is a sexually transmitted infection, yet there is a sizeable amount of research indicating that HPV can also be transmitted by means other than penetrative sexual acts (i.e., through non-sexual means or non-penetrative sex).[105] This may be especially applicable to the spread of HPV infection between genital and anal sites within individuals. Anal HPV infection has been found to occur in individuals without a history of anal sex, and genital HPV infection is an important risk factor for anal HPV infection.[106] For example, in a cohort study of females

aged 18-85 recruited 1998-2008 in Hawaii, Goodman and colleagues found that the relative risk of incident HPV-type specific concordant infections in the cervix or anus was elevated in participants if that HPV type had been previously detected at the other site.[107] Because cervical/anal type-specific concordance also occurred in participants who did not report practicing anal sex, the authors hypothesized that autoinoculation may play a role in intra-individual concordance.[107] In the same cohort, Hernandez and colleagues found that cervical and anal HPV prevalence at baseline were similar, at 29% and 27%, respectively.[108] Furthermore, looking at concurrent anal and genital HPV infections in females, they found substantial HPV type-specific concordance; 26% of participants were concordant for all HPV types detected in their cervical and anal samples, and 53% of participants were concordant at the two sites for some HPV types but not others.[108]

Similarly, in an analysis of women in the control arm of the Costa Rica vaccine trial, 32% of participants were positive for anal infection with any HPV, and genital HPV positivity (any type) at the same visit was a strong predictor of anal HPV positivity, with an OR of 4.8.[109] A 2019 pooled analysis, which assessed whether cervical cancer screening results predicted anal HPV infection and associated disease, demonstrated that HPV-type specific anal prevalence was substantially higher among females in whom the HPV type in question was detected concurrently at the cervix.[104] Additionally, in an analysis of women referred for colposcopy (n=118) in Greece, Nasioutziki and colleagues found significant HPV type-specific intra-individual concordance between cervical and anal sites.[110] It has also been observed that females with cervical precancer are at higher risk for anal, vaginal, and vulvar cancer.[111,112]

A Dutch study looking at HPV type-specific concordance between genital and anal sites in both women, and men who have sex with men, found that anal/genital concordance was likely

in females (i.e., Cohen's kappa was greater than 0.4 for 20 of the 25 HPV types assessed), but not in males (i.e., Cohen's kappa below 0.4 all HPV types).[113] However, in other studies, sequential genital/anal concordance has been observed in males. For instance, in men who have sex with women from the HPV Infection in Men (HIM) study, which includes participants from Brazil, Mexico, and the USA, Pamnani and colleagues found that the risk of an HPV type-specific anal infection was higher after a genital infection with that HPV type but previous anal HPV infection was not associated with a higher risk of type-specific genital HPV infection.[114] Likewise, in a study in China, the hazard ratio in males for a concordant anal HPV infection following a genital infection was 2.6 (95% CI: 1.4 - 4.6), but a previous anal infection was not associated with a higher risk of genital infection.[115]

Studies on oral/genital concordance within individuals have yielded inconclusive results. For example, among females, a large study using NHANES data from the USA (N= 3463) found oral HPV positivity in 4.1% of participants, vaginal HPV positivity in 42.5% of participants, and type-specific oral/genital HPV concordance in only 1.1% of participants.[116] Similarly, a study in Pakistan that looked at cervical and oral HPV (for 14 high-risk types) in 170 females found oral HPV positivity in 11.2% of participants, cervical HPV positivity in 48.8% of participants, and type-specific concurrent cervical/oral positivity in 5.9% of participants.[117] A Brazilian study in healthy females (N=76) found oral HPV positivity in 5.3% of participants and cervical HPV positivity in 9.2% of participants but did not detect any type-specific concordance between oral and genital sites.[118] The authors concluded that HPV infections at the two sites are unlikely to be related and that autoinoculation was improbable.[118] However, an Italian study comparing oral samples from females with (N=100) and without (N=25) HPV-related cervical lesions found that

oral HPV was more common among those with HPV-related cervical lesions (i.e., 24% vs. 8%), leading the authors to conclude that HPV infections at the two sites are correlated.[119]

Looking at oral/anogenital concordance in men who have sex with men, King and colleagues found that 13.7% of participants were positive for oral HPV and 64.9% were positive for anogenital HPV.[120] However, none of the 151 participants with both oral and genital samples tested positive for the same HPV type (of 21 types tested) in both samples.[120] On the other hand, among males, Dahlstrom and colleagues found an unadjusted prevalence ratio for type-specific oral HPV infection of 26.93 (95% CI: 11.33–64.03) in those with genital infections compared with those without.[69]

1.3.10 Concordance and Transmission Between Partners

A 2010 meta-analysis of 33 studies on anogenital HPV concordance between heterosexual partners concluded that 25.5% (95% CI: 17.2%-36.1%) of couples, were concordant for at least one HPV type.[121] Considering only couples wherein both partners tested positive for any HPV, type-specific concordance for at least one type was observed in 63.2% (95% CI: 49.1%-75.3%) of couples.[121]

Some previous research has indicated that genital-to-hand and hand-to-genital transmission could lead to transmission between individuals.[105] However, looking at type-specific concordance between hand and genital sites within individuals and between partners, Malagón and colleagues concluded that hand-to-genital transmission between partners was unlikely and transmission was primarily genital-to-genital.[122] A 2013 study that examined HPV type-specific concordance and transmission in heterosexual couples at genital, hand, and oral sites found that genital/genital concordance for at least one of HPV was between 64% and

95%.[123] The same study also found that throughout follow-up, female-to-male transmission was consistently higher than male-to-female transmission.[123] Similarly, A 2008 study of HPV transmission considering multiple anatomical sites within 25 heterosexual couples found that the female-to-male transmission rates were generally higher than male-to-female transmission rates, and the highest site-specific transmission rate was from the female anus to male genitals.[124] A review and meta-analysis of 7 studies on heterosexual genital HPV transmission found that most studies indicated that transmission is higher from females to males than vice-versa.[1]

CHAPTER 2: MANUSCRIPT 1 – SEX-SPECIFIC HPV INCIDENCE AND TRANSMISSION BY VACCINATION STATUS IN THE TRANSMISSION REDUCTION AND PREVENTION WITH HPV VACCINATION STUDY

2.1 PREFACE

This chapter comprises a manuscript that analyzes results from the Transmission Reduction and Prevention with HPV vaccination (TRAP-HPV) study with regards to the outcomes of genital HPV incidence and transmission, considering sex-specific rates across the four arms of the study for vaccine-targeted HPVs, HPV types that are phylogenetically closely related to vaccine-targeted HPVs, and HPV types that are relatively unrelated to vaccine-targeted HPVs. This manuscript was submitted to the *Journal of Infectious Diseases* on August 5th, 2024, and is formatted accordingly.

T-Table 1. Distribution of analytical sample in Manuscript 1 (n=154 couples) across the four arms of the TRAP-HPV study.

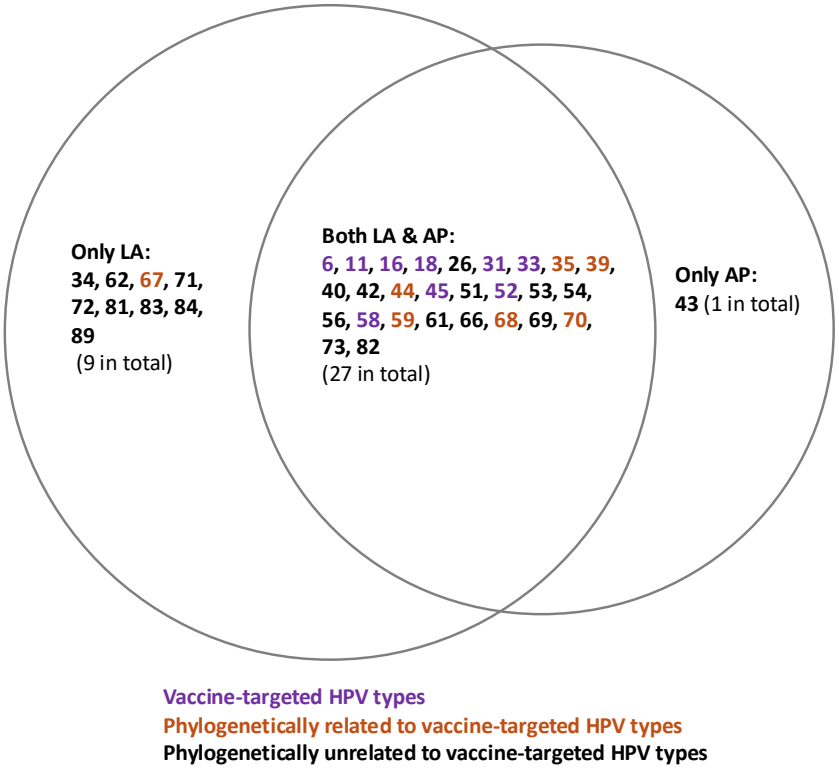
Female Vaccination	**Male Vaccination**	
	Active Control (U)	**HPV (V)**
Active Control (U)	M_uF_u: 40 couples	M_vF_u: 31 couples
HPV (V)	M_uF_v: 39 couples	M_vF_v: 44 couples

Abbreviations: HPV, human papillomavirus; TRAP-HPV Transmission Reduction and Prevention with HPV vaccination.

An interim analysis of results from the Transmission Reduction and Prevention with HPV vaccination (TRAP-HPV) has been previously published.[125] The previous study found lower rates of incident HPV infection among participants who received the intervention vaccine, but did not find evidence of protection to one's partner from recent vaccination. The analytical

samples (in the interim vs current analyses) are different, as the previous analysis was conducted and published while study participants were still being recruited. Furthermore, the analytical approaches are different; the previous study collapsed the study arms and conducted a cohort analysis, whereas the current study maintains the four-arm factorial design of the TRAP-HPV study.

As discussed in the manuscripts, most samples were genotyped using the Linear Array HPV Genotyping Test (Roche Diagnostic, Laval, Canada), which tests for 36 HPV types. However, the necessary reagents became unavailable; hence, later samples (those collected from February 11, 2020 onwards) were genotyped by Anyplex II HPV28 Detection Assay (Seegene, Seoul, Korea), which tests for 28 HPV types. Person-time at risk was adjusted accordingly in the analyses. T-Figure 1 illustrates the overlap of the HPV types tested via the two methods, as well as which HPV types are targeted by the 9-valent HPV vaccine, phylogenetically related to vaccine-targeted types, or relatively unrelated to vaccine-targeted types. It is worth noting that the change in HPV types detected between the two assays (and thus, the truncation of person-time at risk) pertained to 8 HPV types (HPVs 34, 62, 71, 72, 81, 83, 84, and 89) that are phylogenetically unrelated to vaccine-targeted types, and only one HPV type (HPV 67) that is phylogenetically closely related to vaccine-targeted types. For vaccine-targeted HPV types (the main group of interest), there was no change in the types detected and no person-time at risk truncated as a result of the change in assays.



T-Figure 1. HPV types tested via Linear Array (LA) and Anyplex II (AP)

2.2 HUMAN PAPILLOMAVIRUS INCIDENCE AND TRANSMISSION BY VACCINATION STATUS AMONG HETEROSEXUAL COUPLES

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Manuscript word count: 3427
Abstract word count: 250

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Author contributions:
ELF and ANB conceived the study design. ELF and MZ planned and supervised the study. MZ oversaw data collection and management. AM carried out analyses and drafted the manuscript with support from MZ and ELF. FC supervised HPV genotyping. PPT supervised the study nurses and advised on participant recruitment and sexual health. All authors reviewed, provided critical feedback, and approved the final version of the manuscript.

Footnote page:

Conflict of interest statement:

AM and PPT have no conflicts of interest to disclose.

ELF reports grants and personal fees from Merck; grants, personal fees, and nonfinancial support from Roche; and personal fees from GSK, all outside the submitted work.

ELF & MZ hold a patent related to the discovery “DNA methylation markers for early detection of cervical cancer”.

FC received grants or free reagents through his institution from Merck Sharp and Dome, Becton Dickinson, and Roche, as well as honoraria from Merck and Roche for lectures on HPV.

ANB is a Canada Research Chair in Sexually Transmitted Infection Prevention (Tier 2) and also receives support from a University of Toronto Department of Family and Community Medicine Non-clinician Scientist Award.

Funding Statement:
The TRAP-HPV study was funded by the Canadian Institutes of Health Research (CIHR) (grant MOP-125949 and grant FDN-143347 to ELF). It was also supported in part by a research grant from Investigator-Initiated Studies Program of Merck Canada Inc. The opinions expressed are those of the authors and do not necessarily represent those of Merck Canada Inc. HPV genotyping quality control was supported by the Réseau sida et maladies infectieuses (SIDA/MI) du Fonds de recherche du Québec–Santé (FRQS). AM was funded by a Canada Graduate Scholarship-Master’s, CIHR award and a McGill Faculty of Medicine Internal Studentship (in part through C. Epstein Fellowship in Women's Health).

Meeting(s) where the information has previously been presented:
Portions of this manuscript's results have been previously presented at a McGill University Division of Cancer Epidemiology monthly seminar on March 5, 2024, Montreal, Canada; EUROGIN 2024: International Multidisciplinary HPV Congress, March 13-16, 2024, Stockholm, Sweden; the Gerald Bronfman Department of Oncology 3rd annual Celebration of Research and Training in Oncology (CORTO) on May 14, 2024, Montreal, Canada; and the 2nd Epidemiology Symposium presented by students of l'ESPUM (École de santé publique de l’Université de Montréal) on May 21, 2024, Montreal, Canada. Abstract accepted for oral presentation at IPVC 2024 conference, November 12-15, 2024, Edinburgh, UK.

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2.2.1 Abstract

Background: Understanding human papillomavirus (HPV) transmission dynamics within couples is necessary for optimal vaccine strategies. We used data from the Transmission Reduction and Prevention with HPV Vaccination (TRAP-HPV) study to estimate sex-specific incidence and transmission rates.

Methods: The TRAP-HPV study enrolled (2014–2022) new (≤6 months) heterosexual couples aged 18+ in Montreal, Canada. Participants (n=308) were randomized into four comparison groups (both partners vaccinated against HPV or hepatitis A, or each received one or the other vaccine). Genital samples, collected at 0, 2, 4, 6, 9, and 12 months, were genotyped for 36 HPV types. We performed time-to-event analyses for vaccine-targeted HPVs (6/11/16/18/31/33/45/52/58) and HPVs phylogenetically related (35/39/44/59/67/68/70) and unrelated (26/34/40/42/51/53/54/56/61/62/66/69/71/72/73/81/82/83/84/89) to vaccine-targeted types, using type-specific HPV infections as the unit of analysis.

Results: Vaccination was weakly associated with lower incidence of vaccine-targeted HPV for males; incidence rates (in events/1000 months) were 0.99 (95%CI: 0.17, 3.07) and 1.67 (95%CI: 0.75, 3.51) in the two groups with vaccinated males versus 2.42 (95%CI: 0.97, 7.63) and 3.35 (95%CI: 1.95, 6.30) in the two groups with unvaccinated males. There was no consistent pattern of protection against incident HPV detection in females and no indication that recent vaccination was associated with lower transmission in discordant couples or with protection for one's partner. Results were similar for the three HPV groups.

Conclusions: In this population of sexually active adults, we did not find conclusive evidence that recent vaccination was associated with protection for oneself or one's partner. Findings should not be generalized to younger populations.

Keywords: Human papillomavirus, HPV, sexually transmitted infections, vaccination, incidence, transmission

2.2.2 Introduction

Human papillomavirus (HPV) infections are common, with an estimated worldwide prevalence of 11.7% [1]; three-quarters of sexually active adults contract at least one HPV infection throughout their lives [2]. There are over 40 types of HPV that can infect the genital mucosal epithelium [3], and co-infections with more than one HPV type are common [4,5]. Many HPV infections are asymptomatic, and most become undetectable and/or are cleared by the immune system within two years [4,6]. However, persistent infections with high-risk HPV types (particularly HPVs 16 and 18) can lead to oncogenesis [1,4,7]. Infections with low-risk HPVs (6 or 11) are the cause of genital warts and recurrent respiratory papillomatosis [4].

The Merck Gardasil quadrivalent vaccine protects against HPVs 6, 11, 16 & 18 [8,9], whereas Gardasil 9 (a nine-valent vaccine) protects against an additional five oncogenic types (HPVs 31/33/45/52/58) [10]. HPV vaccination is effective in preventing vaccine-targeted type infections in both females and males, [10–13] especially when administered at a young age and before exposure to HPV. Vaccination after the onset of sexual activity can still be beneficial, especially with Gardasil 9, as individuals may not yet have been exposed to all the vaccine-targeted HPV types [2].

Previous research has found sex-specific differences in the epidemiology and natural history of HPV infections; the rates of new infections remain relatively constant throughout adulthood in males, whereas in females, these are highest in young adults <25 years and then decline [1,17,18] with a second smaller peak in middle age [3,6]. Many seemingly incident detections of HPV in middle-aged females may be re-detections of latent infections [6,19,20]. It is unknown whether vaccination can reduce this re-emergence of latent infections [21].

Studies on HPV acquisition or prevention are often conducted with individuals as the unit of observation. However, as HPV is a sexually transmitted infection, optimal vaccination strategies require understanding transmission dynamics within couples [22,23]. Sexual contact with a new partner is a recognized risk factor for incident HPV infection [4], and the transmission of HPV is most likely to occur early in a relationship [24,25]. Yet, couple-based studies tend to include participants who have been together for a considerable length of time or do not report information on the duration of the relationship [23], making it challenging to elucidate transmission patterns. Another challenge to understanding the effects of vaccination on transmission dynamics is that HPV vaccination is often self-reported in epidemiological studies.

We previously showed, using data from an observational cohort study, that self-reported vaccination reduces transmission within couples [26]. A preliminary cohort analysis from a randomized controlled trial by our group, the Transmission Reduction and Prevention with HPV Vaccination (TRAP-HPV) designed to determine the efficacy of HPV vaccination in reducing transmission of HPV to the partners of vaccinated participants [27], found a lower risk of incident infections in vaccinated participants but no evidence of protection for one's partner [22]. Here, we used data from the final enrolled sample of the TRAP-HPV study to estimate sex-specific patterns of HPV transmission in newly formed heterosexual couples according to the four arms of the study, i.e., considering the vaccination status of the male and female partners.

2.2.3 Methods

Study design and procedures

Details of the TRAP-HPV study (registered at ClinicalTrial.gov; ID number: NCT01824537) have been described previously [22,27]. Briefly, the study enrolled couples in Montreal, Canada (January 2014- February 2022) if they fulfilled the following inclusion criteria: participants were cisgender, heterosexual couples (aged 18+) who had not received the intervention vaccine, had no anogenital cancer history, had been together for six months or less, were planning to stay in the Montreal area for at least a year and to have ongoing sexual contact, and were not pregnant or planning to become pregnant in the next year [27]. Participants were randomized individually to receive the intervention HPV vaccine (Gardasil before July 15, 2015, or Gardasil 9 thereafter) or a hepatitis A vaccine as the active control (Havrix before June 12, 2018, or Avaxim thereafter). This randomization created four trial arms in terms of who received the intervention vaccine: neither partner (M_uF_u), only the male partner (M_vF_u), only the female partner (M_uF_v), and both partners (M_vF_v). At the end of follow-up, participants were informed which vaccine they had received and offered the other vaccine. Genital samples were collected at enrolment and at 2, 4, 6, 9, and 12 months. Study procedures were interrupted by the COVID-19 pandemic, resulting in more than 12 months follow-up time for some participants; this affected control and intervention-vaccinated participants equally.

Participants were requested not to engage in sexual activity for at least 48 hours before each clinic visit. Female genital samples were self-collected after receiving written and verbal instructions from a research nurse and in accordance with a previously validated protocol [28–30]. Male genital samples were collected by a research nurse in accordance with a previously validated protocol [31,32]. At each visit, participants individually filled out self-administered

electronic questionnaires, providing information on sociodemographic factors, sexual behaviors, and sexual health history.

The Institutional Review Boards of McGill University (A04- M37-12A), Concordia University (30001405), and Centre Hospitalier de l'Université de Montréal (2014–2019, CE 13.016) approved the TRAP-HPV study. Written informed consent was collected from all participants.

HPV genotyping

HPV genotyping was done by polymerase chain reaction (PCR). Most samples (83%) were assayed for 36 HPV genotypes (6/11/16/18/26/31/33/34/35/39/40/42/44/45/51/52/53/54/ 56/58/59/61/62/66/67/68/69/70/71/72/ 73/81/82/83/84/89) via the Linear Array HPV Genotyping Test (Roche Diagnostic, Laval, Canada). Due to unavailability of Linear Array reagents, later samples (17%) were tested via the Anyplex II HPV28 Detection assay (Seegene, Seoul, Korea) for 28 HPV types (6/11/16/18/26/31/33/35/39/40/42/43/44/45/51/52/53/54/56/58/59/61/66/68/ 69/70/73/82). Both assays have very good agreement, with Anyplex II being more likely to detect multiple genotypes in the same sample [33]. Regardless of the assay used, co-amplification of the human β-globin gene was conducted to determine if samples were valid (i.e., contained sufficient intact human DNA) for HPV genotyping. Additionally, the Anyplex II samples were run with positive and negative controls from the manufacturer.

Statistical analysis

As shown in M1-Figure 1, the current analysis includes 82.8% of the enrolled participants, consisting of 154 couples who had i) at least one follow-up visit, and ii) valid baseline genital samples from both partners, or imputation was possible based on subsequent visits in case a baseline genital sample was invalid. For females and males within each study

arm, we calculated, via time-to-event and Kaplan Meier analyses, the incidence and transmission rates (and their 95% confidence intervals, CI) in events/1000 infection-months at risk (each participant could contribute time at risk for up to 36 type-specific HPV-level infections). Participants contributed time at risk for incidence of type-specific HPV-level infections if they had not previously tested positive for that HPV type (Supplementary M1-Figure S1A). Participants contributed time at risk for transmission if they had not previously tested positive for that HPV type and their partner had previously tested positive for that HPV type (Supplementary M1-Figure S1B).

We considered three groups of HPVs: (1) vaccine-targeted types, against which we would expect to see protection from recent vaccination; (2) types phylogenetically related (HPVs 35/39/44/59/67/68/70) to vaccine-targeted types [34], against which we might expect to see a limited amount of protection from recent vaccination [35,36]; and (3) other mucosotropic HPV types phylogenetically unrelated to the previous two groups (HPVs 26/34/40/42/51/53/54/56/61/62/66/69/71/72/73/81/82/83/84/89), against which we would not expect to see protection from recent vaccination. For the 9 HPV types detectable by Linear Array but not Anyplex II (HPVs 34/62/67/71/72/81/83/84/89), time at risk was included for samples tested via the former and truncated if later samples were tested via the latter assay. HPV 43, which is only detectable by Anyplex II, was not considered in the analyses.

Jackknife CIs are reported wherever possible to account for intraparticipant correlation; if there were insufficient events, exact Fisher's 95% CIs were calculated using WinPepi (version 11.65, J.H. Abramson, 2016). We conducted a sensitivity analysis, restricting to couples wherein both partners reported no outside sexual contact for the duration of their relationship. Most analyses were conducted in Stata (version 18.0, StataCorp LLC., TX).

2.2.4 Results

M1-Table 1 presents baseline characteristics of participants by sex and vaccination status; These did not vary markedly between the groups. Overall, the mean age was 25.5 years (SD 6.0). Almost 50% of participants were Canadian born, with 7.5% born in the US and 42.8% born elsewhere. The majority of participants had some post-secondary education, with only 17.5% reporting high school as their highest level of education. Most had never been smokers, while 21.4% and 7.8% were former or current smokers, respectively. The median number of lifetime vaginal sexual partners was 6 (interquartile range: 2-15), and the median age at coitarche was 18 years (interquartile range: 16-19 years). The median time since coitarche was 5.8 years (interquartile range: 2.7-10.5 years). Overall, 43.5% of participants were positive for at least one HPV type at baseline, 17.9% were positive for one or more vaccine-preventable HPV types, 13% were positive for one or more HPV types that are phylogenetically related to vaccine-preventable HPV types, and 39% were positive for one or more HPV types phylogenetically unrelated to vaccine-preventable HPV types. Female participants were, on average, slightly younger than males, and ages were similar between vaccinated and unvaccinated participants. Positivity for any of the nine vaccine-targeted HPVs was slightly higher in females; 18.3% and 19.3% for vaccinated and unvaccinated participants, respectively; among males, it was 17.7% and 16.0% for vaccinated and unvaccinated participants, respectively. Unvaccinated males had a slightly lower prevalence of any HPV than those vaccinated (38.0% vs 44.0%). Conversely, unvaccinated females had a slightly higher prevalence of any HPV than vaccinated females (47.9% vs 44.6%). Unvaccinated males were less likely than vaccinated males to report having concurrent sexual partners (6.3 % vs 18.7%). Vaccinated males included a higher percentage of participants without post-secondary education than unvaccinated males (28.0% vs.15.2%).

M1-Table 2 shows the sex-specific incidence and transmission rates of the three outcomes among the four study arms. For vaccine-targeted HPV in females, there was no consistent pattern of protection (to oneself) against incident infection through recent vaccination. While the M_uF_v group had the lowest point estimate for the incidence rate (1.05, 95% CI: 0.42, 3.45), the second lowest was in the M_uF_u group (1.40, 95% CI: 0.51, 5.34), whereas the M_vF_u and M_vF_v groups had the highest point estimates for the incidence rates (1.58, 95% CI: 0.55, 6.17 and 1.58, 95% CI: 0.71, 4.25, respectively). The lowest point estimates for incidence rates were for phylogenetically related and unrelated HPVs in the M_uF_u group. For the vaccine-targeted HPVs in males, there was a pattern consistent with protection to self from recent vaccination; the point estimates for the incidence rates were lower in the groups with vaccinated males than those with unvaccinated males: 0.99 (95% CI: 0.17, 3.07) and 1.67 (95% CI: 0.75, 3.51) in the M_vF_u and M_vF_v groups, respectively, versus 2.42 (95% CI: 0.97, 7.63) and 3.35 (95% CI: 1.95, 6.30) in the M_uF_u and M_uF_v groups, respectively. As expected, this pattern was not seen for phylogenetically related and unrelated HPVs. For vaccine-targeted HPVs, there was no indication that recent vaccination of oneself or one's partner is associated with protection against transmission to females. The point estimate for the transmission rate was lowest in the group with neither partner vaccinated and highest in the group with both partners vaccinated (7.07, 95% CI: 0.18, 39.37 vs. 29.83, 95% CI: 7.26, 145.53). For phylogenetically related HPVs, the point estimate for the transmission rate to females was also lowest in the group with neither partner vaccinated. There was also no consistent pattern indicating that recent vaccination of either oneself or one's partner is associated with lower transmission of vaccine-targeted HPV to males. Although the lowest point estimate for the transmission rate was observed in the M_vF_v group (0, 95% CI: 0.00, 45.22), the second lowest was in the M_uF_u group (15.90, 95% CI: 0.40,

88.61). Furthermore, the lowest point estimates for transmission rates for phylogenetically related and unrelated HPVs were also in the M_vF_v group.

M1-Figures 2 and 3 show the respective Kaplan-Meier failure curves for incidence and transmission across the 3 HPV groups within the 4 study arms. As expected, the M_vF_u group had a lower proportion of incident infections in males compared with females, while the M_uF_v group had a lower proportion of incident infections in females compared with males. However, these differences were slight, and the results were similar within the M_vF_u group for all three HPV groups. Contrary to expectations, the group with both partners vaccinated had a particularly high proportion of transmissions of vaccine-targeted HPV to females.

Restricting the analysis to couples who consistently reported no outside sexual contact (n= 87) showed similar findings of protection to oneself from recent vaccination in males but not in females (M1-Table S1). For transmission, the restricted sample had too few events to either support or contradict the findings from the main analysis.

2.2.5 Discussion

We described the sex-specific incidence and transmission rates in the TRAP-HPV study according to the couple-level vaccination assignment group. Other than a weak indication of vaccine protection from incident detection amongst males, our findings are not consistent with protection, in terms of incidence or transmission, from recent vaccination for oneself or for one's partner. This is contrary to expectations and inconsistent with previous studies [22,26].

In this study, follow-up visits to detect incident infections started two months after the first vaccine dose. Previous research indicates that protection could have been observed by that time. Research from the Costa Rica vaccine trial, which found a persistent antibody response

after one dose of the bivalent vaccine, showed that antibody titers at one month were above the subsequent plateau [37].

The previous cohort analysis of the TRAP-HPV data found indications of protection to oneself, reporting an overall hazard ratio (HR) of 0.47 (95% CI: 0.23, 0.97) for incident infections of vaccine-targeted HPV types among vaccinated compared with unvaccinated participants, with HRs of 0.45 (95% CI: 0.15, 1.35) and 0.51 (95% CI: 0.19, 1.34) in females and males, respectively, for participants who had received at least one vaccination dose [22]. A couple of factors may have contributed to the differences between the previous and current findings. First, the previous analysis collapsed the study arms, resulting in larger groups with more events per group and, hence, more statistical power to detect an effect. Second, the follow-up time is longer in the current analysis, which could contribute to a different apparent distribution of events between the study arms.

There are few couple-based studies of HPV transmission and even fewer which also considered vaccination [23]. One previous couple-based study which did consider vaccination was the HPV Infection and Transmission among Couples through Heterosexual Activity (HITCH) cohort study (Montreal, Canada, 2005 – 2011) [26]. Participants were females aged 18-24 and their male partners aged 18 and over. Some of the female participants in the HITCH study elected to be vaccinated against HPV before or during the study, and self-reported vaccination of females reduced transmission of vaccine-targeted and phylogenetically related HPV types to their male partners [26]. However, the effect was entirely due to a reduced number of infections in vaccinated females, i.e., there was no evidence that vaccination reduced the transmissibility of pre-existing infections [26]. Several methodological characteristics inherent to the HITCH study [26] may explain the difference in findings. First, that study was an observational cohort; hence,

participants who opted to receive the HPV vaccine may have differed from those who did not in other ways that contributed to lower HPV transmission. Second, receipt of the first vaccine dose preceded the start of the partnership for a minimum of 35 of the 63 vaccinated participants in the HITCH study [26], while in the TRAP-HPV study, the start of the relationship preceded vaccination [27]. Finally, the median age at self-reported vaccination in the HITCH study was 18 years [26], whereas in the current study, the median age for receiving the first vaccine dose (equivalent to the median age of participants) was 25.5 years. Thus, participants in the current study may have been exposed to more HPV types prior to receiving the vaccine.

In another analysis of the HITCH cohort, Malagón and colleagues estimated that 43% of putative incident infections could be latent infections becoming detectable again [38]. The TRAP-HPV study included older participants compared to females in HITCH [38]. Hence, the proportion of detections attributable to re-emerging latent infections could be even greater in the TRAP-HPV study. Redetection of latent infections is likely not preventable through vaccination and, thus, would be equally likely to occur in all study arms, which could partially explain the lack of observed protection from recent vaccination.

Another factor that may contribute to the lack of observed protection in the current analysis is the age of participants (mean 25.5 years). A 2020 US study in females 20-29 years of age found that the prevalence of quadrivalent vaccine-targeted HPVs was reduced in those vaccinated with 1, 2, or 3 doses compared to unvaccinated females [14]. However, the reduction in prevalence compared to unvaccinated females was much greater for those vaccinated by age 18 than for those vaccinated after [14]. Furthermore, a systematic review of the effectiveness of HPV vaccination found that studies reporting HPV infection as the endpoint consistently showed lower effectiveness with increasing age at vaccination [15]. For example, a 2017 study from

Scotland found that while the bivalent vaccine was 89.1% effective if given between the ages of 12 and 13, it was only 28.9% effective if given after the age of 18 [16].

Several limitations to the current study and analyses need to be acknowledged. First, loss to follow-up was 31.5% overall, likely due in part to the couple-based nature of the study, as couples were censored from the study if they broke up. However, given that only couples in new relationships were eligible and the follow-up of one year was twice as long as the maximum pre-enrollment relationship duration, it is not surprising that many couples terminated their relationship during the study. Second, although couples were asked not to engage in sexual activity for 48 hours prior to clinic visits, some detections could be due to residual depositions of biological material from one's partner rather than actual incident infections [39]. As it would equally be likely to occur in any of the trial arms, it could have obscured any underlying pattern. Third, because of the relatively small sample size, few events were observed in each study arm, making it difficult to detect an effect. Based on an estimated effect size of 40%, we had initially calculated that 90% power to detect an effect would require 125 couples per study arm and planned to recruit 500 couples [27]. However, given the necessarily stringent recruitment criteria regarding relationship status, duration, and stability, the recruitment of eligible couples proved challenging. The COVID-19 pandemic also exacerbated pre-existing recruitment challenges. Thus, to maintain the scientific value and timeliness of the results, the decision was made to close the study prior to reaching the target sample size.

The above limitations notwithstanding, a unique strength of the TRAP-HPV study is that participants are couples in relatively new sexual relationships, when transmission is most likely. Moreover, the innovative 2x2 factorial design allows comparisons by biological sex and vaccination status of both self and partner. Furthermore, since participants were randomized,

there is a reasonable assumption of exchangeability between the study arms. Additionally, the prospective nature of the study and frequent follow-up visits allow for a good level of precision regarding time to events. Importantly, vaccination was administered as part of the study, reducing the chance of misclassification, which is expected with self-reported vaccination status. Finally, the use of type-specific HPV-level infections as the unit of analysis allowed for more insight into transmission dynamics between couples since only the negative partner in a type-specific discordant partnership was at risk of transmission. Type-specific HPV-level infections also provide greater statistical power to detect an effect since each participant contributed time at risk for multiple HPV types.

In conclusion, in this study of sexually active adults aged on average 25 years, we did not find conclusive evidence of a protective effect from recent HPV vaccination against either incident infection or transmission for oneself or one's partner. Given the low number of events in each study arm and the well-established efficacy of HPV vaccination in preventing HPV infection, these findings should be interpreted with great caution and should not be generalized to younger or less sexually experienced populations. Future studies with larger sample sizes could yield further insights into the effects of HPV vaccination in sexually active adult populations.

2.2.6 Acknowledgements

The authors wish to thank the participants and employees of the TRAP-HPV study.

Funding statement

The TRAP-HPV study was funded by the Canadian Institutes of Health Research (CIHR) (grant MOP-125949 and grant FDN-143347 to E.L.F.). It was also supported in part by a

research grant from Investigator-Initiated Studies Program of Merck Canada Inc. The opinions expressed are those of the authors and do not necessarily represent those of Merck Canada Inc. HPV genotyping quality control was supported by the Réseau sida et maladies infectieuses (SIDA/MI) du Fonds de recherche du Québec–Santé (FRQS). AM was funded by a Canada Graduate Scholarship-Master's, CIHR award and a McGill Faculty of Medicine Internal Studentship (in part through C. Epstein Fellowship in Women's Health).

2.2.7 Manuscript 1 References

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2.2.8 Manuscript 1 Tables

M1-Table 1. Baseline characteristics of participants in the TRAP-HPV study, overall and by sex and vaccine assignment.

Variables, n (%) unless otherwise indicated	**Overall (n=308)**	Female		Male	
		unvaccinated (n=71)	vaccinated[a] (n=83)	unvaccinated (n=79)	vaccinated[a] (n=75)
Age, mean (SD)	25.5 (6.0)	24.2 (4.7)	25.4 (6.2)	25.7 (5.7)	26.7 (6.9)
Birth country					
Canada	152 (49.4)	39 (54.9)	41 (49.4)	36 (45.6)	36 (48.0)
United States	23 (7.5)	5 (7.0)	7 (8.4)	7 (8.9)	4 (5.3)
Elsewhere[b]	132 (42.9)	27 (38.0)	34 (41.0)	36 (45.6)	35 (46.7)
Missing	1 (0.3)	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Education					
High school	54 (17.5)	10 (14.1)	11 (13.3)	12 (15.2)	21 (28.0)
College or vocational training	55 (17.9)	10 (14.1)	15 (18.1)	16 (20.3)	14 (18.7)
University	198 (64.3)	51 (71.8)	57 (68.7)	50 (63.3)	40 (53.3)
Missing	1 (0.3)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)
Smoking status					
Never	216 (70.1)	52 (73.2)	60 (72.3)	59 (74.7)	45 (60.0)
Former[c]	66 (21.4)	13 (18.3)	16 (19.3)	16 (20.3)	21 (28.0)
Current	24 (7.8)	6 (8.5)	6 (7.2)	3 (3.8)	9 (12.0)
Missing	2 (0.6)	0 (0.0)	1 (1.2)	1 (1.3)	0 (0.0)
Concurrent sex partners					
No	259 (84.1)	58 (81.7)	67 (80.7)	74 (93.7)	60 (80.0)
Yes	47 (15.3)	13 (18.3)	15 (18.1)	5 (6.3)	14 (18.7)
Missing	2 (0.6)	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.3)
Number of lifetime sex vaginal partners, median (Q1, Q3)	6 (2,15)	5 (2, 14)	6 (2,20)	8 (3,15)	7 (3,16)
Age at coitarche, median (Q1, Q3)	18 (16, 19)	17 (16, 19)	17.5 (16, 19)	18 (16, 20)	18 (16, 19)
Years since onset of sexual activity, median (Q1, Q3)[d]	5.8 (2.7, 10.5)	4.8 (2.5, 9.4)	5.4 (2.5, 9.8)	6.0 (2.9, 10.3)	7.6 (3.1, 12.6)
Grouped HPV positivity[e]					
Vaccine-targeted (any 9vHPV)	55 (17.9)	13 (18.3)	16 (19.3)	14 (17.7)	12 (16.0)
Phylogenetically related to vaccine-targeted types	40 (13.0)	10 (14.1)	13 (15.7)	7 (8.9)	10 (13.3)
Phylogenetically unrelated to vaccine-targeted types	120 (39.0)	29 (40.8)	33 (39.8)	28 (35.4)	30 (40.0)
Any HPV	134 (43.5)	34 (47.9)	37 (44.6)	30 (38.0)	33 (44.0)

Abbreviations: HPV, human papillomavirus; Q1, first quartile; Q3, third quartile; SD, standard deviation.

[a] Participants (11 males and 9 females) received Gardasil up until July 8, 2015, after which they received Gardasil 9 (64 males and 74 females) .

[b]Includes: France, India, Iran, Brazil, China, Mexico, South Korea, Russian Federation & grouped regions: East Asia, Southeast Asia, MENA (Middle East and North Africa), Sub-Saharan Africa, Latin America, Europe, Central Asia, and Oceania.

[c]Includes participants who reported not being current smokers but reported smoking regularly in the past and/or reported having smoked at least 100 cigarettes in their lifetime.

[d]Age at baseline minus age at coitarche.

[e]Vaccine-targeted types include any of HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58. Phylogenetically related types include any of HPVs 35, 39, 44, 59, 67, 68, and 70. Phylogenetically unrelated types include any of HPVs 26, 34, 40, 42, 51, 53, 54, 56, 61, 62, 66, 69, 71, 72, 73, 81, 82, 83, 84, and 89. Any HPV includes any of 36 HPV types that were tested for: HPVs 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58 , 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89.

M1-Table 2. HPV incidence and transmission in female and male participants of the TRAP-HPV study, N= 308 (154 couples).

Vaccination assignment	Grouped HPV types[a]	INCIDENCE						TRANSMISSION					
		FEMALE			MALE			MALE-TO-FEMALE			FEMALE-TO-MALE		
		Events	Time[b]	Rate[c] (95% CI)	Events	Time[b]	Rate[c] (95% CI)	Events	Time[b]	Rate[c] (95% CI)	Events	Time[b]	Rate[c] (95% CI)
Male and female unvaccinated n=40 couples	**Vaccine-targeted**	5	3560.54	1.40 (0.51, 5.34)	8	3311.79	2.42 (0.97, 7.63)	1	141.54	7.07 (0.18, 39.37)[d]	1	62.88	15.90 (0.40, 88.61)[d]
	Related	1	2713.97	0.37 (0.01, 2.05)[d]	3	2552.65	1.18 (0.21, 17.18)	0	44.78	0 (0.00, 82.38)[d]	1	8.08	123.73 (3.09, 689.60)[d]
	Unrelated	12	7153.20	1.68 (0.94, 3.29)	15	6784.93	2.21 (1.35, 3.88)	4	172.82	23.15 (7.85, 76.83)	5	93.93	53.23 (28.79, 99.00)
Male vaccinated, female unvaccinated n=31 couples	**Vaccine-targeted**	5	3172.26	1.58 (0.55, 6.17)	3	3034.10	0.99 (0.17, 3.07)	1	47.90	20.88 (0.52, 116.33)[d]	2	50.47	39.63 (4.79, 143.15)[d]
	Related	5	2305.25	2.17 (0.94, 6.02)	5	2219.67	2.25 (1.00, 6.10)	2	34.99	57.16 (11.98, 361.22)	2	38.11	52.48 (13.61, 271.55)
	Unrelated	22	6081.94	3.62 (1.88, 7.80)	17	5887.70	2.89 (1.49, 6.24)	9	147.78	60.90 (28.94, 141.46)	8	239.91	33.35 (14.26, 75.11)
Male unvaccinated, female vaccinated n= 39 couples	**Vaccine-targeted**	4	3793.67	1.05 (0.42, 3.45)	12	3581.69	3.35 (1.95, 6.30)	1	75.40	13.26 (0.33, 73.90)[d]	5	39.10	127.89 (41.84, 354.21)
	Related	3	2877.35	1.04 (0.34, 4.69)	6	2740.19	2.19 (1.05, 5.30)	1	62.49	16.00 (0.40, 89.17)[d]	2	65.25	30.65 (4.56, 245.92)
	Unrelated	22	7499.06	2.93 (1.67, 5.59)	26	7286.07	3.57 (2.28, 5.93)	2	131.78	15.18 (3.70, 108.35)	12	323.72	37.07 (20.92, 70.39)
Male and female vaccinated n=44 couples	**Vaccine-targeted**	7	4431.81	1.58 (0.71, 4.25)	7	4189.61	1.67 (0.75, 3.51)	3	100.57	29.83 (7.26, 145.53)	0	81.58	0 (0.00, 45.22)[d]
	Related	9	3265.27	2.76 (1.43, 6.00)	10	3141.70	3.18 (1.58, 7.26)	2	77.50	25.80 (5.51, 199.62)	2	127.74	15.66 (3.65, 117.67)
	Unrelated	24	8712.42	2.75 (1.50, 5.63)	20	8307.32	2.41 (1.45, 4.26)	5	267.21	18.71 (7.33, 53.62)	7	333.80	20.97 (11.35, 40.69)

Abbreviations: HPV, human papillomavirus; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination.

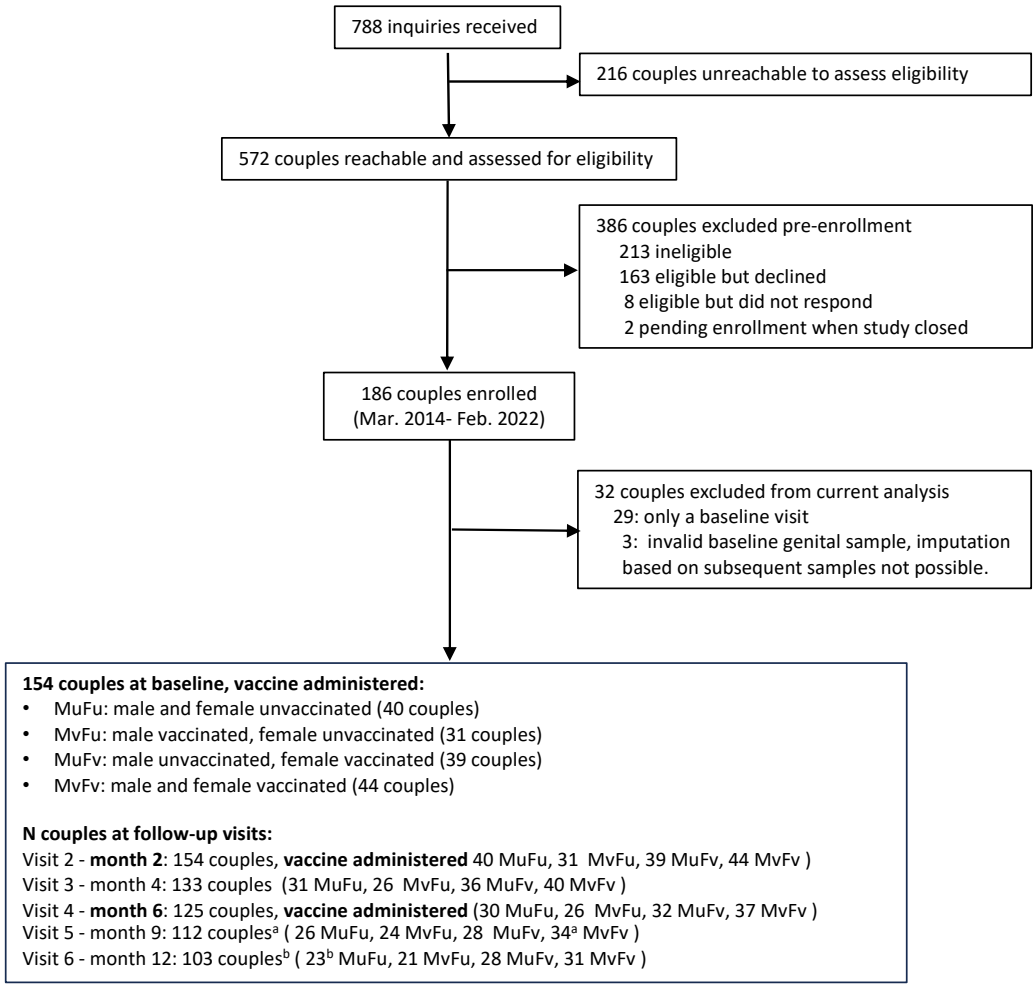
[a]Vaccine-targeted types include any of HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58. Phylogenetically related types include any of HPVs 35, 39, 44, 59, 67, 68, and 70. Phylogenetically unrelated types include any of HPVs 26, 34, 40, 42, 51, 53, 54, 56, 61, 62, 66, 69, 71, 72, 73, 81, 82, 83, 84, and 89.

[b] Indicates infection-months at risk. All analyses were at the HPV-level, meaning that each participant contributed time at risk for up to 36 HPV types. Participants contributed time at risk for incidence if they had not previously tested positive for that HPV type. If a participant tested positive for only 1 HPV type, they would no longer contribute time at risk for that particular type but would continue to contribute time at risk for the other 35 types. Participants contributed time at risk for transmission if they had not previously tested positive for that HPV type and their partner had previously tested positive for that HPV type.

[c]Rates represent events (incidence or transmission) /1000 infection-months at risk. Jackknife confidence intervals are reported wherever possible to account for intra-participant correlation.

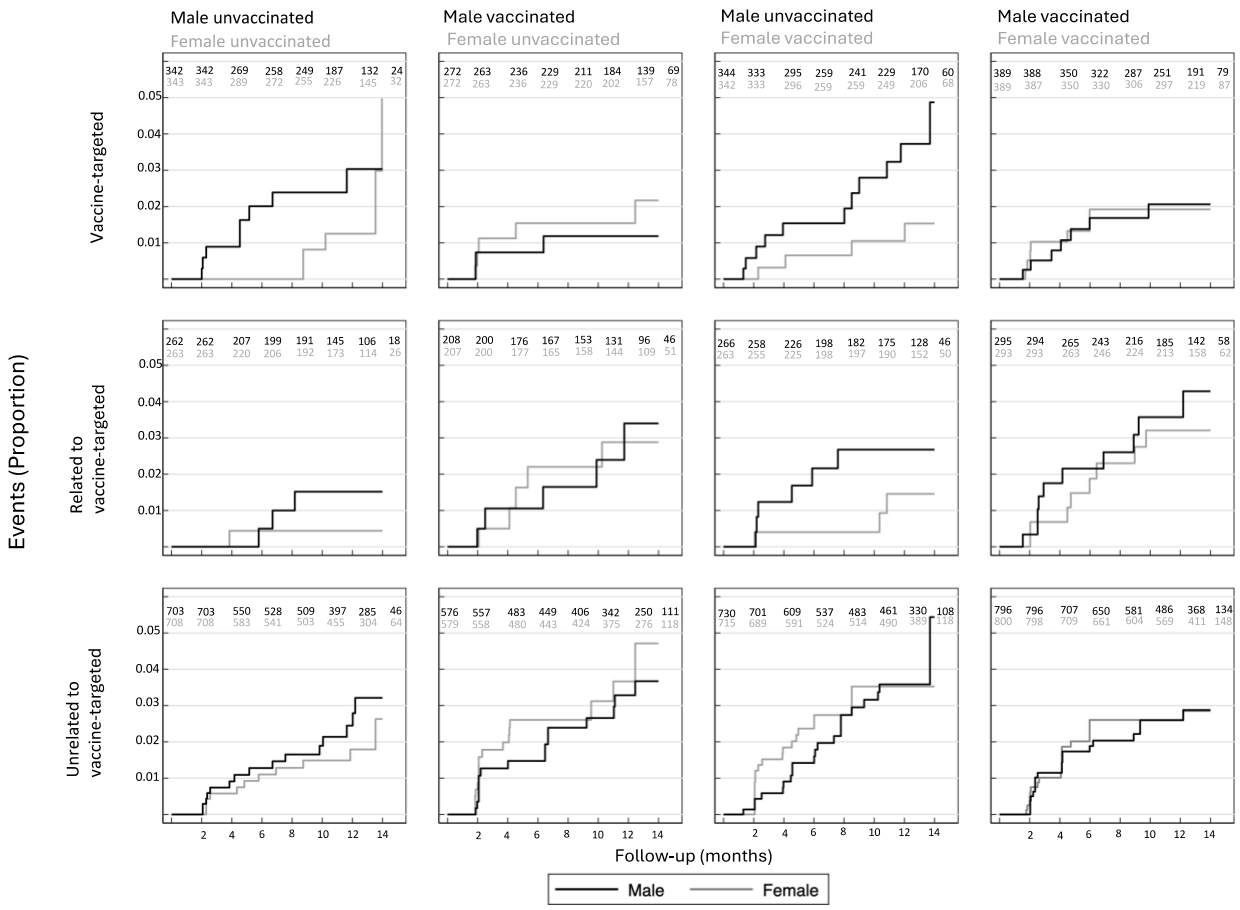
[d] In instances where no events were observed, or there was an insufficient number of failures to calculate jackknife confidence intervals, exact Fisher's 95% confidence intervals were used.

2.2.9 Manuscript 1 Figures



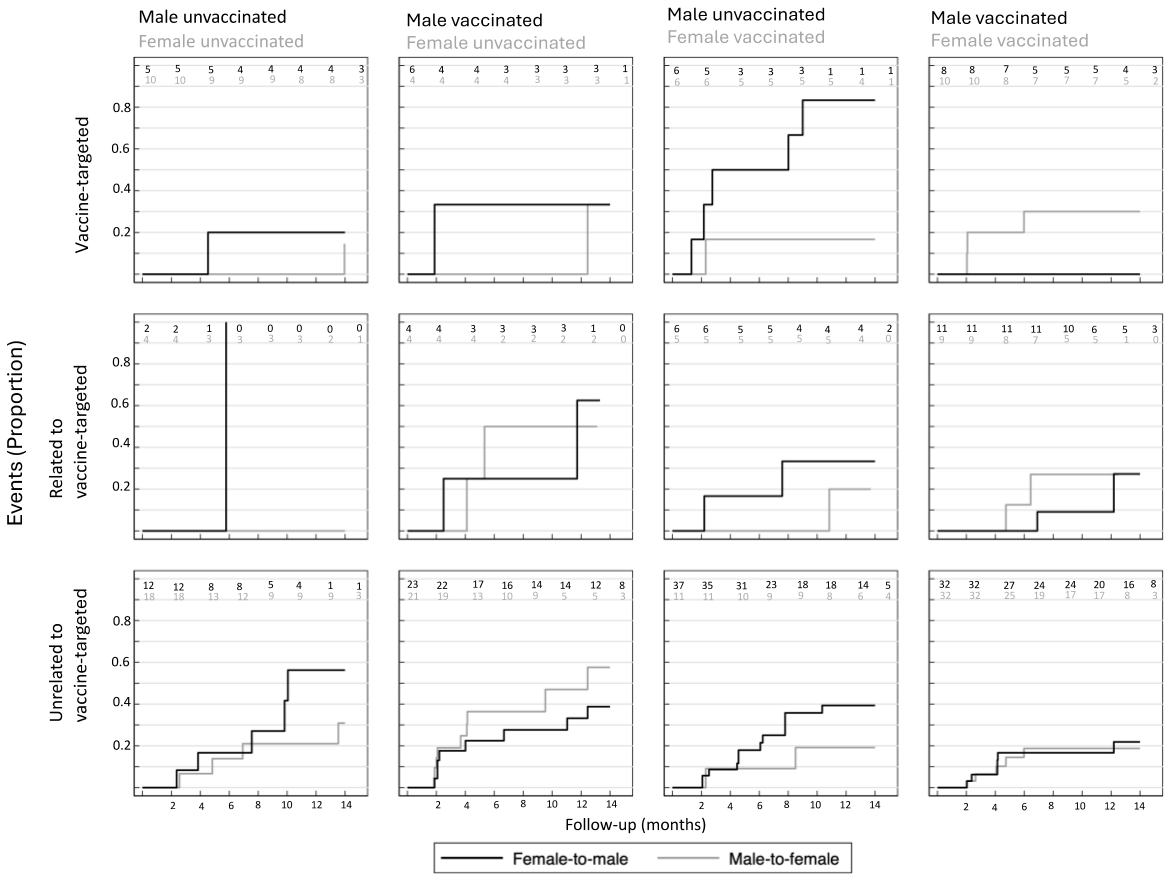
[a]112 females/111 males
[b]103 females/102 males

M1-Figure 1. Enrollment, randomization, and analytical sample in the TRAP-HPV study. In May 2022, the study protocol was amended to make visit 5 the final visit. This shortened follow-up duration affected only three couples.



M1-Figure 2. Incidence of vaccine-targeted HPV types as well as that of HPV types phylogenetically related and unrelated to vaccine-targeted HPV types in males and females among the four vaccination assignment groups. Number of type-specific infections at risk is shown at the top of each graph for males (black) and females (grey). Each participant contributes up to 36 HPV types in total:

9 types for vaccine-targeted infections, 7 types for phylogenetically related, and 20 types for phylogenetically unrelated. These analyses were conducted at the HPV-level, meaning that risk-tables reflect the number or type-specific infections which could occur within each HPV category.



M1-Figure 3. Transmission of vaccine-targeted HPV types as well as that of HPV types phylogenetically related and unrelated to vaccine-targeted HPV types from females-to-males and from males-to-females among the four vaccination assignment groups. Number of type-specific infections at risk is shown at the top of each graph for female-to-male transmission (black) and male-to-female transmission (grey). Each participant contributes up to 36 HPV types in total: 9 types for vaccine-targeted infections, 7 types

for phylogenetically related, and 20 types for phylogenetically unrelated. These analyses were conducted at the HPV-level, meaning that risk-tables reflect the number or type-specific infections which could occur within each HPV category.

CHAPTER 3: MANUSCRIPT 2 – SEX-SPECIFIC & HPV TYPE-SPECIFIC CONCORDANCE AT MULTIPLE ANATOMICAL SITES WITHIN INDIVIDUALS AND BETWEEN PARTNERS IN THE TRANSMISSION REDUCTION AND PREVENTION WITH HPV VACCINATION STUDY

3.1 PREFACE

Manuscript 1, presented in Chapter 2, did not find conclusive evidence of an effect from recent vaccination in the study population (i.e., sexually active adults with a mean age over 25 years). Chapter 3 also comprises a manuscript that analyzes data from the Transmission Reduction and Prevention with HPV vaccination (TRAP-HPV) study. However, the research question no longer relates to the effect of vaccination. In this second manuscript, we assessed the concordance of type-specific HPV infections at genital, oral, and anal sites within individuals and between partners, as well as predictors of genital concordance between partners. This manuscript will be submitted to the journal *Clinical Infectious Diseases* and is formatted accordingly.

3.1.1 Rationale for Using Mixed-Effects Logistic Regression

When using regression analysis to estimate effect sizes, generalized estimating equations (GEEs; also referred to as marginal models) could be used to estimate the average population-level effect of a predictor variable of interest (e.g., one's partner being positive for a given HPV type).[126,127] Alternately, for correlated data, one may use mixed-effect models, which allow for random intercepts (and/or slopes) at the cluster level and produce cluster-specific effect estimates; such estimates tend to be larger than analogous population-level effects that would be estimated by GEEs.[126,127] An individual can be infected with more than one HPV type at the same time.[128,129] Furthermore, given shared demographic risk factors and modes of acquisition,

multiple HPV infections in the same person are not entirely statistically independent.[126,130–132] Because type-specific HPV infections (rather than participants) were the unit of analysis, clustering at the level of the individual participant was appropriate in order to account for correlation in the data. Furthermore, we were interested in estimating how an individual's odds of HPV type-specific positivity at a given site would change if that individual were exposed vs. unexposed to the predictor (e.g., their partner's HPV type-specific positivity at a given site). Therefore, we selected mixed-effects logistic regression as the analytical approach.

3.2 GENITAL, ORAL, AND ANAL TYPE-SPECIFIC HUMAN PAPILLOMAVIRUS CONCORDANCE WITHIN INDIVIDUALS AND BETWEEN PARTNERS

To be submitted to: Clinical Infectious Diseases https://academic.oup.com/cid

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Summary of the article's main point:
Type-specific HPV concordance across anatomical sites within individuals is high, particularly genital/anal concordance in females, supporting previous conclusions that autoinoculation occurs. There is high type-specific genital HPV concordance between partners, especially among couples with greater reported occasions of intimacy.

3.2.1 Abstract

Background: Studies assessing HPV concordance between male and female partners and between multiple anatomical sites are needed.

Methods: Heterosexual couples, aged 18+ formed within the past 6 months, were recruited (2014 -2022) in Montreal, Canada. Participants attended visits at 0, 2, 4, 6, 9, and 12 months. They answered electronic questionnaires and provided biological samples (genital, oral, anal) for HPV genotyping. We calculated observed/expected (O/E) concordance (with 95% confidence intervals) between anatomical sites of HPV genotype-specific infections, across all visits and cumulatively (i.e., ever-positivity). We used mixed-effects logistic regression with random intercepts at the person-level to estimate odds ratios (OR) for concordance and to assess predictors of genital HPV detection and partner concordance.

Results: Within-individual O/E genital/anal concordance was 23.37 (15.55, 38.05) for females and 14.79 (9.20, 43.45) for males, whereas genital/genital O/E concordance between partners was 14.99 (12.47, 18.41). Genital/genital concordance for ever-positivity within couples was substantial: O/E: 10.06 (8.55, 12.12), OR: 70.75 (43.70, 114.56) for females and 67.34 (41.96, 108.06) for males. Significant predictors of genital ever-positivity were one's partner's ever-positivity, OR: 66.2 (40.96, 107.08) in females and 61.53 (38.19, 99.14) in males, and age above the median (23.2 and 24.3 years in females and males, respectively), OR: 1.66 (1.06, 2.59) in females and 1.95 (1.30, 2.91) in males. Concordance doubled (OR: 1.96; 1.12, 3.46) with occasions of intimacy above the median.

Conclusions: Findings indicate that HPV infection at one anatomical site implies an increased risk of a type-specific infection at other sites within individuals and between partners.

Keywords: Human papillomavirus, HPV, sexually transmitted infections, concordance

3.2.2 Introduction

Persistent infection with high-risk human papillomavirus (HPV) types is a necessary cause of cervical cancer and contributes to the burden of other anogenital and head and neck cancers [1,2]. HPV is common among females [3,4] and males [5]. Studies have considered HPV concordance across different anatomical sites within females [6–10] or males [11,12], as well as genital/genital HPV concordance between partners [13,14]. However, there is little research considering HPV infections in males and females from the same population at multiple anatomical sites [15] and over time, as well as concordance between partners.

Concordant type-specific genital HPV infections between heterosexual partners are more common than would be predicted by chance [13], particularly for HPVs 11, 16, and 18 [14]. Within females, high genital/anal concordance of HPV type-specific infections was found at the same study visit [6–8] and from one visit to the next [9]. Similarly, a study among males showed a heightened risk of type-specific anal HPV infection following a genital infection, especially for HPV16 [11]. Evidence for cervical/oral HPV type-specific concordance was found in a meta-analysis conducted in 2011 among 1017 females with genital HPV infections [10], contrary to findings from a study among 118 females referred for colposcopy [7]. A study in 3140 males reported that previous oral infection was associated with an increased risk of subsequent genital infection and previous genital infection was associated with an increased risk of subsequent oral infection [12].

Within heterosexual partnerships, the determinants of HPV prevalence include condom use history and concurrent partners [16]; whereas predictors of transmission include frequency of sexual intercourse and condom use (i.e., those who always used condoms having lower transmission rates than those who never used condoms) [17]. In males, higher oral HPV

prevalence has been associated with smoking, concurrent partnerships, and partner oral or genital HPV positivity [18].

In this context, we aimed to 1) describe sex-specific, within-individual and between-partner HPV concordance between anatomical sites (genital, anal, and oral) and 2) examine predictors of genital/genital concordance between partners in heterosexual couples.

3.2.3 Methods

Study design and procedures

We utilized data from the Transmission Reduction and Prevention with HPV vaccination (TRAP-HPV) study (registered at ClinicalTrial.gov; ID number: NCT01824537), a randomized controlled trial to assess the efficacy of recent HPV vaccination for preventing HPV transmission to the sexual partners of vaccinated individuals. The study protocol [19] and findings on the effect of recent vaccination [20,21] have been previously described.

Briefly, 186 heterosexual couples aged 18+, who had not received the intervention vaccine, and described their relationship as having begun within the past six months were enrolled in Montreal, Canada (2014 - 2022). Participants were randomized to receive the intervention (HPV, i.e., Gardasil until July 15, 2015, Gardasil 9 after) or a control (hepatitis A) vaccine. Couples attended up to six visits at 0, 2, 4, 6, 9, and 12 months. Couples who broke-up were not eligible to attend further visits. Participants were advised to abstain from sexual activity for 48 hours before each visit. At each visit, participants answered electronic questionnaires on sexual behavior, sexual health, and sociodemographic characteristics. Genital (nurse-collected penile; self-collected vaginal) and oral (nurse-collected) samples were obtained. Anal samples were collected from 49 couples recruited before July 14, 2016. Anal sampling was discontinued thereafter as it was a barrier to recruitment [19]. All participants gave informed consent. The

study was approved by the Institutional Review Boards at McGill University (A04- M37-12A), Concordia University (30001405), and Centre Hospitalier de l'Université de Montréal (2014–2019, CE 13.016).

HPV genotyping

Most samples were tested using the Linear Array HPV Genotyping Test (Roche Diagnostic, Laval, Canada) for 36 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89). As this assay has been discontinued, samples (14.6%; 512/3503) collected on or after February 11, 2020 were genotyped using the Anyplex II HPV28 Detection assay (Seegene, Seoul, Korea) for 28 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 69, 70, 73, 82). There is very good agreement between the two assays [22].

Statistical analyses

We summarized characteristics of the study population at enrollment and over follow-up, overall and by sex, using descriptive statistics. All the analyses described hereafter considered type-specific HPV infections within an individual as the unit of analysis (referred to henceforth as HPV-level); each participant could contribute up to 36 HPV types at each anatomical site.

We calculated, at each visit and cumulatively (i.e., over all visits), the sex-specific observed/expected (O/E) concordance of HPV detection at the same visit considering: 1) within individuals between sites (anal and genital, oral and genital), 2) between partners between sites (anal and genital, oral and genital), and 3) between partners at the same site (genital and genital, oral and oral). An O/E above 1 indicates that HPV-level concordance is more frequent than the product of the site-specific probabilities, and thus, the events (positivity at each site) are not independent [23,24]. Corresponding percentile bootstrap 95% confidence intervals (CI) were

calculated by resampling as this method works well for ratios and does not require parametric assumptions [25]. Resampling included clustering at the participant-level to account for correlation in the data.

We examined cumulative concordance, irrespective of study visit, by classifying participants as ever-positive for a given HPV type at an anatomical site (i.e., genital, oral, or anal) if their sample from at least one visit tested positive. We used mixed-effects logistic regression to estimate 1) sex-specific odds ratios (OR) and 95% CIs for ever-positivity, considering partner ever-positivity as the predictor, and 2) univariable and multivariable ORs for the effect of demographic/behavioral variables on genital HPV ever-positivity (to explore possible confounding factors). We considered the following variables: age at baseline (> vs. ≤ sex-specific median), total occasions of sexual intimacy within couples (> vs. ≤ median), concurrency of sexual partners (consistently reporting no concurrent partners vs. reporting concurrent partners or declining to answer), average reported condom use throughout the study (> vs. ≤ 75^{th} percentile), current smoking (ever vs. never smoked during the study), and partner ever-positivity for the HPV type in their genital sample (yes vs. no). To better elucidate the effect of these variables on HPV transmission dynamics, we performed a similar analysis considering HPV-level pairs wherein at least one partner tested ever-positive for genital HPV infection. Furthermore, we assessed effect modification by these variables for genital/genital ever-positivity concordance between partners using logistic regression models in two ways: first, with an interaction term between the variable of interest and partner ever-positivity, and second, by estimating stratum-specific ORs for concordance. For all logistic regression analyses, we used mixed-effects models with exchangeable correlation structures and random intercepts at the person-level, based on the assumption that multiple infections in the same person are not entirely

statistically independent [26]. All statistical analyses were conducted in Stata (version 18.0, StataCorp LLC., TX).

3.2.4 Results

Figure 1 shows the number of participants and valid samples per visit and illustrates the definition of the cumulative "ever-positivity" variable. Analyses were based on valid samples: 806 genital samples from 185 females, 730 genital samples from 184 males, 781 oral samples from 186 females, 787 oral samples from 186 males, 132 anal samples from 48 females, and 98 anal samples from 43 males. Of note, the median follow-up time was 11.7 months (interquartile range: 3.8 -13.3). M2-Table 1 shows participant characteristics. Median age was 23.2 (IQR: 21.0, 26.7) years in females and 24.3 (IQR: 21.5, 29.4) years in males. Most participants (71.5% of females and 65.1% of males) were never-smokers (of tobacco) at baseline. Over follow-up, 68.3% of females and 62.4% of males reported not smoking any cigarettes. The proportion of participants reporting never using condoms with their partner was 26.9% for females and 22.0% for males, whereas 14.5% of females and males reported always using condoms with their partner. Over follow-up, the interquartile range for couple-level averaged condom use frequency score (as a continuous variable from 0 to 4) was 0.17 - 2.5. Most participants reported no concurrent partners at baseline (80.6% of females and 84.9% of males) or over follow-up (65.6% of females and 74.7% of males). The median estimated number of sex acts within couples from relationship debut until the end of the study was 164.6. Based on valid samples at baseline, 51.5% of females and 45.9% of males tested positive for any genital HPV. Equivalent proportions were 3.3% and 2.2% for any oral HPV positivity, and 42.6% and 5.4% for any anal HPV.

M2-Table 2 shows the sex-specific, cumulative, concurrent concordance of HPV infections (refer to M2-Table S1 for concordance by visit). Within individuals, O/E concordance between anal and genital sites was higher for females than males (23.37 vs. 14.79) whereas concordance between oral and genital sites was lower for females than males (4.83 vs. 15.43). Between partners, O/E concordance between an individual's anal sample and their partner's genital sample was higher for females than males (12.91 vs. 6.98), while that between an individual's oral sample and their partner's genital sample was slightly lower for females than males (5.08 vs. 5.95). Considering the same site between partners, O/E concordance was 14.99 for genital samples and 113.07 for oral samples.

M2-Tables S2-S4 detail HPV ever-positivity for the different anatomical sites. Considering couples where both members had at least one valid sample: genital ever-positivity was slightly lower for females than males, 58.5% vs. 60.1% (M2-Table S2); oral ever-positivity was equal between sexes at 5.9% (M2-Table S3); and anal ever-positivity was substantially higher among females than males, 39.5% vs. 7.0% (M2-Table S4). M2-Table 3 presents results from sex-specific concordance and logistic regression analyses considering HPV ever-positivity. For genital/anal concordance, the O/E was fractionally lower for females (15.02 vs. 15.05); however, the OR in females was twice that in males (187.83 vs. 82.84). Between partners, for genital/genital concordance, the O/E was 10.06, and the ORs were 70.75 and 67.34 for females and males, respectively.

M2-Table 4 shows sex-specific estimates for the effect of demographic/behavioral characteristics on genital ever-positivity. The strongest predictor was one's partner's ever-positivity for that HPV type; the effect estimate was larger for females than males (univariable OR: 70.75, 95% CI: 43.70, 114.56 vs. 67.34, 95% CI: 41.96, 108.06; multivariable OR: 66.23,

95% CI: 40.96, 107.08 vs. 61.53, 95% CI: 38.19, 99.14). Age above the median was a significant predictor for both sexes, but the effect estimate was smaller for females than males. Current smoking was a significant predictor in both sexes in univariable analyses; the effect estimate was greater for females than males (univariable OR: 2.25, 95% CI: 1.46, 3.47 vs 1.73, 95% CI: 1.11, 2.69).

M2-Table 5 shows the couple-level effects of demographic/behavioral characteristics on genital/genital ever-positivity concordance when at least one member of the couple is positive for a given HPV type. Total estimated number of occasions of sexual intimacy above the median was a significant predictor in both the univariable (OR: 2,11, 95% CI: 1.25, 3.57) and multivariable (OR: 1.96, 95% CI: 1.12, 3.46) analyses. Current smoking by one partner was significant in the multivariable (OR: 2.31, 95% CI: 1.14, 4.69) but not in the univariable (OR: 2.03, 95% CI: 0.99, 4.17) analysis.

M2-Table 6 shows, for the same set of variables, the adjusted and stratum-specific associations between self and partner genital ever-positivity within couples. This table illustrates how the effect of partner ever-positivity as a predictor of self ever-positivity is modified by other factors. There were statistically significant interaction terms between partner ever-positivity and age above the median for both sexes, with a less pronounced effect estimate in females (OR: 0.31, 95% CI: 0.11, 0.82 vs. 0.23, 95% CI: 0.09, 0.60). The interaction term for occasions of intimacy was slightly larger in females than males (OR: 2.49, 95% CI: 1.01, 6.16 vs. 2.28 95% CI: 0.92, 5.62) and was borderline statistically significant in females only. However, the interaction between partner ever-positivity and concurrency of partners was significant only in males (OR: 0.36, 95% CI: 0.15, 0.85), and the effect was less pronounced in females (OR: 0.53, 95% CI 0.21, 1.32).

3.2.5 Discussion

We found high anal/genital concordance within individuals, especially females; the observed same-visit genital/anal concordance was over 23 times what would be expected if infections were independent. For ever-positivity, the female-specific odds of an anal HPV infection were nearly 187 times higher if that HPV type was ever detected in the individual's vaginal sample. In males, same-visit genital/anal concordance was nearly 15 times what would be expected by chance, and for ever-positivity, the odds of an anal HPV infection were almost 82 times higher if the individual was ever-positive at their genital site. Between partners, observed genital concordance for ever-positivity was over 10 times what would be expected by chance; the person-specific odds of a genital infection in females were nearly 70 times higher if that HPV type was detected in her partner's genital sample, and the person-specific odds for males were over 66 times higher if that HPV type was detected in his partner's genital sample. For all comparisons, O/E concordance was above 1, meaning that concordance occurred more often than by chance. There were few oral HPV detections (i.e., 3.3% of females and 2.2% of males were positive at baseline, and 5.9% of males and females were ever-positive), and few anal HPV detections in males, hence confidence intervals for comparisons involving these sites were wide and frequently included zero. More frequent condom use significantly predicted lower ever-positivity in females. However, in males, the effect of condom use was borderline significant in the univariable analysis and non-significant in the multivariable analysis. Age above the median significantly predicted genital ever-positivity for both sexes. Furthermore, in both males and females, partner ever-positivity was a stronger predictor of ever-positivity among younger participants, perhaps because older participants had more HPV exposure opportunities before their current relationship. Among males, partner ever-positivity was a significantly weaker

predictor of ever-positivity among those who reported concurrent partners, likely because those individuals have more HPV exposures. This differs from previous findings of no significant difference in HPV concordance within couples with and without concurrent partners [27].

Our findings regarding genital/genital concordance between partners are consistent with previous findings of concordant HPV infections between partners more often than would occur by chance [13] and the nature of HPV as a sexually transmitted infection [4]. Our anal/genital concordance results in females align with a study of 751 females, which found that the relative risk of a type-specific anal infection following cervical infection was 20.5 (95% CI: 16.3, 25.7), and the relative risk of cervical infection following an anal infection was 8.8 (95% CI: 6.4,12.2) [9]. Also, a pooled analysis of data from 36 studies (n=13,427 females) found a high correlation between cervical and anal HPV infection [6]. Interestingly, we found that genital/anal concordance within females was higher than genital/genital concordance between partners, supporting previous suggestions that autoinoculation occurs between these sites [8,9]. Our findings regarding genital/anal concordance in males are consistent with a previous study (n=1348 males) which reported an adjusted hazard ratio of 2.80 (95% CI: 1.32, 5.99) for anal HPV infection in males with previous type-specific genital infections compared with those without, for 9-valent vaccine-targeted HPV [11]. Our findings regarding oral HPV prevalence in females align with prior findings; however, estimates for males are usually higher [18,28]. In a cohort study in Canada, oral HPV prevalence was 7.2% and 3.2% among males and females, respectively [18]. According to National Health and Nutrition Examination Survey data from 2011-2014, in the USA, oral HPV prevalence was 11.5% in males and 3.2% in females [28]. Interestingly, in a recent study of gay and bisexual men in Canada, aged 16-30, oral HPV prevalence was only 2.6%; the authors noted that their findings differed from previous research

and hypothesized that the recruitment period (2017-2019) and young age of participants may partially explain their findings of low oral HPV [29]. Similarly, the relatively young median age of participants in the current study may partially explain our findings of low oral HPV males, as oral HPV prevalence in males is higher at older ages [28], and increased herd immunity due to widespread vaccination during the recruitment period may also be a contributing factor.

As expected, when at least one partner was positive for a given HPV type, more occasions of intimacy and lower condom use were significantly associated with both partners being positive. Similarly, a previous study found that more frequent sexual activity was associated with HPV transmission, while consistent condom use was more likely among couples without transmission events [30]. We found that condom use became non-significant in the multivariable analysis, which is not entirely surprising. A review of 8 longitudinal studies reported that condom use was consistently associated with fewer HPV infections, but the effect was only statistically significant in half of the studies [31]. Somewhat surprisingly, in our multivariable analysis, tobacco smoking by one partner, but not both, was significant. Smoking has been associated with cervical HPV in females [32,33] and anal HPV in males [34]. Our finding that smoking in only one partner and not both was associated with concordance could be due to the small sample size and/or residual confounding, even though we explored confounding by selected relevant risk factors. We did not include vaccination as a potential predictor because vaccination would only be pertinent to 9 HPV types [35] of the 36 included in the analyses. Additionally, participants were randomly assigned to receive the intervention vaccine. Therefore, vaccination was unlikely to be associated with other characteristics. Furthermore, previous analyses in this study population did not conclusively indicate a significant effect from vaccination [20,21]. The interim analysis suggested a protective effect to oneself but not to one's

partner from recent vaccination [20], while the subsequent analysis did not conclusively show a protective effect to either oneself or one's partner [21].

This study had several limitations. Most HPV detections were from genital samples; there were few oral and anal detections. Transmission routes other than genital-to-genital may be influenced by different sociodemographic/behavioural variables, but we did not have adequate statistical power to analyze the effects of various predictors (e.g. anal and oral sex behaviours) at non-genital sites. Additionally, because we looked at concordance at the same visit or over the entire study period, we could not assess directionality. Furthermore, some detections may have been due to depositions [36], leading to an over-estimate of concordance. However, to minimize the chances of this, participants were instructed to refrain from sexual activity in the 48 hours before their clinic visits.

Nevertheless, this study had some notable strengths. Couple-based studies often include participants in long-established partnerships [4,37]. However, the beginning of a new sexual relationship is when HPV transmission is most likely [38]. Hence, by looking at concordance in newly formed couples, this study helps to elucidate the interrelated nature of HPV infections within sexual partnerships. Furthermore, by looking at concordance within males and females from the same population, this study contributes to the understanding of HPV epidemiology and sex-specific infection dynamics.

In conclusion, considering males and females in new relationships, HPV infection at one anatomical site implied an increased risk of a type-specific infection at other sites; in addition to finding high genital/anal concordance within individuals (especially among females), we also observed high genital/genital concordance between partners. Genital ever-positivity in females was less likely with more frequent condom use, suggesting that condom use offers some

protection for females against HPV infection. More occasions of intimacy significantly predicted genital/genital type-specific concordance, indicating that HPV transmission between partners is ongoing and concordance of genotype-specific HPV infections increases during a relationship.

3.2.6 Acknowledgements

The authors wish to thank the participants and employees of the TRAP-HPV study.

Author contributions

ELF and ANB conceived the study design. ELF and MZ planned and supervised the study. MZ oversaw data collection and management. AM carried out analyses and drafted the manuscript with support from MZ and ELF. FC supervised HPV genotyping. PPT supervised the study nurses and advised on participant recruitment and sexual health. All authors reviewed, provided critical feedback, and approved the final version of the manuscript.

Funding statement

The TRAP-HPV study was funded by the Canadian Institutes of Health Research (CIHR) (grant MOP-125949 and grant FDN-143347 to E.L.F.). It was also supported in part by a research grant from Investigator-Initiated Studies Program of Merck Canada Inc. The opinions expressed are those of the authors and do not necessarily represent those of Merck Canada Inc. HPV genotyping quality control was supported by the Réseau sida et maladies infectieuses (SIDA/MI) du Fonds de recherche du Québec–Santé (FRQS). AM was funded by a Canada Graduate Scholarship-Master's, CIHR award and a McGill Faculty of Medicine Internal Studentship (in part through C. Epstein Fellowship in Women's Health). ANB is a Canada Research Chair in Sexually Transmitted Infection Prevention (Tier 2) and was supported by a Non-Clinician Scientist award from the Department of Family and Community Medicine, University of Toronto.

3.2.7 Manuscript 2 References

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3.2.8 Manuscript 2 Tables

M2-Table 1. Characteristics of participants in the TRAP-HPV study, overall and by sex.

n (%)	**Overall 372**	**Female 186**	**Male 186**
Age at baseline, median (Q1, Q3)	23.8 (21.2, 28.3)	23.2 (21.0, 26.7)	24.3 (21.5, 29.4)
Age categories at baseline			
18-20	88 (23.7)	49 (26.3)	39 (21.0)
21-24	134 (36.0)	70 (37.6)	64 (34.4)
25-29	77 (20.7)	37 (19.9)	40 (21.5)
>=30	73 (19.6)	30 (16.1)	43 (23.1)
Place of birth			
Canada	186 (50.0)	99 (53.2)	87 (46.8)
United States	33 (8.9)	18 (9.7)	15 (8.1)
France	18 (4.8)	6 (3.2)	12 (6.5)
India	13 (3.5)	5 (2.7)	8 (4.3)
Iran	15 (4.0)	8 (4.3)	7 (3.8)
Brazil	11 (3.0)	7 (3.8)	4 (2.2)
China	9 (2.4)	6 (3.2)	3 (1.6)
Mexico	10 (2.7)	5 (2.7)	5 (2.7)
South Korea	5 (1.3)	1 (0.5)	4 (2.2)
East Asia[a]	7 (1.9)	4 (2.2)	3 (1.6)
Southeast Asia[a]	6 (1.6)	4 (2.2)	2 (1.1)
South Asia[a]	7 (1.9)	2 (1.1)	5 (2.7)
Middle East and North Africa[a]	11 (3.0)	4 (2.2)	7 (3.8)
Sub-Saharan Africa[a]	8 (2.2)	2 (1.1)	6 (3.2)
Latin America[a]	9 (2.4)	3 (1.6)	6 (3.2)
Europe[a]	16 (4.3)	9 (4.8)	7 (3.8)
Other[a]	5 (1.3)	2 (1.1)	3 (1.6)
Missing	3 (0.8)	1 (0.5)	2 (1.1)
Education			
High school or less	69 (18.5)	28 (15.1)	41 (22.0)
College or vocational training	64 (17.2)	30 (16.1)	34 (18.3)
University	235 (63.2)	128 (68.8)	107 (57.5)
Missing	4 (1.1)	0 (0.0)	4 (2.2)
Ever tobacco smoking status at baseline			
Never	254 (68.3)	133 (71.5)	121 (65.1)
Former	79 (21.2)	36 (19.4)	43 (23.1)
Current	35 (9.4)	16 (8.6)	19 (10.2)
Missing	4 (1.1)	1 (0.5)	3 (1.6)
Ever reported smoking over follow-up[b]			
No	243 (65.3)	127 (68.3)	116 (62.4)
Yes	129 (34.7)	59 (31.7)	70 (37.6)
Sexual orientation			
Heterosexual/straight	314 (84.4)	140 (75.3)	174 (93.5)
Other	53 (14.2)	42 (22.6)	11 (5.9)
Missing	5 (1.3)	4 (2.2)	1 (0.5)
Condom use with TRAP-HPV partner at baseline			
0=Never (0)	91 (24.5)	50 (26.9)	41 (22.0)
1= Rarely (1-25)	113 (30.4)	54 (29.0)	59 (31.7)
2=Some of the time (26%-75)	54 (14.5)	26 (14.0)	28 (15.1)

3= Most of the time (76-99)	44 (11.8)	23 (12.4)	21 (11.3)
4= Always (100)	54 (14.5)	27 (14.5)	27 (14.5)
Missing	16 (4.3)	6 (3.2)	10 (5.4)
Condom use with TRAP-HPV over follow-up[c], median (Q1, Q3)	1.0 (0.17, 2.5)	1.0 (0.17, 2.5)	1.0 (0.17, 2.5)
Concurrent sexual partners at baseline			
No	308 (82.8)	150 (80.6)	158 (84.9)
Yes	61 (16.4)	35 (18.8)	26 (14.0)
Missing	3 (0.8)	1 (0.5)	2 (1.1)
Ever reported concurrent partner(s) over follow-up			
No	261 (70.2)	122 (65.6)	139 (74.7)
Yes/missing answers	111 (29.8)	64 (34.4)	47 (25.3)
Number of lifetime sex partners, median (Q1, Q3)	7 (3, 16)	6 (2, 15)	8 (3, 16)
Age coitarche, median (Q1, Q3)	18 (16, 19)	17 (16, 19)	18 (16, 19)
Total estimated number of acts of intimacy since start of relationship, median (Q1, Q3)	164.6 (95.0, 253.3)	164.6 (95.0, 253.3)	164.6 (95.0, 253.3)
Years since onset of sexual activity, median (Q1, Q3)	5.8 (2.8, 10.5)	5.2 (2.5, 9.5)	6.4 (3.2, 12.1)
Positive for any HPV[e] in genital sample at baseline	171 (48.6)	92 (51.1)	79 (45.9)
Positive for any HPV[e] in oral sample at baseline	10 (2.7)	6 (3.3)	4 (2.2)
Positive for any HPV[e] in anal sample at baseline	22 (26.2)	20 (42.6)	2 (5.4)

Abbreviations: HPV, human papillomavirus; Q1, first quartile; Q3, third quartile. TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study.

[a] Countries with less than 5 participants grouped by region. “Other” includes Eastern Europe, Central Asia, and Oceania.

[b] Yes if participant reported being a current smoker at baseline and/or smoking any cigarettes while enrolled.

[c] Couple-level “condom use” variable averaged between partners at each visit, and then averaged over total study visits for the couple (representing a scale value of 2.5); original condom use variable: 0= Never, 1 = Rarely (1-25), 2 = Some of the time (26-75), 3 = Most of the time (76-99), 4 = Always (100). For 80 participants who did not provide information at one or more visits, the frequency reported by the other partner at that visit was used when available. If neither partner provided that information, the visit in question was not included in the average

calculation for that couple; this applied to 3 couples at visit 1, 5 couples at visit 2, 3 couples at visit 3, 6 couples at visit 4, 5 couples at visit 5, and 7 couples at visit 6. These calculations were based on 185 couples (data entirely missing for one couple). For 5 participants who did not give any information about condom use frequency at any visit, the estimated average was based entirely on the information reported by their partner.

[d] Indicates above median couple-level total estimated sex acts, calculated as the sum of averaged (between both partners) sex acts since the previous visit, or since the beginning of the relationship for visit 1. For 131 participants who did not provide information at one or more visits, the number or frequency reported by the other partner at that visit was used when available. If neither partner provided that information, it was counted as zero sex acts for the couple during the time in question; this applied to 2 couples at visit 1, 5 couples at visit 2, 5 couples at visit 3, 4 couples at visit 4, 3 couples at visit 5, and 10 couples at visit 6. There were no couples with zero reported sex acts. However, there were 12 participants who did not provide information on frequency or number of sex acts at any visit, and thus, the estimated was based entirely on the information reported by their partner.

[e] Any HPV includes any of 36 HPV types that were tested for: HPVs 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58 , 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89. HPV71 and HPV 72 were not detected in any sample throughout study. Refer to M2-Figure 1 for number of valid samples at baseline.

M2-Table 2. Sex-specific HPV-level **cumulative** concordance[a] of detection across all visits in the TRAP-HPV study (N=186 couples).

Sex	Concordance within individuals, between sites			Concordance between partners, between sites			Concordance between partners, same site		
	Observed +/+	Expected +/+	O/E (95% CI)[b]	Observed +/+	Expected +/+	O/E (95% CI)[b]	Observed +/+	Expected +/+	O/E (95% CI)[b]
	anal & genital			anal & genital			genital & genital		
Female	86	3.68	23.37 (15.55, 38.05)	59	4.57	12.91 (9.18, 20.33)	314	20.95	14.99 (12.47, 18.41)
Male	15	1.01	14.79 (9.20, 43.45)	6	0.86	6.98 (0, 36.85)	314	20.95	14.99 (12.47, 18.41)
	oral & genital			oral & genital			oral & oral		
Female	2	0.41	4.83 (0, 13.82)	2	0.39	5.08 (0, 14.74)	1	0.01	113.07 (0, 458.15)
Male	6	0.39	15.43 (0, 24.95)	3	0.50	5.95 (0, 15.64)	1	0.01	113.07 (0, 458.15)

+/+ indicates HPV positivity at the two anatomical sites considered.

Abbreviations: CI, confidence interval; HPV, human papillomavirus; O/E, observed/expected; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study.

[a] Considers concordance of type-specific HPV infections at the same study visit (refer to M2-Table S1 for visit-specific results), calculated amongst valid samples (refer to M2-Figure 1). The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample only if the participant's samples for that visit were tested via Linear Array (2,847 tested via Linear Array out of 3,334 total valid samples: 1,278/1,536 genital; 1,339/1,568 oral samples; 230/230 anal).

[b] Represents 95% percentile bootstrap CIs run for 1000 repetitions, clustered at the person-level.

M2-Table 3. Sex-specific HPV-level **ever** concordance[a] of detection and predictor-outcome associations in the TRAP-HPV study.

Concordance	Analytical framework	Sample and observations	-/-	-/+	+/-	+/+ [expected]	O/E (95% CI)[b]	OR (95% CI)[c]
Within individuals, between sites	**anal+ (dependent variable) & genital+ (independent variable)**	**Female (n=47)** N_{HPV} = 1,692	1,591	49	10	42 [2.80]	15.02 (10.68, 23.51)	187.83 (57.11, 617.74)
		Male (n=43) N_{HPV} = 1,548	1,457	83	1	7 [0.47]	15.05 (10.50, 25.80)	82.84 (18.21, 376.85)
	anal+ (dependent variable) & oral+ (independent variable)	**Female (n=48)** N_{HPV} = 1,728	1,673	3	51	1 [0.12]	8.31 (0, 29.79)	33.16 (1.59, 689.98)
		Male (n=43) N_{HPV} = 1,548	1,537	3	6	2 [0.03]	77.40 (0, 387.00)	1172.26 (48.43, 28374.79)
	genital+ (dependent variable) & oral+ (independent variable)	**Female (n=185)** N_{HPV} = 6,327	5,960	353	12	2 [0.79]	2.55 (0, 7.44)	4.87 (0.93, 25.52)
		Male (n=184) N_{HPV} = 6,300	5,958	330	5	7 [0.64]	10.91 (5.73, 16.71)	52.13 (16.85, 161.29)
Between partners, between sites	**anal+ self (dependent variable) & genital+ partner (independent variable)**	**Female (n=48)** N_{HPV}= 1,728	1,599	77	20	32 [3.28]	9.76 (7.15, 14.21)	44.79 (22.46, 89.32)
		Male (n=42) N_{HPV} = 1,512	1,432	72	5	3 [0.40]	7.56 (0, 24.00)	14.22 (3.40, 59.43)
	anal+ self (dependent variable) & oral+ partner (independent variable)	**Female (n=48)** N_{HPV} = 1,728	1,673	3	50	2 [0.15]	13.29 (0, 34.56)	31.94 (2.68, 380.07)
		Male (n=43) N_{HPV} = 1,548	1,537	3	7	1 [0.02]	48.38 (0, 516.00)	3282.36 (3.80, 2.83e+06)
	genital+ self (dependent variable) & oral+ partner (independent variable)	**Female (n=185)** N_{HPV} = 6,327	5,967	5	348	7 [0.67]	10.40 (5.49, 15.34)	39.10 (14.62, 104.61)
		Male (n=184) N_{HPV} = 6,300	5,953	10	333	4 [0.75]	5.34 (1.16, 10.11)	12.74 (3.23, 50.21)
Between partners, same site	**anal+ self (dependent variable) & anal+ partner (independent variable)**	**Female (n=43)** N_{HPV} = 1,548	1,503	6	37	2 [0.20]	9.92 (0, 51.60)	8.87 (0.61, 128.34)
		Male (n=43) N_{HPV} = 1,548	1,503	37	6	2 [0.20]	9.92 (0, 51.60)	6.81 (0.48, 95.74)
	oral+ self (dependent variable) & oral+ partner (independent variable)	**Female (n=186)** N_{HPV} = 6,363	6,338	11	13	1 [0.03]	37.88 (0, 130.36)	78.96 (8.31, 750.48)
		Male (n=186) N_{HPV} = 6,363	6,338	13	11	1 [0.03]	37.88 (0, 130.36)	48.25 (5.48, 425.18)
	genital+ self (dependent variable) & genital+ partner (independent variable)	**Female (n=183[d])** N_{HPV} = 6,264	5,765	146	162	191 [18.99]	10.06 (8.55, 12.12)	70.75 (43.70, 114.56)
		Male (n=183[d])	5,765	162	146	191	10.06	67.34

		N_{HPV} = 6,264				[18.99]	(8.55, 12.12)	(41.96, 108.06)

N_{HPV} indicates the number of observations at the HPV-level (each participant contributed up to 36 observations)

+ indicates HPV positivity at a given site

+/+ indicates HPV positivity at the two anatomical sites considered.

Abbreviations: CI, confidence interval; HPV, human papillomavirus; O/E, observed/expected; OR, odds ratio; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study

[a] Considers type-specific HPV infection-level ever positivity over entire study period, calculated amongst valid samples (refer to M2-Figure 1).

The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample if the participant ever had any valid samples at the relevant site tested via Linear Array (332 genital, 333 oral, 91 anal).

[b] Represents 95% percentile bootstrap CI run for 1000 repetitions, clustered at the person-level.

[c] Odds ratio from mixed effects logistic regression with random intercepts at the person-level using robust 95% CI.

[d] Both members had at least one valid genital sample, considering the 185 females with at least one valid genital sample and the 184 males with at least one genital (refer to M2-Figure 1).

M2-Table 4. Sex-specific HPV-level associations[a] between selected predictors and HPV ever-positivity in genital samples in the TRAP-HPV study.

Predictor	Female (N=183[b])		Male (N=183[b])	
	Univariable OR (95% CI)	**Multivariable OR (95% CI)**	**Univariable OR (95% CI)**	**Multivariable OR (95% CI)**
Age > median[c]	2.08 (1.34, 3.22)	1.66 (1.06, 2.59)	2.45 (1.62, 3.72)	1.95 (1.30, 2.91)
> 164.55 occasions of intimacy[d]	1.32 (0.85, 2.08)	1.00 (0.62, 1.62)	1.20 (0.77, 1.86)	1.13 (0.73, 1.77)
Concurrency of partners or missing answers	1.36 (0.85, 2.16)	1.00 (0.64, 1.59)	2.50 (1.61, 3.88)	1.89 (1.16, 3.08)
Condom use > $75th$ percentile[e]	0.36 (0.21, 0.63)	0.36 (0.20, 0.64)	0.56 (0.32, 1.00)	0.89 (0.51, 1.56)
Current smoking[f]	2.25 (1.46, 3.47)	1.32 (0.83, 2.10)	1.73 (1.11, 2.69)	1.06 (0.66, 1.70)
Partner ever-positivity[g]	70.75 (43.70, 114.56)	66.23 (40.96, 107.08)	67.34 (41.96, 108.06)	61.53 (38.19, 99.14)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study

[a] Modeled considering type-specific HPV infections via mixed effects logistic regression with random intercepts at the person-level using robust 95% confidence intervals (separately for females and males). The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample if the participant ever had any genital samples tested via Linear Array (332 genital, 333 oral, 91 anal).

[b] Includes couples where both members had at least one valid genital sample (as shown in the last 2 rows of M2-Table 3)

[c] Indicates age at baseline: Refer to M2-Table 1

[d] Refer to M2-Table 1 footnote[d].

[e] Refer to M2-Table 1 footnote[c].

[f] Refer to M2-Table 1 footnote[b].

[g] Partner's genital sample ever tested positive for the HPV type of interest.

M2-Table 5. HPV-level associations[a] between selected predictors and HPV ever-positivity in both partners' genital samples given ever-positivity in one partner's genital sample in the TRAP-HPV study (N=125 couples[b]).

Predictor	Univariable OR (95% CI)	Multivariable OR (95% CI)
Averaged couple age > median median[c]	0.62 (0.35, 1.10)	0.67 (0.37, 1.20)
> 164.55 occasions of intimacy[d]	2.11 (1.25, 3.57)	1.96 (1.12, 3.46)
Concurrency of partners or missing answers, one partner	0.73 (0.34, 1.56)	0.66 (0.31, 1.40)
Concurrency of partners or missing answers, both partners	0.87 (0.49, 1.52)	0.65 (0.32, 1.31)
Condom use frequency $> 75th$ percentile[e]	0.43 (0.21, 0.90)	0.62 (0.29, 1.31)
Current smoking[f], one partner	2.03 (0.99, 4.17)	2.31 (1.14, 4.69)
Current smoking[f], both partners	1.72 (0.82, 3.61)	1.80 (0.77, 4.23)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study

[a] Modeled considering type-specific HPV infections via mixed effects logistic regression with random intercepts at the couple-level using robust 95% confidence intervals. The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample if the participants ever had any genital samples tested via Linear Array (332 genital, 333 oral, 91 anal).

[b] Included pairs where at least one member of the couple was positive in their genital sample for that HPV type.

[c] Refer to M2-Table 1.

[d] Refer to M2-Table 1 footnote[d].

[e] Refer to M2-Table 1 footnote[c].

[f] Refer to M2-Table 1 footnote[b].

M2-Table 6. Sex-specific HPV-level associations[a] between genital ever-positivity in individuals and their partner (N=183 couples[b]).

Type of model	Variable[c] chosen as effect modifier	Female HPV positivity as outcome, Male HPV positivity as predictor			Male HPV positivity as outcome, Female HPV positivity as predictor		
		Sample and observations	**OR (95% CI)**	**OR (95% CI) for interaction term**	**Sample and observations**	**OR (95% CI)**	**OR (95% CI) for interaction term**
Model with interaction term	for age $>$ median	**Female (n=183)** N_{HPV} = 6,264	145.84 (61.68, 344.80)	0.31 (0.11, 0.82)[d]	**Male (n=183)** N_{HPV} = 6,264	168.06 (71.83, 393.17)	0.23 (0.09, 0.60)[d]
Stratum-specific models	age $\leq$ median	**Female (n=92)** N_{HPV} = 3,141	154.69 (60.35, 396.50)		**Male (n=93)** N_{HPV} = 3,213	152.16 (67.32, 343.91)	
	age $>$ median	**Female (n=91)** N_{HPV} = 3,123	43.19 (25.13, 74.24)		**Male (n=90)** N_{HPV} = 3,051	39.60 (23.12, 67.83)	
Model with interaction term	occasions of intimacy $>$ median	**Female (n=183)** N_{HPV} = 6,264	43.51 (23.18, 81.66)	2.49 (1.01, 6.16)[d]	**Male (n=183)** N_{HPV} = 6,264	43.29 (23.11, 81.11)	2.28 (0.92, 5.62)
Stratum-specific models	$\leq$164.55 occasions of intimacy	**Female (n=90)** N_{HPV} =3,132	44.08 (23.02, 84.44)		**Male (n=90)** N_{HPV} = 3,132	42.27 (22.48, 79.47)	
	$>$ 164.55 occasions of intimacy	**Female (n=93)** N_{HPV} = 3,132	106.97 (53.36, 214.45)		**Male (n=93)** N_{HPV} = 3,132	101.20 (50.96, 200.99)	
Model with interaction term	Concurrency of partners	**Female (n=183)** N_{HPV} = 6,264	92.49 (47.31, 180.82)	0.53 (0.21, 1.32)	**Male (n=183)** N_{HPV} = 6,264	97.25 (52.60, 179.81)	0.36 (0.15, 0.85)[d]
Stratum-specific models	no concurrency partners	**Female (n=119)** N_{HPV} = 4,068	97.20 (47.61, 198.44)		**Male (n=136)** N_{HPV} = 4,653	97.22 (51.80, 182.46)	
	Concurrency of partners or missing answers	**Female (n=64)** N_{HPV} = 2,196	46.99 (25.17, 87.73)		**Male (n=47)** N_{HPV} = 1,611	34.72 (17.99, 67.00)	
Model with interaction term	Condom use $>$ $75th$ percentile	**Female (n=182)** N_{HPV} = 6,237	75.48 (45.22, 125.99)	0.59 (0.16, 2.15)	**Male (n=182)** N_{HPV} = 6,237	74.16 (44.45, 123.71)	0.53 (0.15, 1.93)
Stratum-specific models	condom use $\leq 75th$ percentile	**Female (n=136)** N_{HPV} = 4,626	76.36 (45.33, 128.62)		**Male (n=136)** N_{HPV} = 4,626	72.22 (43.49, 119.91)	
	condom use $> 75th$ percentile	**Female (n=46)** N_{HPV} = 1,611	43.34 (14.00, 134.12)		**Male (n=46)** N_{HPV} =1,611	41.06 (11.14, 151.29)	
Model with interaction term	Current smoking	**Female (n=183)** N_{HPV} = 6,264	106.26 (61.10, 184.81)	0.42 (0.17, 1.02)	**Male (n=183)** N_{HPV} = 6,264	75.17 (43.01, 131.37)	0.78 (0.31, 1.93)
Stratum-specific models	no current smoking	**Female (n=125)** N_{HPV} = 4,275	125.16 (67.13, 233.35)		**Male (n=115)** N_{HPV} = 3,915	78.62 (43.91, 140.77)	
	current smoking	**Female (n=58)** N_{HPV} = 1,989	39.93 (20.56, 77.53)		**Male (n=68)** N_{HPV} = 2,349	56.56 (27.23, 117.50)	

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study.

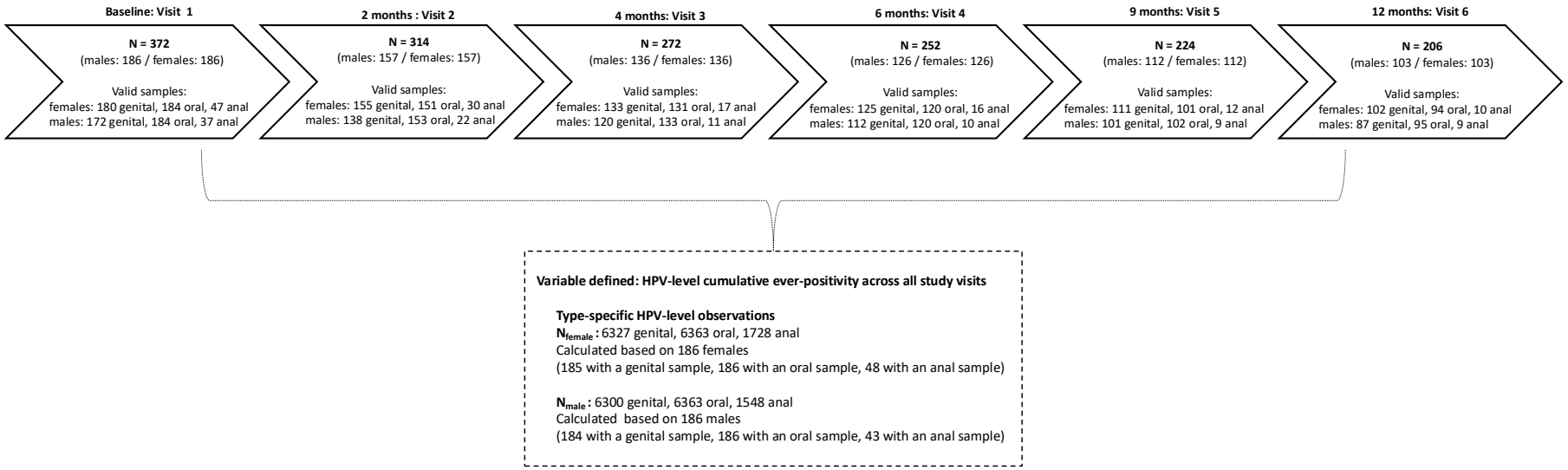
[a] Modeled considering type-specific HPV infections via mixed effects logistic regression with random intercepts at the couple-level using robust 95% confidence intervals. Models with interaction terms included the effect modifier as both a main effect term and an interaction term with partner ever-positivity. The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample if the participants ever had any genital samples tested via Linear Array (332 genital, 333 oral, 91 anal).

[b] Includes couples where both members had at least one valid genital sample (as shown in the last 2 rows of M2-Table 3).

[c] Refer to M2-Table 1.

[d] Indicates statistically significant interaction.

3.2.9 Manuscript 2 Figure



M2-Figure 1. Overview of sample collection at different anatomical sites in the TRAP-HPV study and analytical variable definition.

The upper section shows the study timeline and indicates the number of participants who attended each study visit and number of valid samples collected for each anatomical site. In May 2022, the study protocol was amended, making visit 5 the final visit; this shortened follow-up duration affected three couples.

The lower section describes how the “cumulative ever-positivity” variable was defined. This variable was calculated over the entire study period at the HPV-level based on valid samples. Each participant contributed observations for up to 36 HPV types that were tested for. The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample if the participant ever had any samples at the relevant site tested via Linear Array (332 genital, 333 oral, 91 anal).

Abbreviations: HPV, human papillomavirus, TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study.

CHAPTER 4: DISCUSSION

Manuscript 1 looked at the effect of recent HPV vaccination on genital HPV incidence and transmission, whereas Manuscript 2 focussed on HPV concordance within individuals and between partners. Both manuscripts used data from the TRAP-HPV study. However, the analytical samples for the 2 manuscripts were slightly different. In Manuscript 1, the analytical sample (n=308) consisted of all couples who had at least one follow-up visit and a valid baseline sample from both members of the couple (or imputation of baseline HPV status was possible based on later samples). In Manuscript 2, the analytical sample comprised all participants in the TRAP-HPV study (n=372), although the effective sample sizes for some of the comparisons (e.g., those involving anal samples) were considerably smaller.

4.1 KEY FINDINGS

As a whole, this thesis sheds light on multiple aspects of HPV transmission and infection dynamics within heterosexual couples, as well as the effect of recent vaccination on HPV transmission and infection rates. Therefore, findings are pertinent to clinical decision-making with regard to vaccination of individual adults and couples, vaccination policy, and secondary prevention (i.e., screening) programs.

In Manuscript 1, we described sex-specific incidence and transmission rates across the four arms of the TRAP-HPV study (i.e., neither partner vaccinated against HPV, only the male partner vaccinated against HPV, only the female partner vaccinated against HPV, or both partners vaccinated against HPV). Looking at these 4 study arms, we assessed rates for vaccine-targeted HPVs, as well as HPVs phylogenetically related to vaccine-targeted types, and HPVs that are phylogenetically unrelated to vaccine-targeted types. Overall, the outcomes were similar across the study arms. In males only, there was some indication, although weak, that recent vaccination

was associated with fewer incident detections of HPV. This finding suggests that vaccination may be of clinical interest for adult heterosexual males in terms of preventing incident HPV infections. However, recent vaccination was not associated with fewer incident detections in females, nor was it associated with reduced transmission or a protective effect to one's partner. The effectiveness of HPV vaccination for preventing incident HPV infections in younger populations is well established.[93,133,134] The lack of observed effect in our analyses could have been due to the sample size and the relatively few detections over the four study arms. Furthermore, the age of the participants (mean 25.5 years) may have been a factor, as the effectiveness of HPV vaccination is less pronounced in older populations.[135] Findings from Manuscript 1, therefore, reinforce the importance of early, widespread vaccination programs so that individuals can, ideally, be vaccinated before sexual debut.

In Manuscript 2, we assessed sex-specific, HPV-level concordance within individuals across various anatomical sites (genital, oral, and anal) as well as concordance between partners. For all site combinations, the observed/expected (O/E) concordance ratios for the same study visit and cumulatively over the entire follow-up time were above 1. These O/E concordance ratios above 1 indicate that infections with the same HPV types occurred more frequently than by chance, given the individual type-specific detections at the sites in question. Focusing on genital HPV infections, we explored the effects of behavioural/demographic variables on positivity and concordance. Older age (i.e., above the median) significantly predicted ever-positivity. This finding is interesting given that median age at baseline was 23.2 years in females and 24.3 years in males. Genital HPV prevalence generally peaks close to this age in females while remaining fairly constant in males in adulthood.[2,20] Thus, given the age range of participants, we might have expected the dichotomized age variable not to be predictive of positivity, or for younger age to

be predictive of positivity, especially among females. The most striking result from Manuscript 2, however, was the high concordance between anal and genital sites in females. This agrees with previous findings[108] and suggests autoinoculation between genital and anal sites.[107] The high level of genital/anal HPV concordance in females is a strong argument in favour of introducing HPV DNA testing as the primary method of screening for cervical cancer because detection of high-risk cervical HPV infection could also indicate an increased risk of type-specific anal HPV infection and allow for monitoring to be conducted accordingly.[104]

4.2 STRENGTHS AND LIMITATIONS

Some limitations in the research presented in this thesis should be acknowledged. The total sample size (n=186 couples; i.e., 372 individuals) was smaller than the originally intended 500 couples. However, recruitment was inherently challenging due to the couple-based nature of the study, and was made even more challenging by the COVID-19 pandemic. Thus, in the interest of maintaining the scientific validity and utility of the data, recruitment was closed early. For manuscript 1, which looked at incidence and transmission rates, it was necessary to exclude 29 couples with no follow-up time as well as 3 couples with invalid baseline samples (for whom imputation was not possible based on later results). Thus, the analytical sample was further reduced to 154 couples, however this exclusion was necessary given the nature of the analyses. The smaller than anticipated sample size may have contributed to the lack of observed effect from vaccination in Manuscript 1. In Manuscript 2, the smaller than anticipated sample size likely contributed to the lack of statistical power with regard to oral HPV detections. Additionally, the scheduled follow-up time was one year in total and only six months after the receipt of the third vaccine dose. In Manuscript 1, a longer follow-up time may have affected

findings as it may have led to more overall detections and more statistical power to detect an effect from vaccination.

As noted previously, while the majority of samples were genotyped via the Linear Array HPV Genotyping Test (Roche Diagnostic, Laval, Canada), which tests for 36 HPV types, later samples were genotyped via Anyplex II HPV28 Detection Assay (Seegene, Seoul, Korea), which tests for 28 HPV types. In Manuscript 1, this required that person-time at risk be truncated for 9 HPV types if the individual's samples were tested via Anyplex II. However, this truncation of time at risk did not affect any vaccine-targeted HPV types, and thus, would not have affected the main outcomes of interest in Manuscript 1 (i.e., incidence and transmission of vaccine-targeted HPV-types across the four trial arms). In Manuscript 2, person-time at risk was not a consideration because we looked at concordance either at the same visit or over the entire study period. In this case, the change of assays slightly reduced the HPV-level sample sizes for analyses involving oral and genital samples, and thus may have contributed, to a minor extent, to the low number of oral HPV detections. However, the change in assays did not affect analyses involving anal samples, as anal sampling was discontinued prior to the change in assays. The discontinuation of anal sampling is another potential limitation in Manuscript 2. The relatively small number of anal specimens is unlikely to have affected the results for females. However, among males, because there were few anal detections and a relatively high percentage of invalid anal samples, the smaller sample size may have influenced the point estimates and reduced the statistical significance of our findings.

Despite these limitations, this research also has some important strengths that should be recognized. The TRAP-HPV study was a randomized, controlled, double-blinded trial, meaning that we could be reasonably certain of exchangeability across the study arms. In addition, the

TRAP-HPV study was couple-based, so we were able to investigate sex-specific infection and transmission rates in the context of sexual partnerships. Furthermore, a rare feature of the TRAP-HPV, even among couple-based studies, was that participating couples were required to be early in their sexual relationship, which is a time when there is a relatively high chance of HPV transmission events between partners.[31] Another unique feature of the TRAP-HPV study is the four-arm design, which allowed for comparison of the effects of vaccinating the female vs. male partner in a couple. Additionally, because participants were vaccinated against HPV as part of the study design, misclassification of vaccination status was not a concern. Furthermore, we were able to look at HPV infection dynamics (incidence, transmission, prevalence, concordance) in both males and females from the same population and (for prevalence and concordance) considering multiple anatomical sites. This is an important point, as few studies have reported results for males and females from the same population, which makes comparing HPV-related statistics between sexes difficult and less reliable due to regional variations in HPV epidemiology.[2] Therefore, Manuscript 2 addressed an important gap in the literature as it provides especially valid comparisons of sex-specific concordance across different sites and between partners.

4.3 CONCLUSIONS & DIRECTIONS FOR FUTURE RESEARCH

Viewed together, the two manuscripts presented in this thesis: 1) reinforce the importance of early vaccination through robust gender-neutral vaccination programs delivered at young ages (before sexual exposure to HPV); 2) suggest that there may be clinical benefits to vaccination of adult males; 3) indicate that concurrent concordant infections between various anatomical sites occur more frequently than by chance, and thus, that autoinoculation plays a role in HPV infection dynamics; and 4) support the transition to HPV DNA based cervical screening for

cervical cancer (as opposed to cytology) because the high HPV type-specific genital/anal concordance within females, means that detection of high-risk HPV types at the cervix could also indicate individuals at higher risk for anal cancer.[104]

The research questions addressed in both Manuscripts 1 & 2 could be further examined in future couple-based studies with larger sample size and longer follow-up times. Either of these options would allow for more HPV detections at various anatomical sites, which would increase statistical power and help to assess both the potential effect of vaccination in sexually active adults and the concordance and predictors of concordance between couples, especially at non-genital sites (e.g., predictors of oral/genital or oral/oral concordance). Considering the effect of vaccination in adults, future analyses from the TRAP-HPV study will be able to incorporate baseline serology data to look at antibody titers in participants prior to receipt of the intervention vaccine. This information could yield important insights on the effect of vaccination in participants who were already exposed to vaccine-targeted HPV types vs. those without previous exposure and could also help to distinguish new incident detections from remerging latent infections. With regard to HPV type-specific concordance within individuals and between partners, it will be interesting to see, over the long term, how patterns of concordance change for vaccine-targeted HPV in age cohorts that are vaccinated against HPV at young ages through school-based programs.

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5. APPENDICES

5.1 MANUSCRIPT 1 SUPPLEMENTARY MATERIAL

M1-Table S1. HPV incidence and transmission in female and male participants of the TRAP-HPV study. Sensitivity analysis restricting to 87 couples who did not report outside sexual contact since the beginning of their relationship and for the duration of follow-up.

vaccination assignments	Grouped HPV types[a]	SENSITIVITY ANALYSIS: INCIDENCE						SENSITIVITY ANALYSIS: TRANSMISSION					
		FEMALE			MALE			MALE-TO-FEMALE			FEMALE-TO-MALE		
		Events	Time[b]	Rate[c] (95% CI)	Events	Time[b]	Rate[c] (95% CI)	Events	Time[b]	Rate[c] (95% CI)	Events	Time[b]	Rate[c] (95% CI)
Male, female unvaccinated n=21 couples	**Vaccine-targeted**	2	1885.83	1.06 (0.13, 3.83)[d]	2	1835.56	1.09 (0.26, 9.13)	0	21.49	0 (0.00, 171.66)[d]	0	46.23	0 (0.00, 79.80)[d]
	Related	0	1477.31	0 (0.00, 2.50)[d]	1	1416.76	0.71 (0.20, 3.93)[d]	0	17.58	0 (0.00, 209.84)[d]	0	0	ND
	Unrelated	5	3973.52	1.26 (0.48, 4.36)	5	3826.56	1.31 (0.52, 4.41)	2	76.06	26.30 (4.13, 227.29)	2	47.90	41.75 (17.34, 119.21)
Male vaccinated, female unvaccinated n=18 couples	**Vaccine-targeted**	1	2016.99	0.50 (0.01, 2.76)[d]	0	1945.03	0 (0.00, 1.90)[d]	0	15.77	0 (0.00, 233.93)[d]	0	0	ND
	Related	2	1480.37	1.35 (0.29, 12.07)	1	1443.08	0.69 (0.02, 3.86)[d]	1	5.32	187.88 (4.70, 1047.37)[d]	1	11.73	85.26 (2.13, 475.02)[d]
	Unrelated	14	3921.15	3.57 (1.53, 9.99)	14	3851.10	3.64 (1.71, 8.83)	5	96.76	51.68 (16.09, 218.71)	6	165.82	36.18 (11.35, 98.64)
Male unvaccinated female vaccinated n=24 couples	**Vaccine-targeted**	2	2307.95	0.87 (0.20, 7.39)	3	2182.57	1.37 (0.45, 6.05)	1	27.40	36.50 (0.91, 203.36)[d]	0	0	ND
	Related	2	1710.62	1.17 (0.27, 10.06)	5	1646.52	3.04 (1.38, 7.95)	1	22.97	43.54 (1.09, 242.58)[d]	2	35.09	57.00 (4.62, 539.80)
	Unrelated	13	4329.07	3.00 (1.49, 6.79)	13	4288.99	3.03 (1.66, 6.09)	1	43.83	22.82 (0.57, 127.13)[d]	10	207.91	48.10 (26.73, 92.11)
Male, female vaccinated n=24 couples	**Vaccine-targeted**	2	2365.35	0.85 (0.20, 7.28)	1	2246.38	0.45 (0.01, 2.48)[d]	1	26.28	38.05 (0.95, 212.02)[d]	0	30.88	0 (0.00, 119.46)[d]
	Related	6	1742.62	3.44 (1.46, 10.00)	6	1699.41	3.53 (1.42, 10.84)	1	22.97	43.54 (1.09, 242.58)[d]	1	64.69	15.46 (0.39, 86.13)[d]
	Unrelated	5	4584.13	1.09 (0.50, 2.85)	11	4399.88	2.50 (1.28, 5.41)	1	99.29	10.07 (0.25, 56.12)[d]	3	129.91	23.09 (8.32, 80.29)

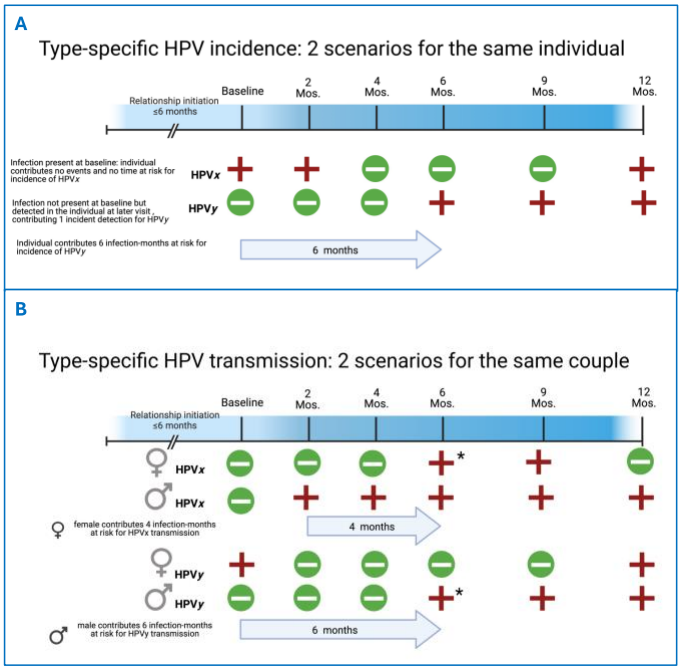
Abbreviations: HPV, human papillomavirus; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination.

[a] Vaccine-targeted types include any of HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58. Phylogenetically related types include any of HPVs 35, 39, 44, 59, 67, 68, and 70. Phylogenetically unrelated types include any of HPVs 26, 34, 40, 42, 51, 53, 54, 56, 61, 62, 66, 69, 71, 72, 73, 81, 82, 83, 84, and 89.

[b] Indicates infection-months at risk. All analyses were done at the HPV-level, meaning that each participant contributed time at risk for up to 36 HPV types. Participants contributed time at risk for incidence if they had not previously tested positive for that HPV type. If a participant tested positive for only 1 HPV type, they would no longer contribute time at risk for that type but would continue to contribute time at risk for the other 35 types. Participants contributed time at risk for transmission if they had not previously tested positive for that HPV type and their partner had previously tested positive for that HPV type.

[c] Rates represent events (incidence or transmission) /1000 infection-months at risk. Jackknife confidence intervals are reported wherever possible to account for intra-participant correlation.

[d] In instances where no events were observed or there was an Insufficient number of failures to calculate jackknife confidence intervals, exact Fisher's 95% confidence intervals were used.



M1-Figure S1. Illustration of outcome definitions: type-specific HPV incidence and transmission, where each participant contributes time at risk for up to 36 HPV types. Only valid samples were included in the calculation of events and time at risk. Image created with Biorender.com.

Panel A shows 2 scenarios for the same individual. **Incidence** was defined as HPV detection when all the participant's previous tests were negative for that HPV type. If a participant tested for HPVx was positive at visit 1, they would not contribute events or time at risk for an HPVx infection. If a participant tested negative at baseline for all HPV types and tested positive at visit 4 (6 months after the baseline visit) for HPVy, that participant would have contributed 6 infection-months at risk for HPVy. After testing positive for HPVy, the participant would still contribute time at risk for the other 35 HPV types tested. The denominator used for incidence rates was HPV type-specific time from baseline until first detection.

Panel B shows 2 scenarios for the same couple. **Transmission** was defined as HPV detection when all the participant's previous tests were negative for that HPV type, and their partner

previously tested positive for that HPV type at any previous visit. As illustrated in the second scenario, the female partner tested positive for HPVy at visit 1, then tested negative for HPVy at visits 2 to 5, and finally tested positive for HPVy again at visit 6, while the male partner tested negative for HPVy at visits 1 through 3, and then tested positive at visits 4 through 6. In this case, the only detection that would be considered a transmission is from the female to the male at visit 4. The denominator used for transmission rates was HPV type-specific time at risk since the participant's partner tested positive for that HPV.

5.2 MANUSCRIPT 2 SUPPLEMENTARY MATERIAL

M2-Table S1: HPV-level concordance[a] (at the same visit), by visit. N=186 couples.

Sex	Visit	Concordance within individuals, between sites					Concordance between partners, between sites					Concordance between partners, same site				
		-/-	-/+	+/-	+/+ [expected]	O/E[a]	-/-	-/+	+/-	+/+ [expected]	O/E[a]	-/-	-/+	+/-	+/+ [expected]	O/E[a]
		Anal & genital					Anal & genital					Genital & genital				
F	1	1,518	28	6	32 [1.44]	22.23	1,530	49	14	27 [1.92]	14.04	5,492	99	126	106 [8.17]	12.98
	2	978	11	6	13 [0.45]	28.74	1,002	23	12	7 [0.55]	12.82	4,546	73	87	55 [3.82]	14.41
	3	550	13	1	12 [0.56]	21.27	584	15	7	6 [0.45]	13.45	3,910	52	70	45 [2.74]	16.45
	4	545	10	6	15 [0.91]	16.46	537	18	9	12 [1.09]	10.97	3,658	57	58	52 [3.13]	16.59
	5	420	4	1	7 [0.20]	34.36	372	16	4	4 [0.40]	9.90	3,256	62	44	31 [2.06]	15.08
	6	348	4	1	7 [0.24]	28.64	306	10	5	3 [0.32]	9.35	2,804	30	57	25 [1.55]	16.16
	All	**4,395**	**70**	**21**	**86 [3.68]**	**23.37**	**4,331**	**131**	**51**	**59 [4.57]**	**12.91**	**23,666**	**373**	**442**	**314 [20.95]**	**14.99**
M	1	1,206	48	3	3 [0.24]	12.35	1,248	42	4	2 [0.20]	9.82	5,492	126	99	106 [8.17]	12.98
	2	729	22	3	2 [0.16]	12.60	693	22	4	1 [0.16]	6.26	4,546	87	73	55 [3.82]	14.41
	3	375	16	2	3 [0.24]	12.51	375	16	4	1 [0.21]	4.66	3,910	70	52	45 [2.74]	16.45
	4	344	12	0	4 [0.18]	22.50	343	13	4	0 [0.14]	0	3,658	58	57	52 [3.13]	16.59
	5	312	10	0	2 [0.07]	27.00	315	7	2	0 [0.04]	0	3,256	44	62	31 [2.06]	15.08
	6	275	9	3	1 [0.14]	7.20	313	7	2	2 [0.11]	18.00	2,804	57	30	25 [1.55]	16.16
	All	**3,241**	**117**	**11**	**15 [1.01]**	**14.79**	**3,287**	**107**	**20**	**6 [0.86]**	**6.98**	**23,666**	**442**	**373**	**314 [20.95]**	**14.99**
		Oral & genital					Oral & genital					Oral & oral				

F	1	6,005	235	4	2 [0.23]	8.78	5,765	205	4	2 [0.21]	9.62	6,380	4	5	1 [0.005]	213.00
	2	5,055	155	1	0 [0.03]	0	4,496	129	1	0 [0.03]	0	5,208	2	1	0 [0.00]	0
	3	4,285	124	1	0 [0.03]	0	3,862	97	1	0 [0.02]	0	4,515	2	1	0 [0.00]	0
	4	3,995	117	1	0 [0.03]	0	3,610	106	1	0 [0.03]	0	4,110	2	1	0 [0.00]	0
	5	3,355	74	0	0 [.]	.	3,051	90	0	0 [.]	.	3,461	4	0	0 [.]	.
	6	3,092	80	5	0 [0.13]	0	2,724	52	5	0 [0.09]	0	3,206	2	5	0 [0.00]	0
	All	**25,787**	**785**	**12**	**2 [0.41]**	**4.83**	**23,508**	**679**	**12**	**2 [0.39]**	**5.08**	**26,880**	**16**	**13**	**1 [0.01]**	**113.07**
M	1	5,791	208	3	1 [0.14]	7.18	5,994	238	4	1 [0.19]	5.22	**6,380**	**5**	**4**	1 [0.005]	213
	2	4,572	125	1	0 [0.03]	0	5,123	158	2	0 [0.06]	0	5,208	1	2	0 [0.00]	0
	3	3,934	96	1	1 [0.05]	20.78	4,356	124	2	0 [0.06]	0	4,515	1	2	0 [0.00]	0
	4	3,611	105	0	1 [0.03]	35.07	3,996	115	1	1 [0.06]	17.73	4,110	1	2	0 [0.00]	0
	5	3,085	88	2	2 [0.11]	17.65	3,383	78	4	0 [0.09]	0	3,461	0	4	0 [.]	.
	6	2,764	51	1	1 [0.04]	27.09	3,132	79	1	1 [0.05]	20.08	3,206	5	2	0 [0.00]	0
	All	**23,757**	**673**	**8**	**6 [0.39]**	**15.43**	**25,984**	**792**	**14**	**3 [0.50]**	**5.95**	**26,880**	**13**	**16**	**1 [0.01]**	**113.07**

+ indicates HPV positivity at a given site.

+/+ indicates HPV positivity at the two anatomical sites considered.

Abbreviations: CI, confidence interval; F, female; HPV, human papillomavirus; M, male; O/E, observed/expected; TRAP-HPV, Transmission Reduction and Prevention with HPV Vaccination study.

[a] Considers concordance of type-specific HPV infection at the same study visit calculated amongst valid samples (refer to M2-Figure 1). The 9 HPV types (34, 62, 67, 71, 72, 81, 83, 84, and 89) detected by Linear Array but not Anyplex II were included in the analytical sample only the participant's samples for that visit were tested via Linear Array (2,847 tested via Linear Array out of 3,334 total valid samples: 1,278/1,536 genital; 1,339/1,568 oral samples; 230/230 anal).

M2-Table S2: **Genital** HPV-level ever-positivity [n (%)] during the study (N=183[a] couples).

HPV type[b]	Female	Male	Either partner	Both partners
HPV6	9 (4.9)	12 (6.6)	15 (8.2)	6 (3.3)
HPV11	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)
HPV16	10 (5.5)	14 (7.7)	19 (10.4)	5 (2.7)
HPV18	6 (3.3)	4 (2.2)	6 (3.3)	4 (2.2)
HPV26	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)
HPV31	3 (1.6)	5 (2.7)	7 (3.8)	1 (0.5)
HPV33	5 (2.7)	1 (0.5)	6 (3.3)	0 (0)
HPV34	1 (0.7)	1 (0.7)	2 (1.4)	0 (0)
HPV35	7 (3.8)	4 (2.2)	8 (4.4)	3 (1.6)
HPV39	13 (7.1)	13 (7.1)	22 (12.0)	4 (2.2)
HPV40	12 (6.6)	12 (6.6)	20 (10.9)	4 (2.2)
HPV42	24 (13.1)	24 (13.1)	37 (20.2)	11 (6.0)
HPV44	6 (3.3)	5 (2.7)	9 (4.9)	2 (1.1)
HPV45	7 (3.8)	6 (3.3)	9 (4.9)	4 (2.2)
HPV51	25 (13.7)	29 (15.8)	35 (19.1)	19 (10.4)
HPV52	15 (8.2)	13 (7.1)	19 (10.4)	9 (4.9)
HPV53	28 (15.3)	26 (14.2)	36 (19.7)	18 (9.8)
HPV54	19 (10.4)	7 (3.8)	22 (12.0)	4 (2.2)
HPV56	12 (6.6)	9 (4.9)	15 (8.2)	6 (3.3)
HPV58	10 (5.5)	13 (7.1)	16 (8.7)	7 (3.8)
HPV59	19 (10.4)	19 (10.4)	24 (13.1)	14 (7.7)
HPV61	8 (4.4)	6 (3.3)	9 (4.9)	5 (2.7)
HPV62	17 (11.6)	22 (15.0)	28 (19.0)	11 (7.5)
HPV66	18 (9.8)	17 (9.3)	24 (13.1)	11 (6.0)
HPV67	6 (4.1)	2 (1.4)	7 (4.8)	1 (0.7)
HPV68	6 (3.3)	7 (3.8)	10 (5.5)	3 (1.6)
HPV69	1 (0.5)	0 (0.0)	1 (0.5)	0 (0)
HPV70	2 (1.1)	3 (1.6)	3 (1.6)	2 (1.1)
HPV73	14 (7.7)	14 (7.7)	19 (10.4)	9 (4.9)
HPV81	5 (3.4)	4 (2.7)	8 (5.4)	1 (0.7)
HPV82	8 (4.4)	6 (3.3)	8 (4.4)	6 (3.3)
HPV83	8 (5.4)	4 (2.7)	9 (6.1)	3 (2.0)
HPV84	13 (8.8)	19 (12.9)	25 (17.0)	7 (4.8)
HPV89	14 (9.5)	14 (9.5)	19 (12.9)	9 (6.1)
Any HPV	107 (58.5)	110 (60.1)	125 (68.3)	85 (46.4)

[a] Includes couples where both members had at least one valid genital sample

(refer to M2-Figure 1).

[b] HPV types ever detected in genital samples. HPV71 and HPV 72 were not detected in any sample throughout study.

M2-Table S3: **Oral** HPV-level ever-positivity [n (%)] during the study (N=186[a] couples).

HPV type[b]	Female	Male	Either partner	Both partners
HPV6	0 (0.0)	2 (1.1)	2 (1.1)	0 (0.0)
HPV16	5 (2.7)	1 (0.5)	5 (2.7)	1 (0.5)
HPV18	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)
HPV31	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV33	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)
HPV39	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)
HPV45	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)
HPV52	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV53	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)
HPV56	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV58	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV59	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV61	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV66	1 (0.5)	1 (0.5)	2 (1.1)	0 (0.0)
HPV73	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)
HPV82	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)
HPV84	0 (0.0)	2 (1.3)	2 (1.3)	0 (0.0)
HPV89	1 (0.7)	0 (0.0)	1 (0.7)	0 (0.0)
Any HPV	11 (5.9)	11 (5.9)	21 (11.3)	1 (0.5)

[a] Includes all couples; all participants had at least one valid oral sample (refer to M2-Figure 1).

[b] HPV types ever detected in oral samples.

M2-Table S4: **Anal** HPV-level ever positivity [n (%)] during the study (N=43[a] couples).

HPV type[b]	Female	Male	Either partner	Both partners
HPV6	0 (0.0)	1 (2.3)	1 (2.3)	0 (0.0)
HPV16	3 (7.0)	2 (4.7)	4 (9.3)	1 (2.3)
HPV18	2 (4.7)	0 (0.0)	2 (4.7)	0 (0.0)
HPV35	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV39	4 (9.3)	1 (2.3)	5 (11.6)	0 (0.0)
HPV40	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV42	3 (7.0)	0 (0.0)	3 (7.0)	0 (0.0)
HPV44	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV51	2 (4.7)	1 (2.3)	2 (4.7)	1 (2.3)
HPV52	1 (2.3)	1 (2.3)	2 (4.7)	0 (0.0)
HPV53	5 (11.6)	0 (0.0)	5 (11.6)	0 (0.0)
HPV54	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV56	0 (0.0)	1 (2.3)	1 (2.3)	0 (0.0)
HPV59	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV62	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV66	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV67	1 (2.3)	1 (2.3)	2 (4.7)	0 (0.0)
HPV68	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV73	4 (9.3)	0 (0.0)	4 (9.3)	0 (0.0)
HPV81	1 (2.3)	0 (0.0)	1 (2.3)	0 (0.0)
HPV84	2 (4.7)	0 (0.0)	2 (4.7)	0 (0.0)
HPV89	3 (7.0)	0 (0.0)	3 (7.0)	0 (0.0)
Any HPV	17 (39.5)	3 (7.0)	18 (41.9)	2 (4.7)

[a] Includes couples where both members had at least one valid anal sample (refer to M2-Figure 1).

[b] HPV types ever detected in anal samples.