PREVENTION OF ANAL HPV INFECTIONS VIA USE OF A CARRAGEENAN-BASED GEL AND HPV VACCINATION IN GAY, BISEXUAL, AND OTHER MEN WHO HAVE SEX WITH MEN

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ABSTRACT

Background

Gay, bisexual, and other men who have sex with men (gbMSM) are disproportionately affected by human papillomavirus (HPV) infection and would benefit from preventive measures. Carrageenan, a red algae derivative, inhibits HPV infection *in vitro* and *in vivo*. However, the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) randomized control trial found that carrageenan use neither influenced anal HPV infection acquisition nor clearance in gbMSM. In addition, although gbMSM are eligible for HPV vaccination in Canada, there is a need for observational studies assessing its real-world impact on anal HPV infections in this at-risk population.

Objectives

Manuscript 1 compared the change in anal HPVs 16 and 18 viral loads following carrageenan use with placebo to investigate its lack of protective effect. Manuscript 2 assessed the association between HPV vaccination and anal HPV incidence.

Methods

In the LIMIT-HPV trial, participants attended 7 visits over 12 months, where they provided a nurse-collected anal sample and self-completed a questionnaire on risk factors and HPV vaccination. In Manuscript 1, analyses included participants who completed the first 4 visits and had a valid baseline sample (n=161; 26/82 in treatment and 28/79 in placebo HIV-positive). The net change in type-specific viral load across visits 1-4 was compared between arms using the Mann-Whitney U test. In Manuscript 2, arms were collapsed, and data were analyzed using a cohort framework (n=258, 69 HIV-positive). The association between HPV vaccination and anal HPV incidence was assessed using Cox regression. Analyses, conducted at the HPV-level (unit of analysis=HPV type), considered vaccine-targeted HPVs (any of HPVs 6/11/16/18) as the outcome and that of non-vaccine-targeted HPVs (within-species: any of HPVs 31/33/35/39/44/45/52/58/59/67/68/70 and cross-species: any of HPVs 26/34/40/42/51/53/54/56/61/62/66/69/71/72/73/81/82/83/84/89) to assess construct validity. Estimates were adjusted for a propensity score to predict HPV positivity across the study based on selected participant and study characteristics.

Results

In Manuscript 1, while the median net change in viral load was higher in the treatment than placebo arm, these differences were not significant (HPV16: 0.68 versus 0.18 copies/cell, *P*=0.60; HPV18: 18.32 versus 10.12 copies/cell, *P*=0.52). In Manuscript 2, 23.3% of participants were HPV vaccinated at baseline. Incidence of vaccine-targeted HPVs was lower among vaccinated than unvaccinated participants (n=754, hazard ratio (HR)=0.22, 95% confidence interval (CI)=0.00-0.79). HPV vaccination was not associated with incidence of within-species (n=2299, HR=0.76, CI=0.42-1.24) or cross-species (n=3774, HR=1.27, CI=0.88-1.83) HPVs.

Conclusions

In Manuscript 1, carrageenan use did not impact anal HPVs 16 or 18 viral load, which may explain its lack of protective effect in gbMSM. In Manuscript 2, results support that HPV vaccination prevents incident anal infections of vaccine-targeted HPV types in gbMSM.

RÉSUMÉ

Contexte

Les hommes gais, bisexuels et autres hommes ayant des relations sexuelles avec des hommes (gbHARSAH) sont touchés de manière disproportionnée par l'infection par le virus du papillome humain (VPH) et bénéficieraient de mesures préventives. Le carraghénane, un dérivé d'algue rouge, inhibe l'infection par le VPH in vitro et in vivo. Cependant, l'essai contrôlé randomisé LIMIT-HPV (*Lubricant Investigation in Men to Inhibit Transmission of HPV Infection*) a montré que l'utilisation de carraghénane n'influençait ni l'acquisition de l'infection anale par le VPH, ni sa clairance chez les gbHARSAH. En outre, bien que les gHARSAH soient admissibles à la vaccination contre le VPH au Canada, il est nécessaire de mener des études d'observation pour évaluer son impact réel sur les infections anales par le VPH au sein de cette population à risque.

Objectifs

Le manuscrit 1 a comparé l'évolution des charges virales des VPH 16 et 18 dans l'anus après l'utilisation de carraghénane avec un placebo pour étudier l'absence d'effet protecteur de cette substance. Le manuscrit 2 a évalué l'association entre la vaccination contre le VPH et l'incidence du VPH anal.

Méthodes

Dans le cadre de l'essai LIMIT-HPV, les participantes ont effectué 7 visites sur 12 mois, au cours desquelles elles ont fourni un échantillon anal prélevé par une infirmière et ont rempli un questionnaire sur les facteurs de risque et la vaccination contre le VPH. Dans le Manuscrit 1, les analyses ont compris les participants qui ont effectué les 4 premières visites et qui avaient un échantillon de base valide (n=161 ; 26/82 dans le traitement et 28/79 dans le placebo séropositifs). Le changement net de la charge virale spécifique au type lors des visites 1 à 4 a été comparé entre les groupes à l'aide du test U de Mann-Whitney. Dans le manuscrit 2, les groupes ont été regroupés et les données ont été analysées dans le cadre d'une cohorte (n=258, 69 séropositifs). L'association entre la vaccination contre le VPH et l'incidence du VPH anal a été évaluée à l'aide de la régression de Cox. Les analyses, effectuées au niveau du VPH (unité d'analyse=type de VPH), ont considéré les VPH ciblés par le vaccin (n'importe lequel des VPH 6/11/16/18) comme le résultat et celui des VPH non ciblés par le vaccin (au sein de l'espèce : tout VPH 31/33/35/39/44/45/52/58/59/67/68/70 et inter-espèces : tout VPH 26/34/40/42/51/53/54/56/61/62/66/69/71/72/73/81/82/83/84/89) pour évaluer la validité de la

construction. Les estimations ont été ajustées en fonction d'un score de propension permettant de prédire la positivité du VPH dans l'ensemble de l'étude, sur la base de certaines caractéristiques des participants et de l'étude.

Résultats

Dans le manuscrit 1, bien que la variation nette médiane de la charge virale ait été plus élevée dans le groupe traité que dans le groupe placebo, ces différences n'étaient pas significatives (VPH16 : 0,68 contre 0,18 copies/cellule, P=0,60 ; VPH18 : 18,32 contre 10,12 copies/cellule, P=0,52). Dans le manuscrit 2, 23,3 % des participants étaient vaccinés contre le VPH au début de l'étude. L'incidence des VPH ciblés par le vaccin était plus faible chez les participantes vaccinées que chez les participantes non vaccinées (n=754, rapport de risques (HR)=0,22, intervalle de confiance à 95% (IC)=0,00-0,79). La vaccination contre le VPH n'a pas été associée à l'incidence au sein d'une même espèce (n=2299, HR=0,76, IC=0,42-1,24) ou entre espèces (n=3774, HR=1,27, IC=0,88-1,83) des VPH.

Conclusions

Dans le manuscrit 1, l'utilisation de carraghénane n'a pas eu d'impact sur la charge virale des VPH 16 ou 18 dans l'anus, ce qui peut expliquer l'absence d'effet protecteur chez les gbHARSAH. Dans le manuscrit 2, les résultats confirment que la vaccination contre le VPH prévient les infections anales incidentes des types de VPH ciblés par le vaccin chez les gbHARSAH.

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PREFACE AND AUTHOR CONTRIBUTIONS

This thesis consists of a literature review, two original manuscripts, a discussion of the key findings, and final conclusions. For clarity, prefixes have been added to distinguish between the tables, figures, and appendices belonging to manuscript 1 (M1-) or manuscript 2 (M2). The contributions of the authors to each of the manuscripts are as follows:

For manuscript 1 titled "Use of a carrageenan-based gel had no impact on anal HPVs 16 and 18 viral loads in gay, bisexual, and other men who have sex with men", Eduardo L. Franco, Alexandra de Pokomandy, Joseph E. Tota, François Coutlée, and Pierre-Paul Tellier conceived and designed the study. Mariam El-Zein managed the study. Pareesa Kassam conducted the statistical analysis and drafted the manuscript under the guidance and supervision of Eduardo L. Franco and Mariam El-Zein. Mariam El-Zein, Cassandra Laurie, Eduardo L. Franco, Alexandra de Pokomandy, Joseph E. Tota, François Coutlée, and Pierre Paul-Tellier all interpreted the data, reviewed the manuscript, and approved the final version.

For manuscript 2 titled "HPV vaccination and anal HPV infection in gay, bisexual, and other men who have sex with men", Eduardo L. Franco, Alexandra de Pokomandy, Joseph E. Tota, François Coutlée, and Pierre-Paul Tellier conceived and designed the study. Mariam El-Zein managed the study. Pareesa Kassam conducted the statistical analysis and drafted the manuscript under the guidance and supervision of Eduardo L. Franco and Mariam El-Zein. Mariam El-Zein, Eduardo L. Franco, Alexandra de Pokomandy, Joseph E. Tota, François Coutlée, and Pierre Paul-Tellier all interpreted the data, reviewed the manuscript, and approved the final version.

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LIST OF ABBREVIATIONS

+	positive
AIDS	acquired immune deficiency syndrome
AIN	anal intraepithelial neoplasia
ANCHOR	Anal Cancer HSIL Outcomes Research
ASCC	anal squamous cell carcinomas
ASCUS	atypical squamous cells of undetermined significance
ASIL	anal squamous intraepithelial lesions
CATCH	Carrageenan gel Against Transmission of Cervical HPV
CI	confidence interval
DNA	deoxyribonucleic acid
gbMSM	gay, bisexual, and other men who have sex with men
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HPV	human papillomavirus
HR	hazard ratio
HSIL	high-grade squamous intraepithelial lesions
HSPG	heparan sulfate proteoglycan
IQR	interquartile range
LIMIT-HPV	Lubricant Investigation in Men to Inhibit Transmission against HPV Infection
LSIL	low-grade squamous intraepithelial lesions
NA	not applicable
ND	not determined
No.	number
OR	odds ratio
SD	standard deviation
STI	sexually transmitted infection
VE	vaccine efficacy

CHAPTER 1: INTRODUCTION

1.1 Rationale

Gay, bisexual, and other men who have sex with men (gbMSM), especially if they are living with human immunodeficiency virus (HIV), have an elevated risk of contracting anal human papillomavirus (HPV) infections and developing related diseases, such as anal precancerous lesions and anal cancer.[1,2] The prevalence of anal high-risk HPV infections is estimated to be 41.2% among HIV-negative gbMSM and 74.3% among gbMSM living with HIV. In contrast, prevalence is estimated to 6.9% and 26.9% among HIV-negative men who have sex with women and men who have sex with women living with HIV, respectively.[1] Furthermore, approximately 88% of anal cancer cases are attributed to HPV infection.[3] While anal cancer is relatively rare among the general population, with an incidence rate of approximately 1-2 cases per 100,000 person-years, among gbMSM, incidence is estimated to be 19 cases per 100,000 person-years if they are HIV-negative and 85 cases per 100,000 person-years if they are living with HIV.[2] Therefore, this population of men would benefit from preventive measures against HPV.

It is well-established that HPV vaccination prevents acquisition of HPV infection and the development of related anogenital warts, precancerous lesions, and cancer in women.[4-8] In gbMSM, randomized control trials have demonstrated that HPV vaccination is safe and efficacious in preventing anal HPV infections and related anal precancerous lesions.[9,10] Although gbMSM are eligible for HPV vaccination in Canada,[11] there is a need for observational studies assessing its real-world impact on anal HPV infections in this at-risk population.

Broad-spectrum measures, like microbicides, offer another potential avenue for HPV prevention.[12] They can be used in conjunction with vaccination to offer robust protection against HPV infection and/or could be used as an alternate prevention strategy, as HPV vaccination coverage among gbMSM is relatively low.[13] Carrageenan, a red algae derivative, has demonstrated anti-HPV activity in *in vivo, in vitro,* and in clinical studies among women.[14-16] However, a recently conducted randomized control trial, the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) trial, among HIV-negative gbMSM and gbMSM living with HIV reported that use of a carrageenan-based gel was not efficacious in preventing acquisition or accelerating clearance of anal HPV infections.[17,18]

1.2 Objectives

The overall purpose of this thesis is to explore prevention of anal HPV infection through use of a carrageenan-based gel and HPV vaccination among HIV-negative gbMSM and gbMSM living with HIV.

Specifically, the main objectives of this thesis are:

1) To compare the change in anal HPV16 and HPV18 viral loads following carrageenan use relative to placebo, to further investigate the lack of protective effect observed in the LIMIT-HPV randomized control trial.
2) To assess the association between HPV vaccination and anal HPV prevalence and incidence.

CHAPTER 2: LITERATURE REVIEW

2.1 HUMAN PAPILLOMAVIRUS

2.1.1 Viral genome & life cycle

HPV is a non-enveloped, double-stranded deoxyribonucleic acid (DNA) virus belonging to the *Papillomaviridae* family. The circular viral genome is made up of about 8000 base pairs, structured into three regions: 1) the early region which encodes functional early proteins (E1, E2, E4, E5, E6, and E7) that facilitate genome replication and gene expression; 2) the late region which encodes two capsid proteins (L1 and L2) that have roles in viral infection, delivery, and packing; and 3) the long control region, a non-coding region which contains the replication origin and transcription-factor binding sites which control gene transcription.[19]

HPV is primarily transmitted through sexual activity involving skin-to-skin or skin-to-mucosa contact.[19,20] HPV virions infect the basal layer of the mucosal or cutaneous stratified epithelium through microtraumas (i.e., which can occur through sexual activity) that compromise the epithelial lining.[19] The L1 capsid protein binds to heparan sulfate proteoglycan (HSPG) located on the target epithelial cell surface and the virion enters the cell through endocytosis.[21,22] Once the cell has been infected, the viral genome is maintained at small numbers of 50 to 100 copies per nucleus.[21] As the epithelial cells differentiate and move through the layers of the stratified epithelium, the viral genome replicates to thousands of copies per cell, the L1 and L2 proteins are expressed, and the virions are assembled. Once at the epithelial surface, infected cells are sloughed off and the newly-assembled virions are released.[19] As the viral lifecycle is intra-epithelial, HPV infections are able to avoid both innate (i.e., non-specific) and adaptive (i.e., specialized) immune responses.[21]

While most infections are transient and cleared within 24 months, some can persist and progress to the development of precancerous lesions and, eventually, cancer.[19] In addition to having roles in the viral lifecycle, the E6 and E7 proteins have a key role in carcinogenesis. To establish a persistent infection, the HPV virion needs to infect basal epithelial cells that display stem-cell-like properties, such as having the ability to proliferate. Following infection of the target epithelial cell, E6 and E7 bind to and inhibit the p53 and retinoblastoma tumour suppressor proteins. This can result in deregulation of the cell cycle, activation of telomerase activity, and

genomic instability – all of which contribute to creating an environment favourable for epithelial cell transformation. When this process occurs alongside an accumulation of mutations in the host cell genome, it can lead to the development of invasive cancer.[19] Overall, carcinogenesis by HPV occurs in four steps: 1) HPV infection, 2) persistence of HPV infection, 3) progression to precancerous lesions, and 4) development of invasive cancer.[23,24]

2.1.2 Phylogenetic classification

Over 200 strains (more commonly called genotypes) of HPV have been identified.[19,22] Genotypes are classified into different genera according to their viral genome structure and epithelial cell tropism. Within each genus, genotypes are classified into species and specific types based on the nucleotide sequence of the L1 gene.[21] The *Alphapapillomavirus*, *Betapapillomavirus*, and *Gammapapillomavirus* genera are the largest, followed by the *Mupapillomavirus* and *Nupapillomavirus* genera.[19,22,25] The *Alphapapillomavirus* genus is made up of HPV genotypes that infect the mucosal and cutaneous epithelium. The other genera consist of genotypes that tend to infect the cutaneous epithelium.[21] The *Alphapapillomavirus* genus is further divided into subgenera according to carcinogenic potential and tissue tropism. Subgenus 1 contains low-risk mucosal HPV types, subgenus 2 contains high-risk mucosal HPV types, and subgenus 3 contains mostly commensal HPV types.[21,26] High-risk HPV types, associated with the development of cancer, include HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Low-risk HPV types, associated with the development of benign diseases such as anogenital warts, include HPVs 6 and 11. Commensal HPV types are typically associated with asymptomatic infections.[19]

2.1.3 Viral load

Viral load is commonly used as an indicator of the productivity and spread of an infection. As a productive and persistent HPV infection is required to develop related diseases, HPV viral load may be important in disease progression. In women, high HPV viral load has consistently been reported to be associated with persistent infections.[20,27] Similarly, an association has been observed between anal HPVs 16 and 18 viral load and persistent infection among gbMSM.[28] The relationship between HPV viral load and precancerous lesions, however, has been inconsistent in the literature thus far. Some studies report that high HPV viral loads, particularly that of HPV16, are associated with the presence of cervical precancerous lesions, with higher loads found among women with higher lesion grades,[29,30] while others do not report an association.[31,32] However,

different methodologies used to measure viral load in the studies may contribute to the inconsistency of results. Among people living with HIV, high anal viral loads of high-risk HPV types have been observed in those with anal cytological abnormalities compared to those without.[33] High anal HPVs 16 and 18 viral loads have also been reported to be associated with anal cytologic abnormalities among gbMSM, with higher loads correlated with higher lesion grades.[34]

2.2 ANAL PRECANCEROUS LESIONS

Anal squamous intraepithelial lesions (ASIL), also referred to as anal intraepithelial neoplasia (AIN), refers to the pre-malignant change of anal squamous cells and is driven by HPV infection.[23] Lesions were previously classified using a three-tiered system similar to nomenclature used for cervical intraepithelial neoplasia: AIN grades I, II, and III. However, AIN II diagnoses by pathologists were variable and often not reproducible.[23] Following the recommendations by the Lower Anogenital Squamous Terminology Standardization Project, anal cytology abnormalities are currently categorized as follows: 1) atypical squamous cells of undetermined significance (ASCUS); 2) low-grade squamous intraepithelial lesions (LSIL); and 3) high-grade squamous intraepithelial lesions (HSIL).[35,36] LSIL include AIN I, condyloma, and mild dysplasia. HSIL includes AIN II, AIN III, and moderate and severe dysplasia.

ASILs are typically diagnosed by anorectal exams, histopathology and p16 immunohistochemistry, anal cytology, or high-resolution anoscopy – considered the gold standard for anal HSIL detection.[35] Persons diagnosed with LSIL are actively monitored, whereas persons diagnosed with HSIL are generally advised to undergo treatment such as surgical excision, ablation, and use of topical agents. However, as some lesions can spontaneously regress, active monitoring is sometimes recommended.[23]

Not a lot is known regarding the natural history of ASIL and the progression from HSIL to anal cancer; progression is thought to occur less often than the progression from cervical intraepithelial neoplasia to cervical cancer. Studies suggest that 10 to 12% of HSIL progress to the development of invasive anal cancer within 5 years.[23,37] Immunosuppression, both related to and unrelated to HIV, smoking status, and having been infected with multiple and/or high-risk HPV types have been reported to increase the risk of progression to anal cancer.[23]

2.3 ANAL CANCER

Anal cancer refers to the cancer of perianal skin and the entire anal canal, including the squamo-columnar junction, where squamous epithelium of the anus transitions to columnar epithelium of the rectum.[24,36] Of note, the squamo-columnar junction is highly susceptible to HPV infection and transformation.[24] Approximately 85% of anal cancers are squamous cell carcinomas (ASCC), 10% are adenocarcinomas, and 5% are rare tumour types such as melanoma, small cell carcinoma, and metastatic tumours from other sites.[38] An estimated 88% of ASCC cases are attributable to anal high-risk HPV infections, particularly that of HPV16.[3,24,36,39] HPV16 is considered to be the most carcinogenic in the anus and is identified in HPV-related anal cancers more often than any other cancer type.[40] The relative contribution of HPV16 and HPV18 to anal cancer is 87%. Furthermore, the relative contribution of HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58 is 96%.[3]

2.4 DESCRIPTIVE EPIDEMIOLOGY

HPV is one of the most common sexually transmitted infections (STI); approximately 80% of sexually active people will contract the virus at least once in their lives. HPV is the cause of about 5% of cancers worldwide. In addition to being responsible for approximately 88% of anal cancer cases, HPV is also causally implicated in the development of all cervical cancers, most anogenital cancers at other sites (i.e., penile, vaginal, and vulvar cancers), and most oropharyngeal cancers.[3,19,41]

In men, the prevalence of anal HPV infections varies according to sexual behaviour, as anal HPV is primarily transmitted through anal sexual intercourse.[42,43] gbMSM, especially if they are living with HIV, have an elevated risk for contracting HPV infections and, consequently, developing ASIL and anal cancer,.[1,2,44-46] A 2021 meta-analysis of 64 studies from North America, Europe, Asia, and Africa, estimated that the pooled prevalence of anal high-risk HPV infection is 41.2% among HIV-negative gbMSM and 74.3% among gbMSM living with HIV. In comparison, pooled prevalence is estimated to be 6.9% among HIV-negative men who have sex with women and 26.9% among men who have sex with women living with HIV.[1] Furthermore, prevalence of anal high-risk HPV types remains high and relatively constant among gbMSM as they age.[1] Other risk factors for anal HPV infection include participating in high-risk sexual

behaviours, such as lack of condom use, having had a high number of sex partners, and having had receptive anal sex, as well as smoking.[45-48]

Consistent with the trends observed for anal HPV infection, the risk of developing anal HSIL and anal cancer is highest among gbMSM, particularly among those living with HIV. A meta-analysis of 32 studies estimated that anal HSIL prevalence is estimated to be 11.3% and 22.4% in HIV-negative gbMSM and gbMSM living with HIV, respectively.[1] A separate meta-analysis estimated that anal cancer incidence among gbMSM is estimated to be 19 cases [95% confidence interval (CI) = 10 – 36] per 100,000 person-years if they are HIV-negative and 85 cases (95% CI: 82 – 89) per 100,000 person-years if they are living with HIV.[2] In contrast, anal cancer among the general population is quite rare - the incidence rate is estimated to be 1-2 cases per 100,000 person-years, though it is rising in middle- and high-income countries.[2,49,50] The recent Anal Cancer HSIL Outcomes Research (ANCHOR) randomized control trial conducted between 2014 and 2021 in the United States among persons living with HIV evaluated the efficacy of ASIL treatment in reducing the progression to anal cancer compared to active monitoring without treatment. This trial reported that 9 of 2227 participants in the treatment and 21 of 2219 participants in the active monitoring arm progressed from HSIL to invasive anal cancer over a median follow-up time of 25.3 [interquartile range (IQR): 11.7-42.0] and 27.2 (IQR: 12.0-42.1) months, respectively.[51]

2.5 HUMAN IMMUNODEFICIENCY VIRUS

HIV primarily targets CD4+ T cells which have an important role in the immune response. HIV may also infect macrophages and dendritic cells.[52,53] HIV has the ability to avoid innate and adaptive immune responses and, consequently, leads to the gradual destruction of naïve and memory CD4+ T cells – a hallmark of infection.[52,53] While most infections are asymptomatic during the early and chronic disease stages, without treatment, HIV can lead to acquired immune deficiency syndrome (AIDS), the last stage of disease. Disease stages are identified by measuring blood CD4+ T cells and plasma HIV viral load, as well as considering any opportunistic infections the person may have.[53] Blood CD4+ T cells can provide an indication of the degree of immunodeficiency and HIV viral load can provide an indication of the rate of the destruction of the immune system. People living with HIV are commonly treated with highly

active antiretroviral therapy (HAART) to suppress the virus, thus, reducing associated morbidity and mortality.[53]

Globally, 1.3 million (95% CI=1 million – 1.7 million) people were diagnosed with HIV in 2022, resulting in a total of 39 million (95% CI=33.1 million – 45.7 million) people living with HIV; 7.5% of these cases were among gbMSM.[54] Specifically in Canada, there were 1833 new HIV diagnoses in 2022 – a 24.9% increase since 2021. 1244 of these diagnoses occurred in males; of that, 51.1% were among gbMSM.[55]

People living with HIV are considered to have a higher risk of HPV infection due to reduced effectiveness in their immune responses. gbMSM living with HIV have been reported to have higher prevalence and less clearance of anal HPV infections compared to HIV-negative gbMSM.[44,56,57] In addition, low CD4 cell counts and high HIV viral load have been reported to be associated with HPV infection.[1,20,47,58] Despite the availability of HAART to control HIV infection, anal cancer incidence remains high, contrary to the risk reductions observed for other AIDS-related opportunistic infections and neoplasia and AIDS-defining cancers.[59] A 2015 meta-analysis of 21 studies reported that the risk of anal cancer was four times higher among people living with HIV compared to before the availability of HAART (risk ratio=4.28, 95% CI= 3.25 – 5.64).[60] This increase may partially be a result of a population-level ageing effect.[2]

2.6 PRIMARY PREVENTION: HPV VACCINATION

Natural infection by HPV induces low levels of antibodies which are often not sufficient to protect against reinfection by the same HPV type. In contrast, HPV vaccines are able to stimulate a strong humoral immune response with long-lasting neutralizing antibodies against HPV.[61] Moreover, HPV vaccination has been shown to induce the highest level of antibody titres when administered in persons less than 16 years of age (i.e., before sexual debut and thus, exposure to HPV).[62]

2.6.1 Recommendations in Canada

There are currently six licensed prophylactic HPV vaccines targeting high-risk HPV types associated with the development of cancer and low-risk HPV types associated with the development of anogenital warts: three bivalent (targets HPVs 16 and 18), two quadrivalent (targets HPVs 6, 11, 16, and 18) and one nonavalent (targets HPVs 6, 11, 16, 18, 31, 33, 45, 52,

and 58).[62] All the vaccines are developed using virus-like particles, empty viral shells composed of the L1 capsid proteins derived from the different HPV types targeted by the vaccine.[61,62]

Three of these HPV vaccines have been licensed for use in Canada – Cervarix®, a bivalent vaccine, Gardasil®, a quadrivalent vaccine that is no longer offered, and Gardasil®9, a nonavalent vaccine.[11] Health Canada initially authorized the use of Gardasil in females in 2006 and expanded to include males in 2010.[63] In the province of Quebec, gbMSM and people living with HIV under the age of 27 years have been eligible for the publicly funded HPV vaccination program since 2014. Since then, the school-based HPV vaccination program expanded to include all genders in 2016, and the publicly funded program expanded to people who are immunocompromised or living with HIV under the age of 46 years in 2022. Furthermore, as of October 2022, two doses are recommended to eligible, healthy youth and adults, and three doses to those who are immunocompromised or living with HIV.[64]

2.6.2 Vaccine efficacy & effectiveness

In women, HPV vaccination has been consistently shown to protect against infection of vaccine-targeted HPV types, and the development of associated anogenital warts and cervical precancerous lesions.[4-7] Furthermore, a 2020 registry-based cohort study of girls and women 10 to 30 years of age in Sweden reported that the quadrivalent vaccine was associated with a lower risk of invasive cervical cancer.[8] Similarly, a 2011 randomized control trial of boys and men between 16 to 26 years demonstrated that the quadrivalent vaccine was safe and efficacious against persistent infection of vaccine-targeted HPV types and associated external genital lesions in both the per-protocol and the intention-to-treat populations.[65] A sub-study of HIV-negative gbMSM with five or less lifetime sex partners from the 2011 randomized control trial, reported that the quadrivalent vaccine was efficacious against persistent anal infections of any of the vaccine-targeted HPV types [per-protocol: vaccine efficacy (VE)= 94.9%, 95% CI= 80.4% - 99.4%; intention-to-treat: VE= 59.4%, 95% CI= 43.0% - 71.4%] and the development of related AIN (per-protocol: VE= 77.5%, 95% CI= 39.6% - 93.3%; intention-to-treat: VE= 50.3%, 95% CI= 25.7% - 67.2%).[9] Furthermore, an extension to the 2011 randomized control trial demonstrated that protection by HPV vaccination against anogenital disease related to any of the vaccine-targeted HPV types lasts for up to ten years.[66] Consistent with results reported among HIV-negative gbMSM, a randomized control trial in the United States among gbMSM living

with HIV, 13 to 26 years of age, and had at least one lifetime male sex partner reported a decrease in HPV16-associated anal HSILs between vaccinated HPV-naïve (i.e., HPV-negative at baseline) and previously-exposed (i.e., HPV-positive at baseline) participants in the per-protocol population (p-value = 0.014).[10] Conversely, a randomized control trial in Spain conducted among gbMSM living with HIV of at least 27 years of age, reported no significant difference between the vaccine and placebo arms in the development of ≥HSILs or external anogenital lesions after 48 months of follow-up.[67]

2.6.3 Uptake among gbMSM

HPV vaccination coverage is relatively low among gbMSM. Most estimates suggest that, on average, HPV vaccine uptake (i.e., received at least one dose of the vaccine) is under 50%.[13,68-71] In Canada, a study of gbMSM enrolled between 2017 and 2019 across three major cities reported that the city-specific vaccine uptake ranged from 26% to 35% among gbMSM under 27 years and 7% to 26% among gbMSM, 27 years and older.[13] In the United States, a study using HIV behavioural surveillance data reported that HPV vaccination uptake among gbMSM, 18 to 26 years, was 32.8% in 2017.[68] Another study conducted across three major cities in the United States between 2016 and 2018 reported vaccine uptake to be 47.5% (range= 33% - 62%).[69] Similarly, a retrospective cohort study in Australia reported a vaccine uptake of 47.8% in 2019 among gbMSM, 16 to 26 years of age.[70] Consistent with these estimates, a 2021 meta-analysis of 33 studies estimated that the pooled average HPV vaccine uptake among gbMSM, majority of whom under 26 years of age, was 37% (median= 26%, range= 5% - 100%).[71]

gbMSM often face barriers which may contribute to the relatively low uptake of the HPV vaccine. These include age, ethnicity, gender identity, lack of access to private health insurance, sexual health education, or health services, and not disclosing their sexual orientation.[69,71-74] Notably, recommendation by a health care provider has consistently been reported to be one of the strongest predictors of HPV vaccination among gbMSM. Other predictors include receiving other vaccinations recommended for gbMSM (i.e., against hepatitis A and B), community involvement, and having knowledge or awareness of STI-related health care.[13,71-74]

2.7 PRIMARY PREVENTION: MALE CONDOMS

When used consistently and correctly, male condoms have demonstrated protection against several STIs such as HIV, chlamydia, gonorrhea, and trichomoniasis.[75] In comparison, use of male condoms has inconsistently demonstrated marginal protection against HPV infections in women.[75,76] Nevertheless, HPV vaccination and condom use is recommended to provide the greatest protection against HPV infection as condoms can provide a broad spectrum of protection against different HPV types, whereas HPV vaccination is targeted to protect against a small number of HPV types that are causally implicated with the development of related diseases.[75] Among men, condom use has been reported to reduce genital HPV infection, particularly for those with multiple sex partners.[77,78] In gbMSM specifically, the odds of having an anal HPV infection was about six times higher in men who never used condoms compared to those who always used condoms.[79] However, consistent condom usage is suboptimal among gbMSM and the rate of gbMSM participating in condomless anal sex is reportedly increasing in high-income countries.[80,81]

2.8 PRIMARY PREVENTION: CARRAGEENAN-BASED GELS

Carrageenan is a class of sulfated polysaccharides derived from red algae. It is commonly used as a thickening agent in food and cosmetic products including sexual lubricants.[12] Carrageenan has demonstrated inhibition of HPV infection in *in vivo* and *in vitro* studies. Furthermore, carrageenan-containing products have been determined to be safe for vaginal and penile use in clinical trials, as well as for rectal use in a mouse model and in a study in humans.[12,14]

2.8.1 Mechanism of action

Carrageenan is structurally similar to HSPG, an HPV cell attachment factor. It is hypothesized to inhibit HPV infection by binding directly to the virion, preventing the virion from binding to HSPG on target epithelial cells. The carrageenan-to-HPV virion interaction is long enough for the innate and adaptive immune responses to be induced.[12] Carrageenan has also shown to prevent the attachment of HPV virions to human sperm, thus preventing the dispersion of virions within the vaginal canal. In addition, carrageenan may inhibit HPV infection through

an HSPG-independent mechanism, where it prevents virions from binding to secondary receptors found on target epithelial cells.[12]

2.8.2 Gel Efficacy

A sub-study of a randomized control trial, originally conducted in South Africa among women to assess the efficacy of a carrageenan-based gel in reducing the risk of HIV infections, reported that carrageenan was associated with a 38% reduction of prevalent HPV infections among the most compliant participants (prevalence ratio= 0.62, 95% CI= 0.41 – 0.94).[82] Similarly, a recent randomized control trial, Carrageenan gel Against Transmission of Cervical HPV (CATCH), reported a 37% protective effect [hazard ratio (HR)= 0.63; 95% CI= 0.49 – 0.81] against incident HPV infections and that the carrageenan-based gel was overall well-tolerated.[15,16] The CATCH trial, however, did not find evidence to support that carrageenan use influenced the clearance of existing infections.

Conversely, the LIMIT-HPV randomized control trial in gbMSM, a sister study to the CATCH trial, did not find any evidence that use of a carrageenan-based gel protected against incident anal HPV infections (HR= 1.21, 95% CI= 0.86 – 1.70) nor did it influence the clearance of baseline infections (HR= 0.84; 95% CI= 0.31 – 2.27).[18] In addition, participants in the carrageenan study arm reported significantly more adverse events compared to participants in the placebo study arm. Following the conclusion of the CATCH and LIMIT-HPV trials, the carrageenan-based gel was found to be hyperosmolal (3790 mOsM/kg), exceeding the lubricant osmolality guidelines set by the World Health Organization (<1200 mOsM/kg), which may have contributed to the adverse events reported by LIMIT-HPV study participants.[15,18] Other hyperosmolar lubricants have been found to cause rectal epithelial injury and are associated with STI acquisition.[18,83-85]

2.9 SECONDARY PREVENTION: SCREENING

While primary prevention measures such as HPV vaccination are likely the most effective at preventing anal cancer, secondary prevention measures such as screening may also provide value for individuals who have been previously exposed to HPV. Due to the rarity of anal cancer in the general population, there is no benefit for a population-based screening program. However, programs for subgroups of the population with a higher risk of developing

anal cancer may be the most effective in contributing to prevention.[23] As anal and cervical carcinogenesis have common disease progressions, similar screening and prevention modalities used for cervical cancer have been proposed for anal cancer screening.[24] These modalities include anal cytology and testing for high-risk HPV types to detect ASIL, followed by high-resolution anoscopy with targeted biopsy for abnormal findings as well as digital anal rectal examinations.[36] However, past screening programs and ASIL treatment guidelines and recommendations were based on expert opinion, as there was a lack of evidence regarding the efficacy of these modalities in preventing the development of anal cancer.[23,35]

The ANCHOR trial reported that treatment of anal HSIL reduced progression by 57% (95% CI= 6% - 80%), p-value= 0.03) compared to active monitoring.[51] Of note, most participants were treated with electrocautery, a type of ablation. The results from the ANCHOR trial provides evidence for which robust anal cancer screening guidelines can be based on.

CHAPTER 3: USE OF A CARRAGEENAN-BASED GEL HAD NO IMPACT ON ANAL HPVS 16 AND 18 VIRAL LOADS IN GAY, BISEXUAL, AND OTHER MEN WHO HAVE SEX WITH MEN

3.1 PREFACE

Carrageenan has demonstrated broad-spectrum anti-HPV activity in *in vivo, in vitro,* and in clinical studies among women.[14,15] Carrageenan-based gels could potentially be used alongside HPV vaccination to provide robust protection against acquisition of HPV infections. In addition, as uptake of HPV vaccination among gbMSM is relatively low,[13,68-71] carrageenan may serve as an alternate HPV preventive measure.

The LIMIT-HPV randomized control trial in HIV-negative gbMSM and gbMSM living with HIV, conducted by our research group, found that use of a carrageenan-based gel neither reduced incident anal HPV infections nor accelerated clearance of existing infections.[17,18] Furthermore, a greater number of adverse events were reported by participants in the treatment than the placebo study arm. Accordingly, the Data Safety and Monitoring board recommended trial termination due to the lack of protective effect by carrageenan and due to safety concerns.[17]

The purpose of this manuscript is to investigate the observed lack of protective effect in gbMSM, by comparing the change in anal HPV16 and HPV18 viral loads following carrageenan use relative to placebo. Our ancillary hypothesis is that if carrageenan does not prevent against anal HPV infection, it may have reduced the viral load (i.e., the productivity and spread) of the infection.

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3.2 TITLE PAGE

USE OF A CARRAGEENAN-BASED GEL HAD NO IMPACT ON ANAL HPVS 16 AND 18 VIRAL LOADS IN GAY, BISEXUAL, AND OTHER MEN WHO HAVE SEX WITH MEN.

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3.3 ABSTRACT

The Lubricant Investigation in Men to Inhibit Transmission of HPV Infection randomized control trial in gay, bisexual, and other men who have sex with men (gbMSM) found that carrageenan use neither reduced acquisition of anal HPV infections nor influenced infection clearance. To investigate carrageenan's lack of protective effect, we compared the change in anal HPV16 and HPV18 viral loads following carrageenan use against placebo. We restricted our analysis to participants who completed the first four visits and had a valid baseline sample (n=161, 54 HIV-positive). Samples were tested for HPV detection using the linear array PCR assay. HPV16- and/or HPV18-positive samples were tested for viral load using real-time PCR. For participants who tested HPV16- (n=29) or HPV18-positive (n=10) at least once across visits 1-4, we compared the change in type-specific viral load between study arms using the Mann-Whitney U test. Although the median net change in HPV16 and HPV18 viral loads across visits 1-4 was higher in the treatment than placebo arm (HPV16: 0.68 versus 0.18 copies/cell, P=0.60; HPV18: 18.32 versus 10.12 copies/cell, P=0.52), these differences were not statistically significant. Results were similar by HIV status. Carrageenan use did not impact anal HPV16 or HPV18 viral loads, which may further explain its lack of protective effect in gbMSM.

Keywords

Human papillomavirus, Carrageenan, Men who have sex with men, Viral load, HIV

3.4 INTRODUCTION

Approximately 80% of sexually active individuals will be infected with human papillomavirus (HPV) at least once in their lives.[1] While most infections are transient, some can persist and progress to precancerous lesions, and eventually cancer.[2] Gay, bisexual, and other men who have sex with men (gbMSM), especially those living with human immunodeficiency virus (HIV), are at an increased risk for HPV-related cancers, particularly anal cancer.[3] In the general population, anal cancer incidence is 1-2 cases per 100,000 individuals, whereas the incidence is estimated at 19 cases per 100,000 individuals in HIV-negative gbMSM and 85 cases per 100,000 individuals in gbMSM living with HIV.[3] Carrageenan, a red algae derivative, has demonstrated inhibition of HPV infection by binding to the viral receptor and preventing attachment onto its epithelial target.[4,5]

We conducted the Lubricant Investigation In Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study in HIV-negative and HIV-positive gbMSM to evaluate the efficacy of a carrageenan-based gel in preventing incidence and accelerating clearance of anal HPV infections.[6] We found that carrageenan use did not prevent the acquisition of incident infections (hazard ratio, 1.21 [95% confidence interval 0.86-1.70]),[7] nor did it affect infection clearance (hazard ratio, 0.84 [95% confidence interval, 0.31-2.27]).[8]

To investigate the lack of protective effect in the LIMIT-HPV study despite prior empirical proof of carrageenan's anti-HPV activity,[4] we compared the change in anal HPVs 16 and 18 viral loads following carrageenan use against placebo, overall and by HIV status. Our ancillary hypothesis is that carrageenan use may reduce anal HPV viral loads, thus reducing infection transmissibility. By interfering with the interaction between the virion and its epithelial target,[5] carrageenan use may reduce the lateral spread of infection foci within the mucosal epithelium, thus reducing the net viral load of the infection. Coherent with this hypothesis, we expected that participants in the treatment arm would have a viral load reduction compared to the placebo arm.

3.5 MATERIALS AND METHODS

Study design & population

The LIMIT-HPV study, a phase 2b, double-blind, randomized control trial, enrolled gbMSM in Montreal, Canada between 2016 and 2020. The trial was registered on ClinicalTrials.gov (identifier NCT02354144). It was terminated due to the carrageenan-based gel's lack of protective effect against acquisition of new anal HPV infections, a low probability of finding a protective effect by the end of the study, and safety concerns due to higher reporting of adverse events among participants in the treatment than placebo arm.[7] The recruitment strategy and study design have previously been reported.[6] Briefly, the eligibility criteria included: ≥18 years of age; lived in Montreal and planned to remain for the next year, had receptive anal intercourse with at least 1 man during the previous 3 months; planned on having receptive anal intercourse with 2-50 partners per year; and willing to complete an HIV test (for those who had never tested seropositive). At enrollment, participants provided written- or e-consent and were randomized 1:1 to receive the carrageenan-based or placebo gel. Participants attended follow-up visits during months 1, 2, 3, 6, 9 and 12, where they self-completed an electronic questionnaire on sociodemographic and behavioral risk factors and provided a nurse-collected anal sample . Nurses used Dacron™ swabs to collect samples and followed the Protocol for Anal Swab Collection.[6] We restricted to participants who had a valid baseline anal sample and completed the first four visits, as we expected to detect the gel's effect on anal HPV viral load within the first 3 months of usage.

HPV DNA genotyping & viral load quantification

Following collection, samples were preserved in PreservCyt and stored at 4°C during laboratory transfer.[6] DNA was purified from samples after centrifugation using a Master-Pure Kit (EpiCentre) and tested in each PCR assay.[9] Most samples were genotyped using the linear array assay (Roche Diagnostics),[10] only 14 were genotyped using the Anyplex II HPV-28 assay (Seegene Inc.).[11] Irrespective of the assay, a β-globin DNA sequence was simultaneously amplified to verify if samples were adequate for polymerase chain reaction (PCR) analysis. HPVs 16 and 18 viral loads were measured using real-time PCR assays in a Light Cycler PCR and detection system (Roche Molecular Systems) by quantifying separately HPV and β-globin copy numbers in 2 μL of processed sample, as described previously[12], after they were shown to

be free of PCR inhibitors by amplification of an internal control, as described previously.[13] Cycle thresholds obtained for each sample were compared to those of a titration curve obtained by serial 10-fold dilutions of HPV16 DNA plasmid or HPV18 plasmid in 75 ng of human genomic DNA (Roche Diagnostics) in 10 mM Tris-HCl (pH 8.2). Viral loads were calculated by dividing the number of HPV DNA copies by the total number of cells, which was estimated from the number of β-globin copies. Results were recorded as copies/cell.

Statistical Analyses

Few samples had missing viral load values. One HPV16-positive sample with a viral load below the assay's detectability threshold was assigned half of the lowest value in the distribution of detectable HPV16 viral loads. Two invalid samples from participants who tested HPV16-positive at least once over visits 1 to 4 were assigned the mean viral load of the two adjacent visits. We assumed that HPV-negative samples from participants who tested HPV16-positive or HPV18-positive at least once over visits 1 to 4 were not truly negative, but that their infection was undetectable by the genotyping assay. Accordingly, we assigned 29 HPV16-negative and 4 HPV18-negative samples half of the lowest type-specific viral load value.

We assessed the participants' baseline characteristics using descriptive statistics. Due to the skewed distribution, we described each study arm's type-specific viral load during visits 1 to 4 using the median, interquartile range, range, and geometric mean. We expected that the difference in viral load would be greatest between visits 1 and 2 as gels were not used at enrollment. For each study arm, we described the change in type-specific viral load between visits 1 and 2 and the net change across visits 1 to 4 using the median and interquartile range. We compared the change in viral load between study arms using the Mann-Whitney U test; p-values <0.05 were considered statistically significant. We conducted the analyses overall and stratified by HIV status. All statistical analyses were conducted in Stata version 18.0.

3.6 RESULTS

Of 258 enrolled participants, 161 completed the first four visits and had a valid baseline anal sample (Figure I). Of these, 29 tested HPV16-positive and 10 tested HPV18-positive at least once over the first 4 visits. Participants completed a median of 7 visits (range, 4-7 visits) over a median of 12.0 months (range, 2.9-40.7 months). Table 1 describes their baseline characteristics.

The mean age of participants was 39.1 years. Most were French-Canadian (37.9%) and attended university (56.5%). The mean age at first sexual activity was 17.8 years. Most reported having had 21 or more lifetime male sexual partners and using condoms for receptive anal sex 75-99% of the time in the past year (22.4%). Less than a quarter of participants had received the HPV vaccine (21.7%) and 54 were HIV-positive (33.5%).

Over visits 1 to 4, 13 participants in the treatment (7 HIV-negative and 6 HIV-positive) and 16 participants in the placebo (10 HIV-negative and 6 HIV-positive) arm tested HPV16-positive at least once. Overall, the geometric mean of HPV16 viral load was lower in the treatment than placebo arm at baseline (0.19 versus 0.44 copies/cell; Table II). At follow-up visits, the geometric mean was higher in the treatment than placebo arm. The interquartile range overlapped between study arms across visits 1 to 4. Among HIV-negative participants, the geometric mean of HPV16 viral load was lower in the treatment than placebo arm at baseline (0.16 versus 0.83 copies/cell) and continued to be lower across visits 2 to 4. Among HIV-positive participants, the geometric mean of HPV16 viral load was slightly higher in the treatment than placebo arm at baseline (0.24 versus 0.16 copies/cell) and continued to be higher across visits 2 to 4. The interquartile range overlapped between study arms across visits 1 to 4 when stratified by HIV status.

Six participants in the treatment (4 HIV-negative and 2 HIV-positive) and 4 participants in the placebo (all HIV-positive) arm tested HPV18-positive at least once over visits 1 to 4. The HPV18 viral load geometric mean was higher overall in the treatment than placebo arm at baseline (0.16 versus 0.07 copies/cell) and continued to be higher across visits 2 to 4. The interquartile range overlapped between study arms at all visits. Among HIV-positive participants, the geometric mean of HPV18 viral load was higher in the treatment than placebo arm at baseline (4.71 versus 0.07 copies/cell) and continued to be higher across visits 2 to 4. The interquartile range also overlapped between study arms at all visits.

As shown in Table III, the median change in HPV16 viral load between visits 1 and 2 was similar between study arms (0.00 versus -0.01 copies/cell) whereas the median net change across visits 1 to 4 was higher in the treatment than placebo arm (0.68 versus 0.18 copies/cell). For HPV18 viral load, the median change between visits 1 and 2 (0.92 versus 0.24 copies/cell) as well as the median net change across visits 1 to 4 (18.32 versus 10.12 copies/cell) was higher in

the treatment than placebo arm. The differences in the change in HPV16 and HPV18 viral load were not statistically different between study arms and by HIV status.

3.7 DISCUSSION

This additional analysis using data from the LIMIT-HPV study, comparing the change in anal HPV viral loads following use of a carrageenan-based gel compared to placebo, is the first to explore if carrageenan influenced anal HPV viral loads in gbMSM. We found small differences in anal HPV16 and HPV18 viral loads between HIV-negative and HIV-positive gbMSM within each study arm at each visit and no evidence that carrageenan use impacts anal HPVs 16 or 18 viral loads in gbMSM.

One factor that may influence the observed range of viral loads is HIV status. While no difference was reported for anal HPV16 viral load by HIV status,[14,15] anal HPV18 viral load was reported to be higher in 49 HIV-positive compared to 122 HIV-negative or unknown status gbMSM in Thailand recruited in 2012-2013,[15] or similar between 88 HIV-positive and 74 HIV-negative gbMSM in the Netherlands recruited in 2010-2011.[14] The variation may reflect differences in antiretroviral therapy access and immune response among gbMSM living with HIV in different time periods and geographical areas.[14] In our study, it is possible that we did not have the power to detect a difference in viral loads by HIV status because of the small number of participants in each comparison group. Our participants living with HIV were receiving HIV care with good antiretroviral therapy access, which is expected to be associated with a good immune response and may explain the absence of difference observed according to HIV status. Furthermore, a higher proportion of HIV-positive participants (47/69, 68%) remained in the study compared to HIV-negative (65/189, 34%; Supplementary Table I) which may also reflect the engagement in care of gbMSM living with HIV, which could have facilitated compliance with the study protocol. The observed range of viral loads may also be explained by the presence of acute versus chronic HPV infections[16] as well as clearing versus productive HPV infections.[17]

Limitations of the analysis include a small number of observations, considerable loss to follow up (57%, Supplementary Table I), and assessing viral load for HPV16 and HPV18. Other common HPV genotypes at enrollment among the 161 participants in this report were HPV53 (18.6%), HPV51 (13.7%), and HPV52 (9.9%; Supplementary Table II). Evaluating these genotypes could reveal additional insights into carrageenan's effect on anal viral loads or the

natural history of anal infection. While HPV53 and HPV51 viral loads have not been studied in gbMSM thus far, anal HPV52 viral load was found not to differ by HIV status.[14] Another limitation is that we assumed that HPV16-negative or HPV18-negative samples from participants who tested positive for either type at least once over visits 1 to 4 had an undetectable infection by the HPV genotyping assay and imputed viral load values for these samples accordingly. It would have been informative to have tested for viral load in these samples instead. However, by using data imputations, we were able to increase the power and provide a reliable comparison of viral load between study arms.

The lack of impact by carrageenan on anal HPVs 16 and 18 viral loads could help explain the lack of protective effect in gbMSM. Larger studies evaluating carrageenan's impact on viral loads of prevalent anal HPV types may be needed to further understand its effect.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author. The data will not be published due to privacy and ethical restrictions; participants of the LIMIT-HPV study did not consent to having their data made publicly available.

CONFLICT OF INTEREST

Outside the submitted work, ELF served as an occasional advisor for companies involved with HPV vaccines (Merck, GSK) and HPV diagnostics (Roche Diagnostics) and as a Steering Committee Member for a publicly funded study in Finland that received support from GSK. MZ and ELF hold a patent related to the discovery "DNA methylation markers for early detection of cervical cancer", registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada (October 2018). AdP received honoraria for consulting on HIV antiretroviral regimen for ViiV Healthcare. FC has received grants through his institution from Merck Sharp & Dohme, Becton Dickinson, and Roche to conduct evaluation of HPV diagnostic tests, as well as honoraria from Merck and Roche for lectures on HPV. JET is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA. PK received the Canada Graduate Scholarship – Master's Award from the Canadian Institute of Health Research. CL has no conflict of interest to disclose.

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AUTHOR CONTRIBUTIONS

ELF, AdP, JET, FC, and P-PT conceived and designed the study. MZ managed the study. PK conducted the statistical analysis and drafted the manuscript under the supervision of ELF and MZ. All authors read, provided feedback, and approved the final manuscript.

ETHICS APPROVAL

The Research Ethics Boards of McGill University, the McGill University Health Centre, Concordia University and Centre Hospitalier de l'Université de Montréal approved the study. Health Canada (file number 169160) authorised use of the study gel.
Ethics committee: A10-M98-14B.

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3.9 TABLES & FIGURES

M1 - Table I: Baseline characteristics of the included LIMIT-HPV study participants, overall and by study arm.

	Overall (n = 161)	**Treatment (n=82)**	**Placebo (n=79)**
Age, years			
Mean ± SD	39.1 ± 13.9	40.2 ± 14.1	38.1 ± 13.7
Median (IQR)	38.7 (26.5-51.3)	41.1 (26.6-51.7)	37.1 (25.7-48.3)
Range	18.2-71.7	18.4-71.7	18.2-68.1
Ethnicity, n (%)			
French Canadian	61 (37.8)	29 (35.4)	32 (40.5)
English Canadian	24 (14.9)	11 (13.4)	13 (16.5)
European	22 (13.7)	14 (17.1)	8 (10.1)
Latin American	21 (13.0)	9 (11.0)	12 (15.2)
Other	33 (20.5)	19 (23.2)	14 (17.7)
Education, n (%)			
Elementary	2 (1.2)	0 (0.0)	2 (2.5)
Secondary	26 (16.2)	12 (14.6)	14 (17.7)
College	42 (26.1)	20 (24.4)	22 (27.9)
University	91 (56.5)	50 (61.0)	41 (51.9)
Age at first sexual activity, years			
Mean ± SD	17.8 ± 6.4	17.6 ± 6.5	18.0 ± 6.3
Median (IQR)	17.0 (14.0-20.0)	17.0 (14.0-20.0)	18.0 (15.0-21.0)
Range	4.0-42.0	4.0-42.0	5.0-42.0
Number of lifetime male sex partners, n (%)			
1-5	11 (6.8)	5 (6.1)	6 (7.6)
6-10	9 (5.6)	1 (1.2)	8 (10.1)
11-20	16 (9.9)	11 (13.4)	5 (6.3)
21-60	41 (25.5)	20 (24.4)	21 (26.6)
61-300	39 (24.2)	20 (24.4)	19 (24.1)
301-1000	32 (19.9)	17 (20.7)	15 (19.0)
>1000	13 (8.1)	8 (9.8)	5 (6.3)
Condom usage for receptive anal sex in past year, n (%)			
Never (0%)	27 (16.8)	13 (15.9)	14 (17.7)
Rarely (1-24%)	26 (16.2)	13 (15.9)	13 (16.5)
Occasionally (25-49%)	18 (11.2)	9 (11.0)	9 (11.4)
Often (50-74%)	24 (14.9)	15 (18.3)	9 (11.4)
Almost always (75-99%)	36 (22.4)	17 (20.7)	19 (24.1)
Always (100%)	27 (16.8)	13 (15.9)	14 (17.7)
Missing	3 (1.9)	2 (2.4)	1 (1.3)

HPV vaccination status, n (%)			
Yes	35 (21.7)	16 (19.5)	19 (24.1)
No	126 (78.3)	66 (80.5)	60 (76.0)
HIV status, n (%)			
Positive	54 (33.5)	26 (31.7)	28 (35.4)
Negative	107 (66.5)	56 (68.3)	51 (64.6)
HPV DNA Status, n (%)[a]			
HPV Negative	48 (29.8)	24 (29.3)	24 (30.4)
Any HPV[b]	113 (70.2)	58 (70.7)	55 (69.6)
HPV16+	21 (13.0)	9 (11.0)	12 (15.2)
HPV18+	10 (6.2)	6 (7.3)	4 (5.1)
Other types[c]	112 (69.6)	58 (70.7)	54 (68.4)

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; IQR, interquartile range; LIMIT-HPV, Lubricant Investigation in Men to Inhibit Transmission of HPV Infection; SD, standard deviation; +, positive.

[a] Percentages exceeded 100% as some participants tested positive for more than one HPV type.

[b] Positive for any of the 36 HPV types (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).

[c] Positive for any of the 36 HPV types other than HPV16 and/or HPV18.

M1 - Table II: Anal HPV16 and HPV18 viral load (copies/cell) at visits 1 to 4 by study arm, overall and by HIV status.

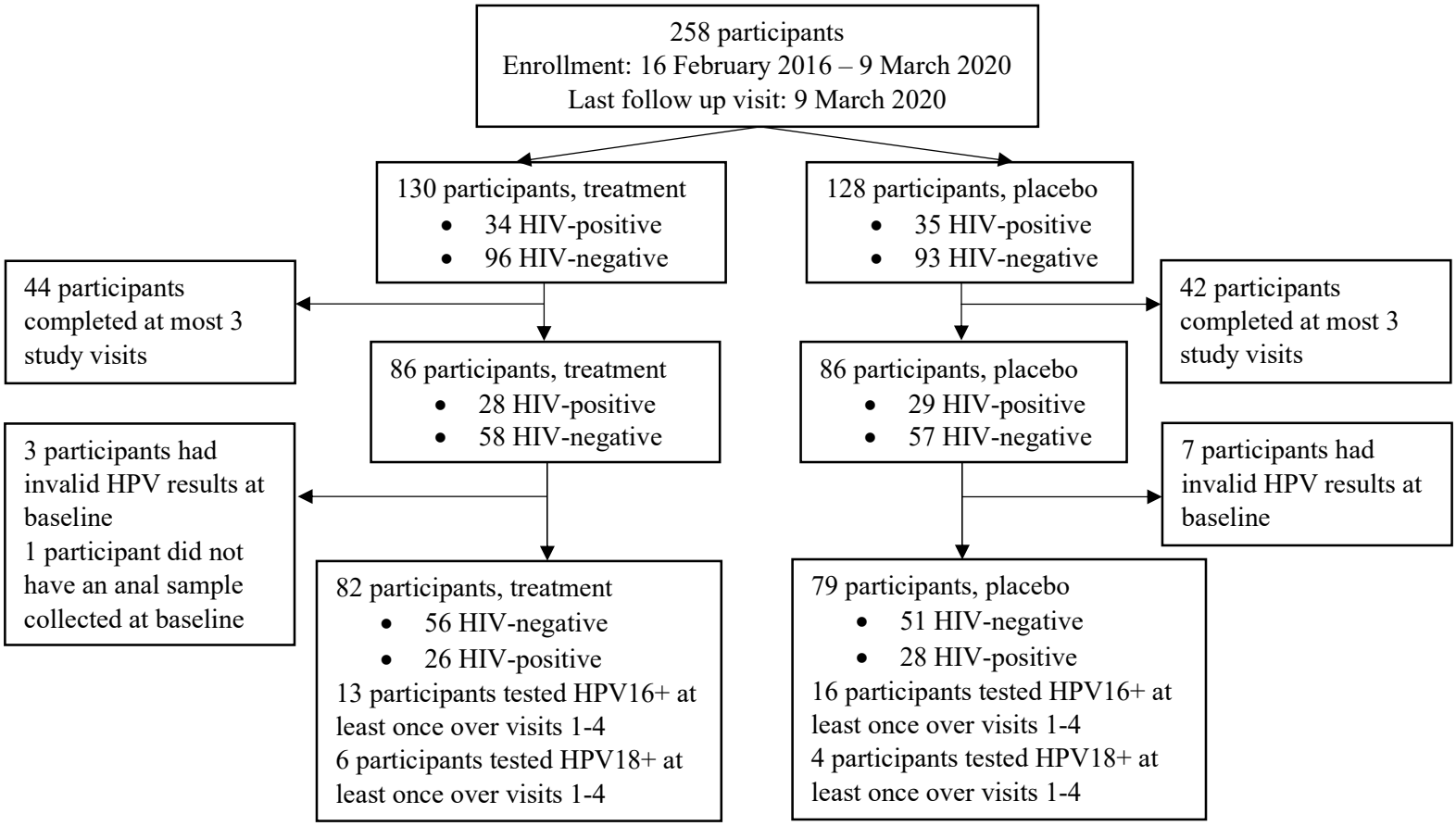
	Visit 1		Visit 2		Visit 3		Visit 4	
	Treatment	**Placebo**	**Treatment**	**Placebo**	**Treatment**	**Placebo**	**Treatment**	**Placebo**
HPV16, all participants								
Median (IQR)	0.63 ($4.27x10^{-3}$-1.43)	0.31 (0.02-4.79)	0.31 ($4.27x10^{-3}$-4.73)	0.20 ($4.27x10^{-3}$-42.45)	1.03 (0.07-12.99)	0.11 ($4.27x10^{-3}$-8.25)	0.39 (0.12-5.92)	0.21 (0.02-26.58)
Range	$4.27x10^{-3}$-120.41	$4.27x10^{-3}$-511.08	$4.27x10^{-3}$-161.76	$4.27x10^{-3}$-137.52	$4.27x10^{-3}$-291.07	$4.27x10^{-3}$-626.43	$4.27x10^{-3}$-49.38	$4.27x10^{-3}$-382.97
Geometric mean	0.19	0.44	0.35	0.35	0.96	0.32	0.61	0.49
HPV16, HIV-negative participants								
Median (IQR)	0.29 ($4.27x10^{-3}$-1.62)	0.83 ($4.27x10^{-3}$-15.53)	0.31 ($4.27x10^{-3}$-4.73)	0.25 (0.06-64.18)	0.71 ($4.27x10^{-3}$-12.99)	0.58 ($4.27x10^{-3}$-6.00)	1.17 (0.07-5.92)	1.61 (0.07-43.49)
Range	$4.27x10^{-3}$-120.41	$4.27x10^{-3}$-511.08	$4.27x10^{-3}$-161.76	$4.27x10^{-3}$-137.52)	$4.27x10^{-3}$-76.94	$4.27x10^{-3}$-626.43	0.04-7.18	0.01-90.76
Geometric mean	0.16	0.83	0.46	0.78	0.35	0.41	0.71	1.01
HPV16, HIV-positive participants								
Median (IQR)	0.74 ($4.27x10^{-3}$-1.43)	0.16 (0.03-1.64)	0.46 ($4.27x10^{-3}$-8.11)	$4.27x10^{-3}$ ($4.27x10^{-3}$-20.72)	1.24 (0.54-58.89)	0.10 ($4.27x10^{-3}$-10.50)	0.17 (0.12-24.52)	0.10 ($4.27x10^{-3}$-0.25)
Range	$4.27x10^{-3}$-12.72	$4.27x10^{-3}$-3.46	$4.27x10^{-3}$-22.90	$4.27x10^{-3}$-96.94	0.07-291.07	$4.27x10^{-3}$-92.54	$4.27x10^{-3}$-49.38	$4.27x10^{-3}$-382.97
Geometric mean	0.24	0.16	0.26	0.09	3.12	0.20	0.51	0.15
HPV18, all participants								
Median (IQR)	0.16 (0.01-0.82)	0.11 (0.03-0.17)	4.48 (0.06-143.80)	0.27 (0.04-0.87)	13.85 (0.17-105.56)	1.70 ($3.74x10^{-3}$-9.37)	0.61 (0.01-1.14)	1.48 ($1.81x10^{-5}$-9.37)
Range	$3.63x10^{-5}$ - 2499.50	0.02-0.17	0.23-302.20	$1.81x10^{-5}$-1.27	0.16-15 201.30	$1.81x10^{-5}$-15.35	$4.77x10^{-5}$-8349.26	$1.81x10^{-5}$-15.78
Geometric mean	0.16	0.07	3.08	0.03	13.26	0.05	0.30	0.01
HPV18, HIV-negative participants								
Median (IQR)	0.16 (0.08-0.49)	NA	4.48 (0.99-75.41)	NA	13.85 (2.92-63.80)	NA	0.45 ($2.97x10^{-3}$-1.02)	NA
Range	$3.63x10^{-5}$ - 0.82	NA	0.02-143.80	NA	0.17-105.56	NA	$4.77x10^{-5}$-1.15	NA
Geometric mean	0.03	NA	2.59	NA	6.88	NA	0.02	NA
HPV18, HIV-positive participants								
Median (IQR)	1249.76 (0.01-2499.50)	0.11 (0.03-0.17)	151.13 (0.06-302.20)	0.27 (0.04-0.87)	7600.73 (0.16-15 201.30)	1.70 ($3.74x10^{-3}$-9.37)	4174.79 (0.32-8349.26)	1.48 ($1.81x10^{-5}$-9.37)

Range	0.01-2499.50	0.02-0.17	0.06-302.20	$1.81x10^{-5}$-1.27	0.16-15 201.30	$1.81x10^{-5}$-15.35	0.32-8349.26	$1.81x10^{-5}$-15.78
Geometric mean	4.71	0.07	4.35	0.03	49.23	0.05	51.68	0.01
Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; IQR, interquartile range; NA, not applicable.								

M1 - Table III: Change [median (IQR)] in anal HPV16 and HPV18 viral load (copies/cell) over visits by study arm, overall and by HIV status.

HPV type	Participants	Change in viral load between visits 1 and 2			Net change in viral load across visits 1 to 4		
		Treatment	Placebo	*P*-value	Treatment	Placebo	*P*-value
HPV16	All	0.00 (0.00-4.05)	-0.01 (-2.27-5.26)	0.3550	0.68 (-1.04-10.30)	0.18 (-5.55-16.53)	0.5987
	HIV-negative	0.30 (0.00-4.05)	-0.04 (-15.52-0.18)	0.2404	0.68 (-2.67-9.02)	0.18 (-38.64-2.31)	0.6963
	HIV-positive	0.00 (-0.03-7.48)	-0.12 (-0.10-20.50)	1.0000	5.24 (-1.04-58.89)	0.19 (-0.29-30.76)	0.8728
HPV18	All	0.92 (-0.80-6.86)	0.24 (-0.05-0.76)	0.8312	18.32 (0.52-249.36)	10.12 (2.09-16.96)	0.5224
	HIV-negative	4.33 (0.50-75.33)	NA	ND[a]	18.32 (3.01-139.42)	NA	ND[a]
	HIV-positive	-1098.63 (-2197.30-0.05)	0.24 (-0.05-0.76)	0.1649	8177.38 (0.52-16 354.25)	10.12 (2.09-16.96)	0.6434

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; IQR, interquartile range; NA, not applicable; ND, not determined.
[a] Mann-Whitney U Test not conducted as there were no participants in the placebo arm.



M1 - Figure I: The LIMIT-HPV study population and selection of analytic sample.

Figure I Legend: The flowchart details selection of the analytical population comprised of participants who completed at the first four study visits and tested positive for HPV16 or HPV18 at least once over visits 1 to 4. Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; +, positive; LIMIT-HPV, Lubricant Investigation in Men to Inhibit Transmission of HPV.

CHAPTER 4: HPV VACCINATION AND ANAL HPV INFECTION IN GAY, BISEXUAL, AND OTHER MEN WHO HAVE SEX WITH MEN

4.1 PREFACE

Although gbMSM are eligible for the publicly funded HPV vaccination program in the province of Quebec,[64] few observational studies have assessed the real-world effectiveness of HPV vaccination on anal HPV infections in this at-risk population. Those that have report that HPV vaccination protects against anal HPV infection.[86-89]

Leveraging the questionnaire and genotyping data collected from the gbMSM of the LIMIT-HPV trial, we assessed the association between HPV vaccination and anal HPV prevalence and incidence. To do so, we collapsed the study arms and analyzed data using an observational cohort framework. While past observational studies focused on evaluating HPV vaccination at the individual-level (i.e., unit of analysis is the participant) and pooling estimates between gbMSM of different HIV statuses, we conducted analyses using several analytical frameworks including 1) analyses at the individual-level, 2) analyses at the HPV-level (i.e., unit of analysis is the HPV type) to allow for multiple infections to be accounted for, and 3) analyses to assess construct validity by assessing the association between HPV vaccination and incident anal HPV infections of non-vaccine-targeted types. In addition, all analyses were conducted considering all participants and stratifying by HIV status.

Findings from this manuscript were presented at the McGill University's Division of Cancer Epidemiology Monthly Seminar on February 27th, 2024, at the EUROGIN International Multidisciplinary HPV Congress on March 13th, 2024, and at the McGill University's Gerald Bronfman Department of Oncology Celebration of Research and Training in Oncology on May 14th, 2024. The manuscript has been submitted for peer review to *Vaccine* on July 23rd, 2024.

4.2 TITLE PAGE

HPV VACCINATION AND ANAL HPV INFECTION IN GAY, BISEXUAL, AND OTHER MEN WHO HAVE SEX WITH MEN

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4.3 ABSTRACT

Background: Gay, bisexual, and other men who have sex with men (gbMSM) have a higher risk of human papillomavirus (HPV) infection and related diseases and would benefit from preventive measures such as HPV vaccination. We assessed the association between HPV vaccination and anal HPV infection in HIV-negative gbMSM and gbMSM living with HIV from the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection study.

Methods: Participants attended 7 visits over 12 months where they provided a nurse-collected anal sample and self-completed a questionnaire on risk factors and HPV vaccination. Samples were tested for HPV using polymerase chain reaction assays. We assessed the association with HPV vaccination and anal HPV prevalence and incidence using logistic and Cox regression, respectively. Analyses at the individual- (unit of analysis=participant) and HPV-level (unit of analysis=HPV type) considered vaccine-targeted types (any of HPVs 6/11/16/18) as the outcome. To assess construct validity, we repeated analyses considering incidence of non-vaccine-targeted (within- and cross-species) HPV types at the HPV-level. Estimates were adjusted for a propensity score to predict HPV positivity across the study based on selected study and participant characteristics.

Results: Of 258 enrolled participants (69 HIV-positive), 60 (23.3%) were vaccinated at baseline. At the individual-level, there was no association between vaccination and HPVs 6/11/16/18 prevalence (n=250, aOR=1.12, 95% CI= 0.56-2.22) or incidence (n=152, aHR=0.34, 95% CI=2.19x10^{-18}-1.38). At the HPV-level, while there was no association with HPVs 6/11/16/18 prevalence (n=1000, aOR=0.99, 95% CI=0.57-1.71), vaccination was associated with a reduction in HPVs 6/11/16/18 incidence (n=754, aHR=0.22, 95% CI=6.01x10^{-18}-0.79). Vaccination was not associated with incidence of within-species (n=2299, HR=0.76, CI=0.42-1.24) or cross-species (n=3774, HR=1.27, CI=0.88-1.83) HPV types. Results were similar by HIV status.

Conclusion: Our findings support that HPV vaccination protects against incident anal infection of vaccine-targeted HPV types, thus, gbMSM should be encouraged to get vaccinated against HPV.

Keywords

Human papillomavirus, Prevalence, Incidence, HPV vaccination, Men who have sex with men, HIV

4.4 INTRODUCTION

Gay, bisexual, and other men who have sex with men (gbMSM), especially if they are living with human immunodeficiency virus (HIV), are disproportionately affected by human papillomavirus (HPV) infection.[1,2] The prevalence of anal high-risk HPV infections is estimated to be 41.2% among HIV-negative gbMSM and 74.3% among gbMSM living with HIV.[1] In comparison, prevalence is estimated to be 6.9% among HIV-negative men who have sex with women and 26.9% among men who have sex with women living with HIV.[1] Furthermore, approximately 90% of anal cancer cases are attributed to HPV.[3] The incidence of anal cancer in HIV-negative gbMSM and gbMSM living with HIV is estimated to be 19 and 85 cases/100,000 person-years, respectively, whereas incidence is estimated to be 1-2 cases/100,000 person-years in the general population.[2] As such, gbMSM would benefit from HPV preventive methods.

In Canada, HPV vaccination was first approved for use in men in 2010.[4] Specifically in the province of Quebec, gbMSM and men living with HIV under the age of 27 years have been eligible for the publicly funded HPV vaccination program since 2014.[4] Currently, three of six licensed prophylactic HPV vaccines, targeting high-risk HPV types associated with the development of cancer and low-risk HPV types associated with the development of anogenital warts, have been authorized for use.[5,6] These include Cervarix, a bivalent vaccine targeting HPVs 16 and 18; Gardasil, a quadrivalent vaccine targeting HPVs 6, 11, 16, and 18, though no longer offered; and Gardasil-9, a nonavalent vaccine targeting HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58.[5] A sub-study of HIV-negative gbMSM, 16-26 years of age, and with five or less lifetime sex partners, from a randomized control trial assessing the efficacy of the quadrivalent HPV vaccine in men, reported that the HPV vaccine protects against anal HPVs 6, 11, 16, and 18 infections and the development of related anal intraepithelial neoplasia.[7] Other randomized control trials conducted among gbMSM living with HIV reported that the quadrivalent HPV vaccine protects against the development of related anal intraepithelial neoplasia if they were vaccinated between the ages of 13-26 years,[8] however, no protection was reported if they were 27 years or older at the time of vaccination.[9] In addition, no prior studies have assessed the effect of HPV vaccination and viral load in gbMSM or men in general. To the best of our knowledge, the few observational studies in women reported that the bivalent vaccine reduced genital HPV viral load in infections in the process of being cleared by the immune system.[10,11] While HPV vaccination

has demonstrated protection against anal HPV infections in a randomized control trial setting, there is a need for observational studies on the real-world effect of HPV vaccination on anal HPV prevalence, incidence, and viral load in gbMSM. It is also important to assess its effect according to HIV status as gbMSM living with HIV carry a greater burden of anal HPV infection and related diseases than HIV-negative gbMSM.[1,2]

In this context and among HIV-negative gbMSM and gbMSM living with HIV, we 1) compared anal HPVs 16 and 18 viral loads by HPV vaccination status and 2) assessed the association of HPV vaccination with prevalent and incident anal infections of vaccine-targeted HPV types (HPVs 6, 11, 16, and 18). To verify the construct validity of findings related to our second objective, we also assessed the association between HPV vaccination and incident anal infections of non-vaccine-targeted HPV types.

4.5 MATERIALS & METHODS

Study design & participants

We used data from the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study, a randomized control trial (ClinicalTrials.gov identifier NCT02354144) that was conducted in gbMSM in Montreal, Canada from 2016 to 2020 to evaluate the efficacy of a carrageenan-based gel in preventing the acquisition of new anal HPV infections.[12] For the current analysis, we collapsed the study arms and analyzed the data as an observational cohort study, treating the intervention as a covariate in adjusted analyses.

Briefly, the eligibility criteria included men 18 years or older, living in Montreal and planning to remain in the city for the next 12 months, had receptive anal sex with at least one man in the previous three months and expected to be sexually active for the duration of their involvement in the study, planned to have receptive anal sex with more than one but less than 50 partners per year, understood French or English, willing to comply with study instructions and follow-up, and willing to undertake an HIV test for those who had never tested seropositive for HIV. Participants attended up to seven study visits over twelve months. At each visit, they provided a nurse-collected anal sample and self-completed an electronic questionnaire on sociodemographic and behavioural HPV risk factors as well as on HPV vaccination. Nurses advised participants who reported being unvaccinated that the HPV vaccine provides

prophylactic protection against nine HPV types and that they were eligible to receive the vaccine if they were between 9 to 26 years of age.

HPV genotyping and viral load determination

Most anal samples were genotyped using the linear array polymerase chain reaction (PCR) assay (Roche Diagnostics), which detects 36 different HPV types;[13] only 14 samples were genotyped using the Anyplex II PCR assay (Seegene Inc), capable of detecting 28 different HPV types.[14] Irrespective of the assay, a β-globin DNA sequence was simultaneously amplified to verify if samples were adequate for PCR analysis.

HPV16 and HPV18 viral loads were quantified using real-time PCR assays in a Light Cycler PCR and detection system (Roche Molecular Systems) by measuring HPV16, HPV18 and β-globin copy numbers in 2 μL of processed sample, as previously described.[15] Briefly, HPV16-positive and HPV18-positive samples were first screened for the presence of PCR inhibitors by amplification of an internal control.[16] All samples were shown to be free of inhibitor activity. HPV16 E6 DNA and HPV18 E7 DNA were then quantified using a standard protocol.[15] Cycle thresholds obtained for each sample were compared to those of a titration curve obtained by serial 10-fold dilutions of HPV16 DNA plasmid or HPV18 plasmid in 75 ng of human genomic DNA (Roche Diagnostics) in 10 mM Tris-HCl (pH 8.2). Processed samples were also tested for quantification of β-globin DNA to estimate the cell content of samples. Viral loads were calculated by dividing the number of HPV DNA copies by the total number of cells, which was estimated with the number of β-globin copies. Results were recorded as copy numbers per cell.

Statistical analysis

HPV vaccination status was defined based on information reported in baseline and follow-up questionnaires. Participants who reported at their enrollment visit that they had received an HPV vaccine were considered as vaccinated. Those who reported being unvaccinated at baseline but reported receiving an HPV vaccine during a follow-up visit were considered to be vaccinated from that visit onwards.

We conducted several analyses to compare vaccinated with unvaccinated participants. First, we described the baseline characteristics of study participants, overall and by vaccination status. Second, we compared HPV16 and HPV18 viral loads between vaccinated participants at

baseline and those unvaccinated throughout the study (i.e., we excluded participants who reported having received the vaccine for the first time at any follow-up visit). Of note, two HPV16-positive samples with a corresponding viral load value of 0 copies/cell were assigned half of the next lowest value in the distribution of HPV16 viral load values. We summarized the viral load data for HPV16, HPV18, and HPV16/18 (sum of HPV16 and HPV18 viral load) at each study visit using descriptive statistics and compared these between vaccinated and unvaccinated participants. We also described and compared the sum of HPV16 and HPV18 viral load across all study visits between vaccinated and unvaccinated participants. Comparisons were evaluated using the Mann-Whitney U test; p-values <0.05 were considered statistically significant.

Lastly, we assessed the association between HPV vaccination and anal HPV infection by conducting analyses at both the individual-level, where participants were the unit of analysis, and the HPV-level, which considered episodes of HPV type detection as the unit of analysis. As schematically represented in Supplementary Figure 1, each participant in individual-level analyses could contribute a single observation corresponding to the detection of a given outcome of interest [HPV6, HPV11, HPV16, HPV18, or any of HPVs 6, 11, 16, or 18 (6/11/16/18)]. In HPV-level analyses (considering only HPVs 6/11/16/18 as the outcome), each participant could contribute up to four observations, corresponding to the potential detection of any of these four HPV types, allowing for greater statistical precision.

We estimated odds ratios (OR) and 95% confidence intervals (CI) for the cross-sectional association between HPV vaccination and HPV prevalence at baseline using logistic regression. Specifically, logistic regression models for analyses conducted at the HPV-level were clustered by participant.[17] We plotted cumulative incidence curves and estimated hazard ratios (HR) and bootstrapped bias-corrected 95% CI,[18] by generating 1000 replications, for the longitudinal association between HPV vaccination (treated as a time-varying exposure) and HPV incidence using the Kaplan Meier failure function and Cox proportional hazards regression, respectively. The Cox proportional hazards regression models for analyses at the HPV-level were stratified by HPV type and clustered by participant.[17] To estimate new detections, we excluded participants who tested positive for the HPV type(s) of interest at baseline (in individual-level analyses) or the HPV type(s) of interest the participant tested positive for at baseline (in HPV-level analyses).

We repeated the longitudinal analysis at the HPV-level considering only new detections of non-vaccine-targeted HPV types as the outcomes of interest to evaluate construct validity. Non-vaccine-targeted HPV types were classified into two groups based on their phylogenetic relatedness to HPVs 6, 11, 16 and 18: 1) HPV types within the same alpha species (i.e., alpha 7, 9, and 10, referred to as within-species HPV types); and 2) HPV types belonging to different alpha species (referred to as cross-species HPV types). Within-species HPV types included HPVs 31, 33, 35, 39, 44, 45, 52, 58, 59, 67, 68, and 70, whereas cross-species HPV types included HPVs 26, 34, 40, 42, 51, 53, 54, 56, 61, 62, 66, 69, 71, 72, 73, 81, 82, 83, 84, and 89.[19,20]

All estimates were adjusted for a propensity score, generated using logistic regression to predict any HPV positivity across the study participation based on selected (with no missingness) baseline sociodemographic and behavioural HPV risk factors and study characteristics. These included age, ethnicity, education, smoking status, age at first sexual activity, number of lifetime sex partners, new sex partner in the last month, and stable sex partner, as well as treatment assignment. Although available, the variable "number of lifetime male sex partners" was not included in the model due to its collinearity with the number of lifetime sex partners. However, for education, categories that perfectly predicted the outcome (secondary-level education) or were collinear (university-level education) were forced in the model.

Analyses were conducted considering all participants and stratified by HIV status. All statistical analyses were conducted in Stata version 18.0.

4.6 RESULTS

HPV vaccination status and analytical samples

As shown in Figure 1, a total of 258 participants were enrolled in the LIMIT-HPV study. The definition of their HPV vaccination status is detailed in Supplementary Tables 1-2. A total of 60 participants were vaccinated at baseline (23.3%) while 12 additional participants were vaccinated exclusively over follow-up (7.1%). Thus, a total of 72 participants had a history of vaccination over the course of the study (27.9%). Among participants who self-reported being vaccinated at baseline (n=60), none indicated receipt of Cervarix and most indicated having received Gardasil or Gardasil-9 (38.3% and 28.3%, respectively) as well as having had 3 doses of the vaccine (65.0%). Among those who self-reported being vaccinated over follow-up (n=48),

2.1% reported receipt of Cervarix, 43.8% reported receipt of Gardasil, and 33.3% reported receipt of Gardasil-9; however, most did not specify the number of doses received (83.3%). The cross-sectional analytical sample included 250 participants (58 vaccinated and 192 unvaccinated); 235 participants had a valid baseline sample whereas we imputed the genotyping data from visit 2 for 15 participants with missing HPV results at baseline (due to invalid or not collected samples). The longitudinal analytical sample included 207 participants (44 vaccinated, 163 unvaccinated) who completed at least two study visits and had at least two valid samples.

Baseline characteristics of the study population

Overall, the mean age of participants was 36.6 years (Table 1). Most were French-Canadian (38.4%), had received some kind of post-secondary schooling (college: 25.2%, university: 51.9%) and had never smoked (62.0%). On average, the age at first sexual activity was 17.3 years. Most reported having had 26 or more lifetime sex partners (26-60: 21.7%, 61-200: 19.0%, 201-500: 12.4%, ≥501: 16.3%), having had a new sex partner in the past month (63.2%), and having a stable sex partner (61.6%). There were 69 HIV-positive participants (26.7%), and most participants tested positive for any HPV type (67.2%). There were imbalances in these characteristics between vaccinated and unvaccinated participants. Those vaccinated were younger than their unvaccinated counterparts (mean age: 27.3 years versus 39.3 years). More vaccinated participants were current smokers (21.7%), whereas more unvaccinated participants were former smokers (23.2%). More vaccinated participants reported having had between 26-60 lifetime male sex partners (30.0%) whereas more unvaccinated participants reported having had between 61-200 (20.2%). Lastly, when comparing positivity for vaccine-targeted types, more vaccinated participants were HPV6-positive (14.3%) whereas more unvaccinated participants were HPV16-positive (14.0%).

Comparison of viral load between vaccinated and unvaccinated participants

For HPV16, HPV18, and HPV16/18 viral load, the overall geometric mean was non-significantly lower among vaccinated compared to unvaccinated participants at each visit (Supplementary Tables 3-5). Similarly, the geometric mean of the sum of HPV16/18 viral load across all visits was non-significantly (p-value=0.1236) lower among vaccinated compared to unvaccinated participants (Supplementary Table 6). Likewise, no significant differences in viral

load were observed between vaccinated and unvaccinated participants when stratified by HIV status.

Cross-sectional analysis

Table 2 displays the crude and adjusted ORs for the association between HPV vaccination and prevalent anal HPV infections. Supplementary table 7 describes the coefficients of the variables for the fitted propensity score to predict any HPV positivity across the study. The score was included in the models with adjusted risk estimates. Overall, ORs were similar after adjusting for the propensity score. In general, no statistically significant associations were observed, overall and by HIV status. The odds of having a prevalent infection with HPVs 6/11/16/18 was higher at the individual-level among vaccinated than unvaccinated participants [27.6% versus 28.1%, adjusted OR (aOR): 1.12, 95% CI: 0.56 – 2.22]. However, at the HPV-level, the odds were similar between vaccinated and unvaccinated participants (7.8% versus 8.9%, aOR=0.99, 95% CI: 0.57 – 1.71). Among HIV-negative participants, the odds of having a prevalent infection with HPVs 6/11/16/18 were lower for those vaccinated compared to unvaccinated at the individual- (21.3% versus 23.5%, aOR=0.94, 95% CI: 0.41 – 2.16) and HPV- (5.3% versus 6.6%, aOR=0.85, 95% CI: 0.44 – 1.63) level. Conversely, among HIV-positive participants, the odds of having a prevalent infection with HPVs 6/11/16/18 were higher for those vaccinated compared to unvaccinated at the individual- (54.5% versus 39.3%, aOR=1.94, 95% CI: 0.52 – 7.20) and HPV- (18.2% versus 14.3%, aOR=1.39, 95% CI: 0.56 – 3.45) level.

Longitudinal analysis

As illustrated in Figure 2, the cumulative incidence of HPVs 6/11/16/18 at the individual-level was lower among vaccinated than unvaccinated participants, considering all participants (Figure 2A) and when stratified by HIV status (Figure 2B-C). Similar results were observed in HPV-level analyses considering all participants (Figure 3A) and by HIV status (Figures 3B-3C). The acquisition of within-species HPV types was slightly lower among vaccinated than unvaccinated participants, considering all participants (Figure 3D) and those HIV-negative (Figure 3E). Conversely, among HIV-positive participants, the acquisition of within-species HPV types was similar between those who were vaccinated and unvaccinated (Figure 3F). The acquisition of cross-species HPV types was comparable between vaccinated and unvaccinated participants, considering all participants (Figure 3G) and by HIV status (Figure 3H-I).

Table 3 presents the crude and adjusted HRs for the association between HPV vaccination and incident anal HPV infections. HRs were also similar after adjustment for the propensity score. In individual-level analyses, while vaccinated participants had a lower risk of incident infections of vaccine-targeted HPV types compared to unvaccinated participants, not all HRs reached statistical significance. In HPV-level analyses, HPV vaccination was associated with a lower risk of incident infections of vaccine-targeted HPV types. The risk of new detections of HPVs 6/11/16/18 was lower among vaccinated than unvaccinated participants in individual- [adjusted HR (aHR)=0.34] and HPV- (aHR=0.22) level analyses. Similarly, among HIV-negative and HIV-positive participants, the risk of new detections of HPVs 6/11/16/18 was lower among those vaccinated compared to unvaccinated at the individual- (HIV-negative: aHR=0.37; HIV-positive: aHR=$3.33x10^{-19}$) and HPV- (HIV-negative: aHR=0.26; HIV-positive: aHR=$5.40x10^{-17}$) levels. Considering within- and cross-species HPV types, the risk of incident infections did not significantly differ between vaccinated and unvaccinated participants, considering all participants and by HIV status.

4.7 DISCUSSION

Using data from the LIMIT-HPV study, conducted among sexually active HIV-negative gbMSM and gbMSM living with HIV, and employing different analytical approaches (individual-level, HPV-level, construct validity), we did not observe that HPV vaccination influenced anal HPV16 or HPV18 viral loads nor was it associated with prevalent anal infections of vaccine-targeted HPV types or, as expected, incident anal infections of non-vaccine-targeted HPV types. However, we found evidence to support that HPV vaccination protects against incident anal infections of vaccine-targeted HPV types.

Based on self-reported HPV vaccination status, the HPV vaccine uptake in our study population was higher than that reported (27.9% vs 14.8%) among the subset of participants enrolled between 2017 and 2019 from the ENGAGE cohort study of gbMSM 16 years and older in Montreal, Canada.[21] This difference may be a reflection of differences in the age distribution (affecting the number of gbMSM eligible to receive the vaccine) and/or recruitment strategy (LIMIT-HPV study recruited participants from the community through promotional materials or from clinical sites,[12] whereas the ENGAGE cohort study used respondent-driven sampling).[21]

Nonetheless, with the inclusion of boys in school-based HPV vaccination programs in 2016 and people who are immunocompromised or are living with HIV under the age of 46 years in the publicly-funded HPV vaccination program in 2022 in Quebec, Canada,[4] these estimates may no longer reflect the present regional HPV vaccine uptake among gbMSM.

The lack of impact by HPV vaccination on anal HPVs 16 and 18 viral loads that we found in gbMSM is contrary to the reduction of genital HPV16 and HPV18 viral load of transient infections following use of the bivalent vaccine reported in women who were vaccinated at an early age (i.e., before 16 years of age).[10,11] This discrepancy could be due to differences in age at the time of vaccination, in the vaccine types received as well as the possibility that our analysis may have been underpowered to detect differences in viral load by HPV vaccination status and/or could not distinguish between viral loads of transient versus persistent infections,[22] as few vaccinated participants tested positive for HPV16 and/or HPV18 at each visit.

The observed lack of association between HPV vaccination and prevalent anal infection of vaccine-targeted HPV types is contrary to previous findings by the few other observational studies carried out in North America that have evaluated HPV vaccination in gbMSM. Of note, these studies used different inclusion criteria for age and different methodologies, making it difficult to directly compare their findings with ours. Protection against prevalent anal infection of vaccine-targeted HPV types was reported among 645 gbMSM (40.3% vaccinated) 16-30 years, enrolled between 2017 and 2019 in the ENGAGE cohort study in Canada,[23] and among 1767 gbMSM and transgender women 18-26 years of age (39.8% vaccinated) enrolled between 2016 and 2018 in the United States.[24] Protection against prevalent anal infection of vaccine-targeted HPV types was also demonstrated comparing birth cohorts, of 200 gbMSM 16-20 years each, before (enrolled between 2010 and 2012) and after (enrolled between 2017 and 2018) the implementation of gender-neutral HPV vaccination in Australia.[25] Taking into consideration that the LIMIT-HPV study participants had an average age of 37 years, an average age at first sexual activity of 17 years, and reported having had multiple lifetime sex partners, the lack of association observed in the current analysis is likely due to participants having prior exposure to vaccine-targeted HPV types before receiving the vaccine. Due to the prophylactic capabilities of the HPV vaccines, they would not be able to protect against existing infections.[26]

Consistent with other findings reported by the ENGAGE cohort study of 248 gbMSM (44% vaccinated) 16-30 years recruited between 2017 and 2019 in Canada,[27] we also found that HPV vaccination reduces the risk of incident anal infections of vaccine-targeted HPV types. Even though not all models in the current analysis reached statistical significance, all point estimates were lower than one, suggestive of protection by HPV vaccination. In comparison, the ENGAGE cohort study reported significant reductions in incidence only if participants had received the HPV vaccine under the age of 24 years or within 5 years of their sexual debut.[27] Due to the high number of missing responses regarding the date of HPV vaccination in the LIMIT-HPV study, we were not able to incorporate this information into our analyses. In addition, contrary to the reduction in incidence we observed among participants living with HIV, although there were no incident infections among those vaccinated, a randomized control trial conducted between 2012 and 2014 in Spain among 129 gbMSM living with HIV (51.2% vaccinated) and aged 27 years and older reported no significant difference between the vaccine and placebo study arms in the acquisition of HPVs 6, 11, 16, or 18 after 48 months of follow-up.[9] Similarly, a randomized control trial conducted between 2012 and 2016 in the United States and Brazil among 472 gbMSM and 103 women living with HIV (50.0% vaccinated) and aged 27 years and older reported that HPV vaccination did not protect against incident persistent infections of vaccine-targeted HPV types.[28] However, the non-significant differences in incidence may be a result of participants in these two trials having previous exposure to vaccine-targeted HPV types as they were past their sexual debut and of an older age at the time of vaccination. Additional studies evaluating HPV vaccination in gbMSM living with HIV are likely needed to further understand its impact on anal HPV incidence.

As expected, the lack of protection by HPV vaccination against incident anal infections of non-vaccine-targeted HPV types verified the validity of our analytical approach. It also provided additional evidence of protection offered by HPV vaccination against new infections of vaccine-targeted HPV types. Curiously, we did observe a non-significant reduction in risk of within-species HPV types among all participants and those that were HIV-negative. To the best of our knowledge, there are no other studies assessing any potential cross-protective effects of the vaccine on anal HPV infections in gbMSM. However, in women, while the bivalent and quadrivalent HPV vaccines have inconsistently demonstrated cross-protection against some non-vaccine-targeted genital HPV types belonging to same alpha species as the ones targeted by the

vaccines, particularly against HPV31 and HPV45,[29-37] these types are targeted by the nonavalent vaccine. Thus, the observed reduction in risk is likely due to the number of LIMIT-HPV study participants who reported receiving the nonavalent vaccine rather than any cross-protection effect due to the phylogenetic relatedness of within-species HPV types to vaccine-targeted HPV types.[19,20]

Our work had limitations. Firstly, it was based on a relatively small sample size and, consequently, few incident infections of HPVs 6, 11, 16, and 18, especially among vaccinated participants and participants living with HIV, potentially contributing to the observed non-significant reductions in risk. The few incident infections also led to some risk estimates and confidence interval boundaries being close to 0, particularly when considering HPV11 and HPV18 and in subgroup analyses among participants living with HIV. However, findings from our construct validity exercise corroborated the overall protective effect of HPV vaccination. Nevertheless, future studies with a larger sample size are needed to further understand the impact of HPV vaccination, especially on HPV11, HPV18, and among gbMSM living with HIV. Also, due to the relatively small numbers, we were not able to stratify the analyses by the number of doses received or vaccine type. Secondly, potential misclassification may have biased our results towards the null as HPV vaccination status was self-reported. In addition, we considered a participant to be vaccinated if they reported receiving an HPV vaccine rather than if they reported completion of the HPV vaccination series (i.e., received three doses of the HPV vaccine) as not all participants reported the number of doses they had received, which may have also biased our results towards the null. Thirdly, though participants' baseline characteristics were balanced between study arms as a result of the randomized control trial design of the LIMIT-HPV study, this was not necessarily the case between vaccinated and unvaccinated participants. To account for this, we adjusted our risk estimates for a propensity score to predict any HPV positivity across the study participation (refer to methods section) and confirmed that it followed a dose-response relationship, where each increase in the propensity score corresponded to an increase in the prevalence of participants who tested HPV-positive. This enabled us to control for any confounding that may have arisen due to imbalances in baseline characteristics by vaccination status. Lastly, as most study participants were young, university-level students, our findings may not be generalizable to all gbMSM.

Our finding of an association between HPV vaccination and protection against incident anal infections of vaccine-targeted HPV types emphasizes the need for gbMSM to get vaccinated against HPV due to their elevated risk of contracting HPV and developing related diseases.[1,2,5] As the global HPV vaccine supply increases due to greater manufacturing capacity, more licensed prophylactic vaccine types, and different dosage schedules,[38] various measures should be explored to encourage HPV vaccination among gbMSM.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author. The data will not be published due to privacy and ethical restrictions; participants of the LIMIT-HPV study did not consent to having their data made publicly available.

CONFLICT OF INTEREST

Outside the submitted work, ELF served as an occasional advisor for companies involved with HPV vaccines (Merck, GSK) and HPV diagnostics (Roche Diagnostics) and as a Steering Committee Member for a publicly funded study in Finland that received support from GSK. MZ and ELF hold a patent related to the discovery "DNA methylation markers for early detection of cervical cancer", registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada (October 2018). AdP received honoraria for consulting on HIV antiretroviral regimen for ViiV Healthcare. FC has received grants through his institution from Merck Sharp & Dohme, Becton Dickinson, and Roche to conduct evaluation of HPV diagnostic tests, as well as honoraria from Merck and Roche for lectures on HPV. JET is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.. PK has no conflict of interest to disclose.

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ETHICS APPROVAL

The Research Ethics Boards of McGill University, the McGill University Health Centre, Concordia University and Centre Hospitalier de l'Université de Montréal approved the study. Health Canada (file number 169160) authorised use of the study gel.

Ethics committee: A10-M98-14B.

CRediT AUTHOR STATEMENT

Pareesa Kassam: Conceptualization, methodology, formal analysis, writing – original draft; Mariam El-Zein: Conceptualization, data curation, writing – review & editing, supervision, project administration, funding acquisition; Joseph E. Tota: Writing – review & editing, funding acquisition; Pierre-Paul Tellier: Writing – review & editing, funding acquisition; François Coutlée: Writing – review & editing, funding acquisition; Alexandra de Pokomandy: Writing – review & editing, funding acquisition; Eduardo L. Franco: Conceptualization, writing – review & editing, supervision, project administration, funding acquisition.

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All authors attest they meet the ICMJE criteria for authorship.

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4.9 TABLES AND FIGURES

M2 - Table 1: Baseline characteristics of participants in the LIMIT-HPV study, overall and by self-reported HPV vaccination status

Variables	Categories	Overall (N = 258)	Vaccinated (n = 60)	Unvaccinated (n = 198)
Age, years	Mean ± SD	36.6 ± 14.3	27.3 ± 8.4	39.3 ± 14.6
	Median (IQR)	33.0 (23.9-49.0)	25.6 (21.4-29.1)	39.4 (25.4-51.7)
	Range	18.2-71.7	18.3-56.4	18.2-71.7
Ethnicity, n (%)	English Canadian	38 (14.7)	11 (18.3)	27 (13.6)
	French Canadian	99 (38.4)	22 (36.7)	77 (38.9)
	Latin American	30 (11.6)	5 (8.3)	25 (12.6)
	European	32 (12.4)	4 (6.7)	28 (14.1)
	Other	59 (22.9)	18 (30.0)	41 (20.7)
Education, n (%)	Elementary	2 (0.8)	1 (1.7)	1 (0.5)
	Secondary	57 (22.1)	13 (21.7)	44 (22.2)
	College	65 (25.2)	16 (26.7)	49 (24.8)
	University	134 (51.9)	30 (50.0)	104 (52.5)
Smoking status, n (%)	Never	160 (62.0)	39 (65.0)	121 (61.1)
	Former	54 (20.9)	8 (13.3)	46 (23.2)
	Current	44 (17.1)	13 (21.7)	31 (15.7)
Age at first sexual activity, years	Mean ± SD	17.3 ± 5.5	16.7 ± 4.5	17.5 ± 5.8
	Median (IQR)	17.0 (14.0-19.0)	17.0 (14.5-19.0)	17.0 (14.0-19.0)
	Range	4.0-42.0	5.0-30.0	4.0-42.0
Number of lifetime sex partners, n (%)	1-5	15 (5.8)	3 (5.0)	12 (6.1)
	6-10	19 (7.4)	3 (5.0)	16 (8.1)
	11-25	45 (17.4)	14 (23.3)	31 (15.7)
	26-60	56 (21.7)	16 (26.7)	40 (20.2)
	61-200	49 (19.0)	12 (20.0)	37 (18.7)
	201-500	32 (12.4)	7 (11.7)	25 (12.6)
	≥501	42 (16.3)	5 (8.3)	37 (18.7)
Number of lifetime male sex partners, n (%)	1-5	21 (8.1)	4 (6.7)	17 (8.6)
	6-10	18 (7.0)	5 (8.3)	13 (6.6)
	11-25	43 (16.7)	11 (18.3)	32 (16.2)
	26-60	56 (21.7)	18 (30.0)	38 (19.2)
	61-200	50 (19.4)	10 (16.7)	40 (20.2)
	201-500	29 (11.2)	7 (11.7)	22 (11.1)
	≥501	41 (15.9)	5 (8.3)	36 (18.2)
New sex partner in the last month, n (%)	Yes	163 (63.2)	39 (65.0)	124 (62.6)
	No	95 (36.8)	21 (35.0)	74 (37.4)
Has a stable sex partner, n (%)	Yes	159 (61.6)	35 (58.3)	124 (62.6)
	No	99 (38.4)	25 (41.7)	74 (37.4)
HIV status, n (%)	Positive	69 (26.7)	11 (18.3)	58 (29.3)
	Negative	189 (73.3)	49 (81.7)	140 (70.7)
HPV DNA status, n (%)[a]	Any HPV[b]	158 (67.2)	37 (66.1)	121 (67.6)
	Negative	77 (32.8)	19 (33.9)	58 (32.4)
	HPV6+	30 (12.8)	8 (14.3)	22 (12.3)
	HPV11+	12 (5.1)	2 (3.6)	10 (5.6)
	HPV16+	29 (12.3)	4 (7.1)	25 (14.0)
	HPV18+	13 (5.5)	4 (7.1)	9 (5.0)
	Other types+[c]	153 (65.1)	37 (66.1)	116 (64.8)
	Missing PCR results[d]	23 (8.9)	4 (6.7)	19 (9.6)

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; IQR, interquartile range; LIMIT-HPV, Lubricant Investigation in Men to Inhibit Transmission of HPV Infection; SD, standard deviation; +, positive.

[a] HPV positivity calculated based on the number of valid samples at baseline. Percentages exceeded 100% as some participants tested positive for more than one HPV type.

[b] Positive for any of the 36 HPV types (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).

[c] Positive for any of the 36 HPV types other than HPVs 6, 11, 16, or 18.

[d] Missing PCR results for anal samples that tested invalid (n=20) or were not collected (n=3).

M2 - Table 2: Association between HPV vaccination and prevalent anal HPV infection at baseline, overall and by HIV status.

HPV Type/Group (Unit of analysis[a])	**Participants[b]**	**Vaccinated, n/N (%)**	**Unvaccinated, n/N (%)**	**Crude OR (95% CI)[c]**	**Adjusted OR (95% CI)[c]**
HPV6 (Individual-level)	All	8/58 (13.8)	23/192 (12.0)	1.18 (0.50 – 2.79)	1.33 (0.55 – 3.22)
	HIV-negative	6/47 (12.8)	13/136 (9.6)	1.38 (0.49 – 3.88)	1.49 (0.52 – 4.25)
	HIV-positive	2/11 (18.2)	10/56 (17.9)	1.02 (0.19 – 5.47)	1.09 (0.20 – 5.93)
HPV11 (Individual-level)	All	2/58 (3.5)	10/192 (5.2)	0.65 (0.14 – 3.05)	0.79 (0.16 – 3.79)
	HIV-negative	1/47 (2.1)	3/136 (2.2)	0.96 (0.10 – 9.50)	1.08 (0.11 – 10.84)
	HIV-positive	1/11 (9.1)	7/56 (12.5)	0.70 (0.08 – 6.34)	0.73 (0.08 – 6.72)
HPV16 (Individual-level)	All	4/58 (6.9)	26/192 (13.5)	0.47 (0.16 – 1.42)	0.52 (0.17 – 1.57)
	HIV-negative	2/47 (4.3)	17/136 (12.5)	0.31 (0.07 – 1.40)	0.32 (0.07 – 1.47)
	HIV-positive	2/11 (18.2)	9/56 (16.1)	1.16 (0.21 – 6.29)	1.18 (0.22 – 6.48)
HPV18 (Individual-level)	All	4/58 (6.9)	9/192 (4.7)	1.51 (0.45 – 5.08)	1.92 (0.55 – 6.75)
	HIV-negative	1/47 (2.1)	3/136 (2.2)	0.96 (0.10 – 9.50)	1.12 (0.11 – 11.39)
	HIV-positive	3/11 (27.3)	6/56 (10.7)	3.13 (0.65 – 15.08)	3.29 (0.67 – 16.24)
HPVs 6/11/16/18 (Individual-level)	All	16/58 (27.6)	54/192 (28.1)	0.97 (0.51 – 1.88)	1.12 (0.56 – 2.22)
	HIV-negative	10/47 (21.3)	32/136 (23.5)	0.88 (0.39 – 1.96)	0.94 (0.41 – 2.16)
	HIV-positive	6/11 (54.5)	22/56 (39.3)	1.85 (0.50 – 6.82)	1.94 (0.52 – 7.20)
HPVs 6/11/16/18 (HPV-level)	All	18/232 (7.8)	68/768 (8.9)	0.87 (0.50 – 1.51)	0.99 (0.57 – 1.71)
	HIV-negative	10/188 (5.3)	36/544 (6.6)	0.79 (0.40 – 1.56)	0.85 (0.44 – 1.63)
	HIV-positive	8/44 (18.2)	32/224 (14.3)	1.33 (0.55 – 3.23)	1.39 (0.56 – 3.45)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papillomavirus; OR, odds ratio.

[a] An individual-level analysis considers study participants as the unit of analysis whereas an HPV-level analysis considers the HPV type as the unit of analysis, i.e., a participant can contribute up to 36 observations, corresponding to each HPV type.

[b] Of the 250 included participants, 235 had their first valid anal sample at baseline and 15 had their first valid anal sample at visit 2.

[c] Reference group comprises participants unvaccinated against HPV. Odds ratios were adjusted for a propensity score calculated using a logistic regression model: dependent variable= any HPV positivity over the study duration; independent variables= treatment assignment and selected participant characteristics at baseline, refer to Methods and supplementary table III.

M2 - Table 3: Association between HPV vaccination and incident anal HPV infection, overall and by HIV status.

HPV Type/Group (Type of analysis[a])	Participants[b]	Vaccinated			Unvaccinated			Crude HR (95% CI)[d]	Adjusted HR (95% CI)[d]
		No. at risk	No. of events	Time at risk[c]	No. at risk	No. of events	Time at risk[c]		
HPV6 (Individual-level)	All	48	1	446.22	136	5	1245.69	0.62 ($5.87x10^{-17}$ – 6.14)	0.66 ($5.50x10^{-20}$– 7.96)
	HIV-negative	39	1	365.86	94	5	778.09	0.48 ($5.11x10^{-17}$ – 4.48)	0.55 ($4.39x10^{-20}$ – 5.53)
	HIV-positive	9	0	80.36	42	0	467.60	ND	ND
HPV11 (Individual-level)	All	54	0	515.30	147	4	1339.61	$5.22x10^{-17}$ ($4.34x10^{-17}$ – $1.20x10^{-16}$)	$3.49x10^{-16}$ ($5.15x10^{-17}$ – $1.26x10^{-15}$)
	HIV-negative	44	0	422.99	103	4	879.28	$3.49x10^{-16}$ ($1.14x10^{-16}$ – $9.59x10^{-16}$)	$4.55x10^{-17}$ ($1.70x10^{-20}$ – $3.98x10^{-16}$)
	HIV-positive	10	0	92.30	44	0	460.33	ND	ND
HPV16 (Individual-level)	All	53	1	521.45	134	10	1196.02	0.24 ($5.59x10^{-17}$– 1.57)	0.24 ($4.99x10^{-20}$ – 1.51)
	HIV-negative	44	1	425.56	92	4	773.42	0.50 ($4.24x10^{-17}$– 5.18)	0.49 ($6.63x10^{-20}$ – 5.97)
	HIV-positive	9	0	95.89	42	6	422.60	$4.25x10^{-16}$ ($1.44x10^{-16}$ – $4.27x10^{-15}$)	$4.14x10^{-16}$ ($7.12x10^{-17}$ – $8.92x10^{-15}$)
HPV18 (Individual-level)	All	54	0	506.28	149	4	1364.87	$5.32x10^{-17}$ ($3.95x10^{-17}$– $1.27x10^{-16}$)	$6.45x10^{-17}$ ($5.32x10^{-20}$ – $3.10x10^{-15}$)
	HIV-negative	44	0	417.76	103	3	875.03	$1.26x10^{-16}$ ($3.56x10^{-17}$– $4.18x10^{-16}$)	$1.98x10^{-17}$ ($3.89x10^{-20}$ – $1.36x10^{-15}$)
	HIV-positive	10	0	88.52	46	1	489.84	$1.79x10^{-16}$ ($5.16x10^{-17}$– $5.29x10^{-16}$)	$1.91x10^{-16}$ ($1.39x10^{-20}$– $1.80x10^{-15}$)
HPVs 6/11/16/18[e] (Individual-level)	All	43	2	405.26	109	15	915.23	0.33 ($1.36x10^{-16}$– 1.27)	0.34 ($2.19x10^{-18}$ – 1.38)
	HIV-negative	37	2	349.24	78	11	599.67	0.36 ($9.63x10^{-17}$– 1.40)	0.37 ($1.19x10^{-19}$ – 1.52)
	HIV-positive	6	0	56.02	31	4	315.56	$5.00x10^{-17}$ ($1.44x10^{-17}$– $1.01x10^{-16}$)	$3.33x10^{-19}$ ($7.14x10^{-39}$ – $1.42x10^{-16}$)
HPVs 6/11/16/18[e] (HPV-level)	All	209	2	2091.28	545	23	5044.14	0.22 ($1.34x10^{-16}$– 0.73)	0.22 ($6.01x10^{-18}$ – 0.79)
	HIV-negative	171	2	1710.53	375	16	3227.47	0.26 ($1.17x10^{-16}$– 1.02)	0.26 ($2.06x10^{-18}$ – 1.04)
	HIV-positive	38	0	380.76	170	7	1816.68	$1.57x10^{-16}$ ($4.14x10^{-17}$– $5.22x10^{-16}$)	$5.40x10^{-17}$ ($4.38x10^{-20}$ – $1.53x10^{-15}$)
Within-species HPV types[f] (HPV-level)	All	618	27	5911.09	1681	113	15406.61	0.63 (0.35 – 1.05)	0.76 (0.42 – 1.24)
	HIV-negative	507	15	4878.52	1135	55	9888.65	0.57 (0.21 – 1.12)	0.66 (0.26 – 1.25)
	HIV-positive	111	12	1032.57	546	58	5517.96	1.00 (0.34 – 2.34)	1.05 (0.36 – 2.67)
Cross-species HPV types[g] (HPV-level)	All	994	75	9347.27	2780	181	25 623.95	1.15 (0.79 – 1.65)	1.27 (0.88 – 1.83)
	HIV-negative	821	56	7698.78	1890	89	16 487.96	1.38 (0.79 – 2.32)	1.49 (0.84 – 2.46)
	HIV-positive	173	19	1648.49	890	92	9135.99	1.10 (0.56 – 2.07)	1.07 (0.47 – 2.05)

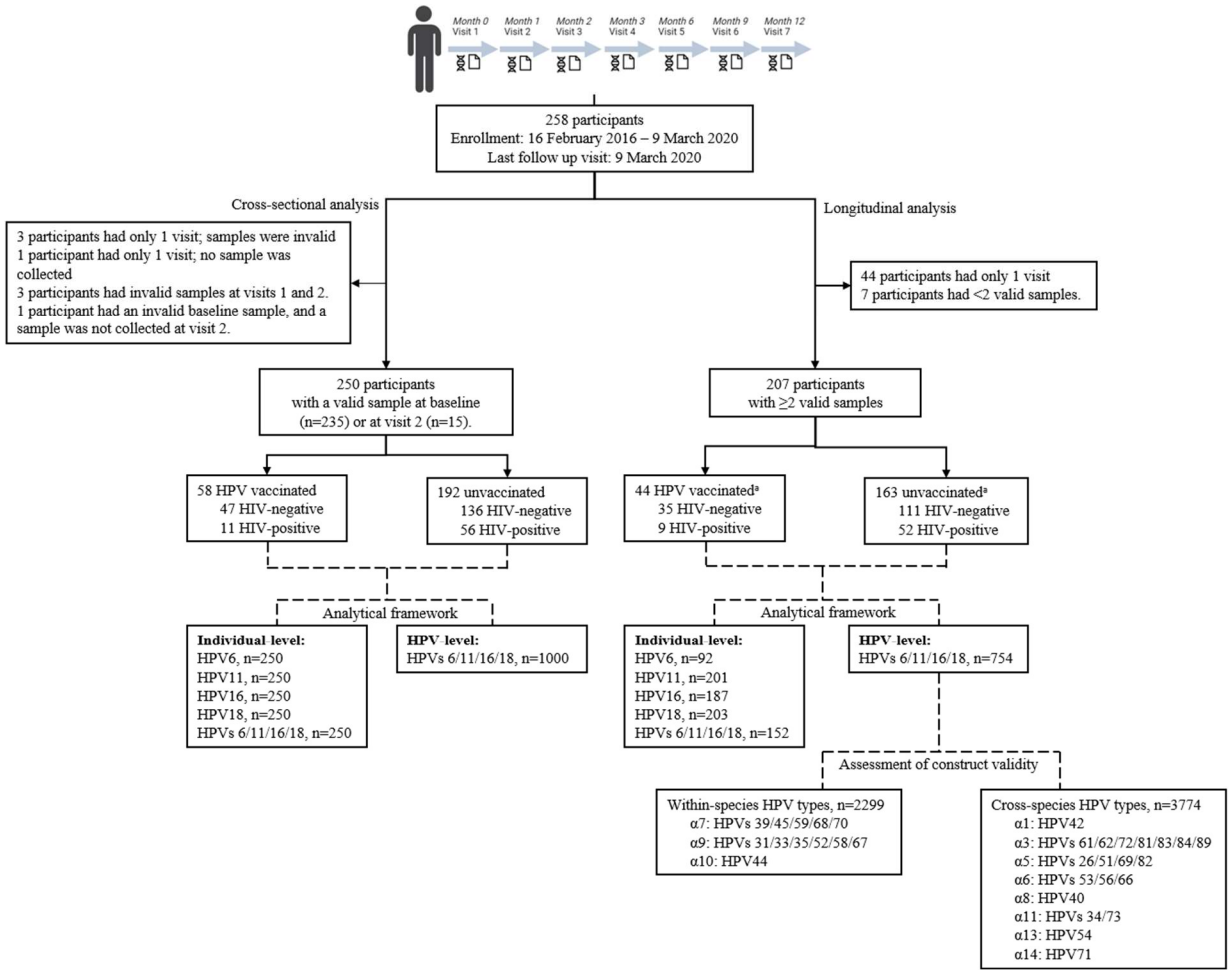
Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papillomavirus; HR, hazard ratio; ND, not determined; No, number.

[a] An individual-level analysis considers study participants as the unit of analysis whereas an HPV-level analysis considers the HPV type as the unit of analysis, i.e., a participant can contribute up to 36 observations, corresponding to each HPV type.

[b] Of the 207 included participants,193 had their first valid anal sample at baseline, 12 had their first valid anal sample at visit 2, 2 had their first valid anal sample at visit 3.

[c] Corresponds to person-months or infection-months depending on the unit of analysis.

[d] Reference group is the cohort of participants unvaccinated for HPV. 95% Confidence Intervals generated using bootstrap resampling. Hazard ratios were adjusted for a propensity score calculated using a logistic regression model: dependent variable= any HPV positivity over the study duration; independent variables= treatment assignment and selected participant characteristics at baseline, refer to Methods and supplementary table III.
[e] One participant reported receipt of the Cervarix vaccine during follow-up (targets HPVs 16 and 18).
[f] Within-species HPV types includes HPVs 31, 33, 35, 39, 44, 45, 52, 58, 59, 67, 68, and 70.
[g] Cross-species HPV types includes HPVs 26, 34, 40, 42, 51, 53, 54, 56, 61, 62, 66, 69, 71, 72, 73, 81, 82, 83, 84, and 89.

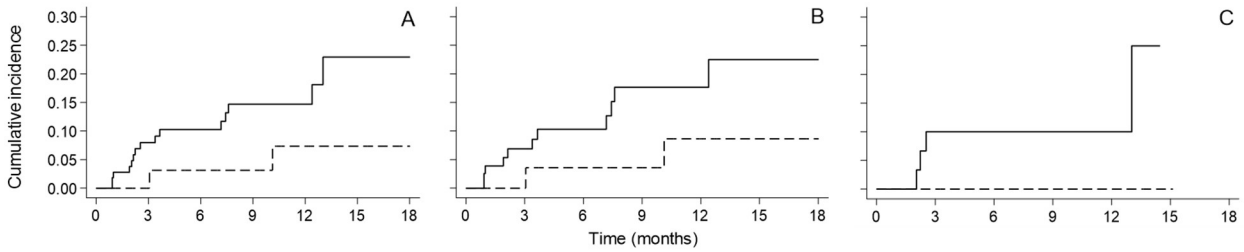


M2 - Figure 1: The LIMIT-HPV study population and analytical samples and frameworks.

Figure 1 Legend: Solid arrows describe selection of the analytical study population for the cross-sectional and longitudinal analyses whereas dashed lines describe the analytical frameworks relating to the type of analysis: 1) individual-level analysis where the participant is the unit of analysis; 2) HPV-level analysis where the HPV type is the unit of analysis, i.e. a participant can contribute up to 36 observations corresponding to each HPV type; 3) construct validity analysis to support the interpretation of the HPV-level analysis of incident HPVs 6/11/16/18, considering non-vaccine-covered (within-species vs cross-species) HPV types.

[a] Numbers represent those at baseline; however, HPV vaccination status was treated as a time-varying exposure in the longitudinal analysis.

HIV, human immunodeficiency virus; HPV, human papillomavirus; LIMIT-HPV, Lubricant Investigation in Men to Inhibit Transmission of HPV.

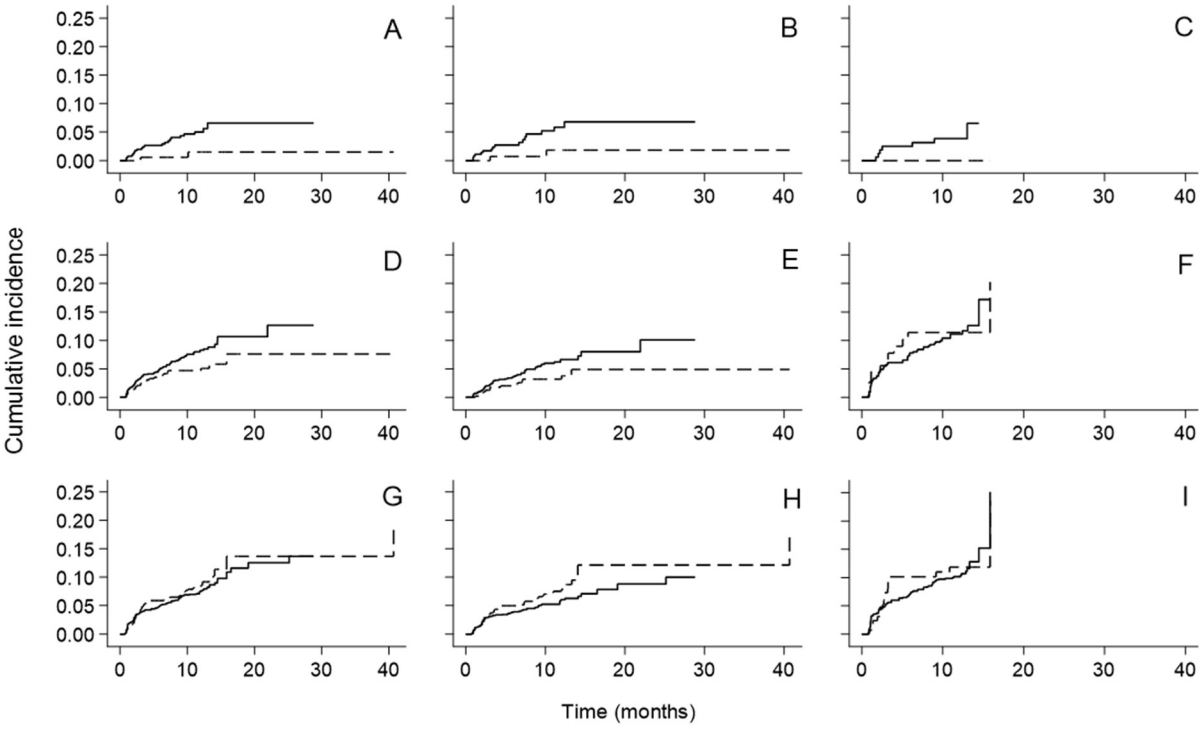


M2 - Figure 2: Cumulative incidence of HPVs 6/11/16/18 at the individual-level, overall and by HIV status.

Figure 2 Legend: Kaplan Meier survival analysis for the acquisition of HPVs 6/11/16/18 at the individual-level (participants being the unit of analysis) in unvaccinated (solid line) and vaccinated (dashed line) participants: panels A (all-participants), B (HIV-negative participants), and C (HIV-positive participants).

[a] One participant reported receipt of the Cervarix vaccine during follow-up (targets HPVs 16 and 18).

HIV, human immunodeficiency virus; HPV, human papillomavirus.



M2 - Figure 3: Cumulative incidence of HPVs 6/11/16/18, within-species HPV types, and cross-species HPV types at the HPV-level, overall and by HIV status.

Figure 3 Legend: Kaplan Meier survival analysis of unvaccinated (solid line) and vaccinated (dashed line) participants considering the following analyses at the HPV-level: 1) acquisition of HPVs 6/11/16/18[a]: panels A (all participants), B (HIV-negative participants), C (HIV-positive participants); 2) acquisition of within-species HPV types[b]: panels D (all participants), E (HIV-negative participants), F (HIV-positive participants); and 3) acquisition of cross-species HPV types[c]: panels G (all participants), H (HIV-negative participants), I (HIV-positive participants). An HPV-level analysis considers the HPV type as the unit of analysis, i.e., a participant can contribute up to 36 observations, corresponding to each HPV type.

[a] One participant reported receipt of the Cervarix vaccine during follow-up (targets HPVs 16 and 18).

[b] Within-species HPV types includes HPVs 31, 33, 35, 39, 44, 45, 52, 58, 59, 67, 68, and 70.

[c] Cross-species HPV types includes HPVs 26, 34, 40, 42, 51, 53, 54, 56, 61, 62, 66, 69, 71, 72, 73, 81, 82, 83, 84, and 89.

HIV, human immunodeficiency virus; HPV, human papillomavirus.

CHAPTER 5: DISCUSSION

5.1 SUMMARY OF KEY FINDINGS

In this thesis, we evaluated the impact of a carrageenan-based gel on anal HPVs 16 and 18 viral loads and the association between HPV vaccination and anal HPV infection using data from HIV-negative gbMSM and gbMSM living with HIV enrolled in the LIMIT-HPV randomized control trial.

In Manuscript 1, we further assessed the lack of a protective effect by carrageenan against anal HPV infection acquisition and clearance, previously observed in the LIMIT-HPV trial,[17,18] by comparing the change in anal HPV16 and HPV18 viral loads following use of a carrageenan-based gel relative to a placebo gel. We found that there was no significant difference in the change in viral loads between visits 1 and 2, where compliance to the study gels was expected to be the highest, or in the net change in viral load across visits 1 to 4, regardless of HIV status. Our findings suggest that carrageenan does not influence anal HPV16 or HPV18 viral loads, which may help explain its null effect in gbMSM. We also observed small differences in HPV16 and HPV18 viral loads by HIV status within each study arm.

Consistent with our results, the CATCH randomized control trial conducted in women, also by our research group, reported that the change in viral load for HPV42, a low-risk HPV type, and HPV51, a high-risk HPV type, did not significantly differ between participants using the carrageenan-based or placebo gels,[90] despite finding that carrageenan use was associated with a reduction of incident genital HPV infections.[15,16] However, the viral load analysis in the CATCH trial was also limited by a small analytical sample.[90] Consequently, larger studies are needed to further understand carrageenan's overall impact on HPV viral loads. Furthermore, the small differences in viral load between HIV-negative participants and participants living with HIV within each study arm of the LIMIT-HPV trial were contrary to what was expected. However, participants living with HIV received HIV care as part of the study,[91] which included access to antiretroviral therapy, expected to increase the effectiveness of immune responses,[53] which may have contributed to the small differences in viral load.

In Manuscript 2, we used data from the LIMIT-HPV study to compare anal HPVs 16 and 18 viral loads between vaccinated and unvaccinated participants as well as assessed the

association with HPV vaccination and anal HPV prevalence and incidence. We observed that anal HPVs 16 and 18 viral loads were similar by HPV vaccination status. We also found that there was no association between HPV vaccination and prevalent infection of vaccine-targeted HPV types or incident infection of non-vaccine-targeted HPV types. In contrast, we found that HPV vaccination was associated with a reduced risk of incident infection of vaccine-targeted HPV types. Results were similar by HIV status.

As HPVs 16 and 18 viral loads were cross-sectionally compared, we were likely comparing viral loads from both transient and persistent infections, which may have contributed to the non-significant differences by HPV vaccination status as high HPV viral load has been shown to be associated with persistent infections.[20,27,28] The non-significant findings may also be a result of a small sample size, especially as few HPV vaccinated participants were HPV16- and/or HPV18-positive at each study visit. Nevertheless, the analysis comparing the sum of HPV16/18 viral loads across all study visits had the highest power and similarly found that the differences in viral load between vaccinated and unvaccinated participants were not statistically significant.

The observed lack of association between HPV vaccination and anal HPV prevalence in the LIMIT-HPV trial is likely a result of participants having had previous exposure to HPVs 6, 11, 16, and/or 18 before receiving the HPV vaccine. Though most participants did not provide the date when they received the HPV vaccine, the average age at first sexual activity (16.7 years), the average age at enrollment (27.3 years), and that gbMSM became eligible for the publicly funded HPV vaccination program in 2014 in Quebec,[64] shortly before the LIMIT-HPV study began enrollment, corroborates that most participants were past their sexual debut and had previous exposure to HPV at the time of vaccination. In contrast, all risk estimates were consistent with protection by HPV vaccination against incidence of vaccine-targeted HPV types, though not all estimates reached statistical significance. Furthermore, the lack of association between HPV vaccination and incidence of non-vaccine-targeted HPV types was expected and helped verify that the analytical approach worked as well as provided additional evidence of the overall protective effect of HPV vaccination against vaccine-targeted HPV types.

5.2 STRENGTHS & LIMITATIONS

A key strength of the LIMIT-HPV trial was the study design.[91] The short intervals between study visits allowed for frequent HPV testing and the comprehensive questionnaires allowed for the collection of data on sociodemographic, behavioural, and clinical risk factors from an at-risk population that has not been extensively studied in regards to HPV. Furthermore, stratifying all analyses by HIV status allowed for the effects of carrageenan and HPV vaccination among HIV-negative gbMSM and gbMSM living with HIV to be documented as well as allowed for potential effect modification by HIV status to be accounted for. A limitation of the LIMIT-HPV trial was that the study did not reach the target sample size as it was terminated early due to a recommendation from the Data Safety and Monitoring Board.[17] The consequently smaller sample size had implications for both manuscripts. In addition, most participants were young, French-Canadian, and had received some kind of post-secondary education, therefore findings may not be generalizable to all gbMSM.

To the best of our knowledge, manuscript 1 was the first to evaluate if carrageenan had an impact on anal HPV viral loads. The randomization of participants to the treatment and placebo study arms resulted in the balance of measured sociodemographic and behavioural HPV risk factors at baseline, thus controlling for confounding. Limitations included a small analytical sample due to the LIMIT-HPV trial not reaching the target size, as mentioned above, and that HPV16 and HPV18, selected to be measured for viral load due to their high oncogenic risk, were not the most prevalent HPV types among participants. In total, 29 participants were evaluated for the change in HPV16 viral load, and 10 participants were evaluated for the change in HPV18 viral load. In addition, we had made a key assumption that the natural history of anal HPV infections would similarly follow the well-known biological process of cervical HPV infections. Accordingly, we had assumed that considerations such as using viral load as an indicator of infection spread would be the same for the anal canal as what has been previously established for the cervix.

The key strength in manuscript 2 was the analytical framework implemented to evaluate the association between HPV vaccination and anal HPV infection. I included (i) analyses at the individual-level, (ii) analyses at the HPV-level, which accounted for multiple infections by different HPV types and increased statistical precision, and (iii) construct validity analyses to

verify that the expected effects were restricted to comparisons in which vaccination was supposed to be influential. However, we acknowledge limitations with the analysis. Firstly, as participants in the LIMIT-HPV trial were not randomized based on their self-reported HPV vaccination status, there were some imbalances in the baseline characteristics between vaccinated and unvaccinated participants. By adjusting the effect estimates for a propensity score to predict HPV positivity, we were able to control for any confounding that may have arisen due to the imbalances as well as achieve parsimony. However, residual confounding may be present due to unmeasured confounders. Secondly, participants self-reported their HPV vaccination status, which may have resulted in misclassification with its biasing effects attenuating associations. In addition, a participant was considered to be vaccinated if they reported receiving an HPV vaccine, rather than if they reported receiving all three doses of the HPV vaccination series, as not all participants specified the number of doses they had received. As such, this may have also biased effect estimates towards the null. Lastly, the relatively small sample size may have contributed to some of the non-significant effect estimates in the cross-sectional and longitudinal analyses.

5.3 FUTURE RESEARCH

While findings from the LIMIT-HPV study do not provide evidence that carrageenan has an impact on anal HPVs 16 or 18 viral loads, larger studies could be carried out to further understand carrageenan's impact on HPV viral load. Future study designs could consider using the participant as their own comparator by using the period prior to use of the carrageenan-based gel as the control or referent group to account for potential confounding by characteristics that remain relatively constant over a period of time (i.e., sexual behaviour), such as case-crossover studies. Other potential broad-spectrum HPV preventive measures that can be used alongside HPV vaccination should also be explored for gbMSM. For HPV vaccination, larger observational studies are needed to further evaluate its effectiveness against anal HPV prevalence and incidence among gbMSM living with HIV. Future observational studies could also consider HPV vaccination's impact on other important disease endpoints, such as persistent anal HPV infections, development of ASIL, and development of anal cancer. Finally, the questionnaire and HPV genotyping data collected from participants in the LIMIT-HPV trial can be leveraged to further study the natural history of anal HPV infections among gbMSM.

5.4 FINAL CONCLUSIONS

In this thesis, we evaluated two HPV preventive measures, a carrageenan-based gel and HPV vaccination, among HIV-negative gbMSM and gbMSM living with HIV. Adding to previous findings that use of a carrageenan-based gel did not influence anal HPV acquisition or clearance, we did not find evidence to support that use impacted anal HPV16 or HPV18 viral loads. This lack of impact may have contributed to carrageenan's observed lack of protective effect in gbMSM. We also found evidence to support that HPV vaccination protects against incident anal infections of vaccine-targeted HPV types. Our findings add to the body of research on the real-world effectiveness of HPV vaccination in gbMSM as well as underscores the importance of encouraging gbMSM to get vaccinated against HPV.

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APPENDICES

MANUSCRIPT 1 APPENDIX

M1 - Supplementary Table I: Number of participants per visit [n, (%)] by study arm and HIV status

	Visit 1 (Month 0) n=258	**Visit 2 (Month 1) n=214**	**Visit 3 (Month 2) n=195**	**Visit 4 (Month 3) n=172**	**Visit 5 (Month 6) n=149**	**Visit 6 (Month 9) n=131**	**Visit 7 (Month 12) n=112**
Treatment	130 (50.4)	106 (49.5)	97 (49.7)	86 (50.0)	73 (49.0)	64 (48.9)	53 (47.3)
Placebo	128 (49.6)	108 (50.5)	98 (50.3)	86 (50.0)	76 (51.0)	67 (51.1)	59 (52.7)
HIV-positive	69 (26.7)	62 (29.0)	58 (29.7)	57 (33.1)	53 (35.5)	50 (38.2)	47 (42.0)
HIV-negative	189 (73.3)	152 (71.0)	137 (70.3)	115 (66.9)	96 (64.4)	81 (61.8)	65 (58.0)
Abbreviations: HIV, human immunodeficiency virus.							

M1 - Supplementary Table II: HPV positivity [n (%)] according to visit number, overall

HPV type	Visit 1 (n=258)	Visit 2 (n=214)	Visit 3 (n=195)	Visit 4 (n=172)	Visit 5 (n=149)	Visit 6 (n=131)	Visit 7 (n=112)
HPV6	30 (11.6)	24 (11.2)	25 (12.8)	19 (11.1)	20 (13.4)	17 (13.0)	17 (15.2)
HPV11	12 (4.7)	10 (4.7)	8 (4.1)	9 (5.2)	10 (6.7)	9 (6.9)	4 (3.6)
HPV16	29 (11.2)	23 (10.8)	23 (11.8)	25 (14.5)	17 (11.4)	15 (11.5)	14 (12.5)
HPV18	13 (5.0)	9 (4.2)	9 (4.6)	8 (4.7)	11 (7.4)	11 (8.4)	5 (4.5)
HPV26	5 (1.9)	6 (2.8)	3 (1.5)	4 (2.3)	3 (2.0)	1 (0.8)	0 (0.0)
HPV31	17 (6.6)	12 (5.6)	14 (7.2)	14 (8.1)	8 (5.4)	10 (7.6)	8 (7.1)
HPV33	11 (4.3)	10 (4.7)	10 (5.1)	13 (7.6)	10 (6.7)	6 (4.6)	6 (5.4)
HPV34	2 (0.8)	2 (0.9)	2 (1.0)	2 (1.2)	0 (0.0)	1 (0.8)	2 (1.8)
HPV35	14 (5.4)	10 (4.7)	9 (4.6)	9 (5.2)	10 (6.7)	12 (9.2)	6 (5.4)
HPV39	20 (7.8)	20 (9.4)	16 (8.2)	16 (9.3)	19 (12.8)	16 (12.2)	14 (12.5)
HPV40	9 (3.5)	9 (4.2)	8 (4.1)	7 (4.1)	4 (2.7)	3 (2.3)	2 (1.8)
HPV42	27 (10.5)	19 (8.9)	22 (11.3)	21 (12.2)	15 (10.1)	15 (11.5)	10 (8.9)
HPV44	19 (7.4)	18 (8.4)	17 (8.7)	16 (9.3)	12 (8.1)	16 (12.2)	15 (13.4)
HPV45	19 (7.4)	10 (4.7)	14 (7.2)	12 (7.0)	13 (8.7)	13 (9.9)	10 (8.9)
HPV51	26 (10.1)	21 (9.8)	25 (12.8)	20 (11.6)	18 (12.1)	22 (16.8)	18 (16.1)
HPV52	21 (8.1)	18 (8.4)	10 (5.1)	21 (12.2)	17 (11.4)	17 (13.0)	13 (11.6)
HPV53	32 (12.4)	31 (14.5)	25 (12.8)	28 (16.3)	28 (18.8)	21 (16.0)	18 (16.1)
HPV54	18 (7.0)	16 (7.5)	15 (7.7)	13 (7.6)	8 (5.4)	14 (10.7)	7 (6.3)
HPV56	13 (5.0)	5 (2.3)	10 (5.1)	14 (8.1)	9 (6.0)	7 (5.3)	5 (4.5)
HPV58	17 (6.6)	14 (6.5)	17 (8.7)	12 (7.0)	13 (8.7)	13 (9.9)	9 (8.0)
HPV59	18 (7.0)	16 (7.5)	13 (6.7)	17 (9.9)	13 (8.7)	11 (8.4)	7 (6.3)
HPV61	30 (11.6)	21 (9.8)	22 (11.3)	21 (12.2)	21 (14.1)	16 (12.2)	15 (13.4)
HPV62	31 (12.0)	21 (9.8)	27 (13.9)	23 (13.4)	19 (12.8)	21 (16.0)	15 (13.4)
HPV66	22 (8.5)	20 (9.4)	14 (7.2)	21 (12.2)	17 (11.4)	13 (9.9)	9 (8.0)
HPV67	8 (3.1)	3 (1.4)	2 (1.0)	5 (2.9)	1 (0.7)	1 (0.8)	1 (0.9)
HPV68	12 (4.7)	11 (5.1)	8 (4.1)	12 (7.0)	9 (6.0)	11 (8.4)	6 (5.4)
HPV69	7 (2.7)	2 (0.9)	4 (2.1)	5 (2.9)	4 (2.7)	1 (0.8)	3 (2.7)
HPV70	20 (7.8)	20 (9.4)	18 (9.2)	20 (11.6)	14 (9.4)	12 (9.2)	12 (10.7)
HPV71	3 (1.2)	3 (1.4)	1 (0.5)	0 (0.0)	2 (1.3)	0 (0.0)	0 (0.0)

HPV72	12 (4.7)	13 (6.1)	9 (4.6)	6 (3.5)	7 (4.7)	6 (4.6)	7 (6.3)
HPV73	18 (7.0)	16 (7.5)	14 (7.2)	15 (8.7)	11 (7.4)	8 (6.1)	11 (9.8)
HPV81	15 (5.8)	14 (6.5)	18 (9.2)	14 (8.1)	11 (7.4)	11 (8.4)	9 (8.0)
HPV82	11 (4.3)	10 (4.7)	6 (3.1)	9 (5.2)	10 (6.7)	8 (6.1)	5 (4.5)
HPV83	10 (3.9)	12 (5.6)	13 (6.7)	12 (7.0)	10 (6.7)	8 (6.1)	8 (7.1)
HPV84	25 (9.7)	26 (12.2)	24 (12.3)	21 (12.2)	20 (13.4)	15 (11.5)	17 (15.2)
HPV89	25 (9.7)	22 (10.3)	23 (11.8)	20 (11.6)	15 (10.1)	10 (7.6)	18 (16.1)
Any HPV type	159 (61.6)	143 (66.8)	130 (66.7)	117 (68.0)	102 (68.5)	93 (71.0)	81 (72.3)
Missing [a]	24 (9.3)	16 (7.5)	15 (7.7)	11 (6.4)	10 (6.7)	8 (6.1)	7 (6.3)

Abbreviations: HPV, human papillomavirus

[a] Missing include anal samples that were invalid or not collected.

MANUSCRIPT 2 APPENDIX

M2 - Supplementary Table 1: Self-reported HPV vaccination status [n (%)] by the LIMIT-HPV study participants at baseline, exclusively over follow-up, and throughout the entire study, overall and by HIV status.

HPV vaccination status	Baseline			Exclusively over follow-up			Complete history of vaccination (baseline & follow-up)		
	All (n=258)	HIV-negative (n=189)	HIV-positive (n=69)	All (n=168)[a]	HIV-negative (n=115)	HIV-positive (n=53)	All (n=258)	HIV-negative (n=189)	HIV-positive (n=69)
Vaccinated	60 (23.3)	49 (25.9)	11 (15.9)	12 (7.1)	10 (8.7)	2 (3.8)	72 (27.9)	59 (31.2)	13 (18.8)
Unvaccinated	198 (76.7)	140 (74.1)	58 (84.1)	151 (89.9)	100 (87.0)	51 (96.2)	186 (72.1)	130 (68.8)	56 (81.2)
Missing[b]	0 (0.0)	0 (0.0)	0 (0.0)	5 (3.0)	5 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus.

[a] 168/214 participants (of the 258 participants, 44 had only 1 visit) with ≥2 visits did not report being HPV vaccinated at baseline.

[b] Missing includes participants who did not provide an answer or replied that they did not know their vaccination status.

M2 - Supplementary Table 2: Self-reported information [n (%)] by the LIMIT-HPV study participants on the HPV vaccine received and number of doses, overall and by HIV status.

Variable	Categories	Baseline			Follow-up		
		All (n=60)	**HIV-negative (n=49)**	**HIV-positive (n=11)**	**All (n=48)[a]**	**HIV-negative (n=42)**	**HIV-positive (n=6)**
HPV vaccine	Gardasil	23 (38.3)	19 (38.8)	4 (36.4)	21 (43.8)	18 (42.9)	3 (50.0)
	Cervarix	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	1 (2.4)	0 (0.0)
	Gardasil-9	17 (28.3)	13 (26.5)	4 (36.4)	16 (33.3)	14 (33.3)	2 (33.3)
	Missing[b]	20 (33.3)	17 (34.7)	3 (27.3)	10 (20.8)	9 (21.4)	1 (16.7)
Number of HPV vaccine doses	1 dose	4 (6.7)	4 (8.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	2 doses	12 (20.0)	9 (18.4)	3 (27.3)	1 (2.1)	1 (2.4)	0 (0.0)
	3 doses	39 (65.0)	31 (63.3)	8 (72.7)	7 (14.6)	6 (14.3)	1 (16.7)
	Missing[b]	5 (8.3)	5 (10.2)	0 (0.0)	40 (83.3)	35 (83.3)	5 (83.3)

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus

[a] Of the 48 participants who reported receiving the HPV vaccine over follow up, 36 reported receipt of the HPV vaccine at baseline.

[b] Missing includes participants who did not provide an answer or replied that they did not know their vaccination status.

M2 - Supplementary Table 3: Anal HPV16 viral load (copies/cell) in the LIMIT-HPV study participants among those vaccinated against HPV at baseline and those unvaccinated throughout the study duration according to visit number, overall and by HIV status.

Visit Number	Vaccinated					Unvaccinated					*P*-value
	n/N[a] (%)	Median	IQR	Geometric mean	Range	n/N[a] (%)	Median	IQR	Geometric mean	Range	
All participants											
Visit 1	4/56 (7.1)	2.14	0.72 – 123.45	1.91	0.01 – 244.04	25/168 (14.9)	1.62	0.29 – 15.53	2.57	$4.27x10^{-3}$ - 1671.49	0.9496
Visit 2	2/39 (5.1)	1.93	0.05 – 3.80	0.44	0.05 – 3.80	20/147 (13.6)	4.07	0.29 – 43.54	3.82	0.03 – 161.76	0.3040
Visit 3	2/37 (5.4)	2.28	1.45 – 3.10	2.12	1.45 - 3.10	21/131 (16.0)	3.79	0.18 – 58.89	2.96	0.02 – 626.43	0.9131
Visit 4	2/35 (5.7)	3.06	0.19 – 5.92	1.07	0.19 - 5.92	23/116 (19.8)	0.51	0.08 – 24.52	1.07	0.01 – 382.97	0.9202
Visit 5	1/28 (3.6)	0.19	0.19 – 0.19	0.19	0.19 – 0.19	16/105 (15.2)	0.65	0.21 – 3.87	1.25	0.02 – 1365.88	0.4142
Visit 6	0/25 (0.0)	--	--	--	--	15/90 (16.7)	0.63	0.11 – 11.76	1.09	0.02 – 3106.40	ND
Visit 7	0/18 (0.0)	--	--	--	--	14/80 (17.5)	0.58	0.08 – 8.40	0.61	$4.27x10^{-3}$ –861.03	ND
HIV-negative participants											
Visit 1	2/45 (4.4)	122.03	0.01 – 244.04	1.81	0.01 – 244.04	16/116 (13.8)	3.87	0.32 – 74.35	4.35	$4.27x10^{-3}$ - 1671.49	0.7787
Visit 2	1/31 (3.2)	3.80	3.80 – 3.80	3.80	3.80 – 3.80	14/99 (14.1)	2.19	0.28 – 64.18	2.71	0.06 – 161.76	0.8170
Visit 3	1/30 (3.3)	3.10	3.10 -3.10	3.10	3.10 -3.10	12/89 (13.5)	4.51	0.45 – 44.97	3.47	0.02 – 626.43	0.7893
Visit 4	1/28 (3.6)	5.92	5.92 – 5.92	5.92	5.92 -5.92	14/72 (19.4)	1.31	0.07 – 9.67	1.13	0.01 – 90.76	0.6434
Visit 5	0/22 (0.0)	--	--	--	--	9/61 (14.8)	0.59	0.18 – 1.12	0.76	0.02 – 94.70	ND
Visit 6	0/20 (0.0)	--	--	--	--	7/48 (14.6)	0.30	0.07 – 4.33	0.39	0.02 – 11.76	ND
Visit 7	0/13 (0.0)	--	--	--	--	7/41 (17.1)	1.02	0.01 – 15.94	0.40	$4.27x10^{-3}$ – 22.24	ND
HIV-positive participants											
Visit 1	2/11 (18.2)	2.14	1.43 – 2.85	2.02	1.43 – 2.85	9/52 (17.3)	0.85	0.21 – 3.46	1.01	0.03 – 45.08	0.6374
Visit 2	1/8 (12.5)	0.05	0.05 – 0.05	0.05	0.05 – 0.05	6/48 (12.5)	14.41	0.82 – 22.90	4.66	0.03 – 96.94	0.3173
Visit 3	1/7 (14.3)	1.45	1.45 – 1.45	1.45	1.45 – 1.45	9/42 (21.4)	1.03	0.17 – 58.89	2.39	0.02 – 291.07	0.8618
Visit 4	1/7 (14.3)	0.19	0.19 – 0.19	0.19	0.19 – 0.19	9/44 (20.5)	0.18	0.12 – 24.52	0.99	0.03 – 382.97	0.8618
Visit 5	1/6 (16.7)	0.19	0.19 – 0.19	0.19	0.19 – 0.19	7/44 (15.9)	1.14	0.23 – 11.36	2.37	0.14 – 1365.88	0.2752
Visit 6	0/5 (0.0)	--	--	--	--	8/42 (19.0)	1.88	0.23 – 19.46	2.69	0.02 – 3106.40	ND
Visit 7	0/5 (0.0)	--	--	--	--	7/39 (17.9)	0.14	0.09 – 8.40	0.95	0.01 – 861.03	ND

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; ND, not determined.
[a] Denominator based on the number of valid anal samples at each visit.

M2 - Supplementary Table 4: Anal HPV18 viral load (copies/cell) in the LIMIT-HPV study participants among those vaccinated against HPV at baseline and those unvaccinated throughout the study duration according to visit number, overall and by HIV status.

Visit Number	Vaccinated					Unvaccinated					*P*-value
	n/N[a] (%)	Median	IQR	Geometric mean	Range	n/N[a] (%)	Median	IQR	Geometric mean	Range	
All participants											
Visit 1	4/56 (7.1)	0.74	0.09 – 339.55	1.23	0.02 – 677.79	9/168 (5.3)	0.15	0.05 – 0.17	0.13	$3.63x10^{-5}$ – 2499.50	0.4404
Visit 2	2/39 (5.1)	1.02	0.08 – 1.95	0.40	0.08 – 1.95	7/147 (4.8)	1.27	0.06 – 143.80	2.21	0.02 – 302.20	0.7697
Visit 3	2/37 (5.4)	2.84	0.01 – 5.67	0.21	0.01 – 5.67	7/131 (5.3)	15.35	0.17 – 105.56	12.58	0.16 – 15 201.30	0.2416
Visit 4	1/35 (2.9)	0.01	0.01 – 0.01	0.01	0.01 – 0.01	7/116 (6.0)	1.14	0.32 – 15.78	1.29	$4.77x10^{-5}$ - 8349.26	0.2752
Visit 5	2/28 (7.1)	0.48	0.41 – 0.56	0.48	0.41 – 0.56	9/105 (8.6)	2.75	0.26 – 58.68	5.64	0.03 – 27 517.26	0.4795
Visit 6	1/25 (4.0)	0.39	0.39 -0.39	0.39	0.39 – 0.39	10/90 (11.1)	2.90	0.07 – 41.65	2.05	0.01 -1713.10	1.0000
Visit 7	1/18 (5.6)	0.01	0.01 – 0.01	0.01	0.01- 0.01	4/80 (5.0)	22.72	0.48 – 7023.89	6.88	$3.77x10^{-3}$ - 14 003.29	0.4795
HIV-negative participants											
Visit 1	1/45 (2.2)	0.16	0.16 – 0.16	0.16	0.16 – 0.16	3/116 (2.6)	0.15	$3.63x10^{-5}$ – 0.82	0.02	$3.63x10^{-5}$ – 0.82	0.6547
Visit 2	1/31 (3.2)	1.95	1.95 – 1.95	1.95	1.95 – 1.95	3/99 (3.0)	7.02	0.02 – 143.80	2.85	0.02 – 143.80	0.6547
Visit 3	1/30 (3.3)	5.67	5.67 – 5.67	5.67	5.67 – 5.67	3/89 (3.4)	22.03	0.17 – 105.56	7.34	0.17 – 105.56	0.6547
Visit 4	1/28 (3.6)	0.01	0.01 – 0.01	0.01	0.01 – 0.01	3/72 (4.2)	0.89	$4.77x10^{-5}$ - 1.14	0.04	$4.77x10^{-5}$ - 1.14	0.6547
Visit 5	1/22 (4.5)	0.56	0.56 – 0.56	0.56	0.56 – 0.56	4/61 (6.6)	0.64	0.21 – 29.85	1.23	0.15 – 56.68	1.0000
Visit 6	0/20 (0.0)	--	--	--	--	5/48 (10.4)	0.11	0.07 – 14.07	0.76	0.01 – 374.07	ND
Visit 7	0/13 (0.0)	--	--	--	--	1/41 (2.4)	$3.77x10^{-3}$	$3.77x10^{-3}$ - $3.77x10^{-3}$	$3.77x10^{-3}$	$3.77x10^{-3}$ - $3.77x10^{-3}$	ND
HIV-positive participants											
Visit 1	3/11 (27.3)	1.32	0.02 – 677.79	2.45	0.02 – 677.79	6/52 (11.5)	0.11	0.05 – 0.17	0.34	0.01 – 2499.50	0.6056
Visit 2	1/8 (12.5)	0.08	0.08 – 0.08	0.08	0.08 – 0.08	4/48 (8.3)	0.87	0.26 – 151.74	1.83	0.06 – 302.20	0.4795
Visit 3	1/7 (14.3)	0.01	0.01 – 0.01	0.01	0.01 – 0.01	4/42 (9.5)	9.37	1.77 – 7608.32	18.85	0.16 – 15 201.30	0.1573
Visit 4	0/7 (0.0)	--	--	--	--	4/44 (9.1)	9.37	1.64 – 4182.52	18.80	0.32 – 8349.26	ND
Visit 5	1/6 (16.7)	0.41	0.41 – 0.41	0.41	0.41 – 0.41	5/44 (11.4)	8.02	2.75 – 138.55	19.02	0.03 – 27 517.26	0.3798
Visit 6	1/5 (20.0)	0.39	0.39 -0.39	0.39	0.39 -0.39	5/42 (11.9)	5.57	0.23 – 41.65	5.56	0.06 – 1713.10	0.7697
Visit 7	1/5 (20.0)	0.01	0.01	0.01	0.01 – 0.01	3/39 (7.7)	44.49	0.95 – 14 003.29	84.01	0.95 – 14 003.29	0.1797

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable; ND, not determined.
[a] Denominator based on the number of valid anal samples at each visit.

M2 - Supplementary Table 5: Anal HPV16/18 viral load (copies/cell) in the LIMIT-HPV study participants among those vaccinated against HPV at baseline and those unvaccinated throughout the study duration according to visit number, overall and by HIV status.

Visit Number	Vaccinated					Unvaccinated					*P*-value
	n/N[a] (%)	Median	IQR	Geometric mean	Range	n/N[a] (%)	Median	IQR	Geometric mean	Range	
All participants											
Visit 1	8/56 (14.3)	1.38	0.09 – 123.45	1.54	0.01 – 677.79	30/168 (17.9)	1.08	0.21 – 15.53	2.05	$4.27x10^{-3}$ – 2499.50	0.8021
Visit 2	4/39 (10.3)	1.02	0.07 – 2.88	0.42	0.05 – 3.80	24/147 (16.3)	4.07	0.29 – 43.54	3.35	0.03 – 305.57	0.1680
Visit 3	4/37 (10.8)	2.28	0.73 – 4.39	0.66	0.01 – 5.67	26/131 (19.8)	4.51	0.18 – 58.89	4.43	0.02 – 15 201.30	0.3290
Visit 4	3/35 (8.6)	0.19	0.01 – 5.92	0.19	0.01 – 5.92	27/116 (23.3)	0.89	0.12 – 24.52	1.51	0.01 – 8349.26	0.3507
Visit 5	3/28 (10.7)	0.41	0.19 – 0.56	0.35	0.19 – 0.56	22/105 (21.0)	0.86	0.18 – 8.02	2.00	0.02 – 27 517.26	0.4030
Visit 6	1/25 (4.0)	0.39	0.39 – 0.39	0.39	0.39 – 0.39	23/90 (25.6)	0.63	0.07 – 14.07	1.29	0.01 – 3148.05	0.9424
Visit 7	1/18 (5.6)	0.01	0.01 – 0.01	0.01	0.01 – 0.01	17/80 (21.3)	0.95	0.08 – 8.40	0.84	$3.77x10^{-3}$ – 14 003.29	0.2103
HIV-negative participants											
Visit 1	3/45 (6.7)	0.16	0.01 – 244.04	0.80	0.01 – 244.04	17/116 (14.7)	2.44	0.29 – 28.30	3.66	$4.27x10^{-3}$ – 1671.49	0.3683
Visit 2	2/31 (6.5)	2.88	1.95 – 3.80	2.72	1.95 – 3.80	15/99 (15.2)	3.41	0.28 – 64.18	3.02	0.06 – 305.57	1.0000
Visit 3	2/30 (6.7)	4.39	3.10 – 5.67	4.20	3.10 – 5.67	13/89 (14.6)	5.24	0.88 – 22.03	4.35	0.02 – 626.43	0.8651
Visit 4	2/28 (7.1)	2.96	0.01 – 5.92	0.19	0.01 – 5.92	15/72 (20.8)	1.46	0.07 – 9.67	1.16	0.01 – 90.76	0.4561
Visit 5	1/22 (4.5)	0.56	0.56 – 0.56	0.56	0.56 – 0.56	11/61 (18.0)	0.59	0.15 – 6.11	0.94	0.02 – 94.70	0.8848
Visit 6	0/20 (0.0)	--	--	--	--	11/48 (22.9)	0.11	0.07 – 11.76	0.54	0.01 – 374.37	ND
Visit 7	0/13 (0.0)	--	--	--	--	8/41 (19.5)	0.55	0.01 – 8.71	0.22	$3.77x10^{-3}$ – 22.24	ND
HIV-positive participants											
Visit 1	5/11 (45.5)	1.43	1.32 – 2.85	2.27	0.02 – 677.79	13/52 (25.0)	0.63	0.17 – 3.46	0.96	0.01 – 2499.50	0.5877
Visit 2	2/8 (25.0)	0.07	0.05 – 0.08	0.06	0.05 – 0.08	9/48 (18.8)	8.11	0.82 – 22.90	3.97	0.03 – 302.20	0.1573
Visit 3	2/7 (28.6)	0.73	0.01 – 1.45	0.10	0.01 – 1.45	13/42 (31.0)	3.39	0.17 – 58.89	4.51	0.02 – 15 201.30	0.2345
Visit 4	1/7 (14.3)	0.19	0.19 – 0.19	0.19	0.19 – 0.19	12/44 (27.3)	0.28	0.13 – 36.95	2.11	0.03 – 8349.26	0.7893
Visit 5	2/6 (33.3)	0.30	0.19 – 0.41	0.28	0.19 – 0.41	11/44 (25.0)	1.63	0.23 – 11.36	4.25	0.03 – 27 517.26	0.2363
Visit 6	1/5 (20.0)	0.39	0.39 – 0.39	0.39	0.39 – 0.39	12/42 (28.6)	1.88	0.22 – 19.46	2.90	0.02 – 3148.05	0.7893
Visit 7	1/5 (20.0)	0.01	0.01 – 0.01	0.01	0.01 – 0.01	9/39 (23.1)	0.95	0.09 – 8.40	2.77	0.01 – 14 003.29	0.1172

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable; ND, not determined.
[a] Denominator based on the number of valid anal samples at each visit.

M2 - Supplementary Table 6: Total sum of anal HPVs 16 and 18 viral load (copies/cell) across all study visits in the LIMIT-HPV study participants among those vaccinated against HPV at baseline and those unvaccinated throughout the study duration, overall and by HIV status.

Participants	Vaccinated					Unvaccinated					*P*-value
	n/N[a] (%)	Median	IQR	Geometric mean	Range	n/N[a] (%)	Median	IQR	Geometric mean	Range	
All	8/56 (14.3)	3.09	1.12 – 128.09	4.65	0.01 – 677.79	30/168 (17.9)	24.93	4.81 – 134.98	30.11	0.05 – 69 585.92	0.1236
HIV-negative	3/45 (6.7)	8.34	0.01 – 247.84	3.03	0.01 – 247.84	17/116 (14.7)	28.30	6.64 – 134.98	30.33	0.35 – 1671.49	0.5604
HIV-positive	5/11 (45.5)	2.90	1.32 – 3.27	6.01	0.92 – 677.79	13/52 (25.0)	21.56	3.46 – 87.04	29.83	0.05 – 69 585.92	0.1833
Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.											

M2 - Supplementary Table 7: Logistic regression coefficients for study and participant characteristics, used to construct a propensity score to detect any HPV positivity over the study participation.

Variable	**Coefficient**	**Standard Error**
Treatment assignment	-0.12	0.39
Age	0.05	0.02
Ethnicity		
French-Canadian	Reference	Reference
English-Canadian	0.63	0.61
European	0.62	0.65
Latin-American	0.52	0.69
Other	0.14	0.54
Education[a]		
Elementary	Reference	Reference
Secondary	-12.77	853.02
College	-12.10	853.02
University	-11.32	853.02
Smoking status		
Never	Reference	Reference
Former	-0.09	0.55
Current	0.96	0.60
Age at first sexual activity	-0.12	0.04
Number of lifetime sex partners		
1-5	Reference	Reference
6-10	-0.70	0.85
11-25	0.75	0.71
26-60	1.31	0.75
61-200	1.27	0.80
201-500	1.30	0.97
≥501	2.16	1.33
New sex partner in the last month	0.82	0.42
Has a stable sex partner	0.74	0.42
Constant	11.60	853.02
Abbreviations: HPV, human papillomavirus. [a] The categories secondary (variable perfectly predicted the outcome) and university (due to collinearity) level education were forced into the model		

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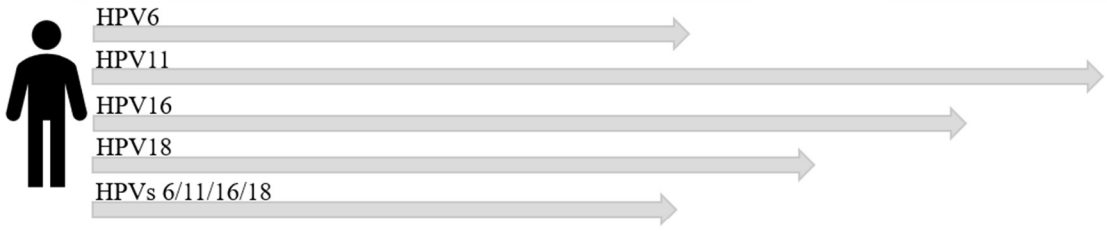
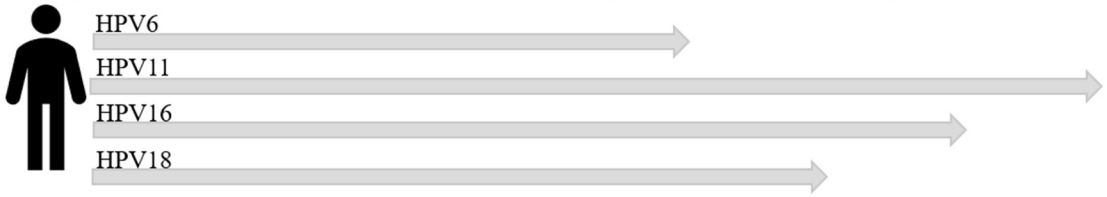
	Visit 1 *Month 0*	Visit 2 *Month 1*	Visit 3 *Month 2*	Visit 4 *Month 3*	Visit 5 *Month 6*	Visit 6 *Month 9*	Visit 7 *Month 12*
HPV6	-	-	-	+	+	+	+
HPV11	-	-	-	-	-	-	-
HPV16	-	-	-	-	-	+	+
HPV18	-	-	-	-	+	+	+



B

	Visit 1 *Month 0*	Visit 2 *Month 1*	Visit 3 *Month 2*	Visit 4 *Month 3*	Visit 5 *Month 6*	Visit 6 *Month 9*	Visit 7 *Month 12*
HPV6	-	-	-	+	+	+	+
HPV11	-	-	-	-	-	-	-
HPV16	-	-	-	-	-	+	+
HPV18	-	-	-	-	+	+	+



M2 - Supplementary Figure 1: Individual-level (Panel A) versus HPV-level (Panel B) analysis of HPVs 6, 11, 16, and 18 as outcomes of interest at different timepoints for the same participant.

Supplementary Figure 1 Legend: - and + indicate the participant tested negative or positive, respectively, for a given HPV type at the specified visit. Grey shaded cells indicate the first visit the participant tested positive for a given HPV type. Grey horizontal arrows represent the person-time the participant contributed to the analysis. In Panel A, the participant contributes an individual person-time up until the first detection of HPVs 6, 11, 16, or 18, depending on the listed HPV type/group. In Panel B, the participant contributes person-time equal to the sum for all four observations, each corresponding to the first detections of HPVs 6, 11, 16, and 18. HPV, human papillomavirus.