EVALUATION OF HUMAN PAPILLOMAVIRUS TYPE COMPETITION AND THE POTENTIAL FOR TYPE REPLACEMENT POST-VACCINATION

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ABSTRACT

Introduction: Infection with certain genotypes of human papillomavirus (HPV) is necessary in the development of cervical cancer. This discovery has led to the establishment of two vaccines that prevent the HPV genotypes that cause the majority (~70%) of cervical cancer cases (HPVs 16 and 18). However, other oncogenic HPV genotypes exist, which could increase in prevalence following reductions in HPV vaccine target genotypes 6, 11, 16 and 18, post-vaccination (i.e., “type replacement”), if certain conditions apply. For instance, if natural type competition exists between these vaccine and other genotypes, then type replacement may be more likely. The main objectives of this project were to evaluate HPV genotype competition and the potential for diagnostic artifacts, which could inhibit our ability to accurately compare pre- and post-vaccination HPV prevalence in vaccinated populations.

Methodology: Different statistical approaches were used to evaluate HPV genotype competition, with subject and HPV DNA information coming from five epidemiological studies conducted among females in Canada and Brazil. These approaches involved: 1) construction of hierarchical logistic regression models for each vaccine-targeted genotype and analyses to explore whether infection with these genotypes may be associated with infection with other HPV genotypes; and 2) construction of Kaplan-Meier curves and Cox models to evaluate sequential acquisition and clearance of HPV genotypes according to HPV status with vaccine-targeted genotypes. To evaluate unmasking of HPV52 that may be caused by elimination of HPV16, we also reanalyzed

1000 cervical specimens (from the same five studies) plus an additional 200 anal specimens (from a Montreal study conducted among HIV infected males). These specimens, which were all HPV52 negative according to consensus PCR assays (200 specimens/study; 100 HPV16+/study) were retested using highly sensitive type-specific real-time HPV52 PCR.

Results: In our pooled analyses comparing risk of infection with vaccine-targeted HPV genotypes according to infection with other genotypes (regression approach), only one negative association was observed (between HPVs 18 and 89), but was not statistically significant. Similarly, in our analyses comparing rates of acquisition or clearance of other HPV genotypes according to infection with vaccine-targeted genotypes (cohort approach), no statistically significant negative or positive associations were observed (once accounting for multiple comparisons), respectively. In our analyses of unmasking, presence of HPV16 was positively associated with HPV52 detection, particularly in the single study that included HIV infected males (adjusted OR=3.82, 95%CI: 1.19-12.26). Although substantial heterogeneity was observed across studies (P value=0.08), there was a positive association between HPV16 viral load (tertiles) and detection of HPV52 (P for trend=0.003).

Conclusion: No clear or consistent evidence of genotype competition was observed across our regression or cohort analyses. Unmasking of HPV52 should be considered in future surveillance studies comparing pre- and post-vaccination HPV prevalence, but may be a greater issue among those with high viral load HPV16 infections (e.g.,

immunosuppressed populations, such as those with high HIV prevalence). These results suggest that HPV type replacement is unlikely to occur, which may help guide decisions regarding vaccination programs.

RÉSUMÉ

Introduction: La découverte que l'infection par certains types de virus du papillome humain (VPH) est nécessaire pour le développement du cancer du col de l'utérus a mené à la création de deux vaccins spécifiquement contre les types de VPH (16 et 18) responsables pour la plupart des cas du cancer cervical (~70%). Il existe cependant d'autres types de VPH, qui, suite à la vaccination pour les sortes cibles 6, 11, 16 et 18, peuvent néanmoins augmenter. Si une compétition naturelle existe entre ces vaccins et d'autres types de VPH, le remplacement de types peut être plus probable. Les objectifs principaux de ce projet étaient d'évaluer la compétition entre les types de VPH et d'estimer le potentiel de création d'artefacts diagnostiques, lesquels pourraient réduire notre capacité de comparer avec précision la prévalence du VPH chez les populations vaccinées avant et après la vaccination.

Méthodes: Diverses approches statistiques furent considérées pour l'évaluation du remplacement de type de VPH. Les données sur les sujets ainsi que celles décrivant l'ADN du VPH provenaient de cinq études épidémiologiques de femmes au Canada et au Brésil. Ces approches incluaient : 1) la construction de modèles de régression logistique hiérarchiques pour chaque type ciblé par les vaccins, et des analyses pour déterminer si une infection avec ces types pourrait être associée à une infection par d'autres types de VPH; et 2) la construction d'estimateurs de Kaplan-Meier et de régressions de Cox pour évaluer l'acquisition séquentielle et l'élimination des types de VPH par rapport au statut d'infection au VPH avec les types ciblés par les vaccins. Pour

évaluer le démasquage du VPH 52 qui pourrait être attribuable à l'élimination du VPH 16, nous avons également refait l'analyse de 1000 spécimens du col de l'utérus (provenant des cinq études mentionnées ci-dessus) et de 200 spécimens anaux supplémentaires (d'une étude basée à Montréal auprès des hommes atteints du VIH). Ces échantillons, qui étaient tous initialement marqués par l'absence du VPH 52 selon PCR (200 spécimens par étude; 100 VPH16+ par étude) furent ré-analysés pour le VPH 52 par un travail de PCR en temps réel, très sensible, et spécifique aux types.
Résultats: Lors de nos analyses combinées qui comparaient le risque d'infection par un type de VPH contenu dans le vaccin avec celui d'autres types de VPH (approche de régression), une seule association négative fut observé (entre les VPH 18 et 89), mais elle n'était pas statistiquement significative. De même, nos analyses examinant le taux d'acquisition ou d'élimination d'autres types de VPH selon l'infection par les types vaccins (approche de cohorte) n'ont rapporté aucune association positive ou négative statistiquement significative (après avoir tenu compte de plusieurs comparaisons). Nos analyses de démasquages ont démontré que la présence du VPH 16 était positivement associée avec la détection du VPH 52, particulièrement dans l'étude des hommes infectés par le VIH (OR=3.82, 95%CI: 1.19-12.26). Malgré une hétérogénéité substantielle entre les études (P=0.08), une association positive entre la charge virale de VPH 16 (terciles) et la détection de VPH 52 (P=0.003) fut observée.

Conclusions: Aucunes preuves claires ou cohérentes ne furent observées dans nos analyses de régression ou de cohorte. Il serait utile de démasquer pour le VPH de type 52 au cours des prochaines études de surveillances cherchant à comparer la

prévalence du VPH avant et suite à la vaccination. Ceci aurait un grand impact pour les individus ayant une infection au VPH 16 à charge virale élevée (par exemple, les populations des personnes immunosupprimés avec une haute prévalence de VIH). Ces résultats indiquent que le remplacement de type de VPH est peu probable, ce qui pourrait aider à aviser certaines décisions sur les programmes de vaccination.

PREFACE

The format of this thesis follows that of a manuscript-based thesis. This dissertation consists of a collection of papers of which the student is the author or co-author. According to McGill University guidelines, the papers must have a cohesive, unitary character making them a report of a single program of research. The structure for the manuscript-based thesis must conform to the following:

1. Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly duplicated text (not the reprints) of one or more published papers. These texts must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.)
2. The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory.
3. The thesis must conform to all other requirements of the “Guidelines for Thesis Preparation” in addition to the manuscripts. The thesis must include the following: (a) a table of contents; (b) an abstract in English and French; (c) an introduction which clearly states the rationale and objectives of the research; (d) a comprehensive review of the literature (in addition to that covered in the introduction to each paper); (e) a final conclusion and summary.

4. As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.
5. When co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. The supervisor must attest to the accuracy of this statement at the doctoral oral defense.

Contribution of co-authors

Manuscript #1:

EPIDEMIOLOGY AND BURDEN OF HPV INFECTION AND RELATED DISEASES: IMPLICATIONS FOR PREVENTION STRATEGIES

Published in Preventive Medicine 2011;53:S12-S21

Joseph E. Tota, Ph.D. Candidate:

Conducted the literature review, prepared table for this manuscript, designed and assisted with the preparation of all figures, wrote the manuscript.

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Manuscript #2:

EPIDEMIOLOGICAL APPROACH TO EVALUATE THE POTENTIAL FOR HUMAN PAPILLOMAVIRUS TYPE REPLACEMENT POST-VACCINATION

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Contributed to the design of the statistical analysis, provided statistical expertise, provided comments on the manuscript.

François Coutlée, Professor, Université de Montréal, Département de Microbiologie et Infectiologie, Montreal, Québec, Canada:
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Luisa L. Villa, Professor, Universidade de São Paulo, Department of Radiology and Oncology, School of Medicine, São Paulo, Brazil:
Co-designed the original study, coordinated and designed the laboratory testing procedures, provided comments on the manuscript.

Eduardo L. Franco, Chair, Department of Oncology, Director, Division of Cancer Epidemiology, and James McGill Professor, Department of Epidemiology, Biostatistics and Occupational Health, McGill University:

Designed, supervised and obtained funding for the original study, supervised the design of the statistical analyses, contributed to manuscript writing.

Manuscript #3:

EVALUATION OF HUMAN PAPILLOMAVIRUS TYPE COMPETITION AND THE POTENTIAL FOR TYPE REPLACEMENT POST-VACCINATION

Submitted to The American Journal of Epidemiology

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Manuscript #4:

CERVICAL INFECTION WITH VACCINE HUMAN PAPILLOMAVIRUS (HPV) TYPES AS A PREDICTOR OF ACQUISITION OR CLEARANCE OF OTHER HPV INFECTIONS

Submitted to The Journal of Infectious Diseases

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Manuscript #5:

EVALUATION OF HUMAN PAPILLOMAVIRUS TYPE REPLACEMENT POST-VACCINATION MUST ACCOUNT FOR DIAGNOSTIC ARTIFACTS: MASKING OF HPV52 BY HPV16 IN ANOGENITAL SPECIMENS

Submitted to Cancer Epidemiology, Biomarkers & Prevention (under revision)

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Luisa L. Villa, Professor, Universidade de São Paulo, Department of Radiology and Oncology, School of Medicine, São Paulo, Brazil:

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Anita Koushik, Associate Professor, Université de Montréal, Département *de* médecine sociale *et* préventive, and Université de Montréal Hospital Research Centre, Montreal, Québec, Canada:
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Marie-Hélène Mayrand, Associate Professor, Université de Montréal Hospital Research Centre, and Département d'obstétrique-gynécologie et Médecine Sociale et Préventive, Montreal, Québec, Canada:
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Eduardo L. Franco, Chair, Department of Oncology, Director, Division of Cancer Epidemiology, and James McGill Professor, Department of Epidemiology, Biostatistics and Occupational Health, McGill University:
Designed and supervised all of the original studies, obtained funding for the investigations (covering all costs associated with parent studies, as well as costs associated with HPV52 retesting in the current unmasking study), participated in the design of the statistical analyses, contributed to manuscript writing.

STATEMENT OF ORIGINALITY

The project described in this thesis represents original research. Although many prior studies have focussed on human papillomavirus (HPV) infection and co-infections, at the time when the idea for this project was conceived (late 2009), there were no other studies that specifically evaluated HPV type competition to gain insight regarding the possibility of HPV type replacement. Since this time, I have published a conceptual paper describing this issue of HPV type replacement, as well as different epidemiological approaches to evaluate HPV type competition. I have also presented preliminary results from my thesis at numerous national/international scientific meetings (including at the 2010 McGill EBOH Research Day Conference). Although others have now evaluated HPV clustering patterns among unvaccinated female populations, none have applied the same (Bayesian) regression or cohort statistical approach that I used, or had access to such a large data set (>38,000 cervical specimens; tested for HPV using similar broad spectrum PCR assays). The final manuscript in this thesis also represents the first study to evaluate whether diagnostic artifacts may inhibit our ability to evaluate type replacement in the future.

For this project, data were available from six studies conducted by members of our division. They included: Ludwig-McGill cohort study, HPV Infection and Transmission among Couples through Heterosexual Activity (HITCH) study, McGill-Concordia cohort study, Biomarkers of Cervical Cancer Risk (BCCR) case-control study, Canadian Cervical Cancer Screening Trial (CCCaST), and the Human

Immunodeficiency and Papilloma Virus Research Group (HIPVIRG) study. Although none of these parent studies were originally designed with the aim of evaluating HPV type competition (or potential diagnostic issues associated with type replacement assessment), they provided excellent data sets for this project.

Considering the large investment that many governments have already made in HPV vaccination programs, concern about HPV type replacement is important to address. Major contributions of this thesis will be to clearly describe this issue of HPV type replacement and appropriate epidemiological approaches to explore its potential, to advance our knowledge regarding specific HPV type interactions (between vaccine-targeted types and other types), as well as to assess the possibility that diagnostic artifacts may arise in future surveillance studies evaluating HPV type replacement.

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For the past six years, I have been fortunate to make McGill my academic home and to have Dr. Eduardo Franco serve as my mentor. Dr. Franco has been extremely supportive and continues to be a constant source of inspiration. The freedom he has provided and his confidence in my abilities has also helped shape me into the epidemiologist that I have become. I will forever be grateful to him.

This project would not have been possible without the direct and indirect contributions of many individuals. The five parent studies that all HPV and participant data came from were designed and directed by Dr. Franco, or by his students (under his supervision). Dr. Franco served as the principal investigator on all of these studies. The Ludwig-McGill cohort study was jointly led by Drs. Eduardo Franco and Luisa Villa from Brazil, the McGill-Concordia study was led by Dr. Harriet Richardson (student), the HITCH study was led by Dr. Ann Burchell (student), the BCCR study was led by Dr. Anita Koushik (student), and the CCCaST study was led by Dr. Marie-Hélène Mayrand (student).

I would also like to thank Silvaneide Ferreira, José Carlos Prado, Maria C. Costa, Joao S. Sobrinho, Hélène Voyer, Véronique Legault, and Julie Guénoun, for the HPV DNA assays; and Luiza Baggio, Lenice Galan, Gail Kelsall, Suzanne Dumais, Natalia Morykon, Amelia Rocamora, Nathalie Slavtcheva, and Allita Rodrigues, for patient and data management in the parent studies. There were also many other co-investigators

who played key roles in each of these parent studies. Funding for these parent investigations was provided by, the Canadian Institutes of Health Research (grants MT-13649, MOP-53111, MOP-49396, MOP-68893, MOP-42532, MCT-54063, MOP-67155, CRN-83320), Canadian Cancer Society (grant 12030), the US National Institutes of Health (grants CA70269, AI073889), and by the Réseau FRSQ SIDA maladies infectieuses. The Society of Gynecologic Oncology of Canada also provided me with funding for the reanalysis of selected cervical/anal specimens, which was critical for the evaluation of one of my objectives.

I would also like to thank my committee members who provided expert guidance on methodology, statistics, and laboratory HPV testing procedures (Drs. Jay Kaufman, Stephen Walter, and François Coutlée). In addition, I would like to acknowledge the contribution of other scientists, especially Drs. Anil Chaturvedi and Allan Hildeshiem from NCI, Dr. Salvatore Vaccarella from IARC, Joakim Dillner from Karolinska Institute, and Dr. Agnihotram Ramanakumar from our own division at McGill, who all share an interest in HPV type replacement and who have helped guide my approach through numerous insightful discussions.

Over the years, I have also had the distinct pleasure to work and spend time with many other individuals from the Division of Cancer Epidemiology in the Department of Oncology. Every day at 2pm, I could count on Raman to join me for an afternoon coffee, which often led to great discussions about all things - personal and work related. I have fond memories of my interactions with the group and will always cherish the friendships

that I made at the division. I would also like to thank our division's administrative coordinator, Candida Pizzolongo, who has been so meticulous in handling important administrative paperwork related to numerous grant applications, travel, and other items.

Last but not least, I would like to convey my gratitude to my friends and family for their love and support, and who could always be counted on when I needed them. I would especially like to thank my wife, Lindsay, who moved from Niagara to Montreal to be with me while I worked to complete my PhD and for her never ending patience, love and support during this process. I would also like to thank her for bringing us our wonderful daughter, Anise, who has already filled our lives with so much joy and who remains the greatest source of motivation to do better in all things.

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CHAPTER 1: INTRODUCTION

In 2008, the Nobel Prize in Physiology or Medicine was awarded to Dr. Harald zur Hausen for his pioneering role in establishing the causal link between human papillomavirus (HPV) infection and cervical carcinoma [1]. This announcement reflects the importance of the discovery of a sexually-transmitted infection (STI) as a necessary cause of cervical cancer and the enormous opportunity for public health interventions. His work paved the way for two highly efficacious vaccines [2, 3] that prevent the types of HPV infection that cause most cases (~70%) of cervical cancer (HPVs 16 and 18) [4-7]. It is expected that vaccination will eventually have a major impact on the incidence and burden of cervical cancer, as well as other HPV-related diseases [7, 8]. However, other oncogenic HPV types exist [9, 10] and so it is at least conceivable that one of these types may eventually begin to occupy the niche vacated by the gradual eradication of vaccine target types; a concept referred to as “type replacement” [11, 12].

A recent historical example of type replacement was following childhood pneumococcal vaccination against pneumonia. Numerous studies have consistently revealed a substantial reduction in carriage of vaccine serotypes among vaccinated individuals, whereas carriage of nonvaccine serotypes increased for this group [13-16]. Unlike *Streptococcus pneumoniae,* which has a high rate of genetic mutation, HPV types are very genetically stable, and therefore it is unlikely that we will observe escape mutants or entirely new HPV types [12, 17]. However, if competition exists between different HPV types during natural infection, then there is still the theoretical possibility

that HPV type replacement may occur. For instance, if infections with either of the vaccine-targeted types were found to be negatively associated with infection by other (non-vaccine) HPV types, then these types would be flagged as potential candidates for HPV type replacement. In the context of public health, HPV type replacement only poses a problem if it ultimately leads to disease in vaccinated populations. Following vaccination against HPVs 6, 11, 16 and 18, disease burden attributed to other HPV types will either: (i) remain the same, (ii) increase as a result of type replacement, or (iii) decrease as a result of cross protection against nonvaccine types. Figure 1-1 presents these three scenarios using the example of HPV31, an oncogenic type that is phylogenetically related to HPV16 [18].

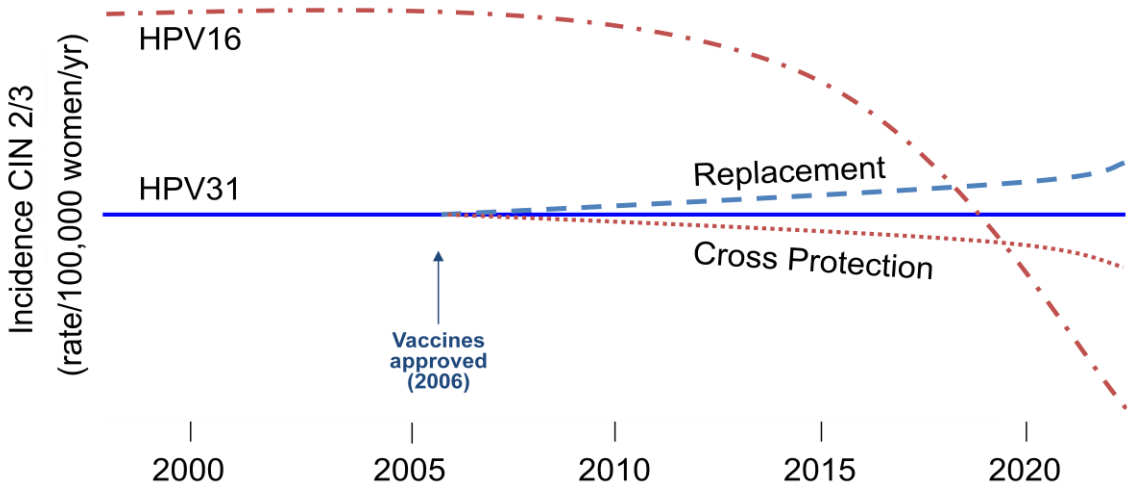

Figure 1-1: Hypothetical changes of HPV types 16 and 31 following HPV vaccination. Incidence of cervical intraepithelial neoplasia (CIN) grade 2/3 attributed to HPV31 in vaccinated populations will either remain constant (solid line), increase (dashed line; indicating type replacement), or decrease (dotted line; indicating cross-protection) as incidence of HPV16 infection declines post-vaccination.

(Courtesy of Joakim Dillner, Karolinska Institute, Sweden)

Alternatively, an apparent post-vaccination increase in some HPV types not targeted by vaccination may be a diagnostic artifact because consensus polymerase chain reaction (PCR) assays may fail to detect HPV types present in low copy numbers in co-infected specimens, such that with a drop in vaccine-preventable types, there may be increased detection of previously masked types. The authors of some studies reporting an increase in certain HPV types post-vaccination (particularly HPV52) have suggested the possibility that this may be caused by diagnostic artifacts. Therefore, just as it is important to identify HPV types that should be monitored for replacement, it is equally important to evaluate the potential for unmasking of specific HPV types. Both replaced and masked types may be more frequent post-vaccination and thus prevent erroneous conclusions concerning the value of this large-scale intervention.

The main objectives of this project were: 1) to evaluate whether infection with any nonvaccine HPV types is associated with infection with vaccine-target types; 2) to evaluate whether pre-existing infection with vaccine-targeted HPV types affects acquisition and/or clearance of other HPV types, and 3) to evaluate the putative masking of HPV52 by HPV16 in amplification HPV DNA assays, which may be helpful in distinguishing artifactual from true type replacement in future surveillance studies.

CHAPTER 2: LITERATURE REVIEW: MANUSCRIPT I

EPIDEMIOLOGY AND BURDEN OF HPV INFECTION AND RELATED DISEASES: IMPLICATIONS FOR PREVENTION STRATEGIES

2.1 Preamble

This first manuscript from my thesis provides a review of the literature pertaining to the epidemiology of HPV infection, including important risk factors; risk of invasive cervical cancer and other diseases associated with HPV infection; the burden of disease attributed to HPV infections; and current/future opportunities for prevention, including cervical cancer screening and HPV vaccination. Although this manuscript provides a fairly comprehensive review of these topics, some topics deserved more attention (in the context of this project), specifically HPV DNA testing, HPV vaccination, and an update on the literature focusing on HPV type interactions. Therefore, at the end of this chapter (following presentation of this manuscript), additional sections focusing on these topics are included.

Epidemiology and Burden of HPV Infection and Related Diseases: Implications for prevention strategies

Running title: HPV and Related Diseases

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Reprint presented in Appendix 1

FOOTNOTES

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Abstract

Human papillomavirus (HPV) infection is a necessary, although not sufficient cause of cervical cancer. Globally, HPV infection accounts for an estimated 530,000 cervical cancer cases (~270,000 deaths) annually, with the majority (86% of cases, 88% of deaths) occurring in developing countries. Approximately 90% of anal cancers and a smaller subset (< 50%) of other cancers (oropharyngeal, penile, vaginal, vulvar) are also attributed to HPV. In total, HPV accounts for 5.2% of the worldwide cancer burden. HPVs 16 and 18 are responsible for 70% of cervical cancer cases and, especially HPV16, for a large proportion of other cancers. Prophylactic vaccination targeting these types is therefore expected to have a major impact on the burden of cervical cancer as well as that of other HPV-related cancers. Over the past 50 years, organized or opportunistic screening with Papanicolaou (Pap) cytology has led to major reductions in cervical cancer in most developed countries. However, due to lack of resources or inadequate infrastructure, many countries have failed to reduce cervical cancer mortality through screening. HPV DNA testing recently emerged as a likely candidate to replace Pap cytology for primary screening. It is less prone to human error and more sensitive than Pap in detecting high-grade cervical lesions. For countries with national vaccination programs, HPV testing may also serve as a low cost strategy to monitor long term vaccine efficacy. Introduction of well organized vaccination and screening programs should be a priority for all countries. Increased support from donors is needed to support this cause.

Introduction

Human papillomavirus (HPV) is currently one of the most common sexually transmitted infections worldwide [10, 19, 20]. Most individuals (~75%) who engage in sexual activity will become infected with HPV at some point during their lifetime [19, 21]. For the vast majority these infections will be asymptomatic and clear within 1-2 years [22-27]; however, a substantial increase in risk for cervical cancer exists for women who develop persistent infection with high-oncogenic risk HPV types (HR-HPV) [28-31]. Infection with low-oncogenic risk HPV types (LR-HPV) is also responsible for considerable morbidity associated with benign lesions known as condylomata acuminate (genital warts) as well as a large proportion of low grade squamous intraepithelial cervical lesions. In this review we discuss the burden of HPV infection and related diseases, mainly focusing on cervical cancer and opportunities for prevention.

Epidemiology of HPV Infection

According to a recent meta-analysis that included data from more than one million women in 59 countries, the country-specific prevalence of cervical HPV infection among those with normal cytology ranges from 1.6% to 41.9% [32]. Higher HPV prevalence was observed in African and Latin American regions in comparison to European, North American, and Asian regions. The estimated average global prevalence of HPV in this particular study was 11.7%, which is similar to previous reports focusing on women [33, 34]. This study, along with others [34-38], reported an

interesting trend in the female age-specific distribution of HPV whereby there is a first peak at younger ages (<25 years) in all regions; and in the Americas, Africa and Europe, a clear second peak among individuals 45 years or older. The first peak, which comes shortly after sexual debut for most women, is generally attributed to higher levels of sexual activity with multiple partners and low viral immunity. After the first peak, a consistent age-related decline in HPV prevalence has been documented in numerous epidemiological studies. This trend was also observed in one study of female sex workers in Denmark, i.e., a population with high lifelong levels of sexual activity [39], which underscores the importance of naturally acquired immunity in protecting against HPV infection. Although the reason for the smaller second peak at middle age still remains unclear, possible explanations include immuno-senescence, hormonal changes prior to menopause, changes in male/female sexual behaviour, cohort effects, or perhaps higher rates of HPV persistence at older ages [32, 34, 40, 41].

Among sexually active males, genital HPV infection is also very common; however, prevalence varies widely depending on geographic region, risk group, anatomical site (glans/corona, penile shaft, urethra, prepuce, or scrotum), sampling method (cytology brush, wet or dry swab), and HPV testing methodology (general or type specific primer systems) [10, 42]. In a recent systematic review, Smith and colleagues [43] estimated HPV prevalence to be between 1% and 84% in low-risk sexually active men, and between 2% and 93% in high risk men. Unlike the situation among females where cervical HPV infection declines substantially after about 30 years of age, prevalence of HPV infection in males generally remains constant or declines

only slightly with age after peak prevalence [43]. One possible explanation for this is that men experience a higher rate of reinfection compared to women. Among circumcised men, the penile shaft and glans are often the most common sites of genital HPV infection [44, 45] whereas for uncircumcised men, it is the foreskin [46].

In addition to penile and cervical HPV, anal HPV is also very common in both genders. In a recent study of Human Immunodeficiency virus (HIV) negative men who have sex with men (MSM), anal HPV infection was detected in 57% of participants [47]. However, among heterosexual men, prevalence of anal HPV infection is generally less than 10% [48]. In studies focusing on women, prevalence and incidence of anal HPV infection is often equal to, or greater than infection with cervical HPV [49-51]. Finally, oral HPV, despite being less common than anogenital infections in adults, should not be overlooked as an important infection site due to its frequent association with many oropharyngeal cancers [52, 53].

Sexual Activity and Other Risk Factors for HPV Infection

Mucosotropic HPVs are highly sexually transmissible in both genders [54-56]. The median per-act transmission probability is estimated to be 40%, which suggests that an infected partner will almost definitely transmit their infection over multiple rounds of sexual intercourse (99.6% probability within 11 sex acts) [56]. Epidemiologic studies have consistently reported markers of sexual activity, including number of recent/lifetime sexual partners and age at sexual debut to be among the most important risk factors for

HPV infection [24, 57-59]. Although age at sexual debut is often strongly associated with other sexual behaviours, it may also be a true causal risk factor for HPV due to greater cervical ectopy during adolescence [60]. In addition to peno-vaginal intercourse, HPV is also transmitted by other sexual practices, including peno-anal intercourse, oral sex, and digital-vaginal sex [59, 61, 62]. There is some evidence that transmissibility may vary by HPV genotype, with HR-HPV types being more strongly associated with sexual behaviour than LR-HPV types [58, 63-65]. HPV may also be transmitted during childbirth from the cervix of infected mothers to the oropharyngeal mucosa of their children, with higher likelihood of transmission occurring for vaginal delivery compared with cesarean section [66].

Independent of sexual activity markers, other factors related to HPV infection or persistence include young age, socioeconomic status, multiparity, circumcision, condom use, oral contraceptive use, smoking, nutrition, immune suppression, viral load, certain genetic polymorphisms in the human leukocyte antigen system, as well as factors associated with the virus itself (e.g., type, variant, methylation status) [19, 22, 49, 51, 57, 59, 60, 65, 67-75]. Presence of pre-existing HPV infection(s) is also associated with increased risk of acquiring infection with other HPV types [76-78]. With regard to condoms, a paradoxical effect has sometimes been reported such that condom use appears to increase risk of HPV infection [58, 79, 80], likely a result of higher probability of infection from partners with whom condoms are used and higher probability of transmission per single act of intercourse [56, 81-83]. However, when used consistently among partners of newly sexually active women, recent data suggest that condoms

may reduce (but not eliminate) the risk of male-to-female genital HPV transmission [84]. There is also evidence that male circumcision reduces the risk of HPV infection among men, which in turn lowers the risk of subsequent transmission and infection in their partners [75, 85-87].

Using an experimental system, investigators at the National Institute of Health (NIH) recently demonstrated that Papanicolaou (Pap) cytology, a common test that is used in screening for cervical cancer, actually increased the number of HPV (pseudovirus) infectious events in a series of female rhesus macaques [88]. Despite histological similarities between the macaque and human cervix and strong biologic rationale to support their findings, i.e., physical trauma that occurs during specimen collection leading to exposure of the basal layer of the genital tract to HPV, results from this study should not immediately be interpreted as evidence that Pap tests increase the risk of HPV infection. As the study authors point out, it is possible that the increase may be transient and that the same trauma which leads to an increase in HPV infection also stimulates the immune system to recognize and combat these infections [88]. Ultimately, further studies are needed to adequately address this question and to determine whether frequent Pap testing has contributed to the rise in cervical adenocarcinoma that has been observed in many screened populations [89, 90].

In recent years, the incidence of oral HPV and related cancers has also increased in the United States [91]. Researchers have identified a strong association between lifetime oral HPV and oral/oropharyngeal cancers [53, 92]. Since oral HPV is

generally transmitted through oral sex or open mouth kissing [93], it is common to presume that this increase in disease burden is related to an increase in oral sexual behaviours (e.g., lifetime number of oral sex partners) among adolescents [94, 95]. Unfortunately, data on these types of sexual behaviours are largely unavailable, which makes it difficult to empirically verify this time trend assumption.

HPV Infection and Risk of Cervical Cancer and Other Diseases

In 1995, the International Agency for Research on Cancer (IARC) first classified HPV types 16 and 18 as carcinogenic to humans, but based on more recent evidence, the list of carcinogenic HPV types has been expanded to include a total of 13 mucosotropic anogenital HPV types as being definite or probable carcinogens (grade 1 or 2a) based on their frequent association with invasive cervical cancer (ICC) and cervical intraepithelial neoplasia (CIN) (see Table 1 for HR-HPVs) [9]. The oncogenic types (mostly HPV16) are also causally implicated in other cancers, including penile, anal, vulvar and vaginal cancers [96, 97]. The remaining genital types (e.g., HPV types 6, 11, 42, 43, 44, and some rarer types) are considered to be of low or no oncogenic risk [98, 99]. However, these types may cause subclinical and clinically visible benign lesions known as flat and acuminate condylomata, respectively.

In descending order, the most common HPV types implicated in cervical cancer globally are: 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, and 56 [5]. HPV types 16 and 18 are the most dominant types implicated in cervical cancer in all continents, being

responsible for ~70% of ICC cases globally. Substantial variation exists between regions for the other HR-HPV types listed here. In many studies, estimating the fraction of cervical cancer cases attributable to the different HPV types is difficult due to the high prevalence of multiple type infections. For example, a recent meta-analysis based on genotyping information from 30,848 cases of ICC, estimated the prevalence of co-infection (≥ 2 HPV types) in tumour specimens at 15.7% [5]. Other recent meta-analyses and cross sectional studies evaluating the worldwide distribution of HPV infections consistently reveal the same HPV prevalence patterns (Figure 1) [32, 100]. This widespread circulation of HR-HPV types strengthens the potential for a phenomenon known as HPV type-replacement, i.e., an increase in other non-vaccine types following HPV vaccination. However, based on evidence that HPVs evolve very slowly and that HPV types do not normally compete with one another during natural infection [76-78, 101-109], it is still unlikely that some other HPV type(s) will evolve to fill the niche currently occupied by vaccine target types. Furthermore, phase III trials evaluating both bivalent and quadrivalent vaccines indicate partial cross-type protection (cross-immunity) against many phylogenetically related HPV types [3, 110, 111], suggesting that the benefit from vaccination may be even greater than expected.

Infection with oral HPV is also now recognized as an important cause of oral and oropharyngeal cancers [91]. However, unlike cervical cancer in which 100% of cases are attributable to infection with HPV, only 25-35% of these cancers are attributable to HPV [112, 113]; the major risk factors being alcohol and tobacco use. Among cases of oral/oropharyngeal cancer linked to HPV infection, HPV16 is by far the most common

type detected in tumour specimens [53, 114]. In the largest study conducted on this topic to date (a case-control study that included 1 600 cases and 1 700 controls from 9 countries), HPV16 was found in 95% of HPV positive cases [114]. Based on evaluation of risk factor profiles for cancers of the head and neck, comparing HPV16-positive and HPV16-negative cases, some researchers have decided that these should actually be considered distinct cancers [115]. In their study, sexual behaviour (but not alcohol or tobacco use) was an important predictor of head and neck cancers among HPV16-postive subjects, meanwhile the opposite was observed for HPV16-negative subjects. In addition to oral and oropharyngeal cancers, HPV is also an important, albeit not a necessary cause of other cancers, e.g., 90% of anal cancers, 40% of penile cancers, and 40% of vaginal or vulvar cancers are attributable to HPV [112, 113].

Persistent HPV Infection and Cervical Carcinogenesis

Most cervical HPV infections clear spontaneously without ever causing lesions. Only a small proportion of infections (10-30%) will persistent beyond 1 or 2 years. Data from cohort studies indicate that the average length of infection is between 4 and 20 months, with HR-HPV types lasting longer than LR-HPV types [22-27]. Numerous cohort studies have confirmed that risk of CIN and ICC is strongly associated with persistent infection with HR-HPV types [30, 116-120]. As a result, persistent infection with at least one HR-HPV type is now well established as a key intermediate step in the etiologic pathway to cervical carcinogenesis.

Following persistent infection with HPV, the process of carcinogenesis progresses with disruption of the normal maturation of the transformation zone epithelium of the uterine cervix. These abnormal changes lead to pre-invasive lesions (dysplasia) that are often asymptomatic and discovered only by cytological examination during Pap smear screening. If these low- and high-grade lesions are left untreated they may grow and eventually cross the epithelium to connective tissue border formed by the basement membrane to become invasive. But until invasion occurs, the entire stepwise precancerous lesion process is reversible. In fact, for the majority of women infected with HPV the infection will clear and precancerous lesions will regress; only approximately 1% of low-grade lesions (CIN1) and 12% of high-grade lesions (CIN3) will progress to become invasive if left untreated [121]. However, in the event that precancerous lesions are not detected by screening or do not regress on their own, without effective treatment the invasive cancer will invariably grow to reach blood and lymphatic vessels and become metastatic. Unfortunately, we are unable to predict with certainty which individuals with high-grade lesions will progress to invasive cancer; therefore, despite the low progression rate, all females with high-grade lesions should be treated.

Currently, much less is known about the natural history of other HPV related cancers in comparison to cervical cancer. But when considering the worldwide burden of cervical cancer compared to other HPV related cancers and that HPV infection is often just as common at other sites (e.g., the anus, penis, vulva, and vagina), this

suggests that the cervix is much more susceptible to HPV-induced carcinogenesis [122].

Burden of Cancer Caused by HPV: Cervix and other sites

After breast and colorectal cancer, cervical cancer is the 3rd leading cancer site worldwide irrespective of gender and second among women. In 2008, there were an estimated 530,000 cases and 270,000 deaths attributed to ICC, with 86% of cases and 88% of deaths occurring in developing countries [123]. In these developing countries, the age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) were 18 and 10 per 100,000 women, respectively; whereas in more developed countries, the ASIR and ASMR were 9 and 3 per 100,000 women, respectively. Globally, incidence of ICC ranges from < 3 to > 50 cases per 100,000 women for low- and high-burden countries, respectively (Figure 2) [123]. These differences between countries are believed to reflect protection from screening, and variance in exposure to HPV and other cofactors like smoking and oral contraceptive use, and other sexually transmitted infections such as human immunodeficiency virus [123].

The global burden of other HPV related cancers is also substantial. Worldwide, approximately 97,215 cases of noncervical cancers for which HPV infection may be an etiologic factor are diagnosed annually; roughly 50,780 in men (520 penile, 26,775 oropharyngeal, and 13,485 anal cancers) and 46,435 in women (25,600 vaginal/vulvar, 6048 oropharyngeal, and 14,787 anal cancers) [124]. However, it is important to

recognize that not all of these cases are attributable to HPV and that these estimates represent the upper limit for the annual burden of cancers caused by HPV. Recall that roughly only a quarter of oropharyngeal cancers are attributable to HPV; meanwhile approximately 90% of anal cancers, and 40% of penile, vaginal or vulvar cancers are attributable to the virus. Although there is some evidence implicating HPV with several other cancers (e.g., lung, colon, ovary, breast, prostate, urinary bladder and nasal/sinonasal cancers), current molecular and epidemiological data are sparse and do not yet support a causal role for HPV in the etiology of these cancers [112, 113, 125].

Globally, HPV accounts for roughly 5.2% of the total cancer burden –the highest among all infectious agents. However, as may be expected, the distribution varies considerably according to country development status, where HPV accounts for approximately 7.7% and 2.2% of all cancer cases in developing and developed countries, respectively [113]. Cervical cancer is the major HPV related cancer contributing to cancer burden in developing countries whereas in more developed countries such as the United States, the burden of non-cervical cancers now approximates that of cervical cancer only (Figure 3) [124].

Current and Future Opportunities for Prevention

The discovery of HPV infection as a necessary cause of cervical cancer has created many new paths for prevention. The most promising strategies include

screening for infection with HR-HPV types and immunization to prevent infection with HR-HPV types.

Pap cytology screening, which has over 50 years of history in medicine, is considered the primary reason we have witnessed a major reduction in cervical cancer mortality in most high-income countries [126, 127]. But in spite of its successes, the Pap test is far from perfect. The average sensitivity of cytology to detect CIN is 51% and its average specificity is 98% [128, 129]. The Pap test's low sensitivity is a reflection of its highly subjective nature, as it is based on interpretation of morphologic alterations present in cervical samples. The high false-negative rate is a severe limitation which has important medical, financial, and legal implications. In the United States, false-negative Pap smears are one of the most common reasons for medical malpractice litigation [130]. Liquid based cytology (LBC) has improved the efficiency of smear processing, but does not improve sensitivity of the test [131]. To bring screening program sensitivity to an acceptable level, Pap tests in Canada and the United States tend to be done annually, which is a costly endeavour. For nations with opportunistic or organized cervical cancer screening programs, management and follow-up of patients with detected abnormalities places a substantial burden to the health care system. At this time, roughly one in ten Pap smears processed by cytotechnicians in the United States is positive for abnormalities, which require either additional follow-up or treatment [130]. Unfortunately, many developing countries that have invested into screening programs have yet to witness a substantial reduction in cervical cancer. Poor education

of healthcare workers, and a lack of costly safeguards to ensure high coverage, compliance and quality are often cited as the cause for this failure [132].

Recently, HPV DNA testing has been suggested as an alternative to primary screening using Pap cytology, perhaps reserving the latter for the triage of HPV positive cases [133]. Compared to Pap, HPV DNA testing is less dependent on quality of personnel training and is much more objective. Individual randomized controlled trials (RCTs) [134-145] and two recent pooled analyses [129, 146] comparing the accuracy of HPV testing against Pap cytology found the former to be much more sensitive but less specific in detecting high grade cervical precancerous lesions (CIN grade 2 or higher). Although results from these and other ongoing RCTs provide the information that is necessary to compare accuracy, it will probably not be sufficient at this point in time to convince most policymakers to adopt HPV testing as the primary screening test. Eventually, results from demonstration projects evaluating the safety of extending screening intervals using HPV testing, as well as lowered HPV test costs resulting from high volume testing and market expansion, may eventually be enough to persuade policymakers to make the change. Also, with the added cost of vaccination to cervical cancer prevention programs there will be added pressure to recommend HPV testing in order to maintain cost-effective cervical cancer screening in the era of HPV vaccination [133].

In developing countries, where Pap screening has had little success, there is renewed hope that less frequent screening using HPV DNA testing may finally help

reduce mortality from ICC [147]. In a large RCT conducted in rural India, investigators found that a single round of screening using HPV DNA testing was sufficient to reduce the incidence of advanced cervical cancer and mortality by about half, providing a solid evidence base to support its implementation in other low resource settings. Ultimately, integration of screening with prophylactic HPV vaccination, which currently protects against the most common LR- (HPVs 6 and 11) and HR-HPV types (HPVs 16 and 18), offers the greatest potential to reduce the burden of ICC and other HPV related diseases (Figure 4) [2, 3, 110, 148, 149]. Unfortunately, for many of these nations where the burden of HPV and cervical cancer is the highest, vaccination and HPV testing remains too expensive. Vaccination uptake has also been slow in some developed countries where cost is not as much of a barrier. According to data from the National Health Interview Survey, less than one quarter of preadolescent and adolescent girls (aged 9-17) in the United States had initiated the HPV vaccination series by the end of 2008 [150].

A novel approach to prevention also lies in the potential to inhibit prevalent HPV infection. Recently, investigators from the NIH identified that the compound *carrageenan*, a safe and inexpensive gelling agent derived naturally from seaweed, serves as a potent HPV infection inhibitor [88, 151, 152]. There has been interest in carrageenan as a vaginal microbicide targeting HIV and herpes viruses, but cell culture tests have found that it is a thousand times more effective against HPV than against HIV [151]. Carrageenan has already been shown to inhibit HPV infection in mice [152] and monkeys [88]. In the same set of experiments described above in which Pap was

shown to increase HPV infection in rhesus monkeys, lubrication with carrageenan gel during an internal digital exam following specimen collection greatly reduced risk of infection [88]. RCTs are currently planned in Canada and the United States to evaluate the efficacy of carrageenan against HPV in human populations (deliverable as a topical microbicide prior to sex) [153].

Prevention Strategies in Developed and Developing Countries

Despite breakthroughs in screening and prevention, cervical cancer remains an important cause of cancer death globally, especially in developing countries where the majority of the burden lies. Although prophylactic vaccination is expected to substantially reduce HPV-associated morbidity and mortality, it currently remains too expensive for introduction in most resource-poor countries [154, 155]. Rwanda recently became the first African country to introduce a national prevention program that includes both HPV vaccination and testing, made possible by donations from the vaccine and HPV test manufacturers, i.e., Merck and Qiagen, respectively [155]. If good vaccine and screening coverage is attainable in this setting, then it would serve as a useful model for other parts of sub-Saharan Africa. However, unless other financing mechanisms become available, it may take many years before similar prevention strategies are introduced in other countries that could benefit the most.

In high-resource nations that already have successful cervical cancer screening programs in place, the addition of vaccination programs is expected to have a major

impact. Pap screening currently leads to the detection and treatment of a large number of low- and high-grade cervical lesions, especially in young women, for whom ablative treatment carries substantial risk of adverse reproductive outcomes including preterm delivery and miscarriage. Prophylactic vaccination of females prior to their sexual debut would prevent a large proportion of these precancerous lesions, cervical cancer, and some other non-cervical HPV-related cancers. Australia recently became the first country to witness a significant decline in the rate of high-grade cervical lesions following implementation of HPV vaccination [156]. However, this reduction may simply be a reflection of lowered screening uptake among young vaccinated women. Future studies that involve linkage between registries are expected to provide us with a better estimate of the benefits of vaccination. Australia is also one of the few countries with a community based catch-up program targeting women up to the age of 26 for vaccination. Most other government programs are exclusively targeting females 12 years of age at this time, and in these settings it is expected to take longer before there is a noticeable decline in cervical abnormalities and HPV related cancers.

In the post-vaccination era, HPV DNA testing will also serve an important second purpose by providing a low cost surveillance approach to monitor vaccine efficacy, protection duration, and cross protection or type replacement. Integration of primary and secondary cervical cancer prevention strategies via record linkage and shared resources inherently lends itself to being treated best as a single prevention strategy. Linkage of registries also provides the necessary data to evaluate the success of prevention strategies and to inform international policies, hopefully putting pressure on

high-income countries, non-governmental organizations, pharmaceutical companies and other donors to provide their support.

Table 2-1: HPV types categorized as carcinogenic in representative studies and reviews

HPV Type	Original taxonomic designation	Lorincz et al., 1992 [98]	Bauer et al., 1993 [157]	Hybrid Capture 2 (Lorincz, 1996[1]) [158]	GP5/6+ (Walboomers et al., 1999[2]) [159]	Roche's line blot assay (Bosch, 1995[3]) [99]	Munoz et al., 2003 [160]	IARC Monograph, Vol. 90, 2005[4] [161]	IARC Monograph Vol. 100B, 2011[5] [162]
16		X	X	X	X	X	X	1	1
18		X	X	X	X	X	X	1	1
26						X	Probable		2B
30									2B (analog)
31		X	X	X	X	X	X	1	1
33		X	X	X	X	X	X	1	1
34									2B (analog)
35		X	X	X	X	X	X	1	1
39			X	X	X	X	X	1	1
45		X	X	X	X	X	X	1	1
51		X	X	X	X	X	X	1	1
52		X	X	X	X	X	X	1	1
53							Probable		2B
55						X			
56		X	X	X	X	X	X	1	1
58			X	X	X	X	X	1	1
59				X	X	X	X	1	1
66					X		Probable	1	2B
67									2B
68				X	X	X	X		2A
69									2B (analog)
70									2B
73	Pap238A, MM9 W13B, MM4, IS39					X	X		2B
82	(subtype)					X	X		2B
83	Pap291, MM7					X			
85									2B (analog)
97									2B (analog)
# types		9	11	13	14	17	15-18	13	25

[1] This HR HPV classification is applied in the Hybrid Capture 2 assay (Digene Co.), a validated diagnostic assay widely used in epidemiologic and clinical studies.

[2] This HR HPV classification is the one used in the polymerase chain reaction (PCR) with GP5/6+ primers, which is used in many international studies of cervical cancer etiology and screening.

[3] This HR HPV classification forms the basis of the PGMY line blot PCR protocol.

[4] This HR HPV classification follows the International Agency for Research on Cancer (IARC) Carcinogenicity Evaluation Monograph, vol. 90.

[5] This HR HPV classification follows the IARC Carcinogenicity Evaluation Monograph, vol. 100 (the indices denote the degree of empirical evidence for carcinogenicity: 1, definite carcinogens, 2A, probable carcinogens, 2B, possible carcinogens)

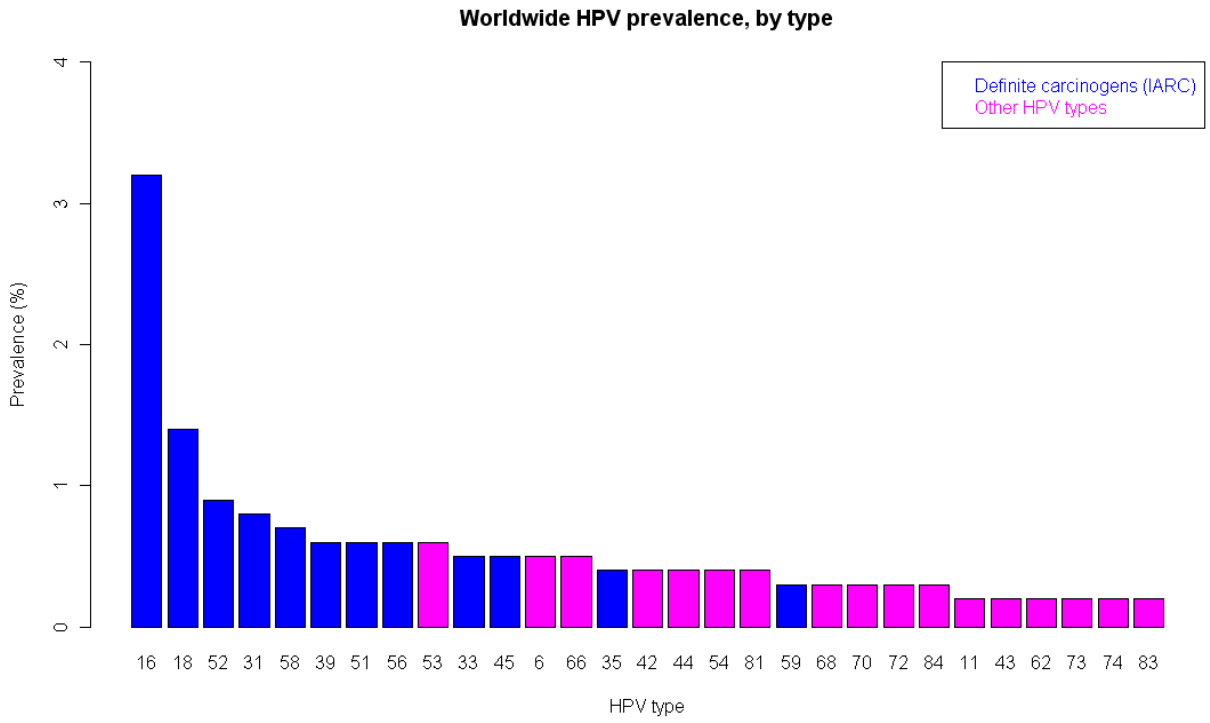

Figure 2-1:

Worldwide Human Papillomavirus (HPV) prevalence, by type, among females with normal cytological findings. Bars in blue indicate HPV types recognised by the International Agency for Research on Cancer (IARC) as definite carcinogens, whereas the other HPV types are depicted with pink bars. Data source: Bruni et al., 2010, JID [32].

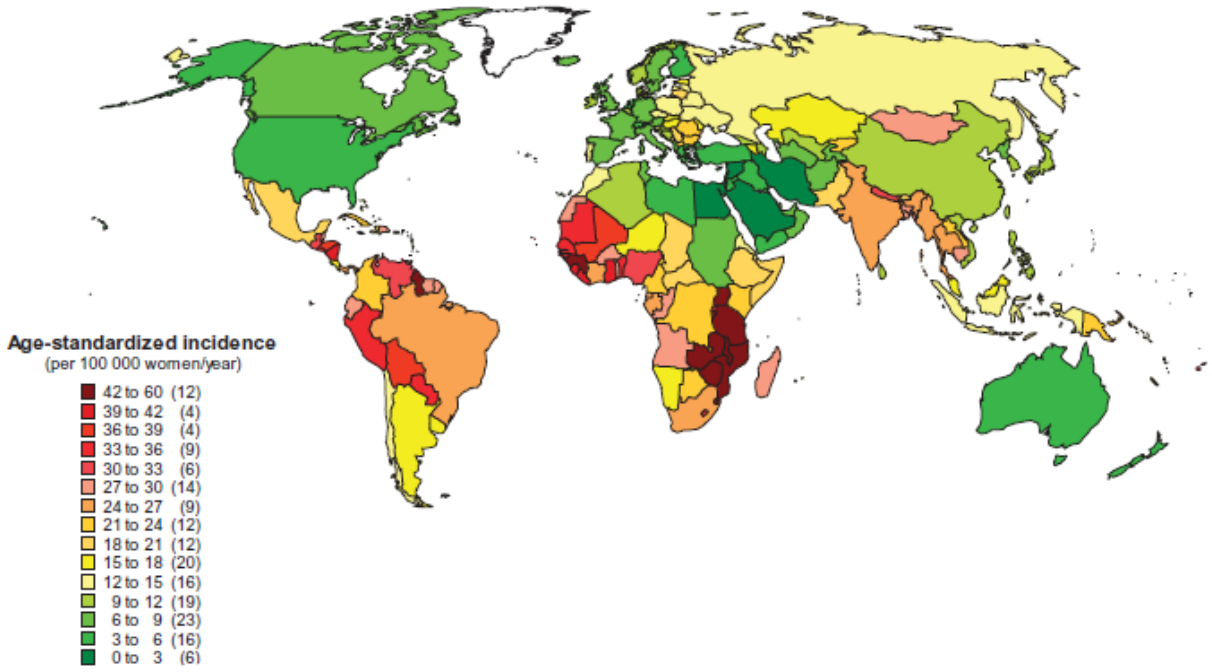

Figure 2-2:

Global view of the age-standardized incidence rates (ASIRs) of cervical cancer within each country as estimated by GLOBOCAN 2008. In the legend, the numbers in parentheses indicate the number of countries in each range of ASIRs. Adapted with permission from reference 116 (Arbyn et al., Ann Oncol, 2011).

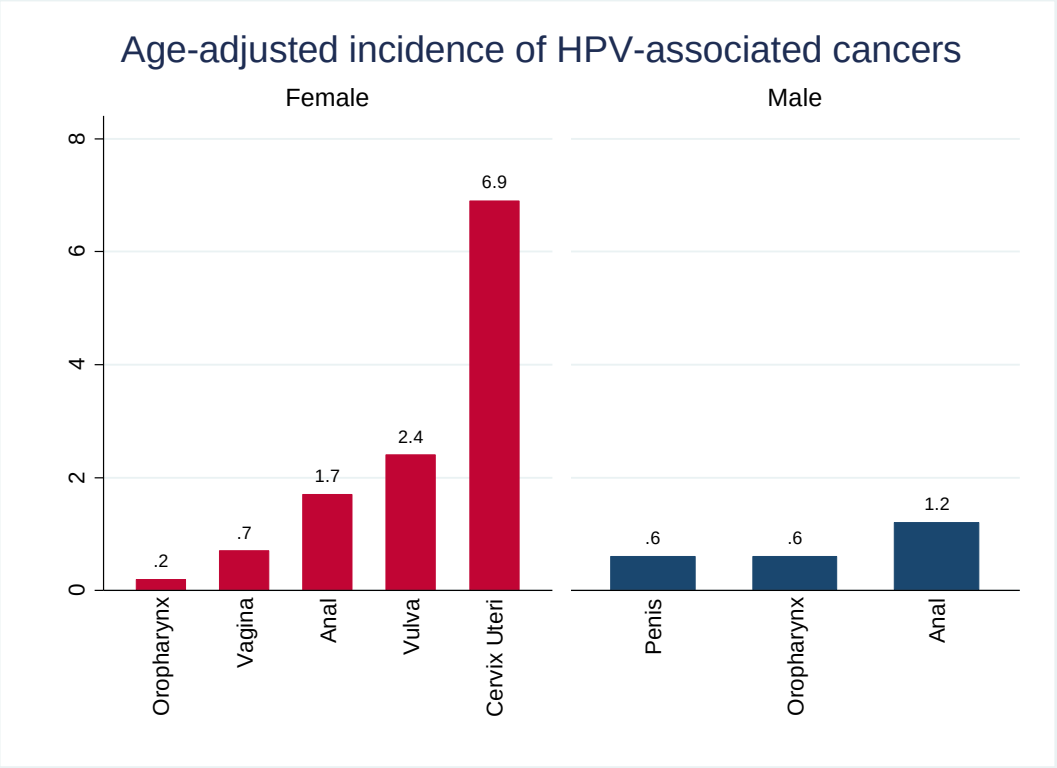

Figure 2-3:

Age-adjusted incidence rates (rate per 100 000) for malignant HPV-associated cancers in men and women for the year 2006. Rates are standardized to the 2000 United States population and include all ages and races. Data obtained from the Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER 9 Regs Research Data, Nov 2010 Sub (1973-2008), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission.

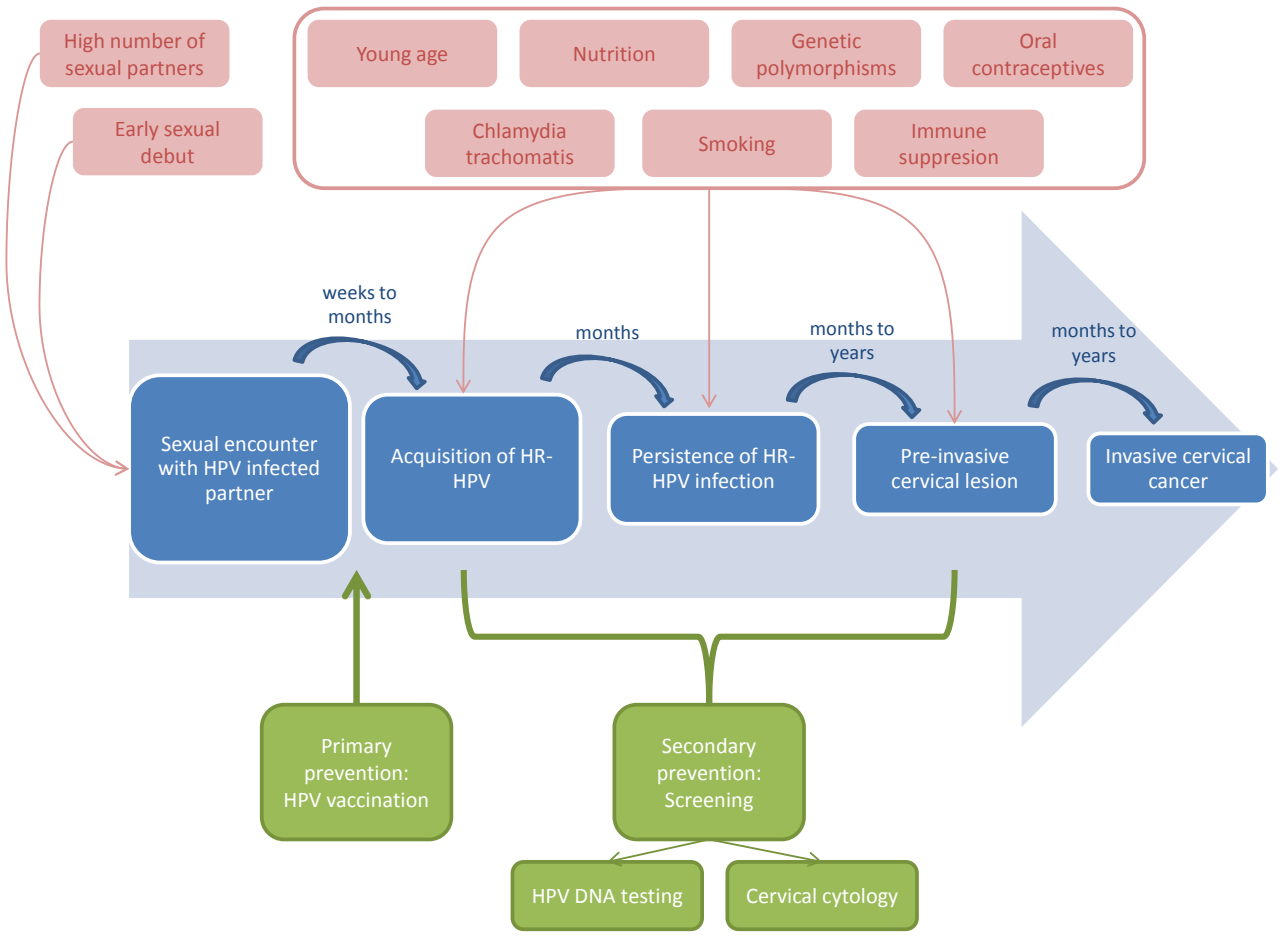

Figure 2-4:

The natural history, risk factors, and opportunities for prevention of cervical cancer (HPV: Human Papillomavirus; HR-HPV: High oncogenic risk HPV). The blue boxes depict the natural history of cervical cancer carcinogenesis, from exposure to HPV, to acquisition and persistence of the infection, to pre-invasive lesions that may progress to invasive cervical cancer (ICC). For each step leading to ICC, only a fraction of cases progresses to the next step, whereas the majority will regress. The salmon boxes above highlight some of the major risk factors for the initial contact with HPV and for the progression to subsequent steps. The green boxes below indicate where opportunities for prevention lie.

2.2 HPV DNA and RNA testing

In the context of this project, high accuracy of HPV DNA testing is required to prevent information bias (measurement error) in our evaluation of HPV type interactions. At this point it is important to distinguish microbiologic sensitivity and specificity as it applies here (i.e., our ability to accurately detect or not detect HPV type(s) using a particular PCR assay when specific virus type(s) are present or not present, respectively) from screening sensitivity and sensitivity. In screening, sensitivity and specificity of HPV DNA testing generally refers to our ability to accurately detect or not detect high-grade precancerous cervical lesions using clinically-calibrated assays for high-risk HPV genotypes when these lesions are present or not present, respectively.

The Hybrid Capture® 2 assay (HCII, Qiagen, Gaithersburg, MD) is currently most common commercial HPV test being used in clinical and screening settings. It is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection in cervical specimens of HPV DNA of 13 high oncogenic risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). If positive, the HCII assay does not permit ascertaining the individual type or types that are present in the specimen, i.e., it does not permit HPV typing. All HPV screening assays detect only HR-HPV genotypes, with some, such as the Cobas® test (Roche Molecular Systems, Pleasanton, CA) and the Cervista® HPV16/18 test (Hologic, Inc., Bedford, MA) capable of detecting HPVs 16 and 18 individually, and a pool of 12 other

HR-HPV types. Different PCR protocols have also been used to detect HPV and permit typing. PCR protocols are based on target amplification with type-specific or consensus primers followed by hybridization with specific oligoprobes. Unfortunately, PCR does not always amplify different DNA segments with equal efficiency and as a result, reduced sensitivity of consensus primer PCR for detection of certain HPV types (e.g., HPV52) in co-infected specimens has been reported [163-166]. This limitation of consensus PCR is what motivated us to evaluate masking of HPV52 by HPV16 (objective 3), using type-specific real-time HPV52 PCR, which is capable of detecting as few as 10 HPV52 copies per assay [167].

In epidemiologic studies, the most widely used PCR-based methods employ consensus primers that are capable of amplifying a wide spectrum of HPV types. The MY09/MY11 protocol consists of a set of degenerate primers complementary to sequences in the L1 gene. Detection of HPV DNA using this particular PCR protocol is very sensitive, especially in comparison with older HPV DNA tests (ViraPap® and Southern Blot) [168], and has very good specificity and reproducibility [169]. Inter-laboratory and intra-laboratory agreement (based on repeat analysis of the same specimen) was observed to be 88% and 96%, respectively [169]. Recently, the MY09/MY11 primers were redesigned to improve the amplification of some HPV types and increase the sensitivity and reproducibility of the method [163, 170, 171]. While this revised PGMY09/PGMY11 PCR protocol did lead to a considerable increase in the detection of multiple infections, overall agreement between these two methods was still found to be good to very good (kappa range= 0.68-0.83) [163, 170, 171]. It should be

noted that PCR assays based on MY09/11 are not currently being used in clinical or public health practice, but only for research.

In addition to HPV DNA tests, commercial HPV RNA tests are also now available to detect HPV mRNA transcripts coding for E6/E7 and presence of oncogene activity [172, 173]. For example, the APTIMA® HPV assay (Hologic Gen-Probe, San Diego, CA) targets E6/E7 mRNA of 14 high-risk HPV types and has already received regulatory FDA and Health Canada approval. Recently, APTIMA® was compared with other approved tests (e.g., HCII, PCR genotyping, liquid based cytology) for the detection of high-grade precancerous cervical lesions and was found to have the best sensitivity/specificity balance, as measured by area under ROC curve [174]. Due to its greater sensitivity (compared to cytology), HPV E6/E7 mRNA testing is now being considered as an alternative to Pap for primary cervical screening, and due to its greater specificity (compared to DNA testing), it is also now being considered as a triage test for HPV DNA testing [174-176].

2.3. Prophylactic HPV vaccination

Vaccination against HPV types 16 and 18 is highly effective in preventing infection by these types and cervical lesions in previously-uninfected females [2, 3, 177-179]. Two vaccines [Gardasil® (Merck and Co., Inc., Whitehouse Station, NJ, USA) and Cervarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium)] were evaluated in randomized controlled trials, and both have now been approved for use in Canada. Both

were nearly 100% effective in preventing new infections and the cervical precancerous lesions associated with the vaccine-target types in susceptible women [122, 179, 180]. Only Gardasil targets additional HPV types 6 and 11, which cause most cases of anogenital warts [179].

There is now compelling evidence in support of universal vaccination of pre-teen girls in high-income countries [181-184]. Furthermore, results from another clinical trial also support vaccination of males at high risk for HPV infection (e.g., men who have sex with men) for prevention of external genital lesions and other HPV related diseases, including cancer [148, 185]. Unfortunately, for nations where the burden of HPV and cervical cancer is the highest, vaccination is still not readily available for most individuals [186-189]. In addition, HPV vaccination is exclusively prophylactic, i.e., it will prevent infections by the vaccine-target types in those who have not yet been sexually exposed, particularly pre-adolescent women [190].

The possibility of type replacement is an important concern; however, if cross-protection against phylogenetically related HPV types not being targeted by vaccination is observed to be strong and long lasting, then it is unlikely that these types will increase in prevalence, regardless of whether they naturally compete with vaccine-targeted types [12, 191]. In a recent large meta-analysis evaluating cross-protection of the two vaccines, both were shown to offer protection against one or more non-vaccine HPV types among the HPV-naive subjects [192]. In general, greater efficacy estimates against persistent infection and pre-invasive cervical lesions associated with HPVs 31,

33, and 45 were observed for Cervarix than for Gardasil [192]. Little evidence of cross-protective efficacy was established against the remaining HPV types evaluated (i.e., HPV types 52 and 58). Additional studies evaluating the long-term duration of cross-protection will be important to determine the ultimate benefits of vaccination against oncogenic HPV types not being targeted by either of the current vaccines.

Previous studies evaluating clustering patterns between different HPV types, or that have used cohort data to assess sequential acquisition/clearance of different HPV types according to infection with other HPV types, have not provided any compelling evidence to support the idea of type competition (refer to section 2.4, below) [12]. Since this biologic prerequisite appears to be missing, the risk of type replacement may be considered low at this time. In addition, there have already been reports of reductions in vaccine-targeted HPV types in the U.S., Scotland and Australia, as well as cross-protection against related types in vaccinated populations; however, these same studies also revealed increases in other types [193, 194]. Focussing on HPV types classified as definite or probable carcinogens [9], the American study reported slight increases in the population prevalence of HPV types 52 and 68 [193], whereas the Scottish study revealed increases in types 51, 56, 59, and 68, with types 51, 52, and 56 becoming the most prevalent among vaccinated individuals [194]. It is important to point out that all increases were minor, not statistically significant, and that ‘unmasking’ (as described in the previous section) may have actually been the cause [12]. Continued surveillance of the prevalence of different HPV types (pre- versus post-vaccination) will be important.

2.4. Update on the literature focusing on HPV type interactions among unvaccinated females

Until recently, very few studies had investigated HPV type interactions in cross-sectional studies, or the natural history of infection with individual HPV types (i.e., whether infection with one HPV type modifies the risk of acquisition or clearance of another HPV type) in cohort studies to gain insight regarding the likelihood of HPV type replacement [76-78, 101-105, 195-199]. Table 2-2 provides a summary of relevant cross-sectional and cohort studies focusing on associations between individual HPV types or between HPV types and subsequent acquisition/clearance of other types, respectively. Many of these studies are also described in the second manuscript of this thesis.

Based on evidence from vaccine trials and other epidemiologic studies (published prior to February/2010), the general consensus among experts at the 2010 EUROGIN conference, which I attended in Monaco, was that type replacement is unlikely to occur [200]. Since then, many scientists have continued to evaluate HPV co-infection patterns in the context of type replacement, i.e., by focussing on co-infection patterns involving vaccine types. However, no cohort studies have been published designed specifically to evaluate HPV type competition between all vaccine-targeted HPV types (6, 11, 16 and 18 separately) with other types among females. In addition, none of the prior cross-sectional studies utilized the same Bayesian analytic approach (incorporating shrinkage to improve precision of pairwise associations) [12] that I

applied in the current project, or had access to as large of a sample (>38,000 cervical specimens with valid HPV testing results).

As we continue to wait on results from long-term surveillance studies comparing the HPV type distribution in vaccinated populations (before and after vaccination), careful analysis of epidemiological study data may help to identify HPV types that should be considered suspicious for replacement and perhaps targeted by second generation HPV vaccines. But more likely, these studies will provide us with additional reassurance that type replacement should not be expected, and shed light on the possibility that any apparent increases in HPV types post-vaccination may be attributed to diagnostic artifacts [12, 153].

Table 2-2: Summary of previous cross-sectional and cohort studies evaluating associations between HPV types

Reference (study design)	Population (sample size)	Association	Results	Conclusions or Additional notes
Thomas, 2000 (cohort)	U.S. female university students (n=518)	Association between types acquired sequentially (only tested for HPV types 6, 11, 16, 18, 31, and 45	No negative associations between current infection with any HPV type and acquisition of others	-Observed concurrent acquisition occurred more than expected, but no differences by HPV type
		Concurrent acquisition of multiple HPV types	No. visits females concurrently acquired 2 or 3 types was greater than expected	-Risk of acquiring new HPV types not associated with previous infection type
		Associations between types acquired together	Associations between HPVs 18, 31 and 6 were all positive	
		Sequential acquisition of multiple HPV types	No difference between observed and expected acquisition among females infected with another type	
Liaw, 2001 (cohort)	Females attending gynecology clinics in the U.S; all with normal cytology (n=1124)	Type specific comparison of acquisition/clearance of new/existing HPV infections among females HPV16+ vs. HPV16- at baseline	Infection with HPV16 at baseline was positively associated with acquisition of others, but not associated with clearance	-HPV16 infection was generally associated with increased risk of subsequent infections

Rousseau, 2001 (cohort)	Brazilian females (n=1860)	Comparison of acquisition/clearance of new/existing HPV infections among females infected with other HPV types at baseline	Highest risk of HPV acquisition among those with baseline HPV16/18 infection. In type specific analyses, no significant negative associations reported (acquisition), or positive associations (clearance).	-Acquisition of HPV (any and HR types) was more likely among those with HPV infection at baseline. Persistence of HPV infection was independent of co-infection with other HPV types
Mendez, 2005 (cohort)	Colombian females (n=1857)	Observed versus expected number of HPV types at each visit	Concurrent acquisition of multiple types occurred > expected	-No evidence of type competition in cohort analysis, but study lacked power
		Association between baseline HPV infection and type specific acquisition of certain other HPV types: 16,18,31,33,39,45,52,58	No significant negative associations. Infection with HPVs 6/11/16/18 (grouped together) sig. associated with higher risk of HPVs 18 and 58	
Chaturvedi, 2005 (cross-sectional)	U.S. females (n=854 HIV-; n=275 HIV+)	Involvement of specific species groups in co-infections	HPV types from α-9 species less likely than others to be a part of co-infection: OR=0.68, 95%CI 0.48-0.95	-α-9: only species that was negatively associated with multiple infections
		Ratio of observed versus expected (O/E) for single and multiple (2, 3 and 4+) infections	1: O/E=.67(.59-.76) 2: O/E=.78(.64-.95) 3: O/E=1.56(1.15-2.13) 4+: O/E=6.91(4.8-9.7)	

Plummer, 2007 (cohort)	Females from ASCUS/LSIL triage study (n=4504)	Comparison of acquisition of new HPV infections among females infected with other HPV types at baseline	No significant negative associations. Significant positive association observed betweens HPV16/31	-HPV infections generally occur independent of one another
Mejlhede, 2010 (cross-sectional)	Danish females with suspected cervical HPV infection (abnormal cytology results) (n=3588)	Association between HPV infection (>350 HPV combinations evaluated)	Many statistically significant positive and negative associations ($p<0.05$); HPV16/51 only combination significant at 0.01 level	-Negative association between HPV16 and 51 suggests that this non-vaccine type should be monitored for replacement
Vaccarella, 2010 (cross-sectional)	Pooled (female) IARC prevalence survey results (n=13961)	Pairwise associations between HPV infections ($p<0.01$) Ratio of observed versus expected (O/E) for single and multiple (2, 3+) infections	Positive associations: HPVs 33/35, 33/58, 33/39, 18/45, 31/35 Negative associations: HPVs 16/81 1: O/E=.67(.65-.69) 2: O/E=1.62(1.49-1.74) 3+: O/E=6.43(5.3-7.6)	-Results differed by genotyping method (EIA vs. line blot), therefore clustering of these HPV types was attributed to a diagnostic artifact and not true biological interaction
Vaccarella, 2011 (cross-sectional)	Female (baseline) data from Guanacaste cohort study (n=8365)	Pairwise associations between HPV infections ($p<0.01$) Ratio of observed versus expected (O/E) for single	Positive associations: HPVs 62/81 Negative associations: HPVs 51/71 1: O/E=.66(.64-.68) 2: O/E=1.17(1.09-1.24)	-Single observed positive association may be explained by diagnostic artifacts (cross-hybridization)

		and multiple (2, 3+) infections	3+: O/E=3.61(3.2-4.1)	
Chaturvedi, 2011 (cross-sectional)	Baseline data from females enrolled in Costa Rica vaccine trial (n=5871)	Pairwise associations between HPV infections (Bonferroni-corrected: p<0.0001)	Positive associations: HPVs 11/53, 31/33, 34/42, 45/68, 45/73 Negative associations: HPVs 44/68, 44/73, 18/33	-Despite the higher frequency of multiple-type HPV infections, the authors concluded that HPV types come together at random i.e., infections occur independently
		Ratio of observed versus expected (O/E) for single and multiple infections	1: O/E=.73(.69-.77) 2: O/E=.86(.79-.93) 3: O/E=1.37(1.21-1.55) 4: O/E=2.7(2.2-3.3) 5: O/E=6.6(4.7-9.1) 6: O/E=15.8(8.2-27.6) 7: O/E=23.7(2.9-85.6) 8: O/E=243.9(30-881)	
Vaccarella, 2013 (cross-sectional) *Published in IAC* *Note: I reviewed this manuscript and pre-publication history is available online*	HIV+ females from Kenya (n=498)	Pairwise associations between HPV infections (p<0.00005 or p<0.01)	Positive/negative associations: none	-HPV co-infections occur at random among HIV-positive women, therefore type replacement is not suspected
		Ratio of observed versus expected (O/E) for single and multiple (2, 3+) infections	1: O/E=.86(.84-.89) 2: O/E=.90(.86-.96) 3+: O/E=1.07(.93-1.22)	
Vaccarella, 2013 (cross-sectional)	Females from the Swedish High Throughput HPV	Pairwise associations between HPV infections (Bonferroni adjusted	Positive associations: HPVs 6/18 (p<.00004); HPVs 56/66 (p<.01)	-Significant negative associations involving HPV68 may be

Published in PLoS One *Note: I originally reviewed this manuscript for JID*	Monitoring (HT-HPV) study (n=33137 specimens; number of females unknown)	p<0.00004; or p<0.01) Ratio of observed versus expected (O/E) for single and multiple (2, 3+) infections	Negative associations: HPVs 51/68, 6/68 (p<.00004); HPVs 18/35, 35/66, 6/58 (p<.01) 1: O/E=.70(.69-.70) 2: O/E=.95(.93-.97) 3+: O/E=1.95(1.9-2.0)	attributed to diagnostic artifacts -Lack of negative and positive clustering suggests elimination of some HPV types is unlikely to have major effect on occurrence of other types
Mollers, 2013 (cross-sectional)	Pooled analysis of 3 female HPV monitoring studies (Nijmegen population study; Chlamydia Screening Intervention (CSI) study; STI clinics study) conducted in the Netherlands (n=3874)	Pairwise associations between HPV infections (p<0.05) Ratio of observed versus expected (O/E) for single and multiple (2, 3, 4+) infections	Positive/negative associations: HPVs 31/33, 31/44, 31/58, 42/70, 43/58, 58/59, 68/74, 53/54 Nijmegen: 1: O/E=.69(.51-.96) 2: O/E=1.82(.92-3.70) 3: O/E=2.53(.89-7.60) 4+: O/E=28.7(6.5-128) CSI study: 1: O/E=.72(.70-.76) 2: O/E=.80(.72-.91) 3: O/E=1.11(.89-1.42) 4+: O/E=1.84(1.2-2.8) STI clinics: 1: O/E=.82(.82-.99) 2: O/E=.70(.70-.77) 3: O/E=1.02(.86-1.24) 4+: O/E=1.37(.95-2.02)	-HPV genotyping algorithm (diagnostic artifacts) may explain some of the type-specific differences in their affinity to cluster -High-risk HPV types more likely to cluster together than low-risk types -Authors suggest there is no reason to suspect negative effects of vaccinating against only a limited set of HPV types

CHAPTER 3: STUDY OBJECTIVES

This project focuses on evaluating HPV type competition (using different epidemiological approaches) to inform the possibility of HPV type replacement post-vaccination. Another focus is to explore the potential for diagnostic artifacts in future surveillance studies evaluating HPV type replacement. Specifically, the objectives of this project were:

- To evaluate whether infection with any nonvaccine HPV types is associated with infection with vaccine-target types (MANUSCRIPT III);

- To evaluate whether pre-existing infection with vaccine-targeted HPV types affects acquisition and/or clearance of other HPV types (MANUSCRIPT IV);

- To evaluate the putative masking of HPV52 by HPV16 in amplification HPV DNA assays, which may be helpful in distinguishing artifactual from true type replacement in future studies (MANUSCRIPT V).

CHAPTER 4: METHODS/MANUSCRIPT II

EPIDEMIOLOGICAL APPROACHES TO EVALUATING THE POTENTIAL FOR HUMAN PAPILLOMAVIRUS TYPE REPLACEMENT POSTVACCINATION

4.1 Preamble

This second manuscript from my thesis provides the epidemiologic basis to assist investigators in different countries to use observational epidemiological data to verify whether different HPV types compete with one another, which may provide useful insights as to the likelihood that type replacement can be expected in the population at this early stage of vaccination rollout. This would allow sentinel systems to be planned and could provide a framework for the design of subsequent vaccine formulations.

The two recommended approaches in this manuscript (regression and cohort) are what I used in this project to evaluate HPV type competition. This manuscript includes examples of these approaches, using data from the Brazilian Ludwig-McGill cohort study, i.e., one of the five epidemiological studies with female HPV testing information that I had access to for the current project. Important methodological aspects of the current project that require further description are provided following presentation of this manuscript, e.g., a brief overview of the parent studies (data sets) available for this analysis, additional details regarding our statistical analysis methods including our approach to investigate masking of HPV52 by HPV16 in amplification HPV DNA assays, as well as sample size considerations.

Epidemiological Approach to Evaluate the Potential for Human Papillomavirus Type Replacement Post-vaccination

Running title: HPV type replacement post-vaccination

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FOOTNOTES

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ABSTRACT

Currently, two vaccines exist that prevent infection by the types of human papillomavirus (HPV) responsible for ~70% of cervical cancer cases worldwide. Although vaccination is expected to reduce the prevalence of these HPV types, there is concern about the effect this could have on the distribution of other oncogenic types. According to basic ecological principles, if competition exists between ≥2 different HPV types for niche occupation during natural infection, then elimination of one type may lead to an increase in other type(s). Here, the authors discuss this issue of "type replacement" and present different epidemiological approaches to evaluate HPV type competition. Briefly, these approaches involve: 1) calculation of expected frequency of co-infection under independence between HPV types for comparison with observed frequency; 2) construction of *hierarchical* logistic regression models for each vaccine-targeted type; and 3) construction of Kaplan-Meier curves and Cox models to evaluate sequential acquisition and clearance of HPV types according to baseline HPV status. A related issue concerning diagnostic artifacts arising when multiple HPV types are present in specific samples (due to broad-spectrum assays inability to detect certain types present in lower concentrations) is also presented. This may result in an apparent increase in previously undetected types, post-vaccination.

The discovery of human papillomavirus (HPV) as a necessary cause of cervical cancer [159] has enormous public health implications and has already led to the establishment of two highly effective HPV vaccines [2, 3]. Both Gardasil© (*Merck & Co.,* Whitehouse Station, New Jersey*)* and Cervarix© (GlaxoSmithKline, London, United Kingdom) prevent the two types of HPV that cause the majority (~70%) of cervical cancer cases (HPVs 16 and 18), but only Gardasil© protects against additional types (HPVs 6 and 11) that are responsible for most cases (~90%) of genital warts [4, 5, 100]. Countries that have implemented HPV vaccination will eventually experience major reductions in the incidence of cervical cancer and other HPV-related diseases. However, the existence of other oncogenic HPV types not targeted by the vaccine raises the concern that one or more of these other types may eventually take over the niches vacated by the eradication of vaccine types; this is a concept referred to as “type replacement” [11, 78, 104, 201]. The important question that remains is: Is it possible to obtain epidemiological insights concerning the likelihood that HPV type replacement may or may not occur?

In this article, we present different epidemiologic approaches to evaluate the potential for HPV type replacement, with examples from the Brazilian Ludwig-McGill cohort study [202]. We also discuss another important issue related to assessing type replacement, namely accuracy of detecting type-specific prevalence when co-infections with multiple HPV types are present.

HPV TYPE REPLACEMENT IN THE POST-VACCINATION ERA

Concern about type replacement is an argument against HPV vaccination used by some policy analysts [203], often citing the pneumococcal vaccine experience as evidence [13-16]. However, unlike pneumococcal infection, in which the pathogen (*S. pneumoniae*) has a high rate of genetic mutation and recombination, HPVs are *deoxyribonucleic acid* viruses that are extremely stable genetically. In fact, the mutation rate for this virus has been estimated at only one base pair every 10,000 years [17]. Emergence of escape mutants that avoid vaccine immunity or entirely new HPV types is therefore unlikely. Emergence of an existing type is also unlikely because of the relatively slower sexual infection dynamics and because the majority of the population is unexposed to specific HPV types (e.g., HPVs 16 or 18), implying that any possible natural competition cannot have greatly impacted the pool of susceptible individuals who may acquire other types. Nonetheless, if it can be demonstrated that HPV types compete with one another during natural infection, then there is still the theoretical possibility that type replacement may occur. Existence of natural type competition is a necessary condition for replacement; the other being that such natural type competition needs to be stronger than the cross-protection afforded by vaccines if type replacement is going to be possible [201].

To date, over 150 HPV types have been identified, including more than 40 anogenital types [6, 18, 204]. Based on the nucleotide sequence of the L1 (late) capsid gene, papillomaviruses have been classified into high and low order clusters, referred to

as genus and species, respectively. Most genital HPV types occupy a single genus, alpha (α), within which there exists 15 species [18, 204, 205]. Types from the same species share at least 60% of their nucleotide sequence identity, and as a result, often exhibit similar biological and pathological properties [18, 204]. Among the 13 HPV types classified by the International Agency for Research on Cancer as definite or probable carcinogens, most belong to two species (α-7 or α-9) [9, 206]. After HPVs 16 and 18, the 10 most common types implicated in cervical cancer globally (in order of decreasing prevalence) are 58, 33, 45, 31, 52, 35, 59, 39, 51, and 56 [5, 100].

According to Gause's ecologic competitive exclusion principle, two species cannot stably coexist when competing for the same niche. If niches overlap and one of the competing species is removed, the remaining one would then take over the available niche space and increase in prevalence. Alternatively, if a symbiotic species is removed, we would expect both to decrease in prevalence [207]. Type replacement after vaccination strongly depends on whether different HPV types interact during natural infection. Plausible competition mechanisms include generation of cross-reactive systemic or local immunity. However, it is well established that if vaccination provides cross-immunity that is at least equivalent to that of natural infection, available niche space will not be increased [208]. Thus far, phase III trials of HPV vaccines have found that vaccination induces antibodies at much higher levels than natural infection. The vaccine-induced partial cross-type protection against certain HPV types, mainly HPVs 31, 33 (α-9) and HPV 45 (α-7) is therefore likely to be well above natural cross-type immunity [3, 209] implying that type replacement is unlikely to occur for these

types. Although negative vaccine efficacy (which could be misconstrued as type replacement) was reported in one of these trials for HPVs 52 and 58 (both from α-9 species) [209], the finding could not have been due to type replacement, because this is a viral dynamics phenomenon that implies within-group transmission. Clinical trial populations do not replicate the transmission conditions seen in entire populations. As discussed later, a diagnostic artifact is a likely explanation.

EPIDEMIOLOGIC APPROACHES TO EVALUATE HPV TYPE COMPETITION

Probabilistic approach

To gain insight on the possibility of type replacement it is useful to evaluate competition between HPV types during natural infection. Competition of this sort may be reflected by a low probability of co-infection between two specific HPV types. For each pair combination involving a vaccine type and a non-vaccine type, we may calculate the expected frequency (E) of co-infection under a model of statistical independence and compare this with the observed frequency (O). This approach was first used in the late 1980's to evaluate multiple HPV infections in a Brazilian population [210] and has since been used by other investigators [77, 78, 101-104, 109, 195, 196, 211].

Table 1 presents a hypothetical example of how one can get insights from epidemiological studies as to whether or not any given HPV type, say type 'X', could occupy the niche vacated by HPV-16. The two sides of the table show what would be a

good clue if one assumes that this type competes with HPV-16 and thus would normally be observed less often than expected by chance alone. On the left, HPV-X was found in 20 women who were also infected by HPV-16 (out of the total 7% HPV-16 positive women among the 10,000 included in the study). Assuming independence between infections, one can calculate what the expected frequency of co-occurrence would be from the product of the two prevalences. The result is 35. In other words, the ratio of observed to the expected number (O/E =20/35=0.57) is less than one and the 95% statistical confidence bounds indicate that this O/E ratio is statistically significant. The conclusion would be that type X tends to occur less frequently than expected in women who are infected with HPV-16. This would be cause for concern because it suggests that HPV-X is suppressed by HPV-16 and thus its frequency could increase in the future post-HPV vaccination. Most epidemiologic studies that have examined the O/E relation for different pairs of HPV types [101-103, 195, 196] seldom found the situation on the left side of the table; rather these studies found a scenario that is comparable to the one on the right hand side of the table. The marginal distributions of HPV types are the same, for type X and for type 16, but the observed frequency is now 40, indicating that HPV-X is actually detected more frequently when HPV-16 is present. However, since there are shared risk factors for HPV infections, O/E ratios > 1.0 do not necessarily rule out the possibility of competition between types.

Using period prevalence data for the first year of subject follow-up from the Ludwig-McGill cohort study (n=2462 women) we compared the observed and expected number of co-infections, focusing on HPV-16 for this example. The Ludwig-McGill study

has been described in detail elsewhere [202]. Briefly, it includes an average of 10 follow-up visits per woman (every 4 months during the first year and twice annually in subsequent years) with questionnaire, Pap cytology, and HPV testing performed at each visit. In figure 1, the majority of log(O/E) ratios were above the null. The average weighted log(O/E) was 0.87 (95%CI: 0.67-1.06). For some types the O/E ratios were zero because it was not observed in co-infection with HPV-16. These types were included in our calculation of average weighted O/E ratio. Previously, others that have evaluated HPV type interactions have restricted their analysis to positive women (i.e., those with ≥1 HPV infection) to ensure that they had focused on a population with sufficient HPV exposure opportunity [101, 109, 210, 212]. This approach leads to higher expected frequencies (reduced O/E ratios) for all pairwise combinations, making results difficult to compare.

Considering that mucosotropic HPV infections share a common route of transmission and many risk factors [65, 213], it is not surprising that infection with multiple HPV types occurs often, in up to 50% of infected women [33, 213] and more frequently than expected by chance [76-78, 101, 103, 104, 195, 196]. Thus in calculating the expected co-infection frequency, our assumption that infections occur independently is a major limitation, leading to biased estimates of the O/E ratio away from zero. To account for correlation between HPV infections, we should therefore attempt to adjust for common risk factors in evaluating pairwise interactions [214], which would reduce most positive associations, thus improving our ability to detect competition between HPV types.

Regression approach

Another approach to evaluate type competition is to construct logistic regression models for each vaccine type separately and calculate the odds ratio (OR) for each pairwise association involving non-vaccine types. Conceptually, the interpretation of ORs is the same as for O/E ratios, i.e., ORs < 1.0 would indicate that the odds of being infected with a particular non-vaccine HPV type is lower among those with a vaccine type compared to those without a vaccine type, and vice versa for ORs > 1.0. A benefit of this approach is that confounding, as described above, may be addressed by the addition of relevant covariates to the model. In particular, factors such as age and number of sexual partners, which are normally predictive of multiple HPV infections, should be included [19, 109, 213, 215-217]. Positive associations that persist after adjustment may indicate synergistic effects between specific HPV types, but more likely either residual confounding or polymerase chain reaction (PCR) cross-reactivity.

A recent pooled analysis of International Agency for Research on Cancer HPV prevalence surveys, evaluated clustering patterns between all HPV types via hierarchical regression models with woman-level random effects, which, presumably, should account for any residual variation in HPV infection risk not captured by covariates in their model [103]. Although only a single statistically significant negative association was observed (between HPVs 16/81), multiple positive associations were observed (between HPVs 33/35, 33/58, 33/39, 18/45, and 31/35). Because results from

this study differed by genotyping method, the authors attributed clustering of these HPV types to a diagnostic artifact and not true biological interaction.

Chaturvedi et al. [195] also examined HPV co-infection patterns among women from a vaccine study in Costa Rica. To account for positive correlation between HPV infections they adjusted for predictors of multiple infection, but also calculated a pooled OR by averaging across all pair-specific ORs (separately for HPV types 6, 11, 16, and 18) and used this to represent the underlying affinity for each of these vaccine types to be involved in co-infection. They calculated the difference between the pair-specific OR and the pooled OR (log-scale) to assess whether any particular pair of types deviated from the pooled OR. This procedure was repeated for a total of 300 type-type combinations (25 HPV types) and statistically significant negative associations were observed between HPVs 44/68, 44/73, and 18/33, whereas HPVs 11/53, 31/33, 34/42, 45/68 and 45/73 were found to be positively associated. In general, HPV types occurred independently and phylogenetic relatedness had no influence.

The two studies described above did not account for the presence of other HPV types in evaluation of pairwise interactions, which according to some [212] may lead to confounding. Another issue is that for rare HPV types, few or no co-infections may be observed, which could lead to non-positivity or wide confidence intervals and extremely limited power to detect competition with these types. Rositch et al. [212] addressed some of these issues using data from a randomized controlled trial of Kenyan males through a semi-Bayesian regression approach. Multivariate hierarchical logistic models

for four outcome types (6, 11, 16, and 18) included variables identified a priori as predictors of multiple HPV infection, and all other HPV types. The hierarchical component was introduced through prior means for type-specific estimates, obtained by calculating the crude average log odds for co-infection for each type. By intentionally introducing some bias by using priors, this produces a shrinkage effect that reduces the overall error across estimates and improves the precision of each estimate [218]. A mix of null and positive associations, but no negative associations, was reported in this study.

Using Ludwig-McGill data, we illustrate the effects of shrinkage and adjustment for confounding in Figure 2. Panel A presents results from a multivariate logistic regression model with HPV-16 as the outcome and all other types as predictor variables. Woman-level clustering was accounted for with woman-specific intercepts. The OR estimates for some rare HPV types were highly unstable and these types were excluded from the model. Panel B presents the results from a similar model, with the addition of age and lifetime number of sexual partners at baseline as covariates. The average weighted log (OR) appears only slightly reduced by adjustment, from 0.38 (95%CI: 0.10-0.62) to 0.37 (95%CI: 0.12-0.58); possibly because these variables were not strong predictors of co-infection in the Ludwig-McGill dataset. Panel C results are from a model similar to B with the addition of a fully Bayesian approach to shrinkage, where the prior distribution for type-specific OR estimates was centered around the pooled estimate. Shrinkage reduced the problem of non-positivity, as unstable estimates were pulled (shrunk) more closely towards the overall mean, which enabled

us to include rare types in the model. The confidence intervals were also narrower compared to panels A and B. The pooled log(OR) from the shrinkage model was 0.53 (95%CI: 0.21-0.77).

By addressing issues of sparse data and confounding by common route of transmission, regression approaches that employ shrinkage to stabilize estimates and include adjustment for confounders may be useful in this context for evaluation of HPV type competition [219].

Cohort approach

When cohort information is available, comparison of sequential acquisition and clearance of HPV types according to infection with vaccine types is another useful approach to evaluate type competition. For acquisition, time to incident HPV infection(s) may be assessed for each of the non-vaccine types separately (or grouped together by species) according to baseline infection with one of the vaccine types. For evaluation of clearance, the approach is similar except that eligible women must be positive for the specific type(s) under study at the baseline. Using Cox regression with adjustment for important confounding factors, we may calculate hazard ratios (HRs) and associated confidence intervals. If we categorize those with a vaccine type as the exposed group, HRs < 1.0 would indicate that the risk of becoming infected with a particular non-vaccine HPV type is lower among those infected with a vaccine type compared with those without, and vice versa for HRs > 1.0. Our interpretation is similar to what we described

for O/E and ORs, except that for clearance it is the opposite, i.e., HRs > 1.0 indicate accelerated clearance of certain HPV types among those with a vaccine genotype and thus, potential type competition.

Previous studies examining the natural history of HPV do not suggest that prior infection with one or more HPV types inhibits acquisition of other types, or facilitates clearance of prevalent types in women [76-78, 104, 105, 109]. Rather, the majority of studies found that presence of pre-existing HPV infection actually increased an individual's risk of acquiring other types, including those from the same species [76-78]. Although these studies do not focus specifically on vaccine target types, they still provide valuable insights concerning type competition in general.

Using Ludwig-McGill cohort data, we prepared Kaplan-Meier curves to compare acquisition and clearance of HPV infection with α-9 types (excluding HPV-16) between women with and without HPV-16 infection at baseline (Figure 3). Despite adjustment for important risk factors of multiple infection (e.g., age, lifetime number of sexual partners), women infected with HPV-16 still appeared more likely to acquire other phylogenetically related HPV types and less likely to clear infections with these types.

Comparing approaches

Based on results presented here from the Ludwig-McGill study, type competition does not appear to exist between HPV-16 and other types, i.e., estimates < 1.0 (O/E

ratios, ORs, and HRs for incidence) or > 1.0 (HRs for clearance) were not statistically significant. Although the probabilistic approach is arguably the most intuitive, it does not permit adjustment for confounding and is more likely to produce biased estimates, making it more difficult to reliably assess type competition. We therefore recommend using regression and cohort approaches. Evidence of type competition that is consistently reported across approaches and studies should be a strong signal to investigators that type replacement is more likely to occur for the flagged HPV type(s).

DIAGNOSTIC ARTIFACTS

An additional concern related to HPV type replacement post-vaccination is the possibility of diagnostic artifacts. Currently, the most common HPV *deoxyribonucleic acid* tests being used for research and surveillance are consensus (or general) primer PCR assays with MY09/11 or GP5+/6+ primer sets. By targeting sequences in the L1 gene of HPV, these assays amplify and detect a broad spectrum of mucosotropic HPV types [220]. However, there may be competition for reagents (e.g., primers) between at least one of the current HPV vaccine types and other prevalent types in consensus PCR assays. The impact of this may be that in the presence of vaccine types, other prevalent HPV types are being missed [221]. For instance, if a specimen contains 1,000,000 HPV-16 genome copies but only 1,000 HPV-31 genome copies, then during amplification the HPV-16 sequences will overwhelm the minority type during the exponential phase of replication and the resulting signal for HPV-16 will be revealed at the expense of HPV-31. Hence this specimen may be erroneously labelled as an HPV-

16 monoinfection. However, if HPV-16 is removed the existing 1,000 molecules will have the entire reagent mixture for their amplification to proceed unhindered and the specimen will be HPV-31 positive.

In the post-vaccination era, surveillance will be necessary to monitor trends in the distribution of HPV types. If an increase in non-vaccine types is observed, it will be important to distinguish whether this resulted from true type replacement or represents a diagnostic artifact. For example, if we observe an increase in the prevalence of HPV-31 post-vaccination, an alternative explanation to type replacement is that HPV-31 had always been present, but that it was underestimated in the presence of vaccine types that were eliminated. In HPV vaccine trials, differential increase in prevalence may occur in the intervention arm, as this group would be protected against future infection by vaccine types, whereas the placebo arm would not. By ignoring this possibility one may arrive at erroneous conclusions when interpreting vaccine efficacy against non-vaccine HPV types.

Numerous studies that have compared PCR methods noted deficiencies in the sensitivity of consensus PCR versus type specific or multiple primer PCR systems (e.g., PGMY09/11 and modified GP5+/6+); particularly in cases of multiple infection and low viral *deoxyribonucleic acid* load [163-165, 170, 222-226]. Recently, Mori et al. [165] found that in samples containing HPV-16 and either HPV18, 51, 52, or 58, these latter types were not sufficiently amplified by consensus PCR at lower viral loads. Consistent with previous reports [163, 164, 225] sensitivity was most severely affected for types 51

and 52. Therefore, negative vaccine efficacy against certain HPV types [209] may simply be a consequence of inadequate test performance and just as it is important to identify types that should be monitored for replacement; it is equally important to evaluate the test used and ensure that it performs adequately. The World Health Organization HPV LabNet provides blinded “proficiency panels” designed to evaluate whether the assays used can detect a monoinfection equally well in the presence of other HPV types. Comparison of results from >100 laboratories worldwide that have used a variety of HPV assays have found that underestimation of some HPV types when other types are present in the same sample is a definite problem for some assays, but not for others [166, 227]. Continued monitoring that assays used for surveillance perform adequately in this regard will be of critical importance.

OTHER ISSUES TO CONSIDER IN THE EVALUATION OF HPV TYPE REPLACEMENT

The term unmasking has previously been used in the pneumococcal vaccine literature as a description for detection of apparent type replacement resulting from misattribution of a strain of microorganism causing disease when multiple strains are present [228, 229]. Because multiple infections with oncogenic HPV types is also common in evaluating cases of cervical cancer, assigning causality to a particular HPV type is often difficult and may also lead to misclassification in this scenario [230]. When investigators are faced with this situation, they often will apply an oncogenic hierarchy where the lesion is attributed to the HPV type present that usually progresses most

rapidly to cause cancer. Often, this will either be HPV16 or 18, which may or may not be present in the actual lesion [231]. When multiple HPVs are present, there could also be different lesions individually caused by different types. Cervical excisional treatment may remove multiple lesions and HPV types simultaneously. However, when excisional procedures for vaccine types detectable by screening are no longer performed in the future, the number of women at risk for non-vaccine type-caused disease may seem to increase. van der Marel et al. [232] used genotyping and laser-capture microdissection PCR analysis to evaluate high-grade cervical lesions with multiple HPV infections (including HPV-16) and found that HPV-16 was the causal type in all cases. We therefore expect that type replacement, observed as a consequence of errors in assigning causality or reduced rates of excisional treatment, will be low.

The possibility that HPV vaccination could lead to an increase in risky sexual behaviour (i.e., “risk compensation”) [233] due to a perceived lower risk of sexually transmitted infections among young vaccinated individuals also has important implications for HPV type replacement. To investigate this, Liddon and colleagues [234] recently evaluated data from a large national U.S. survey and found no association between HPV vaccination and reported risky sexual behaviours. Although these results may provide comfort to concerned parents and health officials, only prospective follow-up studies can provide a definitive answer to this question.

There are so far no indications that the biological prerequisites for type replacement are present in the HPV field and that diagnostic laboratory artifacts may

explain some deviations from random effects. Furthermore, the significant cross-protection seen after vaccination is likely to dwarf possible tendencies for replacement that may not have been possible to detect because of insufficient statistical power. Moreover, even if type replacement is observed, unless it leads to disease, it may not have important public health implications. Because HPV16 and 18 have much higher cancer risks than any other HPV type, replacement by a non-oncogenic type or an oncogenic type that has much lower risk for cancer may not have any major consequences. Results from long-term surveillance studies comparing the prevalence of different HPV types implicated in cervical cancer or high-grade lesions (pre- versus post-vaccination) will eventually provide a clearer estimate of the population level impact of current vaccines. Until then, we may gain valuable insight through evaluation of type competition to identify HPV types considered suspicious for replacement. In the unlikely event that such signals were to be found, types that are flagged could then be included in the new generation multivalent vaccines [153, 235].

Table 4-1: Hypothetical example of analysis of co-occurrence of different types of human papillomavirus in epidemiologic studies[a]

	Type X co-occurs with HPV16 **less** frequently than expected							Type X co-occurs with HPV16 **more** frequently than expected						
	HPV16+ Indv	HPV16- Indv	**Total**	O[b]	E[c]	**O/E Ratio** (95% CI)		HPV16+ Indv	HPV16- Indv	**Total**	O[b]	E[c]	**O/E Ratio** (95% CI)
HPV X+ Indv	20	480	500	20	35	**0.57**[d] (0.35-0.88)	HPV X+ Indv	40	460	500	40	35	**1.14**[e] (0.82-1.56)
HPV X- Indv	680	8820	9500				HPV X- Indv	660	8840	9500			
Total	700	9300	10000				**Total**	700	9300	10000			

Abbreviations: CI, confidence interval; E, expected; HPV, human papillomavirus virus; indv, individuals; O, observed.

[a] Concomitant (cross-sectional) or sequential (cohort) acquisition.

[b] Observed frequency of co-infection with HPV-16 and HPV-X.

[c] Expected frequency of co-infection with HPV-16 and HPV-X.

[d] Interpretation: type X under “suspicion” for replacement.

[e] Interpretation: type X not “suspected” for replacement.

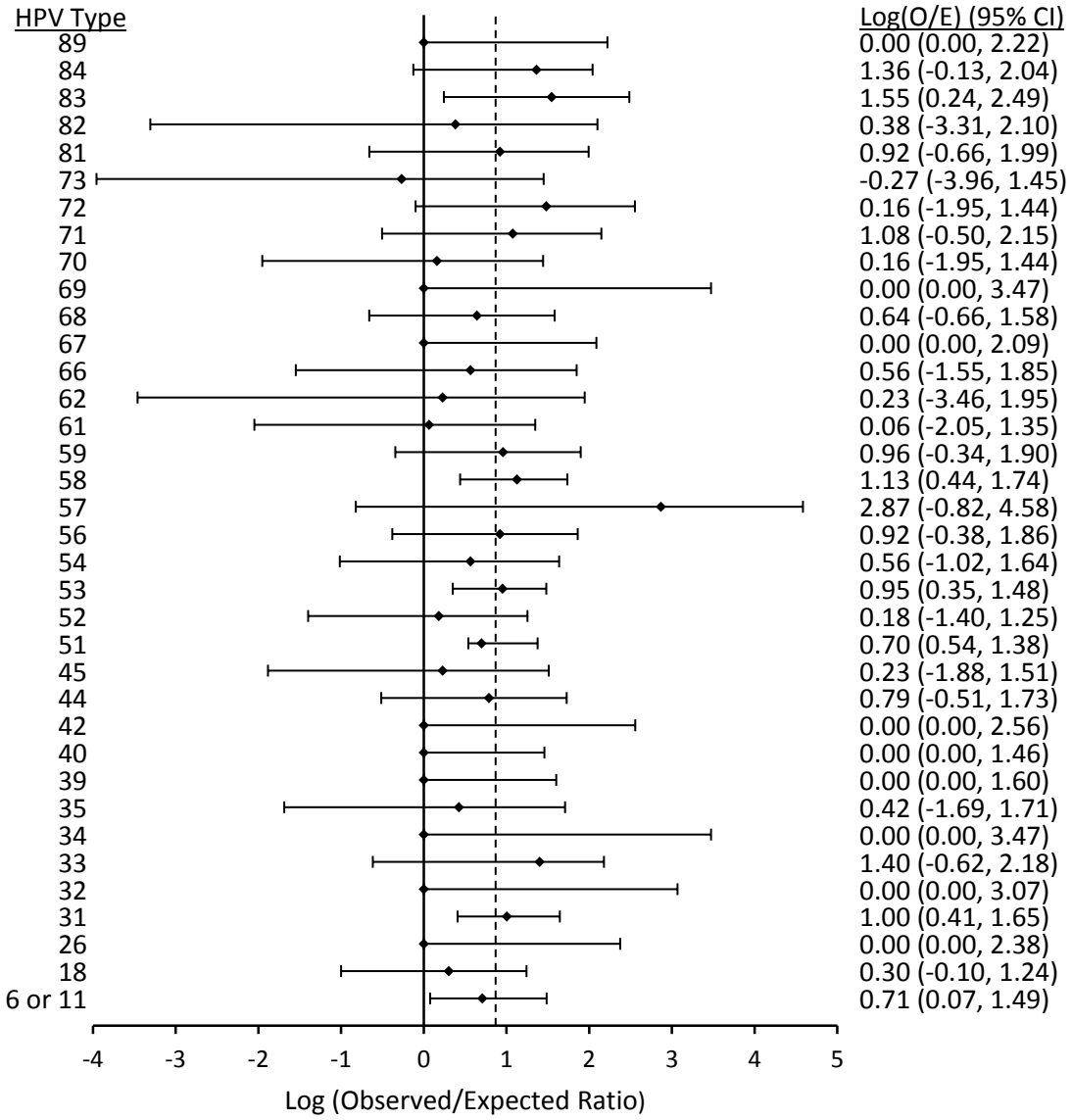
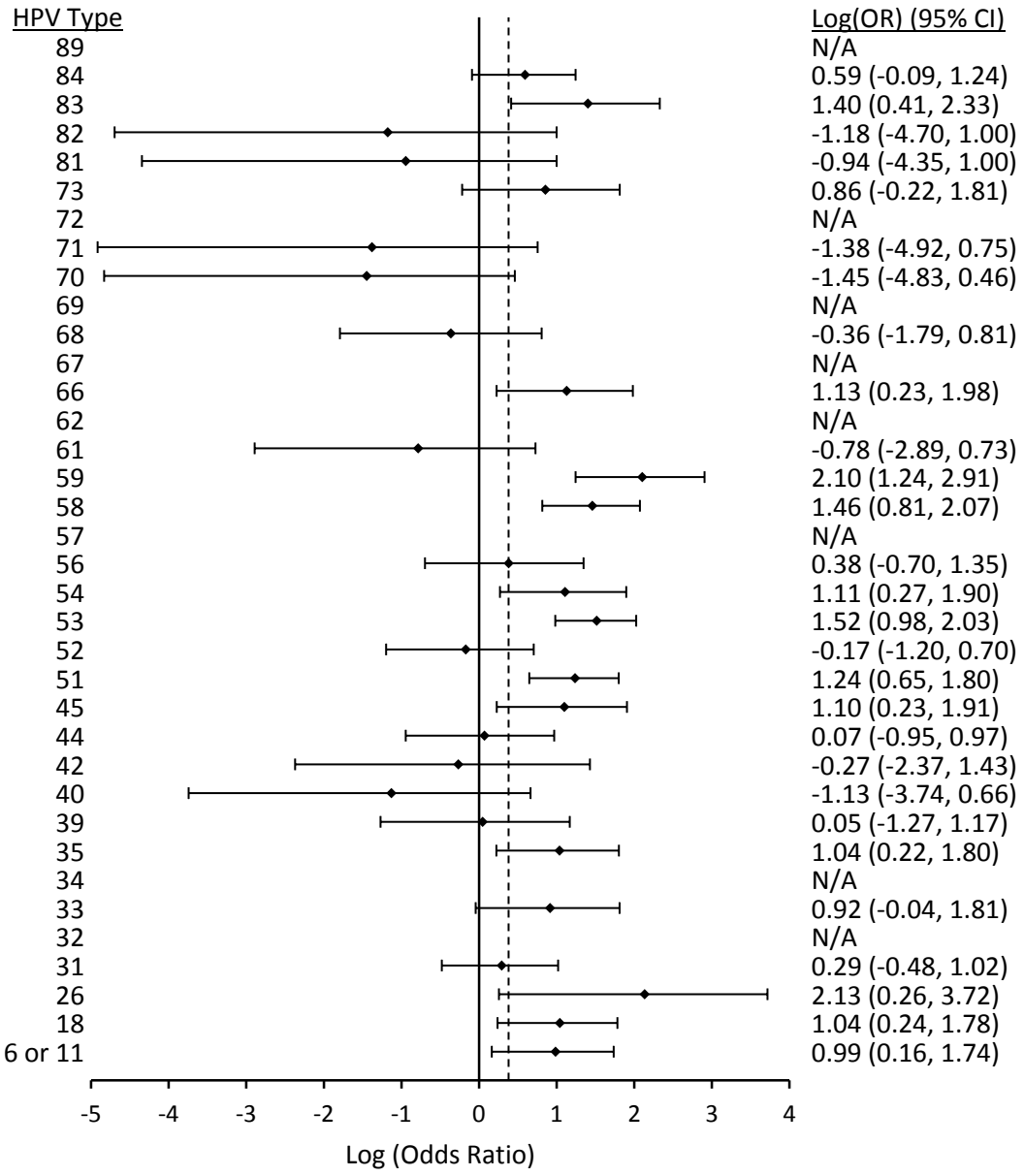
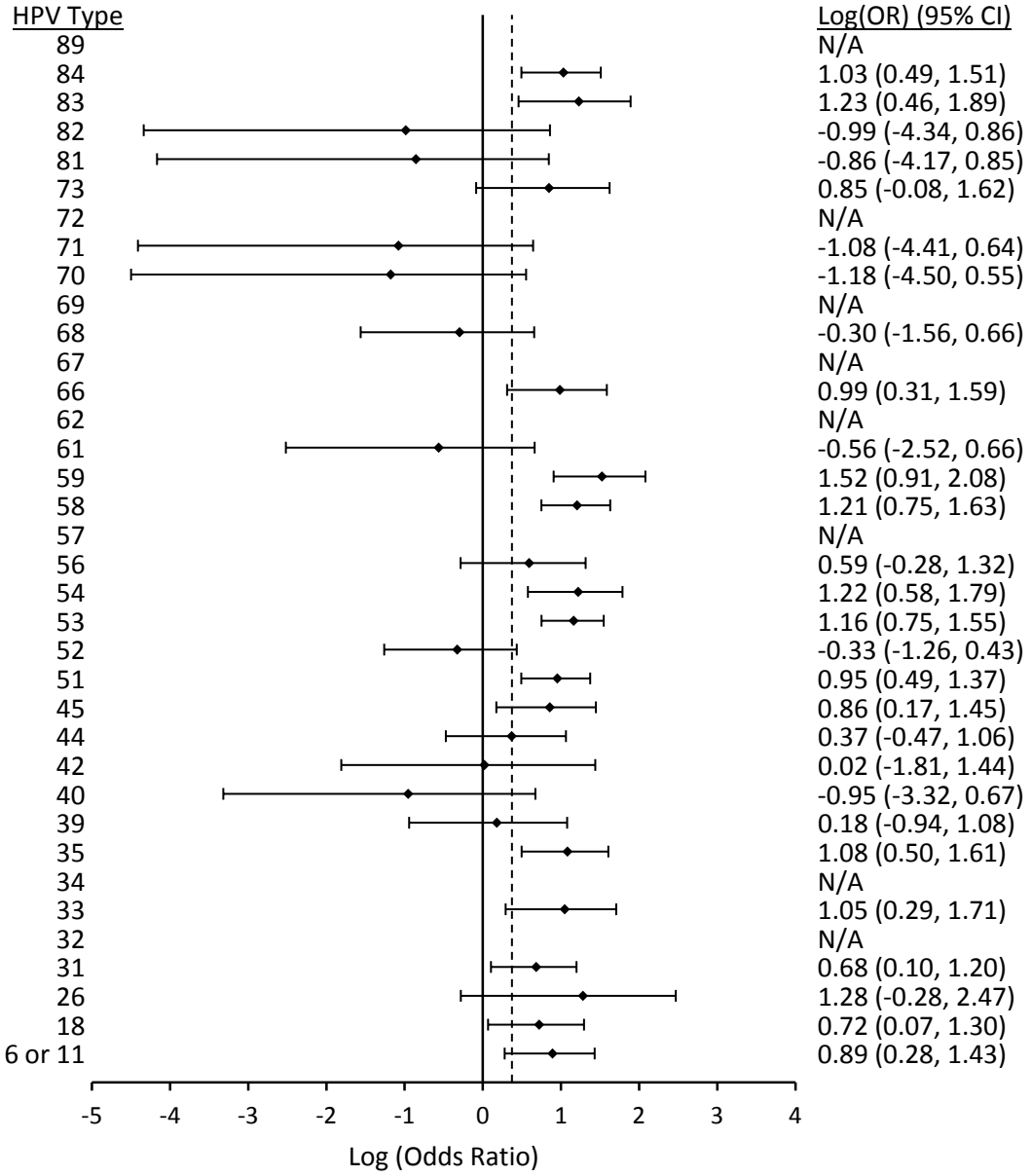


Figure 4:1: Log (observed/expected) ratios (log(O/E)) and 95% confidence intervals (CI) for co-infections involving human papillomavirus (HPV) 16 with other HPV types. Ratios were calculated using one-year period prevalence information. The dashed line represents the average weighted log(O/E) of 0.87 (95%CI: 0.67-1.06). HPV types belonging to the same species as HPV-16 (alpha-9) include 31, 33, 35, 52, 58, and 67. For HPV types with an O/E ratio of 0 (26, 32, 34, 39, 40 42, 67, 69, and 89), 0 was listed for the log and lower range of the 95% CI.

A)


HPV Type
Log(OR) (95% CI)
89 N/A
84 0.59 (-0.09, 1.24)
83 1.40 (0.41, 2.33)
82 -1.18 (-4.70, 1.00)
81 -0.94 (-4.35, 1.00)
73 0.86 (-0.22, 1.81)
72 N/A
71 -1.38 (-4.92, 0.75)
70 -1.45 (-4.83, 0.46)
69 N/A
68 -0.36 (-1.79, 0.81)
67 N/A
66 1.13 (0.23, 1.98)
62 N/A
61 -0.78 (-2.89, 0.73)
59 2.10 (1.24, 2.91)
58 1.46 (0.81, 2.07)
57 N/A
56 0.38 (-0.70, 1.35)
54 1.11 (0.27, 1.90)
53 1.52 (0.98, 2.03)
52 -0.17 (-1.20, 0.70)
51 1.24 (0.65, 1.80)
45 1.10 (0.23, 1.91)
44 0.07 (-0.95, 0.97)
42 -0.27 (-2.37, 1.43)
40 -1.13 (-3.74, 0.66)
39 0.05 (-1.27, 1.17)
35 1.04 (0.22, 1.80)
34 N/A
33 0.92 (-0.04, 1.81)
32 N/A
31 0.29 (-0.48, 1.02)
26 2.13 (0.26, 3.72)
18 1.04 (0.24, 1.78)
6 or 11 0.99 (0.16, 1.74)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

B)


HPV Type
Log(OR) (95% CI)
89 N/A
84 1.03 (0.49, 1.51)
83 1.23 (0.46, 1.89)
82 -0.99 (-4.34, 0.86)
81 -0.86 (-4.17, 0.85)
73 0.85 (-0.08, 1.62)
72 N/A
71 -1.08 (-4.41, 0.64)
70 -1.18 (-4.50, 0.55)
69 N/A
68 -0.30 (-1.56, 0.66)
67 N/A
66 0.99 (0.31, 1.59)
62 N/A
61 -0.56 (-2.52, 0.66)
59 1.52 (0.91, 2.08)
58 1.21 (0.75, 1.63)
57 N/A
56 0.59 (-0.28, 1.32)
54 1.22 (0.58, 1.79)
53 1.16 (0.75, 1.55)
52 -0.33 (-1.26, 0.43)
51 0.95 (0.49, 1.37)
45 0.86 (0.17, 1.45)
44 0.37 (-0.47, 1.06)
42 0.02 (-1.81, 1.44)
40 -0.95 (-3.32, 0.67)
39 0.18 (-0.94, 1.08)
35 1.08 (0.50, 1.61)
34 N/A
33 1.05 (0.29, 1.71)
32 N/A
31 0.68 (0.10, 1.20)
26 1.28 (-0.28, 2.47)
18 0.72 (0.07, 1.30)
6 or 11 0.89 (0.28, 1.43)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

C)

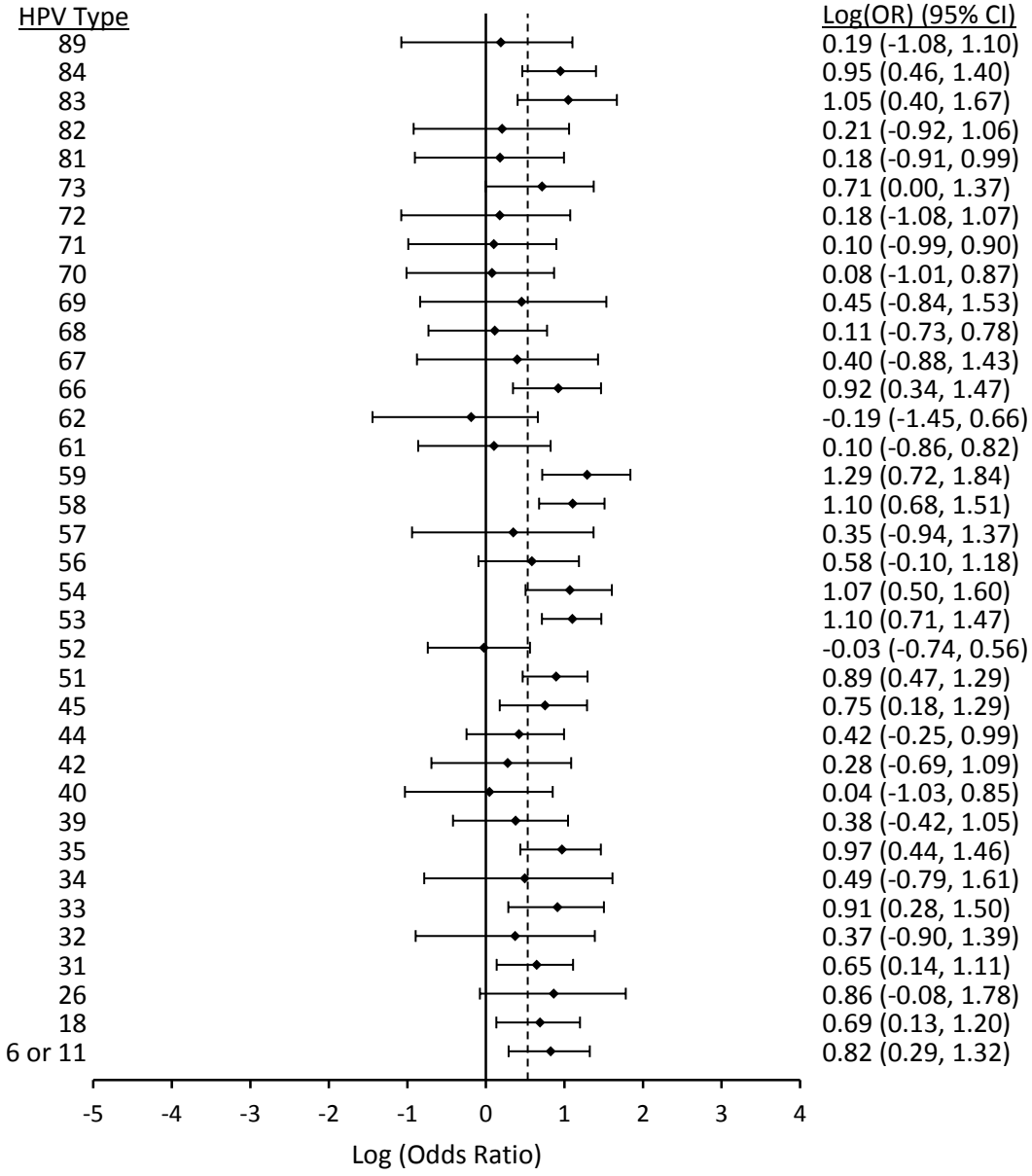
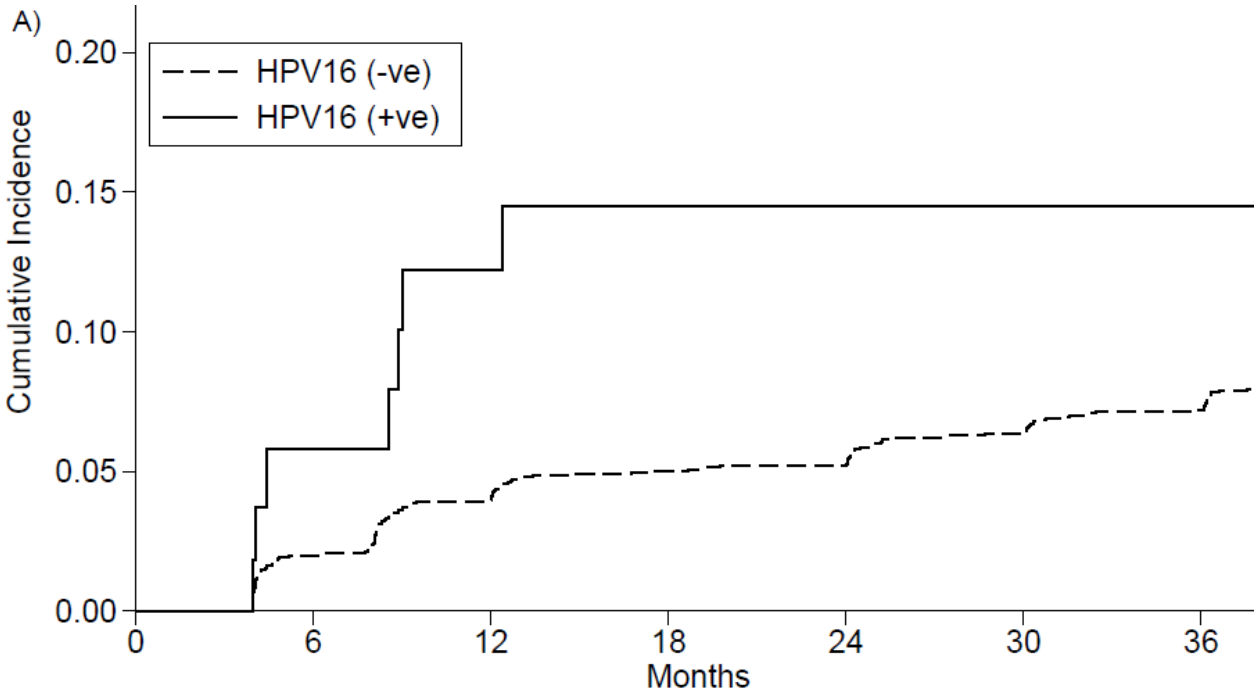

HPV Type
Log(OR) (95% CI)
89
0.19 (-1.08, 1.10)
84
0.95 (0.46, 1.40)
83
1.05 (0.40, 1.67)
82
0.21 (-0.92, 1.06)
81
0.18 (-0.91, 0.99)
73
0.71 (0.00, 1.37)
72
0.18 (-1.08, 1.07)
71
0.10 (-0.99, 0.90)
70
0.08 (-1.01, 0.87)
69
0.45 (-0.84, 1.53)
68
0.11 (-0.73, 0.78)
67
0.40 (-0.88, 1.43)
66
0.92 (0.34, 1.47)
62
-0.19 (-1.45, 0.66)
61
0.10 (-0.86, 0.82)
59
1.29 (0.72, 1.84)
58
1.10 (0.68, 1.51)
57
0.35 (-0.94, 1.37)
56
0.58 (-0.10, 1.18)
54
1.07 (0.50, 1.60)
53
1.10 (0.71, 1.47)
52
-0.03 (-0.74, 0.56)
51
0.89 (0.47, 1.29)
45
0.75 (0.18, 1.29)
44
0.42 (-0.25, 0.99)
42
0.28 (-0.69, 1.09)
40
0.04 (-1.03, 0.85)
39
0.38 (-0.42, 1.05)
35
0.97 (0.44, 1.46)
34
0.49 (-0.79, 1.61)
33
0.91 (0.28, 1.50)
32
0.37 (-0.90, 1.39)
31
0.65 (0.14, 1.11)
26
0.86 (-0.08, 1.78)
18
0.69 (0.13, 1.20)
6 or 11
0.82 (0.29, 1.32)
-5
-4
-3
-2
-1
0
1
2
3
4
Log (Odds Ratio)

Figure 4-2: Log (odds ratios) (log(OR)) and 95% confidence intervals (CI) for human papillomavirus (HPV) 16 for co-infection with other HPV types. Estimates were obtained from logistic regression models A) adjusted for all other HPV types; B) adjusted for all other types, age, and lifetime number of sexual partners at baseline; C) adjusted for all other types, age, lifetime number of sexual partners, and with shrinkage. Dashed lines represent the average weighted log(OR) in A) and B), which were 0.38 (95%CI: 0.10-0.62) and 0.37 (95%CI: 0.12-0.58), respectively; and the pooled log(OR) from hierarchical logistic regression in C), which was 0.53 (95%CI: 0.21-0.77). HPV types belonging to the same species as HPV-16 (alpha-9) include 31, 33, 35, 52, 58, and 67. In A) and B), rare HPV types (32, 34, 57, 62, 67, 69, 72, 89) were excluded from the model because they caused model instability. These types were included in model C) as the hierarchical model is able to stabilize estimates. N/A, not applicable.


A)
0.20
0.15
0.10
0.05
0.00
Cumulative Incidence
HPV16 (-ve)
HPV16 (+ve)
0
6
12
18
24
30
36
Months

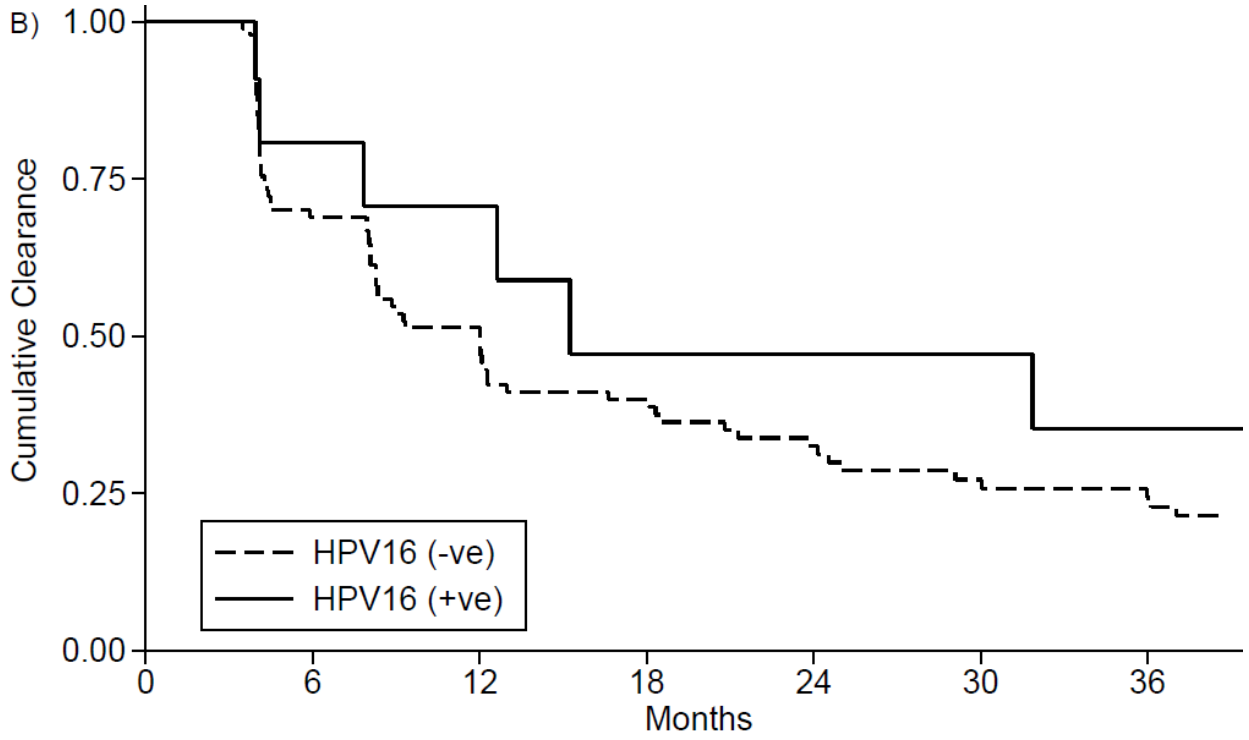

B)
1.00
0.75
0.50
0.25
0.00
Cumulative Clearance
HPV16 (-ve)
HPV16 (+ve)
0
6
12
18
24
30
36
Months

Figure 4-3: Kaplan-Meier curves comparing A) time to incident human papillomavirus (HPV) infection and B) clearance of existing HPV infection (alpha-9 types, excluding HPV-16) according to HPV-16 status at baseline; adjusted for age and lifetime number of sexual partners. Hazard ratios and associated 95% confidence intervals (CI) for curves A) and B) were 1.49 (95%CI: 0.82-2.73) and 0.79 (95%CI: 0.38-1.64), respectively.

4.2 Description of studies included in this project

Female HPV DNA testing and patient information for evaluation of objectives 1-3 were available from five studies conducted by our division. They include: a) the Ludwig-McGill cohort study (n=2462, all with valid HPV results in multiple specimens over a period of up to 10 years; n=22061 valid specimens); b) the HPV Infection and Transmission among Couples through Heterosexual activity (HITCH) cohort study (n=1038, 502 females, 536 males, 452 females with valid HPV results in multiple specimens over a period of two years; n=2203 valid female specimens); c) the McGill-Concordia cohort study (n=636, all with valid HPV results in multiple specimens over a period of two years; n=2689 valid specimens); d) the Biomarkers of Cervical Cancer Risk (BCCR) case-control study (n=1687, 590 cases, 1097 controls; 985 controls had valid HPV results; n=985 valid specimens); and e) the Canadian Cervical Cancer Screening Trial (CCCaST) (n=10154, Pap arm=5059, HPV arm=5095, all with valid HPV results; n=10154 valid specimens). These studies are described in this section, and briefly in manuscripts III and IV as well (chapters 5 and 6, respectively). They are also summarized in Table 4-2.

Ludwig-McGill Cohort Study: Recruitment for this study took place between 1993 and 1997 with follow-up until 2005 in a population of low-income women in São Paulo, Brazil [202]. Eligible women were between 18 and 60 years of age, permanent residents of São Paulo, had an intact uterus and no referral for hysterectomy, not pregnant or planning to become pregnant in the next 12 months, and had not been treated for

cervical disease in the last 6 months prior to enrollment. Participants presented for clinic visits every 4 months (0, 4, 8 and 12 months) during their first year of follow-up and twice annually in subsequent years (maximum 10 years follow-up). At each visit subjects were asked to complete a questionnaire to collect information on sociodemographic, lifestyle, sexual, reproductive, and contraceptive factors; and to provide a cervical sample for Pap cytology and HPV testing. Presence of HPV DNA was determined using a PCR assay employing L1 consensus primers and MY09/11 amplification. Presence of HPV DNA was determined using a PCR assay employing L1 consensus primers and MY09/11 amplification, followed by hybridization with individual oligonucleotide probes and by restriction fragment length polymorphism analysis to identify 40 HPV types. The study was approved by review boards and ethical committees of the participating institutions in Brazil and Canada.

McGill-Concordia Cohort Study: Recruitment and follow-up for this study took place between 1996 and 1999 and included female students attending either the McGill or Concordia University Health Clinic (Montreal, Canada) [22]. The only eligibility criteria were that participants intended to remain in Montreal for the next 2 years and had not been treated for cervical disease in the previous 12 months. All eligible women were asked to return to the clinic every 6 months over a period of 24 months. At each visit subjects completed a questionnaire and provided a cervical sample for Pap cytology and HPV testing. HPV DNA was detected using the L1 consensus HPV primers MY09/11 and HMB01 PCR protocol followed by a line blot assay for the detection of 27

HPV types. The study protocol was approved by the Research Ethics Boards of Concordia University and McGill University.

HPV Infection and Transmission among Couples through Heterosexual activity (HITCH) cohort study: Between 2005 and 2010, young women (aged 18-24) attending a university or junior college in Montreal were recruited to join this study, along with their male partners [236, 237]. Eligible female participants were currently heterosexually active with a male partner (acquired within the previous 6 months) who was also willing to enrol in the study, had an intact uterus, no history of cervical lesions/cancer, not currently pregnant or planning to become pregnant in the next two years, and willing to comply with follow-up for at least two years. Eligible male partners must have been willing to participate in the study for at least 4 months and be at least 18 years of age. All eligible participants were asked to attend clinic visits every 4 months during their first year of follow-up, and every 6 months during their second year of follow-up. At each visit subjects completed an enrolment or follow-up questionnaire and provided cervical samples for HPV testing. HPV detection and typing was done using the PGMY09/11 PCR protocol coupled with the linear array method (commercially available from Roche), which is capable of detecting 37 mucosal HPV types. The study protocol was approved by the ethical review committees at Concordia University, McGill University, and University of Montreal.

Biomarkers of Cervical Cancer Risk (BCCR) Study: This was a hospital based case-control study carried out in Montreal between 2001 and 2009 [238]. Cases were women

referred with an abnormal screening Pap smear to one of five colposcopy clinics of the collaborating hospitals of the McGill University Health Centre and the Centre hospitalier de l'Université de Montréal. Controls were women with a normal screening Pap smear and no history of cervical neoplasia or diagnosed abnormalities, recruited from several family medicine and gynaecology centres that referred patients to the same collaborating hospitals, during the same time period as cases. Exclusion criteria for both cases and controls included current pregnancy, prior hysterectomy or conization surgery, prior history of any cervical abnormalities, and prior history of any cancer (except nonmelanoma skin cancer). Participants completed questionnaires and provided cervical samples for HPV testing. In our regression analyses (manuscript III), only females in the control group with valid HPV testing results were included. HPV DNA was amplified using the L1 consensus primers PGMY09/11 PCR protocol and typed using the reverse line blot assay, with an extended line blot strip capable of identifying up to 37 genital HPV types. The study protocol was approved by the Research Ethics Committees of the Centre hospitalier de l'Université de Montréal, each of the participating hospitals, as well as McGill University's Institutional Review Board.

Canadian Cervical Cancer Screening Trial (CCCaST): CCCaST was the first North American randomized controlled trial designed to compare the performance of two cervical cancer screening strategies, Pap cytology (control) and HPV testing (treatment), to detect high-grade cervical lesions and cancer [145, 239]. Between 2002 and 2005, women aged 30-69 who sought screening for cervical cancer in one of 30 participating clinics in Montreal (Quebec) or St. John's (Newfoundland) were

approached to join the study, unless they met any of the following exclusion criteria: (i) attended a colposcopy clinic for evaluation, treatment or follow-up of a cervical lesion, (ii) were without a cervix, (iii) pregnant, (iv) prior history of invasive cervical cancer, (v) unable to provide informed consent, (vi) or had received a Pap test within 12 months. All women that were eligible to enrol in the trial underwent both tests on two occasions: at enrolment and after 12 months. The difference between arms was the order in which screening tests were administered. Women in both groups were referred for colposcopy if either test was considered to be abnormal, and asked to undergo a second colposcopic examination 6 months later if no disease was identified by either colposcopy or biopsy. A random subsample of 10% of the women in St. John's and 20% of the women in Montreal with a negative index test in each group were invited to undergo colposcopy. Total follow-up, depending on colposcopy results, was between 12 and 18 months. Women completed a questionnaire at every screening and colposcopy visit (when applicable), and provided cervical samples at both screening visits. An important limitation of this study in the context of our investigation is that information on age at sexual debut, number of lifetime sex partners, and number of pregnancies was not collected from participants enrolled at the St. John's study site (n=5754). Consensus primer PGMY09/11 PCR coupled with linear array permitted testing and typing for 37 genital types. The ethical review boards at McGill and Memorial Universities, and all participating hospitals and clinics approved this study.

Table 4-2: Summary of five epidemiological studies on human papillomavirus included for analysis in this project

	Ludwig-McGill	**McGill-Concordia**	**HITCH**	**BCCR**	**CCCaST**
Design	Cohort N=2462	Cohort N=636	Cohort N = 1038	Case-Contol N=1687	RCT N=10154
Population	São Paulo, Brazil - Low-income women	Montreal - Female students	Montreal -Female students and male partners	Montreal Cases: Women with abnormal screening Pap (CIN 2/3) Controls: Women with normal Pap smear	Montreal, St. John's Treat: HPV DNA (index test) followed by Pap Control: Pap test (index test) followed by HPV test
# valid specimens	N=22061	N=2689	N=2203 (females)	N=985 (controls)	N=10154
Recruitment/ Follow-up	1993-1997/up to 10 years	1996-1999/2 year	2005-2010/2 year	2001-2004 /NA	2002-2005/12-18 months
Eligibility Criteria	18-60 yrs, intact uterus/no referral for hysterectomy, no use of vaginal meds, not pregnant, not recent treated for cervical disease	Intend to remain in Montreal for next 2 years, had not been treated for cervical disease in the last 12 months	18-24yrs, sexually active with male partner, intact uterus, no history of cervical lesions/cancer, not pregnant	no prior hysterectomy or conization surgery, not pregnant, no history of cervical disease or cancer	30-60 yrs, not attending colp clinic for treat/evaluation of lesion, not pregnant, no history of invasive cervical cancer, not recently screened using Pap
Clinic Visits	Every 4 months during first yr of follow-up, twice annually after	Every 6 months	Every 4 months (yr 1), every 6 months (yr 2)	Single visit	Screening: Enrolment, 1 year Colposcopy: Post screening, 6 months
Data Collection	Questionnaire, cervical specimen	Questionnaire, cervical specimen	Questionnaire, cervical specimen	Questionnaire, cervical specimen	Questionnaire, cervical specimen (only screen visits)
HPV DNA Testing	Consensus primer PCR - MY09/11 protocol	Consensus primer PCR - MY09/11, HMB01 protocols	Consensus Primer PCR - PGMY protocol	Consensus Primer PCR - PGMY protocol	Consensus Primer PCR -PGMY protocol

4.3 Descriptive Statistics

In all five studies, data were collected for a number of important demographic and lifestyle variables via questionnaire. We used descriptive statistics to summarize these variables (e.g., age, education, income, age at sexual debut, number of lifetime sexual partners, condom use, and oral contraceptive use). We also summarized HPV testing results, e.g., percentage of women with detectable infection at baseline or follow-up, and percentage of women with single versus multiple HPV infection. This information will be reported separately for each study.

4.4 Correlates of multiple HPV infections

Covariates that were investigated as potential risk factors for multiple HPV infections (common to all five studies) are presented in Table 4-3. These covariates were selected based on thorough review of the literature [7] and included: age (continuous: years), marital status (categorical: single, married/common law, widowed/divorced), age at sexual debut (binary: <16, ≥16), lifetime number of sex partners (categorical: 0-1, 2-4, ≥ 5), number of pregnancies (categorical: 0, 1-2, ≥3), oral contraceptive use (binary: never, ever), condom use (categorical: never, rarely or sometimes, regularly or always), and cigarette smoking (categorical: never smoker, former smoker, current smoker). With the exception of the CCCaST trial, which used a checklist to evaluate whether subjects “ever” used oral contraceptives, condoms, or other contraceptives, the questionnaires for each of the five studies (pertaining to each

of eight variables listed here) were very similar. The CCCaST St. John's study site (*n*=5754) also did not collect information on age at sexual debut, number of lifetime sex partners, or number of pregnancies.

To investigate which of these covariates are important predictors of multiple HPV infections, we used multivariate logistic regression (adjusting for all other potential risk factors) to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for multiple versus single HPV infections in each of the five studies. In addition to age and lifetime number of sexual partners (a priori confounders), other variables identified to be independent predictors of multiple HPV infection (and therefore potential confounders) would be included in our analyses of HPV type associations (described, below). Since information for many important covariates (including lifetime number of sexual partners) was not collected in CCCaST (St. John's study site), separate pooled analyses were conducted adjusted for all important covariates of multiple infection (excluding St. John's study site participants), and adjusted for age only (all participants included).

4.5 Missing data

All female participants in the Ludwig-McGill study (n=10154), McGill-Concordia study (n=636), and CCCaST trial (n=10154) provided at least one valid HPV DNA sample for testing. In the HITCH (n=502 females) and BCCR (n=1097 controls) studies, valid HPV DNA results were unavailable for 50 and 112 females, respectively. There is no reason to suspect that these missing results occurred other than randomly;

therefore, the possibility that this may have introduced bias in our analyses of HPV type-type interactions is low. The number and percentage of females in each study with missing covariate information is presented in table 4-3. Since the percentage of participants with incomplete data was generally low (<5%), with the exception of some variables in the CCCaST trial (see explanation, above), we decided to proceed with a complete-case analysis (rather than impute missing values for covariates) in our evaluation of risk factors for multiple HPV infections. Due to such a high amount of missing information for some variables from CCCaST (St. John's site) participants, appropriate sensitivity analyses (including/excluding these individuals) were conducted, as described in the previous section.

For missing HPV DNA results in our time-to-event analyses of HPV acquisition and clearance (manuscript IV), HPV status for visit(s) prior to the first HPV positive visit (or negative visit for evaluation of clearance) were assumed to be negative and positive, respectively. That is, the acquisition or clearance interval was assumed to span the time from the last available HPV negative (or positive) visit to the first HPV positive (or negative) visit, respectively. We explored this assumption through a sensitivity analysis by changing missing values for our acquisition/clearance analyses from negative to positive or positive to negative, respectively. In section 8.1, I also discuss the possibility of selection bias resulting from differential loss to follow-up of females across the three cohort studies.

Table 4-3: Covariates considered as potential correlates of multiple HPV infection among female participants at baseline/enrollment in five epidemiological studies

Study covariates	Missing in Ludwig-McGill *n*=2462 n (%)	Missing in McGill-Concordia *n*=636 n (%)	Missing in HITCH *n*=502 n (%)	Missing in CCCaST [a] *n*=10154 n (%)	Missing in BCCR *n*=985 n (%)
Age (years)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Marital status	2 (0.1%)	13 (2.0%)	0 (0%)	98 (1.0%)	4 (0.4%)
Age at sexual debut	25 (1.0%)	68 (10.7%)	3 (0.6%)	48 (1.1%)	40 (4.0%)
Lifetime # of sex partners	2 (0.1%)	26 (4.1%)	0 (0.0%)	62 (1.4%)	15 (1.5%)
# of pregnancies	19 (0.8%)	22 (3.5%)	1 (0.2%)	61 (1.4%)	5 (0.5%)
Oral contraceptive use	1 (0.0%)	40 (6.3%)	1 (0.4%)	4700 (46.3%)	12 (1.2%)
Condom use	36 (1.5%)	35 (5.5%)	1 (0.2%)	4761 (46.9%)	12 (1.2%)
Cigarette smoking	1 (0.0%)	14 (2.2%)	0 (0.0%)	77 (0.8%)	4 (0.4%)

[a] Checklist was used in CCCaST study only to evaluate whether subjects "ever" used oral contraceptives or condoms. St. John's study site (*n*=5754) did not collect information on age at sexual debut, number of lifetime sex partners, or number of pregnancies. For these variables, percentage missing was based on the number of Montreal study site subjects only (*n*=4400).

4.6 Epidemiologic approach to evaluate HPV type competition and the potential for HPV type replacement in the post-vaccination era

In this section I provide additional information (beyond what is provided in the statistical analysis sections of each manuscript) describing the two approaches used to address the potential for HPV type replacement by specific HPV types (objectives 1 and 2). Because these approaches involve the evaluation of HPV co-infection, it is important to distinguish what is meant by *concurrent, sequential,* and *cumulative co-infection*. Concurrent co-infection refers to the detection of multiple HPV types in the cervical specimen collected at a given visit. Sequential co-infection refers to the detection of multiple HPV types detected at different visits, e.g., HPV16 detected at one visit and HPV31 at another would be considered a sequential infection. Cumulative co-infection refers to co-infection status during all visits completed, i.e., it includes co-infections occurring at the same index visit (concurrent) as well as those occurring over different visits, i.e., prior to or after the index visit over a given time period (sequential) [77, 109, 213].

4.6.1 Objective 1: Regression approach

For objective 1**,** we investigated the association between infection with the vaccine preventable types and infection with each of the other HPV types using data from the five studies, described above. Bayesian hierarchical logistic regression models were constructed for each HPV vaccine type (6, 11, 16, and 18), including all other HPV

types as exposures. This approach allowed us to assess which of the exposure HPV types are associated with the outcome HPV type while adjusting for age and lifetime number of sexual partners, as well as all other HPV types. Not controlling for other HPV types while examining pairwise associations may produce confounded estimates, e.g., if we are examining HPV16 and HPV52, co-infection with HPV31 may explain part (or all) of the observed association. Recent studies focusing on HPV type interactions among females have only considered pairwise combinations without adjustment for other HPV types. To avoid statistical problems (e.g., unstable estimates and estimate inflation) resulting from the inclusion of many covariates in the model, we propose a modern adjustment technique known as shrinkage [218, 219, 240]. Shrinkage is useful to control inflation, as this method pulls (shrinks) coefficient estimates towards expected values estimated from the data (empirical Bayes), with unstable estimates being shrunk more than stable ones. Using this approach, we were able to assess which of the exposure HPV types are associated with the outcome HPV type (6, 11, 16, or 18) without a large number of comparisons, adjusted for all other HPV types, and with stable estimates due to the shrinkage factor. Since we could not distinguish between HPV6 and 11 infections in the Ludwig-McGill study, we also conducted analyses for these two types grouped together (including participant information from all Ludwig-McGill subjects). Below, we provide a description of our hierarchical modelling approach, focusing on HPV16 as the particular outcome type in this example.

The probability of HPV16 infection was modeled in a 2-tier hierarchical model, where individual visits for subjects over time were nested within subjects in order to

account for subject-level clustering. At the visit level, a logistic model was fit with HPV16 infection as the outcome and the presence of each potential co-infection type as predictors, adjusted for age at the time of the visit. The model was:

$$logit(p_{ij}) = \alpha_i + \sum_{q=1}^{a-1} \beta age_q * age_{ijq} + \sum_{r=1}^{h} \beta type_r * type_{ijr}$$

where p_{ij} is the probability of HPV16 infection at visit j for subject i. α_i is the subject-specific intercept. βage_1 to βage_{a-1} are logistic regression coefficients for the a-1 indicator variables for the a age categories. $\beta type_1$ to $\beta type_h$ are coefficients for other HPV types numbered 1 to h.

At the subject level, the subject-specific intercepts were modeled by accounting for the effect of lifetime number of sex partners at baseline, as well as the effect of the different studies that were pooled. The model was:

$$\alpha_i = \alpha + \sum_{u=1}^{p-1} \beta partners_u * partners_{iu} + \sum_{v=1}^{s-1} \beta study_v * study_{iv}$$

where α represent the overall intercept. $\beta partners_1$ to $\beta partners_p$ are logistic regression coefficients for the p-1 indicator variables for the p categories of the number of lifetime sex partners at baseline. $\beta study_1$ to $\beta study_s$ are coefficients for the s-1 indicator variables for the s studies. Thus each subject has her own intercept, which varies around an overall intercept, in a way that is affected by the number of sex partners and the study that she was a subject. Note that we adjusted for age at the visit level and for

sex partners at the subject level. This was due to the fact that only baseline data on the number of sex partners was available for some of the studies. We chose to account for between-study variation by including indicator variables for studies as covariates, this essentially allows for each study to have its own intercept.

In order to improve the precision of the estimates for the effect of the presence of other HPV types on the presence of HPV16, the logistic regression parameters for all the other HPV types were assumed to be normally distributed around an overall mean effect of co-infection μ with variance σ as below:

$$\beta type_t \sim N(\mu, \sigma)$$

This additional hierarchical component on the coefficients produces a shrinkage effect, whereby unstable estimates with large variances are drawn closer to the mean. The assumption introduces a bias in favour of potentially reducing mean squared error. All analyses were conducted using WinBUGS software version 1.4.3 (MRC Biostatistics Unit, Cambridge).

Ultimately, our aim for this analysis was to identify HPV type combinations that are positively or negatively associated. Final model estimates are interpreted as odds ratios and corresponding 95% confidence intervals. Positive associations (OR > 1.0) indicate that the odds of the outcome HPV type are increased when women have the exposure HPV type compared to women without concurrent infection with the HPV exposure type. Oppositely, negative associations (OR < 1.0) indicate the odds of the

outcome type are reduced in women who have the exposure HPV type compared to women who are not concurrently infected with the exposure type (i.e., potential type competition). If one HPV type is prevented by vaccination, the other could increase in prevalence by filling its vacated ecologic niche, resulting in type replacement.

4.6.2 Objective 2: Cohort approach

For objective 2, our analyses were restricted to the three cohort studies involving multiple follow-up visits. They included the Ludwig-McGill, McGill-Concordia, and HITCH cohort studies (for the latter cohort study only, some HPV results continued to arrive after completing our analyses and were therefore not available for inclusion in the current project). We planned a prospective analysis to evaluate the association between type-specific HPV types at baseline (or time of index infection) and acquisition of additional HPV infections (or clearance of existing HPV infections) over a period of 24 months. For our main analyses, we compared time to acquisition/clearance of HPV (both individually, and grouped according to species) according to infection with current generation vaccine HPV types (6, 11, 16 and 18). Type specific acquisition was defined as the detection of a new genotype that was not present in any of the females' previous study visits. As mentioned previously, in the Ludwig-McGill study we were unable to distinguish between HPVs 6 and 11, therefore we decided to group these phylogenetically related types together in all subsequent analyses. But considering that these are among the most closely related HPV types (with indistinguishable biological

and pathological properties) [204], this provided us with some reassurance that it was appropriate to group these two types together for the purpose of this project.

The Kaplan-Meier method and Cox's proportional hazard regression was used for the evaluation of sequential acquisition and clearance of HPV types according to presence/absence of vaccine types. Since the actual time of acquisition/clearance is unknown (i.e., events are only known to have occurred between the last HPV negative or positive visit and the first HPV positive or negative visit, for acquisition and clearance analyses, respectively) we had to rely on interval censored survival methods for each of the respective analyses. For each HPV type where an event was not observed at the final study visit, the data were right censored. For missing visits between two HPV negative visits (or positive visits; for clearance objective), we assumed the participants status to be HPV negative (or positive; for clearance objective). In addition, if HPV DNA results were missing for visit(s) prior to the first HPV positive visit (or negative visit; for evaluation of clearance), then the acquisition or clearance interval was assumed to span the time from the last available HPV negative (or positive) visit to the first HPV positive (or negative) visit, respectively. To explore the potential impact of this assumption, we changed missing values for our acquisition and clearance analyses from negative to positive or positive to negative, respectively.

Assumptions and requirements of the Kaplan-Meir method include: a well defined starting point (time 0), well defined outcome, no secular trend (i.e., risk of outcome is independent of calendar time), and losses to follow-up occur independent of the

outcome. For our analyses, the starting point corresponds to study enrollment (or time of the index HPV infection) and for most subjects this was a different calendar time. The outcome of interest is infection with specific HPV type(s) as determined by viral DNA. For the separate acquisition analyses, all women were negative for the specific type(s) being studied at the baseline visit and all those that were found to be positive for the particular HPV type(s) at enrollment were excluded. Females with less than two visits were also excluded from survival analyses. To explore if losses to follow-up were independent of the study outcome, I compared HPV status (positive versus negative) of females according to their last completed study visit in each of the three studies and found no significant differences (i.e., p-values across studies were all >0.05).

Cox's proportional hazards regression was used to calculate the hazard ratio (HR) and associated 95% CI, estimating the effect of infection with vaccine targeted HPV types (6/11, 16, 18) on time to infection with other types or clearance of existing types (as described, above) [241]. Briefly, the assumptions underlying this method include: independence of observations and proportionally of hazards. The hazard ratio is a constant that does not vary over time; and therefore, it is assumed that at any given time, the hazard in those exposed is a multiple of some underlying hazard. Important bias would result if our data do not satisfy the proportionality assumption [242]. In conducting our analyses, I first evaluated this assumption graphically (i.e., by plotting -ln[-lnS(t)] for each level of the covariate) to check whether the level-specific curves were parallel (roughly equal spacing over time). However, since this approach is generally only useful for detecting major departures from proportional hazards, I also checked this

assumption by introducing time-by-covariate interaction terms in the model (assessed using the likelihood ratio test) and confirmed no statistically significant ($p<0.05$) interaction, which provided additional reassurance that this assumption was satisfied. Finally, since only the first event per subject was considered, there was no reason to suspect that the assumption of independence may be violated.

In each of our manuscripts, important confounding factors that we decided should be adjusted for (based on a priori knowledge) included age and lifetime number of sexual partners. As discussed above, any other independent predictors of multiple infections consistently identified across the studies would be adjusted for in our models. In conducting our analyses, we also explored whether any strong empirical confounders exist, producing >10% change in our effect estimates [243].

4.6.3 Objective 3: Evaluation of unmasking

For objective three, I designed a study to evaluate the potential for unmasking that may result from competition for reagents in PCR assays (e.g., primers) between HPVs 52 and 16. HPV16 is the most common HPV genotype worldwide and therefore most likely to be responsible for any unmasking, compared with the other types currently being targeted by vaccination [7]. The primary reason that I decided to focus on HPV52 (rather than other types) was based on negative vaccine efficacy reported in one trial for some endpoints involving HPV52, which the authors suggested may be attributable to unmasking [209]. In addition, other recent studies evaluating the effect of

HPV vaccination in different populations (United States and Scotland [193, 194]) have reported slight increases in certain HPV types not being targeted by vaccination, including HPV52.

In total, 1,200 anogenital specimens (all HPV52 negative according to consensus-primer PCR) were selected for retesting using type-specific, real-time HPV52 PCR, which is capable of detecting as few as 10 HPV52 copies per assay and is therefore much more sensitive for detecting specific HPV types among co-infected specimens, compared with general primer PCR systems [167]. These specimens were collected from females (n=1000) participating in the five epidemiological studies (all were previously described in this section) [22, 202, 236, 238, 239], as well as from males (n=200) participating in HIPVIRG study, which was designed to evaluate the natural history of type specific anal HPV infection among HIV positive men who have sex with men (MSM) living in Montreal [244]. An equal number of specimens from each of these studies (n=200) were randomly selected according to the following criteria. Half were positive for HPV16 (n=100) and half were negative for HPV16, but among this latter group, half (n=50) were positive for an HPV type phylogenetically related to HPV16 (eligible α-9 species types included: 31, 33, 35 and 58), and the other half (n=50) were positive for some other non α-9 HPV type. Our motivation for selecting only specimens that were positive for some other HPV type (among the HPV16 negative 'control' group) was to eliminate major confounding due to the shared route of transmission for HPV infections. That is, females infected with HPV16 would be expected to be at much higher risk for HPV52 infection than females not infected with

HPV (any type). However, the trade-off of this approach is that ‘masking’ of HPV52 (as we have defined it here) may have also occurred in the HPV16 naïve group by some other HPV type(s) present in higher viral load concentrations. Reanalysis of all specimens for HPV52 was carried out (blinded to HPV status) by Dr. François Coutlée at l'Université de Montréal.

The effect of HPV16 positivity on detection of HPV52 (i.e., of unmasking) was evaluated using logistic regression analysis. ORs and associated 95% CIs were calculatedly separately for each study, as well as pooled (with adjustment for age, lifetime number of sexual partners, as well as study in our pooled analysis). Similar to the situation in our regression and cohort approach analyses, some females from the CCCaST trial (St. John’s study site) did not provide information on sexual history, which led to the exclusion of some specimens in our fully adjusted models (n=76). As part of our sensitivity analyses, we eliminated adjustment for sexual history to include all CCCaST specimens. In our pooled analysis, we also explored the effect of removing specimens collected from male subject specimens, i.e., anal specimens collected in the HIPVIRG study.

In each of the six studies, viral load was quantified according to a well-established real time PCR protocol [245] and expressed as the number of HPV DNA copies per cell. Logistic regression was also used to evaluate the effect of HPV16 viral load on HPV52 detection. Specifically, HPV16 viral load was categorized into study

specific tertiles (low, medium, high) and we estimated ORs for each tertile with the HPV16 negative group once again serving as the reference category.

4.7 Pooling

The relatively large size of pooled analyses is a major benefit that permits rare exposures to be more easily studied. In our investigation, we were interested in evaluating the potential for unmasking as well as associations between vaccine-targeted types with other HPV types, with some HPV types being much less common than others. The hierarchal regression approach we used for combining data from five epidemiological studies has already been described above.

Appropriate methods for pooled analyses of epidemiologic studies have been described by others [246, 247]. Before getting to the step of pooling, we examined the homogeneity of effects across studies. We did this by testing for statistical heterogeneity ($p<0.05$), exploring whether there were any important sources of variance/heterogeneity across the studies (e.g., study design, HPV testing methods), and by inspecting the study specific estimates to determine whether results appear to differ only randomly from each other. Ultimately, no statistical heterogeneity was consistently identified across the studies, and in general, results appeared to differ only randomly from each other. We therefore decided that it was appropriate to pool information from the separate studies into a common database, comparing our results from fixed versus random effects models using the Q-test statistic and Hausman specification test (with

adjustment for study in all our models) [248]. Since we expect HPV type competition to be a biological phenomenon that is consistent across populations (i.e., no important residual differences should exist across studies) we were motivated to report estimates from our fixed effects models, assuming that results were comparable with estimates from our random effects models [249].

Unlike prospectively planned pooled analyses such as the European Investigation on Cancer and Nutrition (EPIC) studies [250] or other studies conducted by IARC as part of the SEARCH program [251], the parent studies in this project were not designed with the intent of pooling. However, a benefit for us is that all of these studies were conducted by researchers at our McGill division, involving similar data collection and HPV testing procedures.

4.8 Power and sample size considerations

Sample size for each parent study was calculated for specific endpoints that were different from those that are relevant for my objectives. As a result, the sample size for the current project (objectives 1 and 2) was effectively fixed a priori. The power and sample size considerations described in this section were therefore intended to provide us with an estimate of the power/precision of estimates that may be expected, and were performed prior to conducting any analyses. The only exception was our sample size calculations performed for objective 3 (i.e., our analysis of unmasking), which required retesting of cervical and anal specimens for HPV52.

For our regression approach (objective 1), our study power depended on a number of factors, including sample size, effect estimate, prevalence of the outcome (i.e., HPV vaccine types), and prevalence of exposure (other HPV types) in the population. According to two major recent studies, the global prevalence of HPV16 and 18 (in women with normal cytology) is approximately 5.0% and 2.0%, respectively [32, 33]. Exploration of our data sets supported these estimates. In table 4-4, we present power estimates based on different levels of our exposure variables (other HPV types; 1% or 3%) and anticipated OR estimates (range: 0.1 – 0.9) for HPVs 16 and 18.

Table 4-4: Power calculation for evaluation of objective 1 †

Prevalence of Outcome HPV Type ‡	**Exposure Rate in Control Group***	**Odds Ratio**	**Estimated Power (%)**
HPV16	1.00%	0.90	5.50
		0.70	16.50
5.00%		0.50	46.40
		0.30	88.50
		0.10	100.00
	3.00%	0.90	8.70
		0.70	49.30
		0.50	96.20
		0.30	100.00
		0.10	100.00
HPV18	1.00%	0.90	4.60
		0.70	7.20
2.00%		0.50	14.80
		0.30	32.20
		0.10	74.70
	3.00%	0.90	5.90
		0.70	20.00
		0.50	57.20
		0.30	95.80
		0.10	100.00

† Two-sided test at a significance level of α=0.05; based on a sample of 29,468 HPV test results from our studies.

‡ Estimated prevalence of HPV types 16 and 18, based on references [32, 33] and our own data.

*Estimated prevalence of exposure of other HPV types, based on references [32, 33] and our own data.

Statistical power for Kaplan-Meier analyses and Cox's proportional hazards regression (objective 2) is mainly determined by the number of events of interest, and is less influenced by the number of subjects or length of follow-up. In our case I was interested in estimating the power for a number of different outcome comparisons. Figure 4-4, below, shows the statistical power obtained with a range of number of events per group [252-254]. When groups with less than an average of 20 events are compared, the statistical power for detecting a HR of less than 2.0 will be below 35%. However, for HR ≥ 4.0, groups with 10 and 20 events provide statistical power of approximately 60% and 90%, respectively. These estimates represent the expected range in power for rarer HPV types (e.g., types 6 and 11) – based on worldwide prevalence statistics [32, 33] and exploration of our own data. For more common types the power would be much greater. For example, in groups with ≥ 60 events (representing common types such as HPVs 16 and 18) the statistical power to detect a HR of 2.0 would be above 80%.

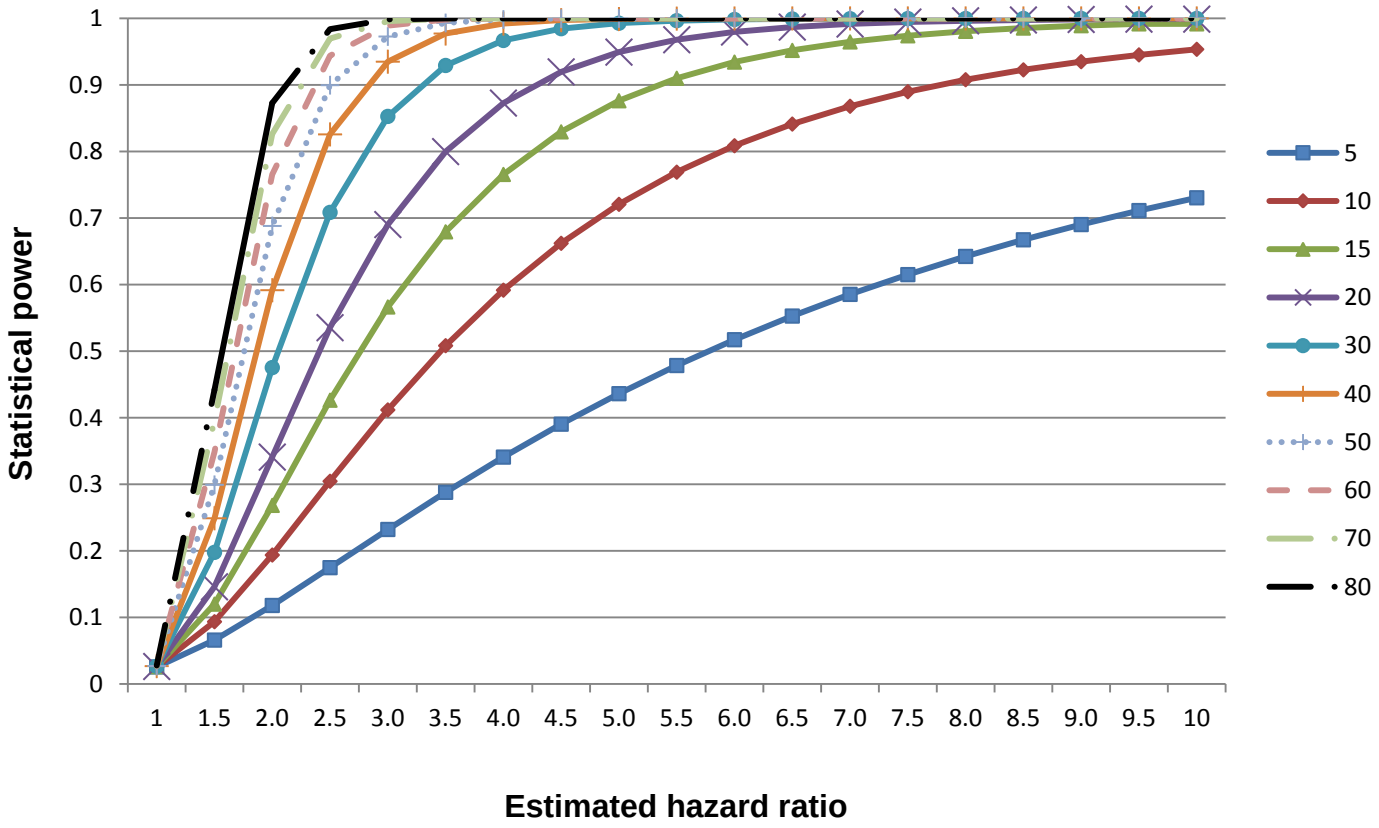


Figure 4-4: Statistical power according to estimates of the hazard ratio, for a range of events per group.

The final objective of this study was to evaluate unmasking. To estimate the sample size required for this analysis we were required to make an educated guess with regard to the amount of unmasking that should be expected. A recent large study (n=15,774) comparing consensus PCR with type-specific PCR reported a false negative detection rate of 10.9%; suggesting that masking may be an important cause [222]. If we assume that masking was the main source of error, then this can be used to inform our sample size calculation [255]. For example, if the probability of unmasking for a candidate HPV type is assumed to be 3% in HPV16 naive subjects (not an unrealistic

assumption for some common HPV types) and we set our α error to be 0.05, we would need to re-test a total of 1656 cervical samples (equal number of cases and controls) using type specific PCR to have 80% power to detect ORs ≥ 2.0. But if masking does exist, we anticipated that effect estimate would be much greater than 2.0. In table 3, we present the sample size needed for varying levels of exposure, effect size, and study power.

Table 4-5: Sample size calculation for evaluation of objective 3 †

Study Power	Exposure Rate in Control Group‡	Odds Ratio	Total Number of Subject Samples Required (cases and controls)*
0.80	0.01	2.0	4796
		5.0	608
		10.0	224
	0.03	2.0	1656
		5.0	216
		10.0	82
	0.05	2.0	1030
		5.0	138
		10.0	54
0.90	0.01	2.0	6420
		5.0	814
		10.0	300
	0.03	2.0	2218
		5.0	288
		10.0	110
	0.05	2.0	1380
		5.0	184
		10.0	72

† Two-sided test at a significance level of α=0.05.

‡ Estimated level of unmasking (exposure) in control group was based on references [32, 33, 222, 223]

* Ratio of cases to controls equal to one.

CHAPTER 5: MANUSCRIPT III

EVALUATION OF HUMAN PAPILLOMAVIRUS TYPE COMPETITION AND THE POTENTIAL FOR TYPE REPLACEMENT POST-VACCINATION

5.1 Preamble

The first recommended approach from manuscript II that we used to evaluate HPV type competition is regression. For each of the vaccine-targeted HPV types (6, 11, 16 and 18), we created separate models that included all other HPV types as predictor variables, along with important confounding factors, such as age and number of lifetime sexual partners. In our analyses of pairwise associations involving vaccine-targeted HPV types, non-vaccine types found to be negatively associated with any of these types (ORs <1.0; 95% CI excluding 1.0) would be flagged as candidates for type replacement.

At the time when this third manuscript was being prepared, few other studies had evaluated interactions between different HPV types; and until recently, none had focussed specifically on individual vaccine-targeted types. With access to >38,000 cervical specimens from five studies conducted by our division, the current study represents the largest study on this topic to date, and is also the only one that applied Bayesian hierarchical regression, which incorporates shrinkage to improve precision. As a result, we were able to include additional rare HPV types in this analysis (compared with previous smaller studies), and our estimates of different type-type associations were generally more precise.

Evaluation of Human Papillomavirus Type Competition and the Potential for Type Replacement Post-vaccination

Running title: Regression approach to evaluate HPV type competition

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FOOTNOTES

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ABSTRACT

Currently, two vaccines are available that prevent the human papillomavirus (HPV) types (16 and 18), which are responsible for the majority of cervical cancer cases worldwide. To explore the potential for type replacement following introduction of vaccination, we assessed natural HPV type competition among unvaccinated females. Valid HPV DNA typing information was available from five epidemiological studies conducted in Canada and Brazil (n=14,685), which used similar consensus-primer PCR assays, capable of detecting up to 40 HPV types. A total of 38,088 cervicovaginal specimens were available for inclusion in our analyses evaluating HPV type-type interactions involving vaccine-targeted types (6, 11, 16, and 18), and infection with each of the other HPV types. Across the studies, the average age of participants ranged from 21.0 to 43.7 years. HPV16 was the most common type (prevalence range: 1.0% to 13.8%), and in general HPV types were more likely to be detected as part of a multiple infection than as single infections. In our analyses focusing on each of the vaccine-targeted HPV types separately, many significant positive associations were observed (particularly involving HPV16); however, we did not observe any statistically significant negative associations, which suggests that HPV type competition is unlikely.

Infection with high-oncogenic risk human papillomavirus (HR-HPV) is a necessary cause of cervical cancer in women [159] and an important cause of other anogenital cancers in both genders [7]. In addition, low-oncogenic risk (LR) HPV infections may cause benign lesions known as acuminate condylomata (genital warts), as well as low grade squamous intraepithelial cervical lesions. Fortunately, there are now two highly effective HPV vaccines available (Merck's Gardasil© and GlaxoSmithKline's Cervarix©) [2, 3] that offer protection against two HR-HPV types (16 and 18), which are responsible for approximately 70% of invasive cervical cancer cases. Only Gardasil protects against additional LR-HPV types (6 and 11) that cause approximately 90% of genital warts cases [4, 5, 100]. Although HPV vaccination is eventually expected to reduce the burden of disease attributable to these HPV types, there is also concern that it may lead to "type replacement", i.e., an increase in the prevalence of other non-vaccine HPV types following the reduction of vaccine targeted types [12, 201].

For type replacement to occur, a biological prerequisite is that different HPV types must compete with one another for niche occupation during natural infection [11, 12, 201]. We recently described different epidemiological approaches to evaluate HPV type competition in order to gain insight regarding the likelihood of type replacement [12]. The two main approaches include construction of Kaplan-Meier curves and Cox models to evaluate sequential acquisition and clearance of HPV types according to HPV status with vaccine-targeted types; and construction of logistic regression models for each vaccine-targeted type to explore whether infection with these types may be

associated with infection with other HPV types. A number of cohort studies evaluating the natural history of HPV infections among females have suggested that those infected with HPV (any type) are generally at higher risk of acquiring other types [76-78], or at about equal risk of acquiring and clearing existing infections [76-78, 104, 105]. Similarly, other recent cross-sectional studies that have investigated clustering patterns of different HPV types have found that females infected with HPV (vaccine or other types) are more likely to be infected with additional HPV types [101-103, 195-199]. These previous studies reported very few negative associations, therefore providing some reassurance that type competition does not exist and that replacement is unlikely. Despite the large sample size of some of these studies, few or no co-infections were observed for rare HPV types, leading to non-positivity or low precision for some comparisons. In addition, evaluation of pairwise interactions in these studies did not account for presence of other HPV types, which may have introduced some confounding [12].

To evaluate HPV type competition in the current study, we applied a unique hierarchical (Bayesian) regression approach that employs shrinkage and adjustment for confounders, as well as other HPV types. Data were available from five pre-vaccination studies conducted among females in Canada and Brazil.

METHODS

Study population and design

Participant data for the current analysis came from five studies conducted by our division. They included: a) the Ludwig-McGill cohort study (São Paulo, Brazil; n=2462) [202], b) the HPV Infection and Transmission among Couples through Heterosexual activity (HITCH) cohort study (Montreal, Canada; n=1038; 502 females, 536 males) [236], c) the McGill-Concordia cohort study (Montreal, Canada; n=636) [22], d) the Biomarkers of Cervical Cancer Risk (BCCR) case-control study (Montreal, Canada; n=1687) [238], and e) the Canadian Cervical Cancer Screening Trial (CCCaST) (Montreal/St. John's, Canada; n=10,154) [239]. Recruitment for these studies took place between 1993 (Ludwig-McGill) and 2010 (HITCH), and age of participants ranged from 18 (Ludwig-McGill, HITCH and McGill-Concordia) to 69 years (CCCaST). Protocols for each of the five studies have been described in detail elsewhere [22, 202, 236, 238, 239]. Briefly, the three cohort studies (Ludwig-McGill, HITCH, and McGill-Concordia) were designed to evaluate the natural history of HPV infection among females, and transmission of HPV among heterosexual couples (HITCH study only; male data from this study was not included in the current analysis). BCCR is a case-control study that was originally designed to evaluate the role of p53 codon 72 polymorphism in the etiology of cervical cancer, and CCCaST was the first North American randomized controlled trial to compare Pap cytology versus HPV testing in screening for cervical cancer. Subjects completed questionnaires to collect information on important demographic and lifestyle variables; and provided cervical samples (self or provider collected) for HPV testing at each of their clinic visits. All participants provided written informed consent prior to joining these studies, and each study was approved by review boards or ethical committees of the participating institutions.

HPV DNA detection and genotyping

In the three cohort studies, cervical specimens were collected and tested for HPV at each clinic visit (every four months during the first year of follow-up/twice annually in subsequent years of follow-up in the Ludwig-McGill and HITCH studies; and twice annually in the McGill-Concordia study). Subjects from the Ludwig-McGill, HITCH, and McGill-Concordia studies contributed an average of 9.0, 4.4, and 4.2 cervical specimens for HPV testing, respectively; whereas subjects from the BCCR and CCCaST studies contributed only one specimen for HPV testing.

Details regarding the specific HPV testing protocols applied for each study (including sample collection) have been described in detail elsewhere [22, 202, 236, 238, 239]. Briefly, all studies employed consensus primer PCR assays (L1 PGMY or MY09/11 and hybridization with oligonucleotide probes and restriction fragment length polymorphism analysis, line blot assay, or linear array), which are capable of detecting between 27 and 40 different HPV types. Although the genotyping procedure in the Ludwig-McGill study (hybridization with individual oligonucleotide probes and restriction fragment-length polymorphism analysis) did not allow us to distinguish between vaccine-targeted HPV types 6 and 11, these are two of the most closely related HPV types (with indistinguishable biological and pathological properties) [204], therefore grouping them was not viewed as a major limitation of our analysis. Nonetheless, we evaluated HPVs 6 and 11 together, as well as separately in the other four studies. Since types that are phylogenetically related (i.e., from the same species) share a large proportion of their nucleotide sequence (≥60%) and display similar properties, we

suspected that types from the same species would be more likely to compete [18, 204]. HPV types belonging to the same species as HPV6/11 (α-10) include 13, 44, and 74; as HPV16 (α-9) include 31, 33, 35, 52, 58, and 67; and as HPV18 (α-7) include 39, 45, 59, 68, and 70.

Statistical analysis

We investigated the association between infection with the vaccine preventable types and infection with each of the other HPV types using pooled data from the five studies. Bayesian hierarchical regression models were constructed for vaccine preventable types 6, 11 (6/11 combined), 16, and 18. Age and lifetime number of sex partners were chosen as covariates a priori, since they are strong predictors of HPV infection [7]. Thus the primary analyses excluded a portion of CCCaST participants who were missing baseline data on lifetime number sex partners. Models for 6/11 combined, 16, and 18 included data from all five studies. Models for 6 and 11 separately excluded the Ludwig-McGill study, as explained above. Secondary analyses included the CCCaST participants with missing information on lifetime number of sex partners by excluding it as a covariate. We also conducted analyses for each study separately.

Specifically, the probability of infection with the vaccine preventable type was modeled in a 2-tier hierarchical model, where individual visits for subjects over time were nested within subjects in order to account for subject-level clustering. At the visit level, a logistic model was fitted with infection with the vaccine preventable type as the

outcome and every other HPV type and age at the time of the visit as predictors. At the subject level, the subject-specific intercepts were modeled by accounting for lifetime number of sex partners at baseline, as well as the study that the subject came from for the pooled models. Thus, the odds ratio estimate for each HPV type represents the odds of the vaccine preventable type being present in the presence of that HPV type compared to the odds of the vaccine preventable type being present in the absence of that particular HPV type, adjusted for all other HPV types, age at visit, lifetime number of sex partners at baseline, and study.

In order to improve the precision of the estimates for the effect of the presence of other HPV types on the presence of the vaccine preventable type, the logistic regression parameters for all the other HPV types were assumed to be normally distributed around an overall mean effect of co-infection. Diffuse or wide prior distributions were used for all other parameters. All analyses were conducted using WinBUGS software version 1.4.3 (MRC Biostatistics Unit, Cambridge).

The additional hierarchical component on the coefficients of other HPV types produces a shrinkage effect, whereby unstable estimates with large variances are drawn closer to the mean. The assumption introduces a bias in favour of reducing variance and potentially reducing mean squared error [218]. To explore the possible effect of this bias, we also compared our results with estimates for HPV type associations calculated using the maximum likelihood method.

RESULTS

Subject characteristics stratified by study population are listed in Table 1. The average age of participants at enrollment across the five studies ranged from 21.0 (HITCH study) to 43.7 years (CCCaST study). HITCH and McGill-Concordia studies included few females that were married/common-law (14.1% and 18.0%, respectively) or that had ever been pregnant (9.8% and 16.2%, respectively). Compared with subjects from the four Canadian studies, Brazilian Ludwig-McGill study participants reported fewer lifetime sexual partners (87% had less than five partners) and the majority rarely used condoms (less than 4% used condoms regularly). Most subjects in the McGill-Concordia, HITCH and BCCR studies indicated that they were never smokers (62.7%, 62.3% and 50.0%, respectively); whereas the majority of Ludwig-McGill and CCCaST participants reported that they were current/former smokers (52.5% and 79.8%, respectively).

Across all studies, HPV16 was the most common type detected among cervical specimens: Ludwig-McGill (n=546, 2.5%), McGill-Concordia (n=220, 8.2%), HITCH (n=305, 13.8%), CCCaST (n=105, 1.0%), and BCCR (n=47, 4.8%) (Figure 5-1). Although the ranking of other common HPV types varied across the studies, most were detected as part of a multiple infection (rather than as single infections), except in the Ludwig-McGill study. Subject characteristics that were commonly associated with multiple HPV infection included younger age and higher number of sexual partners (Table 5-2). CCCaST participants that reported condom use (“ever” versus “never”) and who were widowed/divorced were at higher risk of being infected with multiple HPV types, whereas subjects from the BCCR study who were married/common-law were at

significantly lower risk compared with single individuals. Former smoking status was also associated with greater risk of multiple infections in HITCH and CCCaST studies, but not in the others.

Each of the figures present OR estimates for type-type associations on the natural log scale; therefore, (log)OR estimates greater than zero correspond to ORs greater than one (i.e., positive associations between HPV types), and the opposite for (log)OR estimates below zero. In our pooled regression analyses (including data from all five studies), no statistically significant negative associations were observed between vaccine-targeted HPV types (HPVs 6, 11, 16, and 18) and any other types (Figure 5-2). In fact, the only negative association observed was between HPV18 and 89 (OR=0.92, 95%CI: 0.49-1.52). These analyses included adjustment for other HPV types, age and lifetime number of sexual partners, but excluded over half of CCCaST study participants (n=5754) due to missing sexual history information from St. John's study site participants. In our analyses adjusted for other HPV types and age only (including all CCCaST subjects), results were similar (zero negative associations were observed) and OR estimates were generally higher (Appendix 3, Figure 5-3).

Across the studies with individual typing information for HPVs 6 and 11 (i.e., all other than Ludwig-McGill study), HPV11 was detected in only 23 of 16027 specimens. In our analyses of HPVs 6 and 11 grouped together (Figures 5-2 and 5-3; panel A) and separately (Figures 5-2 and 5-3; panels B and C, respectively), results were similar between HPVs 6/11 and HPV6, but not between HPVs 6/11 and HPV11. In our fully

adjusted pooled analyses (Figure 5-2), many statistically significant positive associations (ORs>1.0, 95% CIs excluded 1.0) were observed between HPVs 6/11 and other types (HPVs 68, 53, 52, 44, 40, 35, 31, 18, and 16), as well as between HPV6 and other types (HPVs 89, 84, 68, 53, 52, 44, 42, 35, 33, 31, and 16); however, no significant positive associations were observed involving HPV11. HPV16 was positively associated with all except for the following HPV types: 71, 70, 69, 68, 61, 57, 40, 34, and 32. Finally, HPV18 was positively associated with HPVs 82, 72, 68, 66, 59, 58, 56, 55, 53, 52, 35, 31, 16, 6/11. In summary, significant positive associations were observed involving one or more vaccine-targeted HPV types, with all except for seven other types (HPVs 71, 70, 69, 61, 57, 34 and 31). In our pooled analyses not controlling for lifetime number of sexual partners (Appendix 3, Figure 5-3; all CCCaST specimens included), all of the HPV types listed above remained statistically significant in each of the respective analyses; and also included additional significant types (ORs>1.0).

In our fully adjusted pooled analyses focusing on HPVS 6/11, 6, 11, 16 and 18 (Figure 5-2), the average pooled (log)ORs for co-infections involving these HPV types estimates (i.e., the value that individual type-type associations were “shrunk” towards in each of the respective analyses) were 0.39 (95%CI: 0.24-0.53), 0.32 (95%CI: 0.20-0.43), 0.26 (95%CI: -0.07-0.50), 0.45 (95%CI: 0.34-0.55), and 0.41 (95%CI: 0.23-0.57), respectively. The average pooled ORs for co-infections involving vaccine-targeted HPV types with other types varied across the five studies; however, no consistent trend of higher or lower pooled ORs was observed for any of the studies (Figures 5-4, 5-5, 5-6, 5-7 and 5-8, respectively; Appendix 3). Because very few HPV11 infections were

observed in the BCCR and CCCaST studies (n=2 and n=1, respectively), individual study results for this vaccine-target type were only presented for the McGill-Concordia and HITCH studies (Figure 5-6; Appendix 3).

DISCUSSION

The concept that pre-vaccine epidemiological data may be useful to evaluate natural HPV type competition and the potential for type replacement is now well established [12]. HPV types that naturally compete with HPVs 6, 11, 16, and/or 18 may be more likely to fill the ecological niches vacated by these vaccine-target types. The present study focussed on female populations and utilized a novel Bayesian analytic approach that incorporates shrinkage to improve precision of pairwise associations [12]. In general, our results support previous studies, which mainly reported null or positive associations between different HPV types [101-103, 195-199].

Recently, Vaccarella and colleagues used a number of different large data sets to evaluate clustering patterns between HPV types (via hierarchical regression models with women-level random effects), identifying few negative associations and some positive associations, which they generally attributed to diagnostic artifacts [103, 196-198]. Similarly, Chaturvedi and colleagues reported very few negative and positive associations in examining HPV co-infection patterns among women from the Costa Rica Vaccine Trial, concluding that HPV infections seemed to occur independently in this population [195]. In a recent pooled analysis, including information from three diverse

study populations in the Netherlands, Mollers and colleagues also reported no significant pairwise interactions, but did suggest that clustering patterns differed across risk groups and across types, particularly between low- and high-risk HPV types [199]. In general, phylogenetic relatedness did not strongly influence clustering patterns in these prior studies; whereas in our study, HPV16 (α-9) was positivity associated with all related types, and HPVs 6/11 and 18 were positively associated with related types 44 (α-10) and 59 (α-7), respectively.

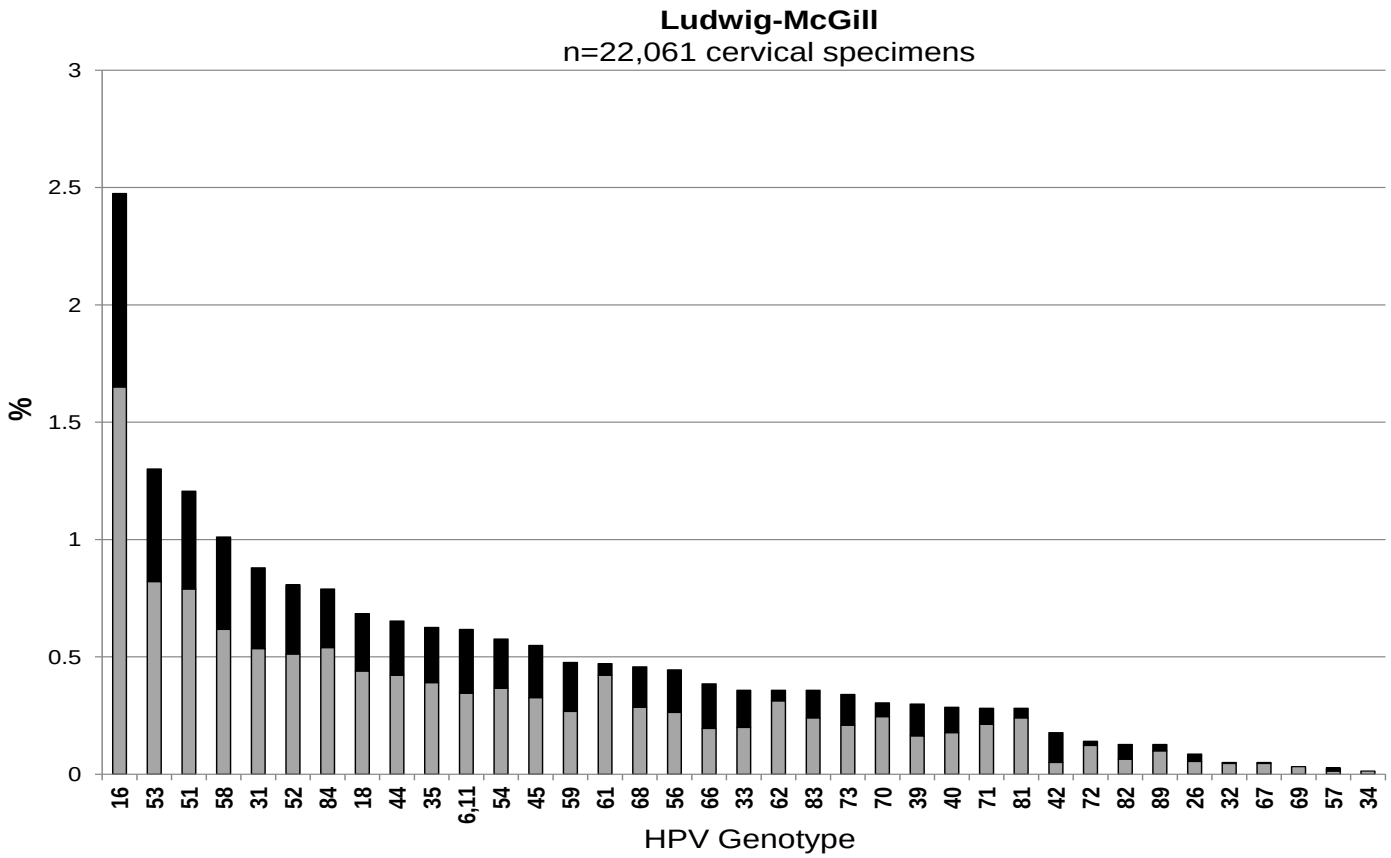
Across the five studies, there were more than 38,000 cervical specimens with valid HPV testing results, which makes the current pooled analysis the largest study on this topic to date. As a result, we were able to evaluate associations between vaccine-targeted HPV types with all others, including rare types. The application of Bayesian methods incorporating shrinkage further improved our precision, and still allowed us to adjust for all relevant covariates and presence of other HPV types in our models. However, any improvement in precision resulting from shrinkage comes at the expense of introducing some bias [218]. To explore if our results may have been meaningfully different according to traditional analytic methods (i.e., without this bias/precision trade-off), we performed sensitivity analyses using maximum likelihood estimation. As expected, this approach led to wider confidence intervals, but importantly it did not lead to any statistically significant ORs less than one (data not shown). Although we did not observe any statistically significant negative associations in our study, we did observe a high number of positive associations. One possible explanation is that some of the significant positive associations may have resulted from residual confounding, due to

our inability to control for all risk factors of multiple-type HPV infection, e.g., host susceptibility, immunological differences, HPV type exposure from male sexual partners, or other unmeasured behaviour risk factors. For example, in our analyses including all CCCaST specimens (i.e., unadjusted for sexual history), confounding may explain the higher OR estimates and greater number of HPV types found to be positively associated with HPVs 6/11, 16 and 18. To ensure that analyses of type interactions are focused among those with sufficient HPV exposure opportunity, others have previously explored the effect of restricting their study sample to individuals with ≥1 HPV infection [101, 109, 210, 212]. However, this approach leads to a form of selection bias, referred to as collider stratification bias [256], and was therefore not applied in the current study.

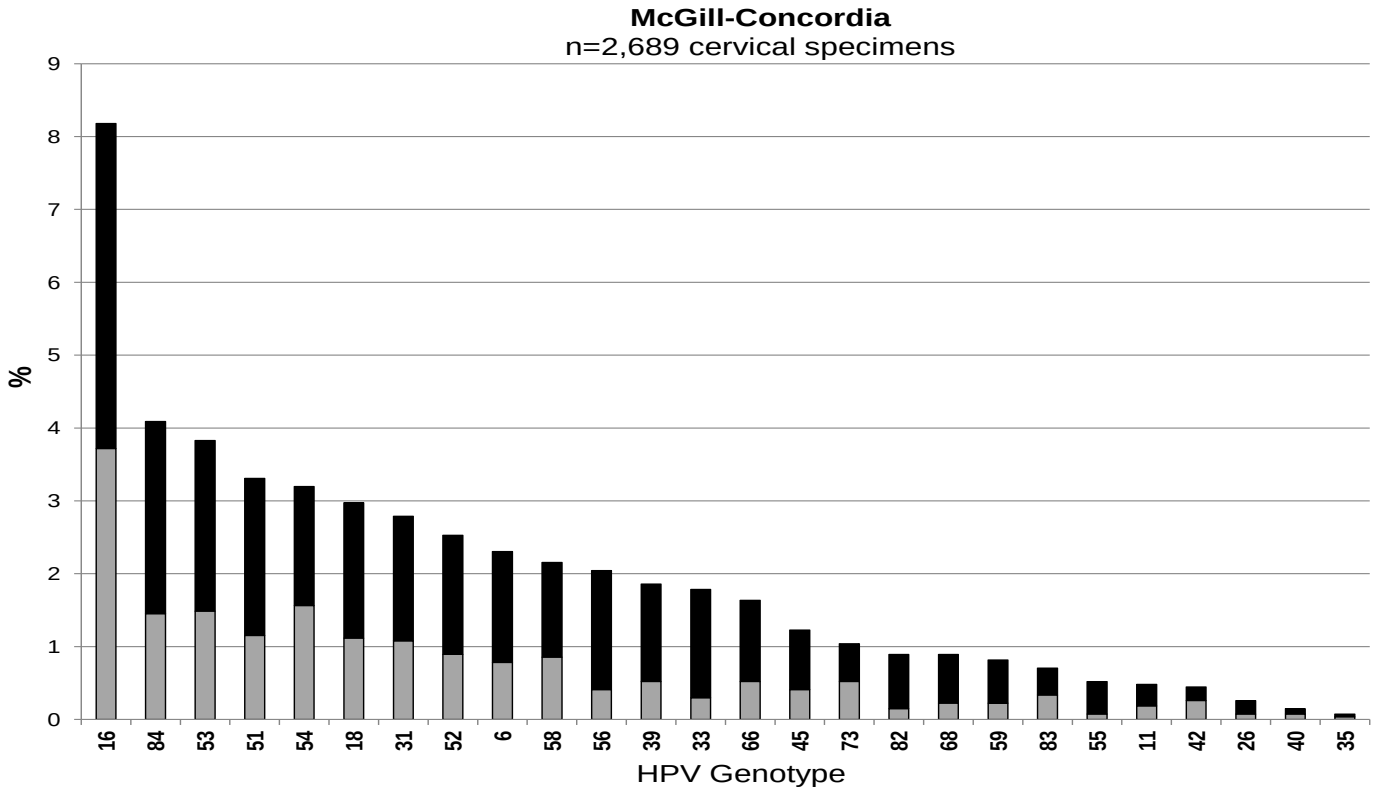
The five parent studies from which specimens were collected all utilized broad spectrum PCR assays to test for the presence of HPV. Previously, we discussed concerns regarding the sensitivity of these assays in the context of type replacement evaluation, particularly in situations where specimens are coinfected with multiple HPV types [12]. In addition, there is also the possibility that specificity may suffer as a consequence of probe cross-reactivity [257], which may explain the tendency for some phylogenetically related types to cluster together. However, considering that most HPV types from the α-9 species are also classified as definite carcinogens by the International Agency for Research on Cancer (all except for HPV67) [9], they are also more likely to persist (than low-risk types) and therefore more likely to be detected together with other types, which has also previously been reported [199].

Previous cross-sectional and cohort studies focusing on different populations and employing unique analytic/genotyping methods have failed to provide consistent or strong evidence that negative pairwise HPV interactions exist [76-78, 101-105, 195-199]. The current study adds to this literature by providing additional reassurance that - owing to the lack of HPV type competition - type replacement appears unlikely. Since we did not include females that received prophylactic HPV vaccines for comparison in this study, we must assume that no major difference in acquiring other types exists among females who are naturally uninfected with vaccine-target types. Eventually, a definitive answer to this question of whether HPV type replacement will (or has) occurred will come from long-term surveillance studies comparing pre- and post-vaccination HPV prevalence rates, which properly account for possible diagnostic artifacts [12]. For now, the absence of HPV type competition (a necessary prerequisite for type replacement) suggests that reductions in vaccine-target HPV types will not be countered by any increase in other HPV types in vaccinated populations.

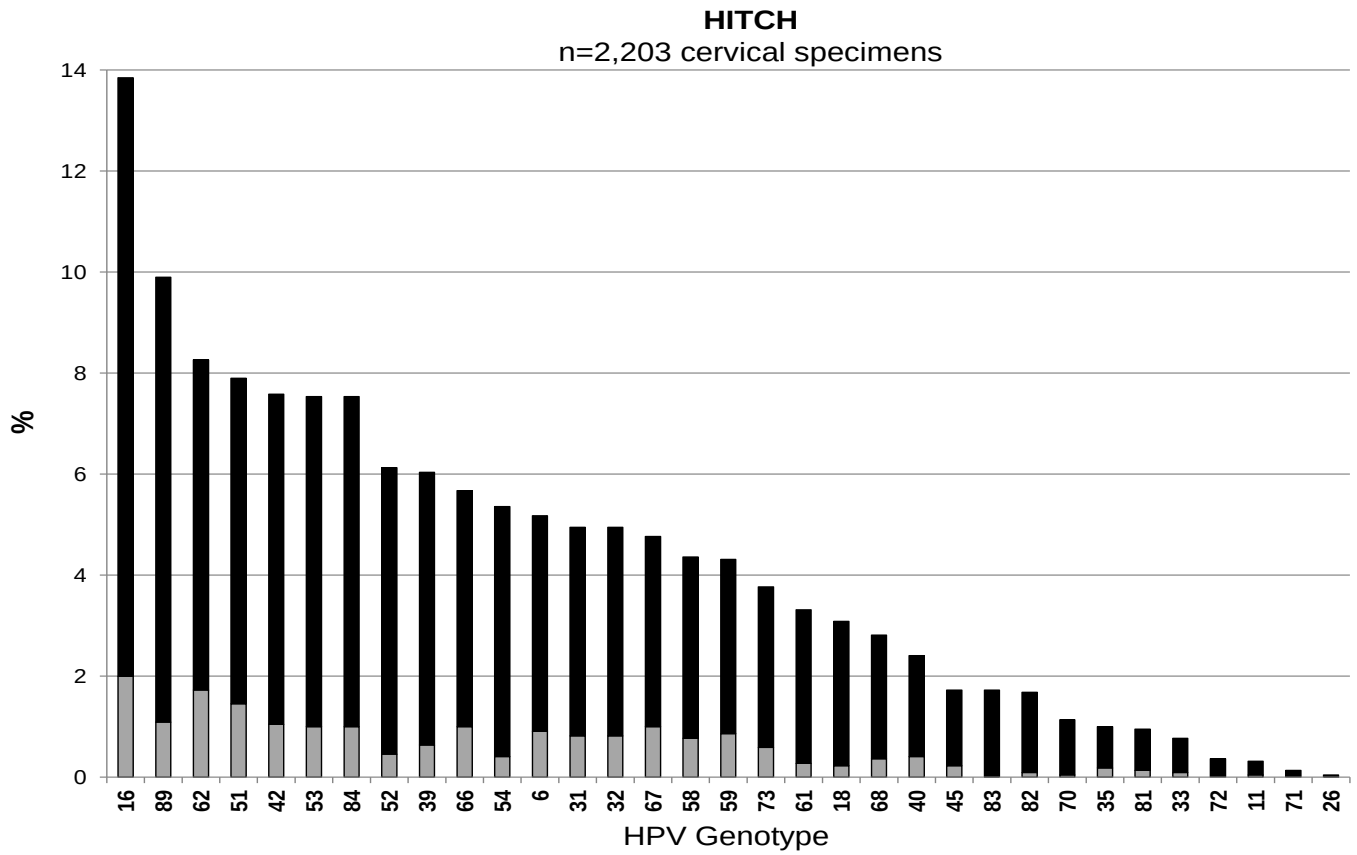
A)


Ludwig-McGill
n=22,061 cervical specimens
%
3
2.5
2
1.5
1
0.5
0
16 53 51 58 31 52 84 18 44 35 6,11 54 45 59 61 68 56 66 33 62 83 73 70 39 40 71 81 42 72 82 89 26 32 67 69 57 34
HPV Genotype

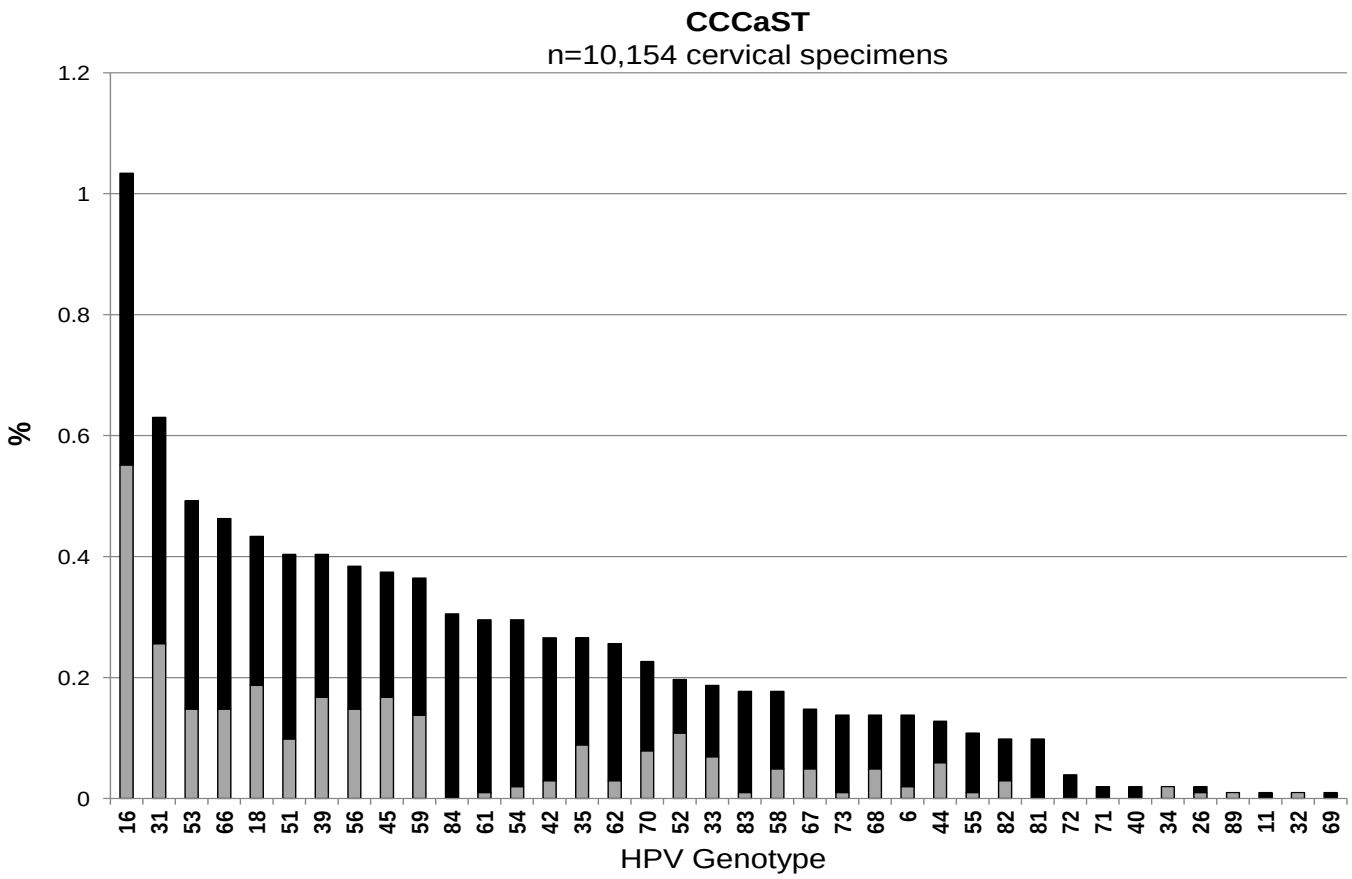
B)


McGill-Concordia
n=2,689 cervical specimens
%
9
8
7
6
5
4
3
2
1
0
16 84 53 51 54 18 31 52 6 58 56 39 33 66 45 73 82 68 59 83 55 11 42 26 40 35
HPV Genotype

C)


HITCH
n=2,203 cervical specimens
%
0
2
4
6
8
10
12
14
16 89 62 51 42 53 84 52 39 66 54 6 31 32 67 58 59 73 61 18 68 40 45 83 82 70 35 81 33 72 11 71 26
HPV Genotype

D)


CCCaST
n=10,154 cervical specimens
%
0
0.2
0.4
0.6
0.8
1
1.2
16 31 53 66 18 51 39 56 45 59 84 61 54 42 35 62 70 52 33 83 58 67 73 68 6 44 55 82 81 72 71 40 34 26 89 11 32 69
HPV Genotype

E)

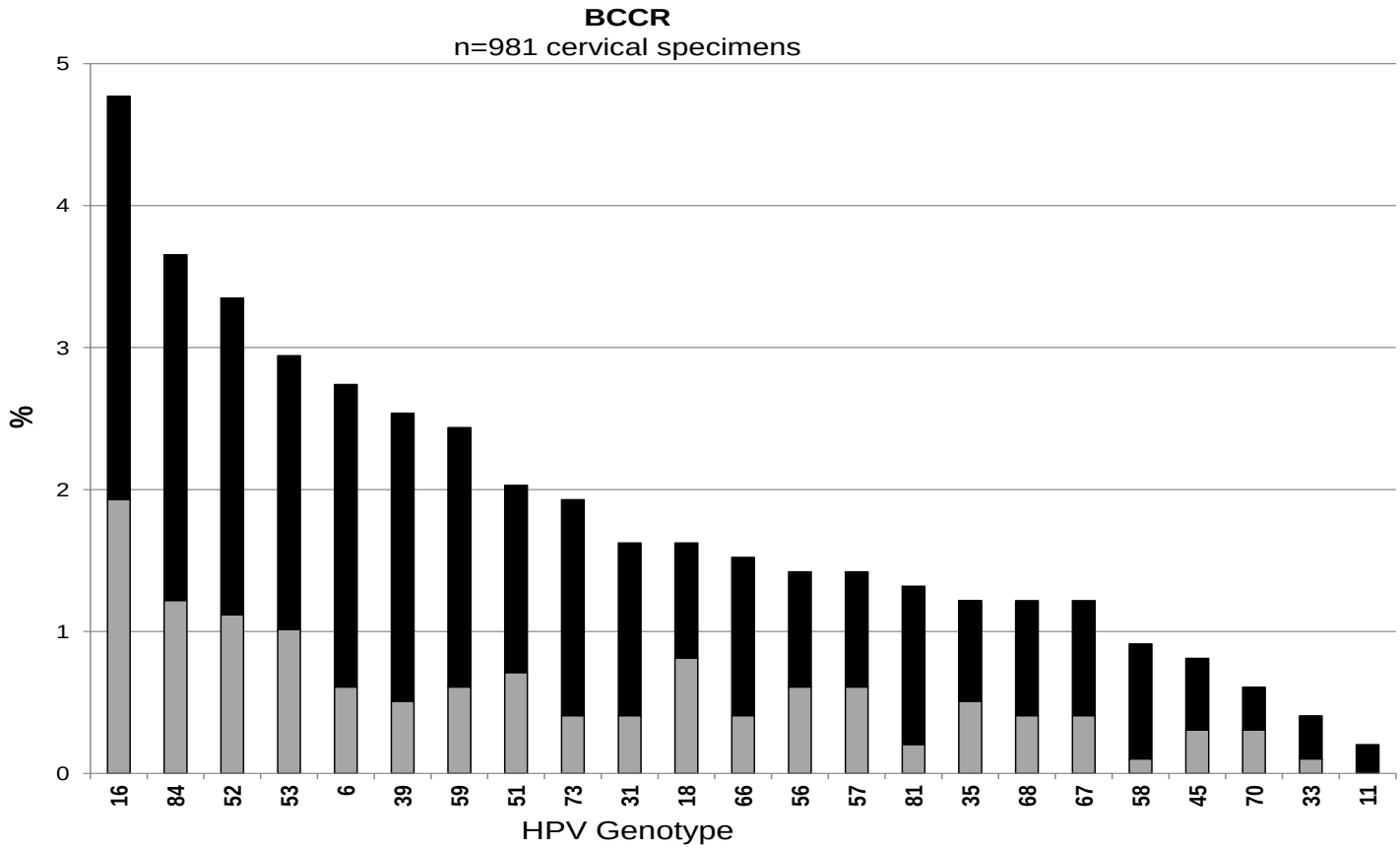


Figure 5-1:

Human papillomavirus (HPV) genotype distribution of single (in light grey) and multiple infections (in black) in order of descending frequency from a) Ludwig-McGill cohort study; b) McGill-Concordia cohort study; c) HITCH cohort study; d) CCCaST study; and e) BCCR case-control study. Note that the scale for the vertical axis ranges across panels.

Table 5-1: Characteristics of female participants at baseline/enrollment in five epidemiological studies

Characteristic	Ludwig-McGill *n*=2462 n (%)	McGill-Concordia *n*=636 n (%)	HITCH *n*=502 n (%)	CCCaST [a] *n*=10154 n (%)	BCCR *n*=985 n (%)
Age, years, mean (SD)	32.7 (8.8)	22.5 (4.0)	21.0 (2.1)	43.7 (9.1)	30.1(9.8)
Marital status					
Single	252 (10.2)	495 (77.8)	425 (84.7)	1262 (12.4)	450 (45.7)
Married/common law	2011 (81.7)	114 (18.0)	71 (14.1)	7441 (73.3)	474 (48.2)
Widowed/divorced	197 (8.0)	14 (2.2)	6 (1.2)	1353 (13.3)	57 (5.8)
Missing	2 (0.1)	13 (2.0)	0 (0.0)	98 (1.0)	4 (0.4)
Age at sexual debut					
< 16	479 (19.5)	125 (19.6)	45 (24.3)	557 (12.7)	243 (24.7)
≥ 16	1958 (79.5)	443 (69.7)	454 (75.1)	3795 (86.2)	702 (71.3)
Missing	25 (1.0)	68 (10.7)	3 (0.6)	48 (1.1)	40 (4.0)
Lifetime # of sex partners					
0-1	1089 (44.2)	135 (22.2)	54 (10.7)	851 (19.3)	163 (16.5)
2-4	1053 (42.8)	198 (32.1)	145 (28.9)	1251 (28.4)	291 (29.5)
≥ 5	318 (12.9)	277 (43.6)	303 (60.4)	2236 (50.8)	516 (52.4)
Missing	2 (0.1)	26 (4.1)	0 (0.0)	62 (1.4)	15 (1.5)
# of pregnancies					
0	47 (1.9)	511 (80.3)	452 (90.0)	806 (18.3)	471 (47.8)
1-2	894 (36.3)	97 (15.2)	47 (9.4)	2113 (48.0)	335 (34.0)
≥ 3	1502 (61.0)	6 (1.0)	2 (0.4)	1420 (32.3)	174 (17.7)
Missing	19 (0.8)	22 (3.5)	1 (0.2)	61 (1.4)	5 (0.5)
Oral contraceptive use					
Never	397 (16.1)	135 (21.2)	80 (16.0)	3958 (39.0)	91 (9.2)
Ever	2064 (83.9)	461 (72.5)	421 (83.9)	1496 (14.7) [b]	882 (89.5)
Missing	1 (0.0)	40 (6.3)	1 (0.4)	4700 (46.3)	12 (1.2)
Condom use					
Never	936 (38.0)	30 (4.7)	16 (3.2)	4206 (41.4)	93 (9.4)
Rarely or sometimes	1398 (56.8)	209 (32.9)	185 (37.0)	1187 (11.7) [b]	344 (34.9)
Regularly or always	92 (3.7)	362 (56.9)	300 (59.6)		536 (54.4)
Missing	36 (1.5)	35 (5.5)	1 (0.2)	4761 (46.9)	12 (1.2)
Cigarette smoking					
Never smoker	1168 (47.5)	399 (62.7)	313 (62.3)	1967 (19.4)	492 (50.0)
Former smoker	429 (17.4)	124 (19.5)	129 (25.7)	4928 (48.5)	189 (19.2)
Current smoker	864 (35.1)	99 (15.6)	60 (12.0)	3182 (31.3)	300 (30.4)
Missing	1 (0.0)	14 (2.2)	0 (0.0)	77 (0.8)	4 (0.4)

Abbreviations: SD, standard deviation.

[a] St. John's study site (*n*=5754) did not collect information on age at sexual debut, number of lifetime sex partners, or number of pregnancies. For these variables, percentage missing was based on the number of Montreal study site subjects only (*n*=4400).

[b] Checklist was used in CCCaST study only to evaluate whether subjects "ever" used oral contraceptives or condoms, along with other contraceptive methods.

Table 5-2: Characteristics of female participants at baseline/enrollment from five epidemiological studies, stratified by HPV status [a]

Characteristic	Ludwig-McGill study *n*=2462			McGill-Concordia study *n*=636			HITCH study *n*=452			CCCaST study *n*=10154			BCCR study *n*=981		
	S n (%)	M n (%)	OR (95% CI) [c]	S n (%)	M n (%)	OR (95% CI) [c]	S n (%)	M n (%)	OR (95% CI) [c]	S n (%)	M n (%)	OR (95% CI) [c]	S n (%)	M n (%)	OR (95% CI) [b]
Age, years, mean (SD) [c]	32.1 (8.7)	29.6 (8.8)	0.96 (.94-.98)	22.9 (4.0)	21.8 (3.1)	0.92 (.87-.98)	21.0 (2.0)	21.3 (2.3)	1.02 (.92-1.14)	40.0 (8.3)	38.3 (7.1)	0.96 (.92-.99)	29.2 (7.6)	26.0 (7.1)	0.94 (.90-.98)
Marital status															
Single	106 (12.7)	72 (22.3)	1.00	118 (76.1)	158 (90.8)	1.00	111 (84.1)	187 (85.8)	1.00	90 (27.3)	86 (31.6)	1.00	95 (54.9)	103 (73.1)	1.00
Married/common law	653 (77.9)	221 (68.4)	0.60 (.34-1.09)	33 (21.3)	14 (8.0)	.96 (.26-3.56)	19 (14.4)	29 (13.3)	0.84 (.44-1.63)	170 (51.7)	94 (34.6)	0.94 (.52-1.71)	70 (40.5)	35 (24.8)	0.51 (.30-.89)
Widowed/divorced	79 (9.4)	30 (9.3)	1.14 (.62-2.11)	4 (2.6)	2 (1.2)	1.68 (.46-6.08)	2 (1.5)	2 (0.9)	0.49 (.03-8.33)	69 (21.0)	92 (33.8)	2.98 (1.48-6.01)	8 (4.6)	3 (2.1)	0.52 (.12-2.37)
Age at sexual debut															
< 16	165 (19.9)	61 (19.2)	1.00	36 (25.7)	44 (26.8)	1.00	37 (28.5)	67 (30.7)	1.00	31 (15.7)	28 (20.4)	1.00	44 (26.4)	52 (38.2)	1.00
≥ 16	665 (80.1)	257 (80.8)	1.31 (.93-1.86)	104 (74.3)	120 (73.2)	1.09 (.67-1.78)	93 (71.5)	151 (69.3)	1.14 (.67-1.93)	166 (84.3)	109 (79.6)	0.70 (.37-1.31)	123 (73.6)	84 (61.8)	0.84 (.48-1.45)
Lifetime # of sex partners															
0-1	320 (38.1)	119 (36.8)	1.00	21 (13.6)	18 (10.4)	1.00	10 (7.6)	5 (2.3)	1.00	8 (4.1)	5 (3.7)	1.00	5 (2.9)	1 (0.7)	1.00
2-4	382 (45.5)	170 (52.6)	1.16 (.82-1.66)	49 (31.8)	56 (32.4)	2.77 (1.64-4.66)	35 (26.5)	44 (20.2)	2.69 (.80-9.02)	44 (22.3)	21 (15.3)	0.59 (.16-2.24)	40 (23.4)	25 (17.7)	2.56 (.24-17.65)
≥ 5	137 (16.3)	34 (10.5)	1.41 (.97-2.07)	84 (54.6)	99 (57.2)	6.71 (3.73-12.07)	87 (65.9)	169 (77.5)	3.88 (1.20-12.57)	145 (73.6)	111 (81.0)	0.95 (.27-3.28)	126 (73.7)	115 (81.6)	4.23 (.40-25.07)
# of pregnancies															
0	19 (2.3)	7 (2.2)	1.00	127 (81.4)	147 (84.5)	1.00	117 (88.6)	193 (88.9)	1.00	47 (23.9)	41 (29.9)	1.00	99 (57.6)	76 (53.9)	1.00
1-2	313 (37.5)	134 (42.0)	1.27 (.49-3.30)	27 (17.3)	25 (14.4)	0.81 (.46-1.42)	13 (9.9)	24 (11.1)	1.05 (.48-2.29)	92 (46.7)	56 (40.9)	0.72 (.40-1.30)	51 (29.6)	51 (36.2)	1.36 (0.79-2.10)
≥ 3	503 (60.2)	178 (55.8)	1.30 (.49-3.41)	2 (1.3)	2 (1.1)	1.09 (.17-7.13)	2 (1.5)	0 (0.0)	N/E N/E	58 (29.4)	40 (29.2)	0.79 (.41-1.51)	22 (12.8)	14 (9.9)	0.88 (0.42-1.84)
OC use															
Never	717 (85.5)	260 (80.5)	1.00	30 (20.0)	39 (22.9)	1.00	20 (15.4)	31 (14.2)	1.00	122 (36.9)	106 (38.7)	1.00	11 (6.4)	5 (3.6)	1.00
Ever [d]	122 (14.5)	63 (19.5)	0.92 (.61-1.39)	120 (80.0)	131 (77.1)	0.95 (.60-1.50)	110 (84.6)	187 (85.8)	0.91 (.46-1.79)	87 (26.3)	66 (24.1)	1.45 (.66-3.20)	161 (93.6)	135 (96.4)	1.26 (.34-4.66)
Missing [e]	- -	- -	- -	- -	- -	- -	- -	- -	- -	122 (36.8)	102 (37.2)	1.11 (.44-2.78)	- -	- -	- -
Condom use															
Never	320 (38.8)	105 (32.7)	1.00	7 (4.6)	7 (4.1)	1.00	4 (3.1)	2 (0.9)	1.00	159 (48.0)	119 (43.4)	1.00	9 (5.2)	5 (3.6)	1.00
Rarely/sometimes/ ever [d]	472 (57.2)	198 (61.7)	1.22 (.91-1.63)	84 (55.6)	105 (61.4)	0.70 (.30-1.66)	46 (35.1)	86 (39.4)	0.62 (.05-8.26)	55 (16.6)	60 (21.9)	2.53 (1.15-5.54)	62 (36.0)	54 (38.6)	1.60 (.45-5.66)
Regularly/always/ missing [e]	33 (4.0)	18 (5.6)	1.39 (.74-2.64)	60 (39.7)	59 (34.5)	0.99 (.41-2.38)	81 (61.8)	130 (59.6)	0.50 (.04-6.60)	117 (35.4)	95 (34.7)	1.77 (.56-5.56)	101 (58.7)	81 (57.8)	0.86 (.52-1.43)
Cigarette smoking															
Never smoker	386 (46.0)	155 (48.0)	1.00	96 (61.9)	96 (55.5)	1.00	85 (64.4)	114 (52.3)	1.00	49 (64.5)	150 (53.6)	1.00	78 (45.1)	56 (39.7)	1.00
Former smoker	145 (17.3)	46 (14.24)	0.91 (.67-1.22)	35 (22.6)	45 (26.0)	1.36 (.83-2.24)	29 (22.0)	76 (34.9)	1.99 (1.13-3.49)	19 (25.0)	90 (32.1)	1.94 (1.10-3.42)	39 (22.5)	25 (17.7)	0.80 (.41-1.56)
Current smoker	308 (36.7)	122 (37.8)	0.74 (.50-1.11)	24 (15.5)	32 (18.5)	1.12 (.67-1.88)	18 (13.6)	28 (12.8)	0.97 (.49-1.94)	8 (10.5)	40 (14.3)	1.85 (.96-3.56)	56 (32.4)	60 (42.6)	1.06 (.61-1.85)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; S, single HPV infection; M, multiple HPV infection; N, number; N/E, not able to estimate; OC, oral contraceptive; OR, odds ratio; Ref, reference; SD, standard deviation.

[a] Subject was assigned to multiple HPV infection category if concurrent HPV co-infection was observed at any clinic visit (baseline or follow-up).

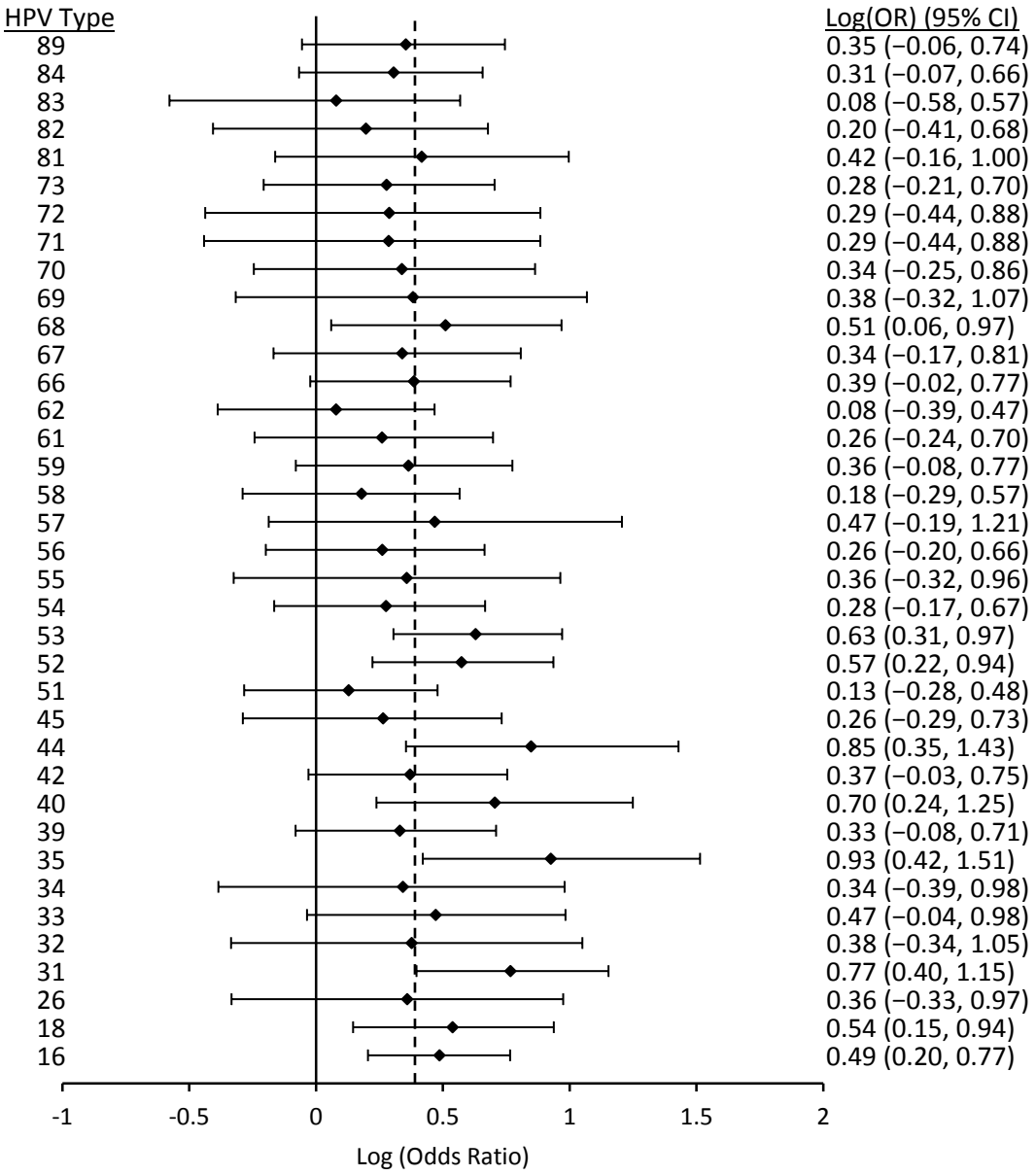
[b] Odds ratios were adjusted for all variables listed in the table.

[c] Age was modeled as a linear variable with 1 degree-of-freedom.

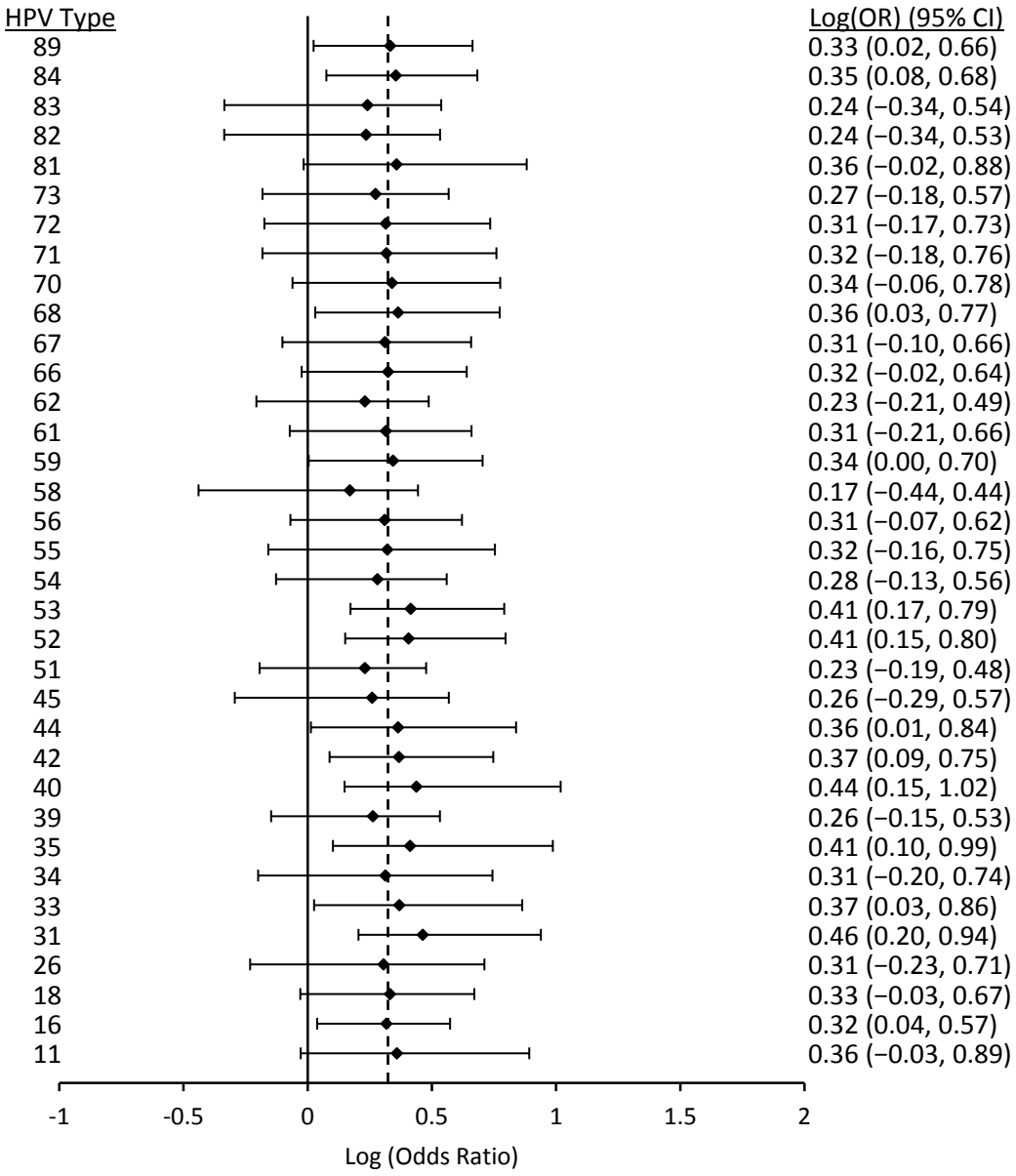
[d] Checklist was used in CCCaST to evaluate whether subjects “ever” used OCs or condoms, along with other contraceptive methods.

[e] For CCCaST only, “missing” was included in analysis for OC and condom use variables.

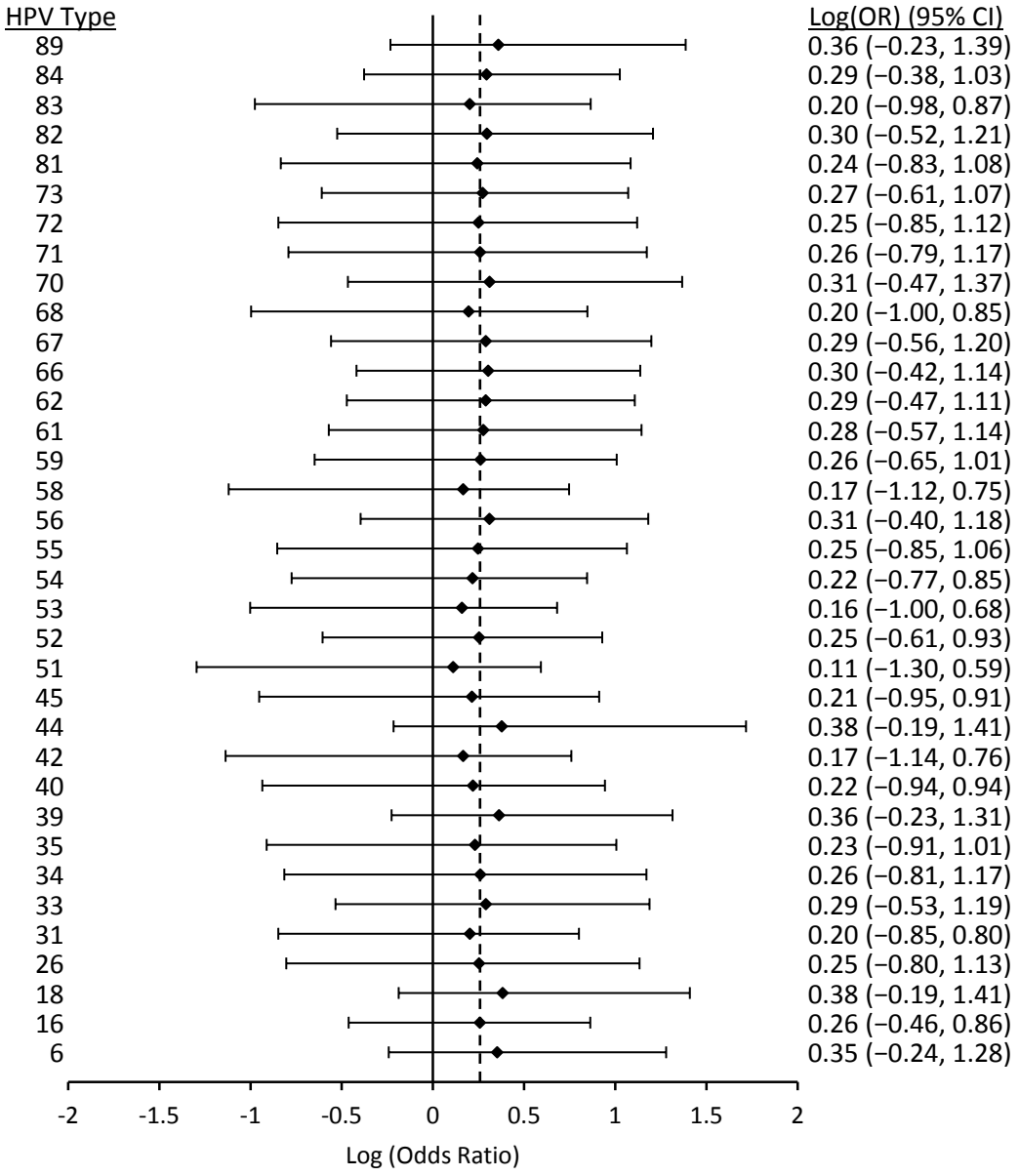
A)


HPV Type
Log(OR) (95% CI)
89 0.35 (−0.06, 0.74)
84 0.31 (−0.07, 0.66)
83 0.08 (−0.58, 0.57)
82 0.20 (−0.41, 0.68)
81 0.42 (−0.16, 1.00)
73 0.28 (−0.21, 0.70)
72 0.29 (−0.44, 0.88)
71 0.29 (−0.44, 0.88)
70 0.34 (−0.25, 0.86)
69 0.38 (−0.32, 1.07)
68 0.51 (0.06, 0.97)
67 0.34 (−0.17, 0.81)
66 0.39 (−0.02, 0.77)
62 0.08 (−0.39, 0.47)
61 0.26 (−0.24, 0.70)
59 0.36 (−0.08, 0.77)
58 0.18 (−0.29, 0.57)
57 0.47 (−0.19, 1.21)
56 0.26 (−0.20, 0.66)
55 0.36 (−0.32, 0.96)
54 0.28 (−0.17, 0.67)
53 0.63 (0.31, 0.97)
52 0.57 (0.22, 0.94)
51 0.13 (−0.28, 0.48)
45 0.26 (−0.29, 0.73)
44 0.85 (0.35, 1.43)
42 0.37 (−0.03, 0.75)
40 0.70 (0.24, 1.25)
39 0.33 (−0.08, 0.71)
35 0.93 (0.42, 1.51)
34 0.34 (−0.39, 0.98)
33 0.47 (−0.04, 0.98)
32 0.38 (−0.34, 1.05)
31 0.77 (0.40, 1.15)
26 0.36 (−0.33, 0.97)
18 0.54 (0.15, 0.94)
16 0.49 (0.20, 0.77)
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

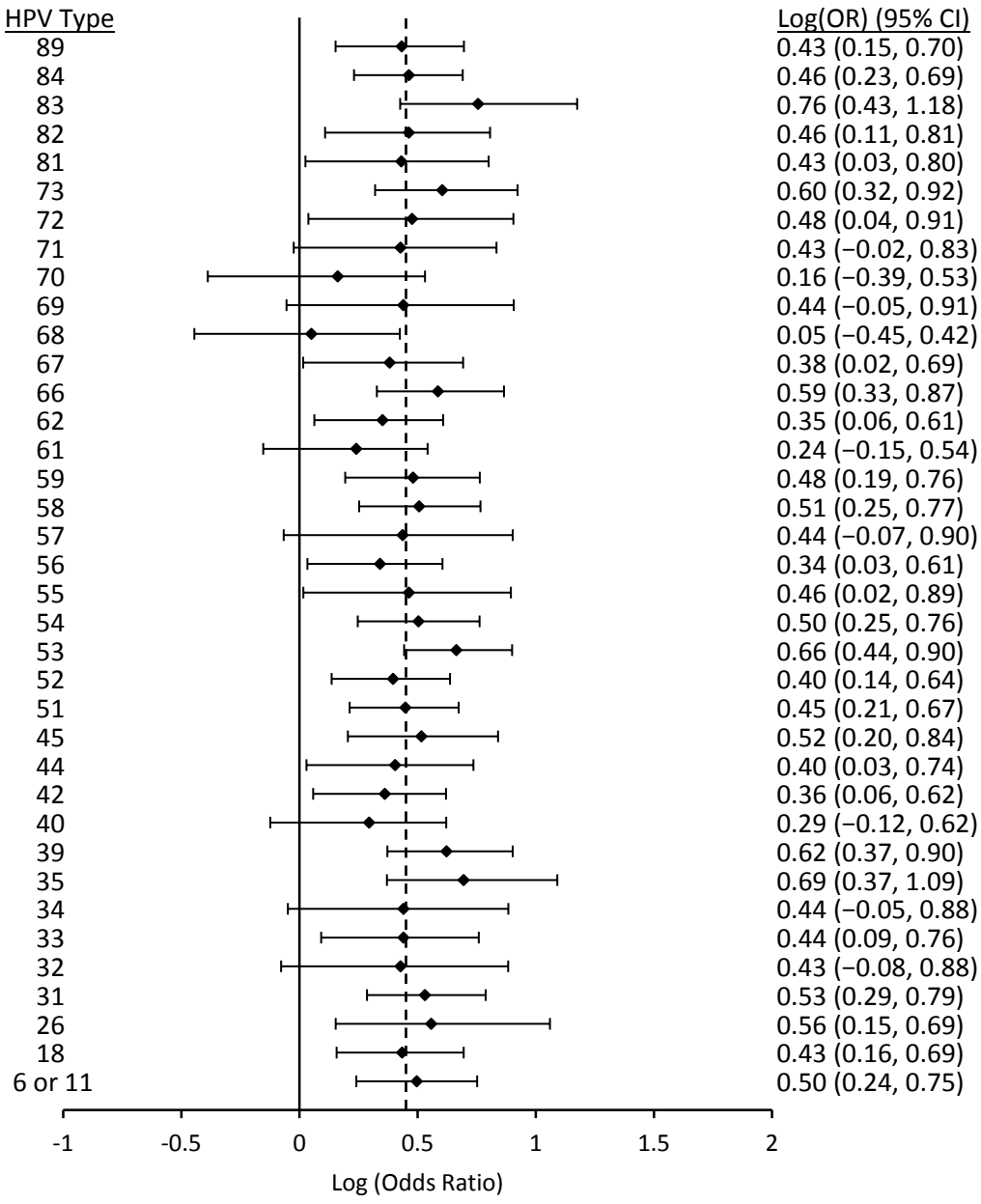
B)


HPV Type
Log(OR) (95% CI)
89 0.33 (0.02, 0.66)
84 0.35 (0.08, 0.68)
83 0.24 (−0.34, 0.54)
82 0.24 (−0.34, 0.53)
81 0.36 (−0.02, 0.88)
73 0.27 (−0.18, 0.57)
72 0.31 (−0.17, 0.73)
71 0.32 (−0.18, 0.76)
70 0.34 (−0.06, 0.78)
68 0.36 (0.03, 0.77)
67 0.31 (−0.10, 0.66)
66 0.32 (−0.02, 0.64)
62 0.23 (−0.21, 0.49)
61 0.31 (−0.21, 0.66)
59 0.34 (0.00, 0.70)
58 0.17 (−0.44, 0.44)
56 0.31 (−0.07, 0.62)
55 0.32 (−0.16, 0.75)
54 0.28 (−0.13, 0.56)
53 0.41 (0.17, 0.79)
52 0.41 (0.15, 0.80)
51 0.23 (−0.19, 0.48)
45 0.26 (−0.29, 0.57)
44 0.36 (0.01, 0.84)
42 0.37 (0.09, 0.75)
40 0.44 (0.15, 1.02)
39 0.26 (−0.15, 0.53)
35 0.41 (0.10, 0.99)
34 0.31 (−0.20, 0.74)
33 0.37 (0.03, 0.86)
31 0.46 (0.20, 0.94)
26 0.31 (−0.23, 0.71)
18 0.33 (−0.03, 0.67)
16 0.32 (0.04, 0.57)
11 0.36 (−0.03, 0.89)
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

C)


HPV Type
Log(OR) (95% CI)
89 0.36 (−0.23, 1.39)
84 0.29 (−0.38, 1.03)
83 0.20 (−0.98, 0.87)
82 0.30 (−0.52, 1.21)
81 0.24 (−0.83, 1.08)
73 0.27 (−0.61, 1.07)
72 0.25 (−0.85, 1.12)
71 0.26 (−0.79, 1.17)
70 0.31 (−0.47, 1.37)
68 0.20 (−1.00, 0.85)
67 0.29 (−0.56, 1.20)
66 0.30 (−0.42, 1.14)
62 0.29 (−0.47, 1.11)
61 0.28 (−0.57, 1.14)
59 0.26 (−0.65, 1.01)
58 0.17 (−1.12, 0.75)
56 0.31 (−0.40, 1.18)
55 0.25 (−0.85, 1.06)
54 0.22 (−0.77, 0.85)
53 0.16 (−1.00, 0.68)
52 0.25 (−0.61, 0.93)
51 0.11 (−1.30, 0.59)
45 0.21 (−0.95, 0.91)
44 0.38 (−0.19, 1.41)
42 0.17 (−1.14, 0.76)
40 0.22 (−0.94, 0.94)
39 0.36 (−0.23, 1.31)
35 0.23 (−0.91, 1.01)
34 0.26 (−0.81, 1.17)
33 0.29 (−0.53, 1.19)
31 0.20 (−0.85, 0.80)
26 0.25 (−0.80, 1.13)
18 0.38 (−0.19, 1.41)
16 0.26 (−0.46, 0.86)
6 0.35 (−0.24, 1.28)
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

D)


HPV Type
Log(OR) (95% CI)
89
0.43 (0.15, 0.70)
84
0.46 (0.23, 0.69)
83
0.76 (0.43, 1.18)
82
0.46 (0.11, 0.81)
81
0.43 (0.03, 0.80)
73
0.60 (0.32, 0.92)
72
0.48 (0.04, 0.91)
71
0.43 (−0.02, 0.83)
70
0.16 (−0.39, 0.53)
69
0.44 (−0.05, 0.91)
68
0.05 (−0.45, 0.42)
67
0.38 (0.02, 0.69)
66
0.59 (0.33, 0.87)
62
0.35 (0.06, 0.61)
61
0.24 (−0.15, 0.54)
59
0.48 (0.19, 0.76)
58
0.51 (0.25, 0.77)
57
0.44 (−0.07, 0.90)
56
0.34 (0.03, 0.61)
55
0.46 (0.02, 0.89)
54
0.50 (0.25, 0.76)
53
0.66 (0.44, 0.90)
52
0.40 (0.14, 0.64)
51
0.45 (0.21, 0.67)
45
0.52 (0.20, 0.84)
44
0.40 (0.03, 0.74)
42
0.36 (0.06, 0.62)
40
0.29 (−0.12, 0.62)
39
0.62 (0.37, 0.90)
35
0.69 (0.37, 1.09)
34
0.44 (−0.05, 0.88)
33
0.44 (0.09, 0.76)
32
0.43 (−0.08, 0.88)
31
0.53 (0.29, 0.79)
26
0.56 (0.15, 0.69)
18
0.43 (0.16, 0.69)
6 or 11
0.50 (0.24, 0.75)
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

E)

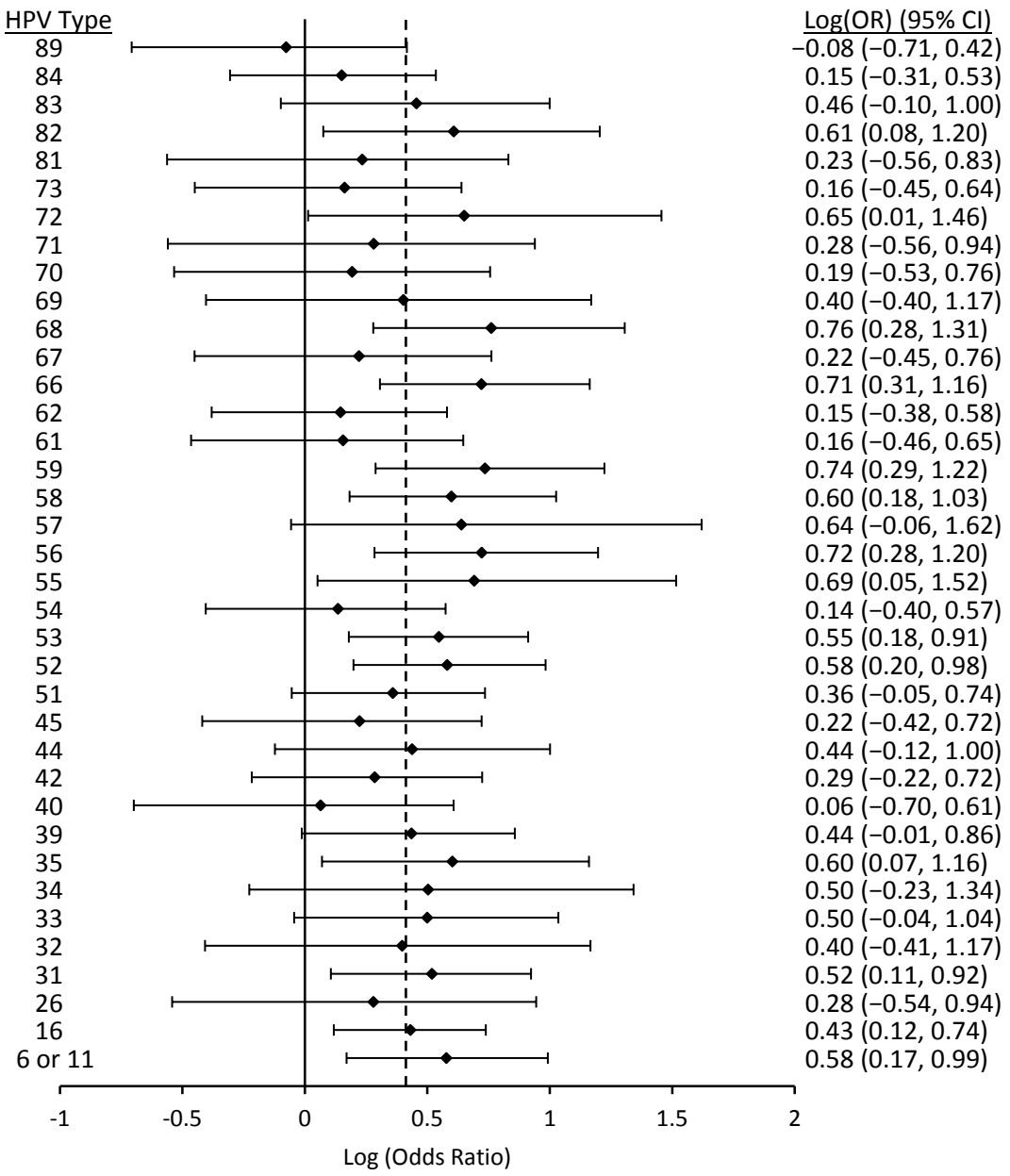


Figure 5-2:

Log (odds ratios) and 95% confidence intervals for HPVs 6/11, 6, 11, 16 and 18 (panels A-E, respectively) for co-infection with other HPV types. Estimates were obtained from logistic regression models adjusted for all other types, age, and lifetime number of

sexual partners. In panels A-E, the dashed lines represent the average pooled log(OR) from hierarchical logistic regression, which were 0.39 (95%CI: 0.24-0.53), 0.32 (95%CI: 0.20-0.43), 0.26 (95%CI: -0.07-0.50), 0.45 (95%CI: 0.34-0.55), and 0.41 (95%CI: 0.23-0.57), respectively. All analyses included pooled results from Ludwig-McGill (except for panels B and C; due to our inability to distinguish between HPVs 6 and 11), McGill-Concordia, HITCH, BCCR, and CCCaST studies. Approximately half of subjects from CCCaST (n=5754; St. John's site) were excluded from these analyses due to missing information regarding lifetime number of sexual partners.

5. 2 Additional analyses to manuscript III

Due to space limitations of the journal, Figures 5-3, 5-4, 5-5, 5-6, 5-7 and 5-8 were submitted for consideration as online supplementary material (presented in Appendix 3). These figures display log (odds ratios) and 95% confidence intervals for HPVs 6/11, 6, 11, 16, and 18 for co-infection with other HPV types without adjustment for lifetime number of sexual partners (i.e., including all specimens collected in CCCaST; Figure 5-3) and for each of the five studies separately (Figures 5-4 to 5-8).

CHAPTER 6: MANUSCRIPT IV

CERVICAL INFECTION WITH VACCINE HUMAN PAPILLOMAVIRUS (HPV) TYPES AS A PREDICTOR OF AQUISITION OR CLEARANCE OF OTHER HPV INFECTIONS

6.1 Preamble

To date, few studies have evaluated the effect of prior HPV infection on the acquisition or clearance of other HPV types. In the context of HPV type replacement, investigating whether risk of acquiring/clearing different HPV types varies according to infection with current vaccine-targeted HPV types (6/11/16/18) may provide some insight regarding HPV type competition and the potential for replacement. 157

Manuscript IV represents the first study focused on a female population that individually looks at HPVs 6/11, 16 and 18 as unique exposure variables in comparing time to acquisition and clearance of other HPV types. With cohort information available from a combined 3,200 subjects, this also represents one of the largest studies conducted on this topic to date. Despite the availability of cohort information, the previous study (regression approach; manuscript III) only focussed on HPV co-infection patterns among specimens to assess type competition. It did not link episodes of infection to one another as we did in this manuscript. Observation of consistent HPV type competition across these complementary regression and cohort approaches for some specific HPV type(s) may be a strong signal to investigators that type replacement is more likely to occur for the indicated HPV type(s).

Cervical Infection with Vaccine Human Papillomavirus (HPV) Types as a Predictor of Acquisition or Clearance of Other HPV Infections

Running title: Cohort approach to evaluate HPV type competition

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FOOTNOTES

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ABSTRACT

Background: Current human papillomavirus (HPV) vaccines target up to four HPV types (6/11/16/18). If natural competition exists between vaccine-targeted HPV types and other types, then prevalence of the latter may increase post-vaccination. Cohort information may be used to evaluate HPV type competition and the potential for type replacement.

Methods: Using data from three cohort studies, we compared acquisition and clearance of 30 different HPV types (prevalence ≥1%) according to infection with vaccine-targeted types at baseline or time of the index infection, respectively. Study specific and pooled analyses were conducted and hazard ratios (HRs) were adjusted for predictors of multiple-type infection.

Results: Across all studies (n=3200), 857 females were infected with HPV at baseline and 994 acquired new infections during follow-up. Females infected with vaccine-targeted types were generally at higher risk of acquiring other types (majority HRs>1.0), and at about equal risk of clearing infections. Accounting for multiple comparisons, none of the HRs<1.0 or >1.0 were statistically significant in our analyses of acquisition or clearance, respectively.

Conclusions: Vaccine-targeted HPV types do not appear to compete with other types. Future studies comparing the distribution of individual HPV types (pre- versus post-vaccination) will be important to definitively address this issue.

INTRODUCTION

The discovery that invasive cervical cancer (ICC) is caused by human papillomavirus (HPV) has led to the establishment of two vaccines targeting the HPV types responsible for ~70% of ICC cases worldwide, i.e., HPVs 16 and 18 [2, 3]. One of these vaccines offers additional protection against HPV types 6 and 11, which are responsible for ~90% of genital warts cases [3]. But the possibility that other oncogenic HPV types may increase in prevalence following a decline in vaccine-targeted types, i.e., take over the ecological niche vacated by these types, remains an important concern. This is a concept referred to as “type replacement” [12].

HPVs are DNA viruses and are extremely stable genetically; therefore, in order for biologic type replacement to occur, different HPV types must compete with one another during natural infection. Recently, we described a number of epidemiologic approaches to evaluate HPV type competition that may provide insight regarding the likelihood of type replacement [12]. One of these approaches involves the evaluation of sequential acquisition or clearance of HPV types according to infection with current generation vaccine types. A number of studies have evaluated the natural history and clustering patterns of HPV to determine whether acquisition or persistence varies according to infection with other types [76-78, 104-106, 258]. However, none of them provided any evidence of HPV type competition; in fact, they all found that prior HPV infection was associated with an increased risk of acquiring additional types during

follow-up, suggesting possible synergistic interactions, or perhaps residual confounding due to incomplete adjustment for risk factors of multiple-type infection.

Recently, Rositch et al. [258] compared acquisition of HPV according to baseline status with vaccine-targeted types in a population of Kenyan males. Among the studies evaluating acquisition or clearance of HPV, this is the only one that specifically compared acquisition according to baseline infection with all vaccine relevant HPV types to evaluate the potential for type replacement. Since the natural history of HPV infection differs between males and females [7], we decided to evaluate time to acquisition and time to clearance of different HPV types (individually, and grouped according to species) among females who were infected compared with those who were not infected with vaccine-targeted HPV types at baseline or time of their index infection, respectively.

METHODS

Study Population and HPV DNA Detection

Subject information for the current analysis came from three cohort studies conducted by our division: Ludwig-McGill, McGill-Concordia, and HITCH. The design and methods for these studies have been described in detail elsewhere [22, 202, 236]. Below we provide a brief description of each study. All were approved by review boards

or ethical committees of the participating institutions, and all participants provided written informed consent.

Ludwig-McGill Cohort Study (n=2462). This study was designed to evaluate the natural history of HPV infection and cervical neoplasia [202]. Recruitment took place between 1993 and 1997 in a population of low-income women in São Paulo, Brazil. Eligible women were between 18 and 60 years of age, permanent residents of São Paulo, had an intact uterus and no referral for hysterectomy, not pregnant or planning to become pregnant in the next 12 months, and had not been treated for cervical disease in the six months prior to enrollment. Participants presented for clinic visits every four months during their first year of follow-up and twice annually in subsequent years (maximum 10 years follow-up). Presence of HPV DNA was determined using a PCR assay employing L1 consensus primers and MY09/11 amplification, followed by hybridization with individual oligonucleotide probes and by restriction fragment length polymorphism analysis to identify 40 HPV types.

McGill-Concordia Cohort Study (n=636). This study was also designed to evaluate the natural history of HPV infection, among a younger population of university students [22]. Recruitment and follow-up took place between 1996 and 1999 and included female students attending either the McGill or Concordia University Health Clinic (Montreal, Canada). The only eligibility criteria were that participants intended to remain in Montreal for the next two years and had not been treated for cervical disease in the previous 12 months. All eligible women were asked to return to the clinic every six

months over a period of two years. HPV DNA was detected using the L1 consensus HPV primers MY09/11 and HMB01 PCR protocol, followed by a line blot assay for the detection of 27 HPV types.

HPV Infection and Transmission among Couples through Heterosexual activity (HITCH) Cohort Study (females: n=502). This study was designed to evaluate issues surrounding HPV transmission and prevention among heterosexual couples [236]. Between 2005 and 2010, young women (aged 18-24) attending a university or junior college in Montreal were recruited, along with their male partners. Eligible female participants were currently heterosexually active with a male partner (acquired within the previous 6 months) who was also willing to enrol in the study, had an intact uterus, no history of cervical lesions/cancer, not currently pregnant or planning to become pregnant in the next two years, and willing to comply with follow-up for at least two years. All eligible participants were asked to attend clinic visits every four months during their first year of follow-up, and every six months during their second year of follow-up. For the current analysis, we only considered information from female participants. HPV detection and typing was done using the PGMY09/11 PCR protocol coupled with the linear array method (commercially available from Roche), which is capable of detecting 36 mucosal HPV types.

At each clinic visit, subjects were asked to complete a questionnaire to collect information on sociodemographic, lifestyle, sexual, reproductive, and contraceptive factors; and to provide a cervical sample for HPV testing. Females were included in our

analysis of acquisition of HPV types if valid HPV DNA results were available at baseline and at least one follow-up visit (Ludwig-McGill: n=2185, McGill-Concordia: n=578, HITCH: n=437). In our analysis of loss of any HPV infection (clearance), females were included if they tested positive for HPV at any visit, followed by a valid HPV DNA testing result in at least one visit (Ludwig-McGill: n=1124, McGill-Concordia: n=279, HITCH: n=249). The number of females included in each of our individual type-specific analyses of HPV clearance was generally much lower because these analyses were restricted to those with the particular HPV types under study, with valid HPV testing results for at least one follow-up visit.

Statistical Analysis

We used the Kaplan-Meier method to present and compare time to acquisition or clearance of HPV (both individually, and grouped by species) according to presence of current generation vaccine types (6/11/16/18) at baseline or time of the index infection, respectively. Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for both acquisition and clearance objectives. By categorizing those with vaccine types as the exposed group, HRs < 1.0 (acquisition objective) would indicate that the risk of becoming infected with a specific non-vaccine HPV type is lower among individuals infected with a particular vaccine HPV type, and thus potential type competition between these types. In our evaluation of clearance, our interpretation is the opposite, i.e., HRs > 1.0 would signal accelerated clearance of certain HPV types among those infected with vaccine types and possible type

competition. In total, there were 720 pre-planned statistical tests; therefore, in addition to presenting 95% CIs to assess statistical significance, we also applied more conservative p-value thresholds of 0.01 and 0.00007 (0.05/720; Bonferroni correction) and tested using the log-rank method. This statistical “cohort approach” to evaluate HPV type competition has recently been described by us elsewhere [12].

Due to our inability to distinguish between HPV6 and 11 infections in the Ludwig-McGill cohort study, and the low number of HPV11 infections observed in the McGill-Concordia and HITCH cohort studies (<10), we decided it was appropriate to group these phylogenetically related types together in all subsequent analyses. Two years was the maximum follow-up we allowed for evaluation of acquisition and clearance, i.e., from the baseline visit or the first visit at which the index infection was detected, respectively. If HPV DNA results were missing for visit(s) prior to the first HPV positive visit (or negative visit for evaluation of clearance), then the acquisition or clearance interval was assumed to span the time from the last available HPV negative (or positive) visit to the first HPV positive (or negative) visit, respectively. To investigate this assumption, we changed missing values for our acquisition/clearance analyses from negative to positive or positive to negative, respectively; but this generally led only slight and unimportant changes in our results (data not shown). We also conducted separate analyses to evaluate whether results differed according to prevalent versus incident HPV infections in comparing time to clearance (i.e., infections detected at baseline versus follow-up only). Despite sparse data for some comparisons, results were very similar and therefore we decided to combine baseline/incident infections in our analysis

for this objective. Important predictors of multiple HPV infection that we adjusted for included age and lifetime number of sexual partners. The possibility of confounding by other factors (e.g., marital status, age at sexual debut, parity, smoking, oral contraceptive and condom use) was also evaluated empirically; however, additional adjustment for these variables generally did not have an important effect on our parameter estimates (<10% change).

Pooling was conducted to improve our precision for both our acquisition and clearance analyses. Since we expect HPV type competition (if it exists) to be a biological phenomenon (i.e., consistent across populations), we were motivated to report estimates from our fixed effects models to evaluate both objectives, assuming that results would be similar to estimates generated from random effects models, i.e., confirming that no important residual differences across studies. Prior to pooling, heterogeneity of effects was compared across studies, and the Q-test statistic and Hausman specification test were used to compare estimates from fixed and random effects models (data not shown). Some very rare HPV types (<1% cumulative incidence across all studies) were excluded from our specific and pooled analyses because they resulted in HRs that were either very imprecise or not estimable (HPVs 26, 32, 34, 57, 69, 71, 72, and 81).

RESULTS

The average age of participants at baseline in the Ludwig-McGill, McGill-Concordia, and HITCH cohort studies was 32.7, 22.5, and 21.0, respectively (Table 6-1). The majority reported that they were married in the Ludwig-McGill study (81%), but single in the McGill-Concordia (77.8%) and HITCH (84.7%) studies. Compared with the latter two studies, a smaller proportion of females in the Ludwig-McGill study reported ≥5 lifetime sexual partners (12.9% versus 43.6% and 60.4%, respectively) and regularly/always using condoms (3.7% versus 56.9% and 59.6%, respectively), but many more reported at least one pregnancy (97.3% versus 16.2% and 9.8%, respectively) (Table6-1). Characteristics associated with multiple HPV infection included age and lifetime number of sexual partners (Table 6-2).

Prevalence of HPV infection (any type) at baseline was 16.4% (403/2462) in Ludwig-McGill, 27.2% (173/636) in McGill-Concordia, and 62.2% (281/452) in the HITCH study (Table 6-2). Among these females with baseline infection, the proportion with multiple HPV infections in each study was 18.4% (n=74), 41.0% (n=71), and 68.0% (n=191), respectively. Baseline prevalence of HPV16 was 2.6% (n=64) in Ludwig-McGill, 6.8% (n=43) in McGill-Concordia, and 18.1% (n=82) in HITCH. Across these same studies, baseline prevalence of HPVs 6/11 was 1.1% (n=28), 3.3% (n=21) and 4.6% (n=21); and for HPV18 was 1.1% (n=26), 2.8% (n=18) and 4.0% (n=18), respectively. The incidence of any new HPV infection (i.e., infections acquired during the entire follow-up period) was 34.7% (758/2185) in Ludwig-McGill, 27.0% (156/578) in

McGill-Concordia, and 18.3% (80/437) in the HITCH study. In the first two years of follow-up, the number of women in each of these studies who acquired new HPV infections from the α-7 species was 123, 64 and 42; from the α-9 species was 209, 90 and 66; and from the α-10 species was 63, 32 and 43, respectively (supplementary Table 1). Similarly, the number of women in each of the respective studies with α-7 species infections (baseline cases included; denominator) who eventually cleared their infection within two years (numerator) was 275/286, 53/84 and 64/98; with α-9 species infections was 497/556, 82/148 and 82/137; and with α-10 species infections was 178/187, 35/42 and 34/55. The most commonly acquired HPV types varied across studies. In Ludwig-McGill, it was HPV16 (n=108), followed by 53 (n=66), 51 (n=57), 58 (n=35), 6/11 (n=35), and 52 (n=34). In McGill-Concordia, HPV16 was also the most common (n=61), followed by 84 (n=47), 51 (n=44), 54 (n=34), 6/11 (n=32), and 53 (n=31). In HITCH, HPV 89 was the most common (n=53), followed by 84 (n=45), 66 (n=44), 42 (n=44), 6/11 (n=40), and 53 (n=33). Clearance patterns also varied across studies, but in general, high oncogenic risk types cleared less frequently compared with other types (Table 6-7; appendix 4) [9].

In our analysis of acquisition, baseline infection with vaccine-targeted HPV types (either 6/11, 16, or 18) was not associated with a statistically significant reduced risk of acquiring other HPV types (individually or grouped by species) in either our study-specific (Table 4), or pooled analyses (Table 6-6). Even after adjustment for risk factors of multiple infection; the hazards of acquiring other HPV types was generally higher among females infected with vaccine-targeted HPV types, compared with those who

were not. Similarly, in our evaluation of clearance, co-infection with a vaccine-targeted HPV type at time of the index infection was not associated with a statistically significant elevated risk of clearing other types after accounting for multiple comparisons (Tables 6-5 and 6-6). However, there were some types that cleared more rapidly (when prevalent as a co-infection with either HPVs 6/11, 16 or 18), which were significant at less conservative levels. Among those co-infected with HPV16, these types included HPVs 6/11 and 45 in the McGill-Concordia and HITCH studies, respectively (Table 6-5). These types, along with HPV18, were also found to clear more rapidly in our pooled analysis (Table 6-6). Among those co-infected with HPV-18, HPVs 16 and 66 cleared more rapidly among HITCH participants and HPV6/11 cleared more rapidly among McGill-Concordia participants. In our pooled analysis, HPV66 plus some additional types (HPVs 44, 33, and 61) were found to clear more rapidly among those infected with HPV18. In our pooled analysis only, clearance of HPV61 was positively associated with HPV6/11 infection. No clear evidence of type competition between HPVs 6/11, 16, and 18 was observed at the species level with phylogenetically related types (α-10, α-9, and α-7, respectively) in either our evaluation of acquisition or clearance (Figure 6-1).

DISCUSSION

To our knowledge, this is the first study among females focusing on type competition and the potential for replacement that specifically evaluates acquisition and clearance of HPV types according to infection with current vaccine-targeted types. Among 3200 females from Canada and Brazil, baseline infection with vaccine-targeted

HPV types (6/11, 16, or 18) was generally associated with a similar or shorter time to acquisition of other HPV types, providing no evidence of HPV type competition. In our evaluation of clearance (study specific/pooled analyses), many positive associations were observed between vaccine-targeted HPVs with other types, some of which included other vaccine-targeted types. Among the eight different HPV types that were statistically significant at less conservative thresholds, HPV66 is the only (possible) oncogenic type [9] not being targeted by current [2, 3] or future generation [259] HPV vaccines, and has been implicated in approximately 0.4% of invasive cervical cancer cases, globally [5]. None of these associations remained statistically significant once accounting for multiple comparisons in our analysis.

The ability to pool information from across three large cohort studies greatly enhanced our precision, and allowed us to estimate associations that were previously not possible due to sparse data in individual studies. Focusing on results from our pooled analysis, we identified nine negative associations (HR<1.0; eight with HR<0.9) between baseline infection with either HPV6/11, 16, or 18 and acquisition of other types (all 95% CIs included 1.0). In our pooled analysis of clearance, we identified a total of 41 positive associations (HR>1.0; 33 with HR>1.1; eight with 95% CIs excluding 1.0). Because HPV types belonging to the same species share at least 60% of their nucleotide sequence identity and exhibit similar biological and pathological properties [18, 204, 205], we expected that types from the same species may be more likely to compete with one another. With the exception of HPV6/11 with α-10 types (clearance analysis: HR>1.0, 95% CI included 1.0), this was not the case in our study. Although we

would have preferred to evaluate HPVs 6 and 11 separately, this probably would not have made much of a difference considering that these are among the two most closely related HPV types with indistinguishable biological and pathological properties [204]. In Plummer and colleagues comparison of time to acquisition/clearance of other HPV types according to infection with HPV16, they also found no evidence of competition according to degree of phylogenetic relatedness (α-9 species types); however, they did find a slight decrease in incidence of α-7 species types, particularly HPVs 59 and 68 [105].

Despite the large sample size of this study, we were still unable to accurately evaluate HPV acquisition/clearance for rare HPV types, which is reflected by wide confidence intervals for some comparisons. Also, despite the use of well established consensus primer PCR assays to detect a broad spectrum of HPV types [220], these assays have been documented to perform with reduced sensitivity in cases of multiple infection and low viral DNA load [163-165, 170, 222-226]. In the context of our investigation, this may explain why those co-infected with vaccine-targeted HPV types appeared to clear certain other types more rapidly. That is, differences in clearance may be attributed to differential PCR sensitivity ("masking") as a result of competition for reagents (e.g., primers) among those co-infected with vaccine-targeted HPV types. Due to this same masking phenomenon, those infected with vaccine-targeted HPV types may be at even greater risk of acquiring other HPV types than what our results suggest. However, there is also the possibility that adjustment for shared HPV risk factors (predictors of multiple infection) was not sufficient, and that some unmeasured variables

(e.g., behavioral, biological, or host immunity factors) may have led to residual confounding, explaining why those infected with vaccine-targeted HPV types were generally at higher risk of acquiring other types [7]. Therefore, in addition to adjusting for important measured predictors of multiple-infection in our analyses, we also performed analyses restricted to females with HPV detected at some point during follow-up (n=1652). This approach often led to attenuated risk associations in our acquisition analyses; however, our HR estimates generally remained above 1.0 and were not meaningfully different for our purpose of evaluating type competition (data not shown). Finally, we also explored whether results varied according to definition of baseline status with HPV vaccine-targeted types, i.e., restricted to those with infection present on the first 2 visits (persistent infection) versus those with infection at baseline but not at the second visit (transient infection), but observed only minor differences according to these two definitions (data not shown). Although it is also possible to model infection with vaccine-targeted HPV types as a time-varying exposure, these models are more challenging to fit and interpret and were therefore excluded from this investigation.

In our analyses of acquisition and clearance, interval length between visits ranged between four and six months. This may have led to slight overestimation of acquisition or clearance time; however, since our objective was to compare groups according to their infection status with vaccine-targeted HPV types (not to estimate the time to an event), we do not suspect any bias was introduced. In addition, we assumed type competition (if it exists) to be a consistent phenomenon, across populations and women of different ages; therefore, despite known differences in risk of HPV acquisition

and persistence due to acquired immunity or other factors [7, 39] pooling was considered appropriate. We also explored the possibility of effect modification according to age in the Ludwig-McGill study, stratifying females into two groups (<26 versus ≥25 at enrollment), but found no difference (data not shown). Finally, compared with their younger counterparts in the McGill-Concordia and HITCH studies, Ludwig-McGill participants were actually more likely to acquire new HPV infections (any type) during follow-up, and often more likely to clear their infections (especially non-oncogenic HPV types) within two years.

No consistent or strong evidence of type competition between specific HPV types was observed across our analyses of acquisition and clearance. Although some types were flagged as possible candidates in our clearance analysis, this may have resulted from the high number of statistical comparisons or from PCR detection issues. In summary, our study provides no clear evidence to suggest that type replacement may occur following vaccination. However, it is possible that risk of acquiring other HPV types differs between those who are vaccinated and protected against certain HPV types, compared with those who are naturally uninfected. Ultimately, the population level impact of vaccines will be determined by comparing prevalence (pre- versus post-vaccination) of different HPV types involved in cancerous/precancerous cervical lesions utilizing long-term surveillance data. However, until these data become available, results from studies like ours that evaluate natural HPV type competition may provide the best clues regarding the likelihood of HPV type replacement.

Table 6-1: Baseline characteristics of female participants in three epidemiologic cohort studies

Characteristic	Ludwig-McGill study n=2462 N (%)	McGill-Concordia study n=636 N (%)	HITCH study n=502 N (%)
Age, years, mean (SD)	32.7 (8.8)	22.5 (4.0)	21.0 (2.1)
Marital status			
Single	252 (10.2)	495 (77.8)	425 (84.7)
Married/common law	2011 (81.7)	114 (18.0)	71 (14.1)
Widowed/divorced	197 (8.0)	14 (2.2)	6 (1.2)
Missing	2 (0.1)	13 (2.0)	0 (0.0)
Age at sexual debut			
< 16	479 (19.5)	125 (19.6)	45 (24.3)
≥ 16	1958 (79.5)	443 (69.7)	454 (75.1)
Missing	25 (1.0)	68 (10.7)	3 (0.6)
Lifetime # of sex partners			
0-1	1089 (44.2)	135 (22.2)	54 (10.7)
2-4	1053 (42.8)	198 (32.1)	145 (28.9)
≥ 5	318 (12.9)	277 (43.6)	303 (60.4)
Missing	2 (0.1)	26 (4.1)	0 (0.0)
# of pregnancies			
0	47 (1.9)	511 (80.3)	452 (90.0)
1-2	894 (36.3)	97 (15.2)	47 (9.4)
≥ 3	1502 (61.0)	6 (1.0)	2 (0.4)
Missing	19 (0.8)	22 (3.5)	1 (0.2)
Oral contraceptive use			
Never	397 (16.1)	135 (21.2)	80 (16.0)
Ever	2064 (83.9)	461 (72.5)	421 (83.9)
Missing	1 (0.0)	40 (6.3)	1 (0.4)
Condom use			
Never	936 (38.0)	30 (4.7)	16 (3.2)
Rarely or sometimes	1398 (56.8)	209 (32.9)	185 (37.0)
Regularly or always	92 (3.7)	362 (56.9)	300 (59.6)
Missing	36 (1.5)	35 (5.5)	1 (0.2)
Cigarette smoking			
Never smoker	1168 (47.5)	399 (62.7)	313 (62.3)
Former smoker	429 (17.4)	124 (19.5)	129 (25.7)
Current smoker	864 (35.1)	99 (15.6)	60 (12.0)
Missing	1 (0.0)	14 (2.2)	0 (0.0)

Abbreviations: SD, standard deviation

Table 6-2: Characteristics of female participants at baseline in three epidemiologic cohort studies, stratified by HPV status[a]

	Ludwig-McGill study n=2462			McGill-Concordia study n=636			HITCH study n=452		
Characteristic	Sgl n=840 N (%)	Mult n=323 N (%)	OR (95% CI)[b] Mult vs. Sgl HPV (ref)	Sgl n=156 N (%)	Mult n=174 N (%)	OR (95% CI)[b] Mult vs. Sgl HPV (ref)	Sgl n=132 N (%)	Mult n=218 N (%)	OR (95% CI)[b] Mult vs. Sgl HPV (ref)
Age, years, mean (SD)	32.1 (8.7)	29.6 (8.8)	0.96 (.94, .98)	22.9 (4.0)	21.8 (3.1)	0.92 (.87, .98)	21.0 (2.0)	21.3 (2.3)	1.02 (.92, 1.14)
Marital status									
Single	106 (12.7)	72 (22.3)	1.00	118 (76.1)	158 (90.8)	1.00	111 (84.1)	187 (85.8)	1.00
Married/common law	653 (77.9)	221 (68.4)	0.60 (.34, 1.09)	33 (21.3)	14(8.0)	.96 (.26, 3.56)	19 (14.4)	29 (13.3)	0.84 (.44, 1.63)
Widowed/divorced	79 (9.4)	30 (9.3)	1.14 (.62, 2.11)	4 (2.6)	2 (1.2)	1.68 (.46, 6.08)	2 (1.5)	2 (0.9)	0.49 (.03, 8.33)
Age at sexual debut									
< 16	165 (19.9)	61 (19.2)	1.00	36 (25.7)	44 (26.8)	1.00	37 (28.5)	67 (30.7)	1.00
≥ 16	665 (80.1)	257 (80.8)	1.31 (.93, 1.86)	104 (74.3)	120 (73.2)	1.09 (.67, 1.78)	93 (71.5)	151 (69.3)	1.14 (.67, 1.93)
Lifetime # of sex partners									
0-1	320 (38.1)	119 (36.8)	1.00	21 (13.6)	18 (10.4)	1.00	10 (7.6)	5 (2.3)	1.00
2-4	382 (45.5)	170 (52.6)	1.16 (.82, 1.66)	49 (31.8)	56 (32.4)	2.77 (1.64, 4.66)	35 (26.5)	44 (20.2)	2.69 (.80, 9.02)
≥ 5	137 (16.3)	34 (10.5)	1.41 (.97, 2.07)	84 (54.6)	99 (57.2)	6.71 (3.73, 12.07)	87 (65.9)	169 (77.5)	3.88 (1.20, 12.57)
# of pregnancies									
0	19 (2.3)	7 (2.2)	1.0	127 (81.4)	147 (84.5)	1.00	117 (88.6)	193 (88.9)	1.00
1-2	313 (37.5)	134 (42.0)	1.27 (.49, 3.30)	27 (17.3)	25 (14.4)	0.81 (.46, 1.42)	13 (9.9)	24 (11.1)	1.05 (.48, 2.29)
≥ 3	53 (60.2)	178 (55.8)	1.30 (.49, 3.41)	2 (1.3)	2 (1.1)	1.09 (.17, 7.13)	2 (1.5)	0 (0.0)	N/E
OC use									
Never	717 (85.5)	260 (80.5)	1.00	30 (20.0)	39 (22.9)	1.00	20 (15.4)	31 (14.2)	1.00
Ever	122 (14.5)	63 (19.5)	0.92 (.61, 1.39)	120 (80.0)	131 (77.1)	0.95 (.60, 1.50)	110 (84.6)	187 (85.8)	0.91 (.46, 1.79)
Condom use									
Never	320 (38.8)	105 (32.7)	1.00	7 (4.6)	7 (4.1)	1.00	4 (3.1)	2 (0.9)	1.00
Rarely/sometimes	472 (57.2)	198 (61.7)	1.22 (.91, 1.63)	84 (55.6)	105 (61.4)	0.70 (.30, 1.66)	46 (35.1)	86 (39.4)	0.62 (.05, 8.26)
Regularly/always	33 (4.0)	18 (5.6)	1.39 (.74, 2.64)	60 (39.7)	59 (34.5)	0.99 (.41, 2.38)	81 (61.8)	130 (59.6)	0.50 (.04, 6.60)
Cigarette smoking									
Never smoker	386 (46.0)	155 (48.0)	1.00	96 (61.9)	96 (55.5)	1.00	85 (64.4)	114 (52.3)	1.00
Former smoker	145 (17.3)	46 (14.24)	0.91 (.67, 1.22)	35 (22.6)	45 (26.0)	1.36 (.83, 2.24)	29 (22.0)	76 (34.9)	1.99 (1.13, 3.49)
Current smoker	308 (36.7)	122 (37.8)	0.74 (.50, 1.11)	24 (15.5)	32 (18.5)	1.12 (.67, 1.88)	18 (13.6)	28 (12.8)	0.97 (.49, 1.94)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; Sgl, single HPV infection; Mult, multiple HPV infection; N, number; OC, oral contraceptive; OR, odds ratio; Ref, reference; SD, standard deviation.

[a] Subject was assigned to multiple HPV infection category if concurrent HPV co-infection was observed at any clinic visit (baseline or follow-up).

[b] Odds ratios were adjusted for all variables listed in the table.

Table 6-3: Prevalence of HPV infection at baseline and cumulative incidence during follow-up among female participants in three epidemiologic cohort studies

	Ludwig-McGill study[a] n=2462		McGill-Concordia study n=636		HITCH study n=452	
	Baseline N (%)	Follow-up[b,c] N (%)	Baseline N (%)	Follow-up[b] N (%)	Baseline N (%)	Follow-up[b] N (%)
Any-HPV	403 (16.4)	758 (34.7)	173 (27.2)	156 (27.0)	281 (62.2)	80 (18.3)
Multiple HPV infections	74 (3.0)	247 (11.3)	71 (11.2)	103 (17.8)	191 (42.2)	33 (7.5)
Number of HPV types[d]	1 (1-5)	1 (1-9)	1 (1-8)	2 (2-6)	2 (1-11)	1 (1-9)
HPV-6 (or 6/11)	28 (1.1)	71 (3.2)	15 (2.4)	29 (5.0)	18 (4.0)	39 (8.9)
HPV-11	N/E	N/E	5 (0.8)	4 (0.7)	3 (0.7)	4 (0.9)
HPV-16	64 (2.6)	215 (9.8)	43 (6.8)	61 (10.6)	82 (18.1)	29 (6.6)
HPV-18	26 (1.1)	60 (2.7)	18 (2.8)	25 (4.3)	18 (4.0)	11 (2.5)
Other α-10 HPV types[e]	13 (0.5)	84 (3.8)	3 (0.5)	11 (1.9)	10 (2.2)	10 (2.3)
Other α-9 HPV types[f]	86 (3.5)	295 (13.5)	48 (7.5)	53 (9.2)	97 (21.5)	68 (15.6)
Other α-7 HPV types[g]	62 (2.5)	197 (9.0)	20 (3.1)	46 (8.0)	76 (16.8)	42 (9.6)

Abbreviations: HPV, human papillomavirus; N/E, not able to estimate.

[a] The HPV test used in the Ludwig-McGill cohort study was unable to discriminate between HPVs 6 and 11.

[b] Subject was counted as having given HPV type(s) if it was acquired at any time during follow-up. The number of subjects in the Ludwig-McGill, McGill-Concordia, and HITCH cohort study with available HPV DNA testing results for at least one follow-up visit were 2185, 578, and 437, respectively.

[c] Follow-up in Ludwig-McGill was truncated after 7 years, at which point sample size reduced to approximately one quarter.

[d] Median (range) among women with detectable HPV infection.

[e] Other α-10 types include HPVs 44 and 55 (HPV-44 was not typed in McGill-Concordia study. HPV-55 was only typed in McGill-Concordia study).

[f] Other α-9 types include HPVs 31, 33, 35, 52, 58, and 67 (HPV-67 was not typed in McGill-Concordia study).

[g] Other α-7 types include HPVs 39, 45, 59, 68, and 70 (HPV-70 was not typed in McGill-Concordia study).

Table 6-4: Association between vaccine type HPV infection at baseline and future acquisition of individual HPV types in three epidemiologic cohort studies[a]

	Hazard ratios (95%CI) according to baseline vaccine HPV type Infection								
	Ludwig-McGill study[b]			McGill-Concordia study			HITCH study		
HPV type acquired	HPV-6/11 n=28	HPV-16 n=64	HPV-18 n=26	HPV-6/11 n=20	HPV-16 n=43	HPV-18 n=18	HPV-6/11 n=21	HPV-16 n=82	HPV-18 n=18
α-10 species	N/E	1.2 (0.3. 5.0)	N/E	1.7 (0.2, 14.2)	0.8 (0.3, 2.1)	0.6 (0.2, 1.9)	7.7 (1.4, 41.0)	1.5 (0.7, 3.1)	1.3 (0.3, 5.4)
HPV-6/11	N/A	N/E	N/E	N/A	0.7 (0.2, 2.9)	N/E	N/A	1.8 (0.9, 3.8)	1.9 (0.6, 6.1)
HPV-44	N/E	3.1 (0.7, 13.1)	N/E	N/D	N/D	N/D	7.7 (1.4, 41.0)	0.6 (0.1, 4.6)	2.5 (0.3, 21.0)
HPV-55	N/D	N/D	N/D	1.7 (0.2, 14.2)	N/E	2.9 (0.4, 23.4)	N/D	N/D	N/D
α-9 species	3.8 (1.7, 8.6)	2.2 (1.0, 4.7)	2.1 (0.7, 6.7)	0.4 (0.1, 2.6)	0.9 (0.3, 2.6)	1.2 (0.4, 3.9)	1.8 (0.6, 5.8)	1.7 (0.9, 3.5)	0.6 (0.1, 4.6)
HPV-16	1.3 (0.3, 5.1)	N/A	0.8 (0.1, 5.7)	0.8 (0.2, 3.3)	N/A	2.1 (0.6, 6.6)	N/E	N/A	N/E
HPV-31	4.8 (1.3, 16.9)	5.2 (1.8, 14.8)	N/E	1.3 (0.2, 9.7)	0.5 (0.1, 4.1)	2.8 (0.6, 12.1)	3.1 (0.7, 14.0)	1.7 (0.6, 5.0)	N/E
HPV-33	3.6 (0.5, 27.4)	4.8 (1.1, 20.9)	3.9 (0.5, 31.0)	7.0 (1.5, 33.4)	1.2 (0.2, 9.7)	N/E	5.9 (0.6, 53.8)	0.9 (0.1, 8.0)	N/E
HPV-35	4.3 (0.9, 20.8)	1.9 (0.3, 14.4)	N/E	N/E	N/E	N/E	4.1 (0.4, 39.9)	N/E	3.5 (0.4, 34.0)
HPV-52	4.9 (1.2, 20.7)	1.2 (0.2, 8.9)	4.2 (1.0, 18.5)	5.4 (1.5, 18.9)	3.1 (1.0, 9.5)	N/E	N/E	3.2 (1.4, 7.4)	2.6 (0.6, 11.2)
HPV-58	N/E	2.2 (0.5, 9.4)	2.7 (0.4, 20.2)	1.5 (0.2, 11.9)	1.6 (0.3, 7.2)	N/E	N/E	0.7 (0.2, 3.1)	1.7 (0.2, 13.3)
HPV-67	N/E	N/E	N/E	N/D	N/D	N/D	2.3 (0.5, 10.0)	2.0 (0.9, 4.8)	N/E
α-7 species	2.5 (0.9, 6.7)	0.7 (0.2, 2.7)	N/E	0.8 (0.2, 3.2)	1.6 (0.7, 3.6)	1.5 (0.4, 6.2)	2.2 (0.7, 7.4)	1.8 (0.9, 3.6)	2.4 (0.7, 8.0)
HPV-18	N/E	N/E	N/A	0.9 (0.1, 6.6)	1.1 (0.2, 4.6)	N/A	N/E	1.9 (0.5, 7.4)	N/A
HPV-39	5.0 (0.6, 39.4)	2.8 (0.4, 21.8)	N/E	1.7 (0.4, 7.5)	2.1 (0.7, 6.2)	2.5 (0.6, 10.8)	2.5 (0.6, 10.7)	1.2 (0.5, 3.2)	2.0 (0.5, 8.6)
HPV-45	3.4 (0.4, 26.3)	2.0 (0.3, 15.2)	N/E	N/E	N/E	N/E	N/E	2.3 (0.7, 7.7)	N/E
HPV-59	2.9 (0.4, 22.0)	1.8 (0.2, 13.6)	N/E	N/E	3.0 (0.6, 14.2)	N/E	3.6 (0.8, 16.0)	0.9 (0.3, 3.4)	2.7 (0.6, 12.0)
HPV-68	N/E	N/E	N/E	N/E	N/E	N/E	N/E	2.3 (0.4, 12.7)	4.7 (0.5, 40.6)
HPV-70	4.4 (0.6, 34.4)	N/E	N/E	N/D	N/D	N/D	6.1 (0.6, 61.0)	1.0 (0.1, 9.7)	5.3 (0.5, 51.5)
Other types									
HPV-40	7.2 (0.9, 57.9)	N/E	N/E	N/E	N/E	N/E	1.4 (0.2, 10.8)	1.4 (0.4, 4.2)	2.3 (0.5, 10.4)
HPV-42	N/E	N/E	N/E	8.3 (0.8, 83.8)	2.8 (0.3, 25.9)	N/E	0.6 (0.1, 4.7)	2.5 (1.3, 4.9)	0.5 (0.1, 3.7)
HPV-51	1.0 (0.1, 7.4)	N/E	N/E	2.5 (0.9, 7.0)	0.5 (0.1, 2.2)	N/E	N/E	0.6 (0.2, 1.8)	0.7 (0.1, 5.3)
HPV-53	2.4 (0.6, 9.8)	1.4 (0.4, 5.9)	1.3 (0.2, 9.2)	0.7 (0.1, 5.3)	1.8 (0.6, 5.2)	N/E	N/E	1.6 (0.7, 3.7)	0.6 (0.1, 4.6)
HPV-54	N/E	8.5 (3.2, 22.3)	N/E	1.9 (0.4, 7.9)	0.7 (0.2, 3.1)	2.0 (0.5, 8.4)	N/E	1.0 (0.7, 5.3)	2.5 (0.5, 11.6)
HPV-56	N/E	2.2 (0.3, 16.8)	N/E	1.0 (0.1, 7.8)	N/E	1.4 (0.2, 10.9)	4.2 (1.2, 14.5)	2.8 (1.1, 6.9)	1.2 (0.2, 8.7)
HPV-61	N/E	2.0 (0.3, 15.1)	8.7 (2.0, 38.1)	N/D	N/D	N/D	1.8 (0.4, 7.9)	1.9 (0.7, 4.9)	1.9 (0.4, 8.4)
HPV-62	3.7 (0.5, 28.1)	1.9 (0.2, 13.9)	N/E	N/D	N/D	N/D	1.8 (0.4, 7.6)	2.1 (0.9, 4.7)	3.8 (1.1, 12.9)
HPV-66	N/E	8.0 (1.7, 37.4)	N/E	1.3 (0.2, 10.0)	1.2 (0.3, 5.3)	1.4 (0.2, 10.5)	1.3 (0.3, 5.3)	1.0 (0.4, 2.2)	2.5 (0.9, 7.1)
HPV-73	2.9 (0.4, 21.7)	N/E	N/E	1.6 (0.2, 12.5)	0.8 (0.1, 6.4)	6.6 (1.8, 23.3)	N/E	3.5 (1.5, 7.8)	1.6 (0.4, 6.7)
HPV-82	7.5 (0.9, 60.5)	4.3 (0.5, 35.0)	N/E	1.6 (0.2, 12.7)	0.8 (0.1, 6.1)	2.0 (0.3, 15.9)	N/E	2.0 (0.5, 7.6)	2.4 (0.3, 18.7)
HPV-83	N/E	6.9 (1.5, 31.0)	N/E	2.5 (0.3, 20.8)	1.1 (0.1, 8.9)	3.0 (0.4, 23.9)	N/E	1.8 (0.3, 9.2)	N/E
HPV-84	2.6 (0.3, 19.2)	4.9 (1.5, 16.3)	N/E	1.9 (0.6, 6.3)	1.6 (0.6, 4.1)	N/E	0.6 (0.1, 4.7)	1.3 (0.6, 2.7)	2.5 (0.9, 7.0)
HPV-89	N/E	4.6 (0.6, 37.4)	N/E	N/D	N/D	N/D	0.9 (0.2, 3.8)	1.8 (1.0, 3.5)	2.8 (1.2, 6.7)

Abbreviations: CI, confidence interval; HR, hazard ratio; HPV, human papillomavirus; N/A, not an applicable outcome type; N/D, presence of HPV type was not determined; N/E, not able to estimate.

[a] Models adjusted for age at baseline, and lifetime number of sexual partners.

[b] Subject follow-up was truncated after two years (from baseline) in the Ludwig-McGill study.

Table 6-5: Association between vaccine type HPV infection at time of index infection and clearance of individual HPV types in three epidemiologic cohort studies[a]

	Hazard ratios (95%CI) according to Index vaccine-type HPV Infection								
	Ludwig-McGill study[b]			McGill-Concordia study			HITCH study		
HPV type cleared	HPV-6/11 N=103	HPV-16 n=276	HPV-18 n=87	HPV-6/11 n=42	HPV-16 n=81	HPV-18 n=39	HPV-6/11 n=49	HPV-16 n=96	HPV-18 n=25
α-10 species	3.0 (0.4, 23.7)	1.8 (0.8, 3.9)	0.7 (0.2, 2.3)	22.3 (0.8, 662.1)	1.7 (0.8, 3.8)	5.8 (0.7, 48.8)	N/E	0.6 (0.3, 1.4)	2.8 (0.7, 11.3)
HPV-6/11	N/A	1.6 (0.6, 4.1)	0.5 (0.1, 1.9)	N/A	3.6 (1.4, 8.9)[c]	5.4 (1.1, 27.3)[c]	N/A	1.3 (0.6, 2.9)	3.0 (0.7, 13.3)
HPV-44	3.0 (0.4, 23.7)	2.1 (0.5, 9.2)	4.3 (0.5, 34.8)	N/D	N/D	N/D	N/E	0.9 (0.1, 5.2)	8.3 (0.1, 532.9)
HPV-55	N/D	N/D	N/D	22.3 (0.8, 662.1)	0.5 (0.1, 2.6)	22.3 (0.8, 662.1)	N/D	N/D	N/D
α-9 species	0.6 (0.2, 1.9)	0.8 (0.4, 1.5)	1.1 (0.4, 3.6)	N/E	0.8 (0.4, 1.5)	1.0 (0.4, 2.9)	1.7 (0.8, 3.4)	1.3 (0.7, 2.4)	0.6 (0.3, 1.2)
HPV-16	0.6 (0.1, 4.1)	N/A	0.8 (0.2, 3.3)	N/E	N/A	2.1 (0.5, 10.1)	1.4 (0.6, 3.1)	N/A	19.5 (1.7, 216.9)[c]
HPV-31	0.4 (0.1, 1.6)	1.5 (0.6, 3.6)	0.9 (0.1, 6.4)	3.6 (0.6, 20.1)	0.6 (0.1, 4.4)	0.8 (0.2, 3.0)	1.4 (0.5, 4.3)	0.7 (0.3, 1.7)	0.6 (0.1, 2.4)
HPV-33	N/E	2.7 (0.6, 11.5)	6.8 (0.5, 42.3)	N/E	N/E	N/E	1.4 (0.1, 17.2)	1.4 (0.1, 17.2)	N/E
HPV-35	N/E	0.1 (0.0, 0.7)	0.7 (0.1, 5.6)	N/E	N/E	N/E	N/E	N/E	N/E
HPV-52	1.8 (0.2, 13.3)	0.6 (0.1, 2.5)	1.8 (0.2, 13.3)	N/E	0.8 (0.3, 2.4)	2.5 (0.3, 21.2)	0.9 (0.3, 2.9)	1.8 (0.8, 3.9)	0.5 (0.2, 1.3)
HPV-58	0.6 (0.1, 2.3)	0.7 (0.2, 3.1)	N/E	N/E	0.6 (0.1, 2.9)	1.0 (0.2, 5.4)	N/E	0.8 (0.2, 2.9)	1.2 (0.1, 11.3)
HPV-67	N/E	N/E	N/E	N/D	N/D	N/D	1.2 (0.5, 2.7)	1.2 (0.5, 2.8)	1.4 (0.5, 3.8)
α-7 species	0.9 (0.3, 2.8)	1.8 (0.7, 4.6)	0.5 (0.1, 3.7)	0.3 (0.1, 1.1)	1.8 (0.7, 4.6)	2.0 (0.5, 8.9)	1.9 (0.9, 4.1)	0.8 (0.5, 1.6)	0.6 (0.1, 2.1)
HPV-18	1.3 (0.2, 10.0)	2.3 (0.7, 7.2)	N/A	0.5 (0.1, 5.2)	3.3 (1.0, 11.0)	N/A	0.9 (0.1, 8.1)	0.4 (0.1, 1.5)	N/A
HPV-39	1.5 (0.2, 12.4)	1.3 (0.2, 10.2)	N/E	N/E	0.6 (0.1, 3.2)	1.0 (0.1, 9.2)	1.5 (0.4, 5.2)	0.8 (0.4, 1.7)	0.3 (0.1, 1.4)
HPV-45	N/E	2.1 (0.3, 16.1)	N/E	1.2 (0.2, 6.1)	0.8 (0.1, 3.4)	4.9 (0.5, 52.1)	0.7 (0.2, 2.9)	49.6 (3.0, 811.6)[d]	1.3 (0.1, 29.3)
HPV-59	N/E	0.4 (0.1, 2.9)	0.4 (0.1, 3.4)	N/E	N/E	N/E	0.9 (0.2, 3.0)	1.2 (0.4, 3.5)	0.9 (0.1, 6.7)
HPV-68	0.6 (0.1, 2.4)	0.4 (0.1, 1.7)	N/E	N/E	N/E	N/E	0.7 (0.1, 7.5)	0.3 (0.1, 2.5)	0.7 (0.0, 50.7)
HPV-70	1.5 (0.2, 12.5)	1.5 (0.2, 12.5)	N/E	N/D	N/D	N/D	N/E	0.7 (0.1, 26.9)	N/E
Other types									
HPV-40	3.8 (0.5, 31.1)	1.0 (0.2, 4.4)	0.8 (0.1, 6.3)	N/E	N/E	N/E	N/E	2.1 (0.6, 7.2)	0.2 (0.0, 1.8)
HPV-42	0.3 (0.0, 22.9)	0.3 (0.0, 22.9)	N/E	N/E	N/E	N/E	0.7 (0.2, 2.0)	0.9 (0.4, 1.9)	1.3 (0.4, 4.7)
HPV-51	0.7 (0.3, 1.9)	0.8 (0.4, 1.8)	1.2 (0.3, 5.0)	2.5 (0.8, 7.4)	0.3 (0.1, 1.1)	1.4 (0.5, 4.0)	2.1 (0.8, 5.3)	1.8 (0.5, 6.1)	N/E
HPV-53	0.8 (0.2, 2.4)	0.6 (0.3, 1.3)	0.8 (0.1, 5.6)	N/E	0.6 (0.2, 1.6)	2.6 (0.3, 20.5)	0.4 (0.1, 2.0)	1.3 (0.5, 3.0)	12.1 (0.9, 156.9)
HPV-54	0.7 (0.1, 3.3)	0.7 (0.2, 2.5)	N/E	1.1 (0.1, 9.5)	1.6 (0.5, 5.2)	N/E	0.4 (0.1, 1.8)	0.5 (0.2, 1.4)	0.8 (0.2, 3.0)
HPV-56	N/E	0.9 (0.3, 2.6)	3.5 (0.5, 26.5)	6.2 (0.6, 62.3)	1.5 (0.4, 5.4)	N/E	3.5 (0.4, 34.6)	0.5 (0.1, 2.6)	0.7 (0.1, 3.7)
HPV-61	3.3 (0.4, 26.8)	3.0 (0.4, 22.4)	5.0 (0.5, 51.7)	N/D	N/D	N/D	4.7 (0.4, 52.8)	0.3 (0.1, 1.4)	N/E
HPV-62	N/E	1.9 (0.5, 6.8)	N/E	N/D	N/D	N/D	2.0 (0.6, 6.8)	0.8 (0.3, 1.9)	0.4 (0.1, 2.0)
HPV-66	N/E	0.8 (0.2, 2.7)	N/E	N/E	0.7 (0.2, 2.2)	N/E	0.8 (0.3, 1.8)	0.8 (0.4, 1.7)	6.1 (1.3, 29.5)[d]
HPV-73	1.5 (0.3, 6.3)	0.8 (0.1, 6.1)	N/E	N/E	0.2 (0.0, 1.6)	0.7 (0.1, 24.8)	0.8 (0.1, 6.8)	0.8 (0.3, 2.1)	0.3 (0.0, 2.2)
HPV-82	N/E	1.5 (0.2, 13.6)	1.7 (0.4, 8.4)	6.3 (0.5, 76.9)	0.4 (0.1, 1.6)	N/E	N/E	0.5 (0.2, 1.3)	1.2 (0.2, 5.5)
HPV-83	N/E	2.9 (0.6, 13.8)	N/E	N/E	19.9 (1.9, 207.0)[c]	N/E	0.5 (0.0, 10.6)	0.1 (0.0, 1.3)	2.4 (0.1, 42.7)
HPV-84	0.7 (0.2, 2.3)	0.9 (0.3, 2.6)	1.3 (0.2, 9.4)	2.2 (0.6, 8.0)	0.8 (0.4, 1.6)	N/E	0.9 (0.3, 2.2)	0.7 (0.3, 1.6)	0.6 (0.1, 2.4)
HPV-89	N/E	N/E	N/E	N/D	N/D	N/D	1.3 (0.6, 3.2)	1.1 (0.6, 1.8)	1.3 (0.5, 3.7)

Abbreviations: CI, confidence interval; HR, hazard ratio; HPV, human papillomavirus; N/A, not an applicable outcome type; N/D, presence of HPV type was not determined; N/E, not able to estimate.

[a] Models adjusted for age at time of index infection, and lifetime number of sexual partners.

[b] Subject follow-up was truncated after two years (from time of index HPV infection) in the Ludwig-McGill study.

[c] Not statistically significant at 0.01 level of testing.

[d] Not statistically significant at 0.00007 level of testing (Bonferroni corrected p-value threshold).

Table 6-6: Pooled analysis of association between vaccine type HPV infection at baseline/time of index infection and acquisition/clearance of individual HPV types

	Hazard ratios (95%CI) according to analytical objective and vaccine-type HPV infection					
	Acquisition Objective			Clearance Objective		
HPV Acquisition/ Clearance	HPV-6/11 n=69 HR (95% CI)	HPV-16 n=189 HR (95% CI)	HPV-18 n=62 HR (95% CI)	HPV-6/11 n=187 HR (95% CI)	HPV-16 n=453 HR (95% CI)	HPV-18 n=151 HR (95% CI)
α-10 species	1.1 (0.3, 4.1)	1.7 (1.0, 2.7)	0.8 (0.3, 2.0)	2.3 (0.8, 6.6)	1.4 (0.8, 2.3)	2.5 (0.8, 7.6)
HPV-6/11	N/A	1.7 (0.9, 3.3)	1.1 (0.4, 3.5)	N/A	1.7 (1.1, 2.6)[c]	0.9 (0.4, 2.0)
HPV-44	1.8 (0.2, 13.1)	2.0 (0.6, 6.7)	2.6 (0.3, 21.1)	2.9 (0.3, 31.3)	1.7 (0.4, 3.8)	6.7 (1.1, 42.4)[c]
HPV-55	1.7 (0.2, 14.2)	N/E	2.9 (0.4, 23.4)	22.3 (0.8, 662.1)	0.5 (0.1, 2.6)	22.3 (0.8, 662.1)
α-9 species	1.9 (1.0, 3.9)	1.9 (1.2, 3.0)	1.5 (0.6, 3.6)	1.1 (0.6, 1.8)	0.9 (0.7, 1.3)	0.8 (0.5, 1.3)
HPV-16	0.9 (0.4, 2.7)	N/A	1.1 (0.4, 3.1)	1.1 (0.6, 2.0)	N/A	1.4 (0.7, 3.0)
HPV-31	3.1 (1.3, 7.2)	2.3 (1.2, 4.6)	1.0 (0.3, 4.2)	1.0 (0.5, 2.1)	0.9 (0.5, 1.5)	0.8 (0.4, 1.7)
HPV-33	5.2 (1.8, 14.8)	2.0 (0.7, 5.8)	1.2 (0.1, 8.6)	0.9 (0.2, 3.7)	1.3 (0.4, 2.5)	9.5 (1.1, 80.2)[c]
HPV-35	4.3 (1.2, 15.4)	0.7 (0.1, 5.0)	1.7 (0.2, 12.5)	N/E	0.6 (0.3, 1.4)	0.9 (0.3, 2.6)
HPV-52	3.6 (1.4, 9.0)	3.8 (2.1, 7.0)	2.6 (0.9, 7.2)	0.8 (0.3, 2.3)	1.0 (0.6, 1.7)	0.8 (0.4, 1.8)
HPV-58	0.7 (0.1, 5.0)	1.7 (0.7, 4.1)	1.6 (0.4, 6.5)	0.6 (0.1, 2.4)	0.7 (0.4, 1.4)	1.0 (0.3, 3.1)
HPV-67	2.6 (0.6, 11.0)	2.8 (1.2, 6.7)	N/E	1.0 (0.4, 2.2)	1.1 (0.5, 2.5)	1.3 (0.5, 3.4)
α-7 species	1.8 (0.9, 3.6)	1.6 (1.0, 2.7)	1.7 (0.8, 3.9)	0.9 (0.5, 1.6)	1.4 (0.9, 2.1)	1.0 (0.4, 2.2)
HPV-18	0.6 (0.1, 4.4)	1.3 (0.5, 3.4)	N/A	1.1 (0.3, 3.4)	2.0 (1.1, 3.7)[c]	N/A
HPV-39	2.6 (0.9, 7.2)	2.3 (1.1, 4.7)	2.7 (1.0, 7.3)	0.7 (0.2, 1.9)	1.0 (0.5, 1.8)	0.8 (0.3, 2.3)
HPV-45	1.3 (0.2, 9.3)	2.2 (0.8, 6.4)	N/E	1.1 (0.4, 3.3)	2.7 (1.2, 5.8)[c]	0.7 (0.1, 4.9)
HPV-59	2.6 (0.8, 8.4)	2.2 (0.9, 5.2)	1.7 (0.4, 7.0)	1.4 (0.4, 4.8)	1.1 (0.4, 3.2)	0.7 (0.2, 3.1)
HPV-68	N/E	0.8 (0.2, 3.1)	2.1 (0.5, 8.8)	0.9 (0.3, 2.9)	0.5 (0.2, 1.4)	2.0 (0.1, 27.6)
HPV-70	5.5 (1.3, 24.4)	1.0 (0.1, 7.9)	2.6 (0.3, 19.7)	1.9 (0.5, 6.7)	2.1 (0.6, 7.7)	N/E
Other types						
HPV-40	3.1 (0.7, 13.3)	2.6 (0.9, 7.6)	1.5 (0.2, 11.1)	6.9 (0.9, 54.9)	1.3 (0.6, 3.0)	0.5 (0.2, 1.7)
HPV-42	1.6 (0.4, 6.5)	6.0 (3.2, 11.6)	0.7 (0.1, 5.4)	0.8 (0.4, 2.0)	0.9 (0.5, 1.8)	1.3 (0.3, 5.5)
HPV-51	1.6 (0.7, 4.0)	0.5 (0.2, 1.3)	0.3 (0.1, 2.2)	1.2 (0.7, 2.0)	0.9 (0.5, 1.5)	1.3 (0.6, 2.9)
HPV-53	1.2 (0.4, 3.6)	2.2 (1.2, 3.9)	0.8 (0.2, 3.1)	0.7 (0.3, 1.8)	0.7 (0.4, 1.1)	1.3 (0.4, 4.1)
HPV-54	1.4 (0.3, 5.9)	2.6 (1.2, 5.9)	1.6 (0.4, 6.5)	0.9 (0.3, 3.1)	1.1 (0.6, 2.2)	0.8 (0.3, 3.2)
HPV-56	2.8 (1.0, 7.7)	2.6 (1.3, 5.4)	1.5 (0.4, 6.1)	1.6 (0.4, 6.8)	0.7 (0.4, 1.4)	0.5 (0.2, 1.4)
HPV-61	1.8 (0.4, 8.0)	1.7 (0.6, 5.0)	5.6 (2.0, 16.0)	4.9 (1.1, 22.3)[c]	0.8 (0.2, 2.7)	17.0 (3.3, 86.5)[d]
HPV-62	3.4 (1.0, 10.9)	3.8 (1.8, 8.0)	3.6 (1.1, 11.7)	1.6 (0.5, 5.1)	0.9 (0.4, 1.7)	0.3 (0.1, 1.3)
HPV-66	1.6 (0.5, 5.1)	2.0 (1.0, 3.9)	3.3 (1.4, 7.7)	0.8 (0.4, 1.8)	0.9 (0.6, 1.5)	12.0 (2.6, 54.7)[d]
HPV-73	1.3 (0.3, 5.2)	3.0 (1.5, 5.9)	2.7 (1.0, 7.5)	1.4 (0.4, 4.6)	0.9 (0.5, 1.7)	0.7 (0.2, 2.3)
HPV-82	2.5 (0.6, 10.5)	2.3 (0.9, 6.2)	2.5 (0.6, 10.4)	1.4 (0.2, 10.6)	0.9 (0.5, 1.9)	1.1 (0.4, 3.1)
HPV-83	1.4 (0.2, 10.5)	3.2 (1.2, 8.4)	1.6 (0.2, 11.4)	1.9 (0.4, 8.2)	1.5 (0.6, 3.6)	2.0 (0.6, 6.6)
HPV-84	1.6 (0.6, 4.2)	2.5 (1.4, 4.3)	1.5 (0.5, 4.0)	1.0 (0.5, 1.8)	1.0 (0.6, 1.5)	0.7 (0.2, 2.2)
HPV-89	1.6 (0.4, 6.5)	3.6 (1.9, 7.2)	4.4 (1.9, 10.2)	1.4 (0.6, 3.2)	1.0 (0.6, 1.7)	2.4 (0.5, 3.9)

Abbreviations: CI, confidence interval; HR, hazard ratio; HPV, human papillomavirus; N/A, not an applicable outcome type; N/E, not able to estimate.

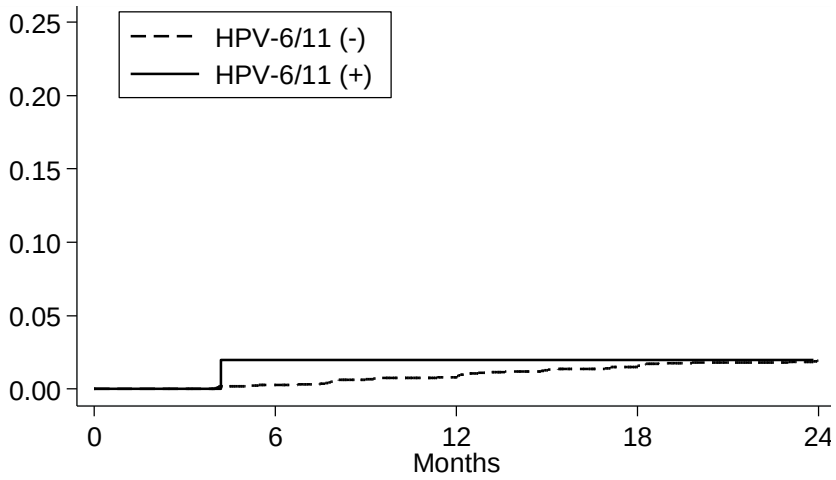
[a] Models adjusted for age at baseline or time of index infection, lifetime number of sexual partners, and study.

[b] Subject follow-up was truncated after two years (from time of index HPV infection) in the Ludwig-McGill study.

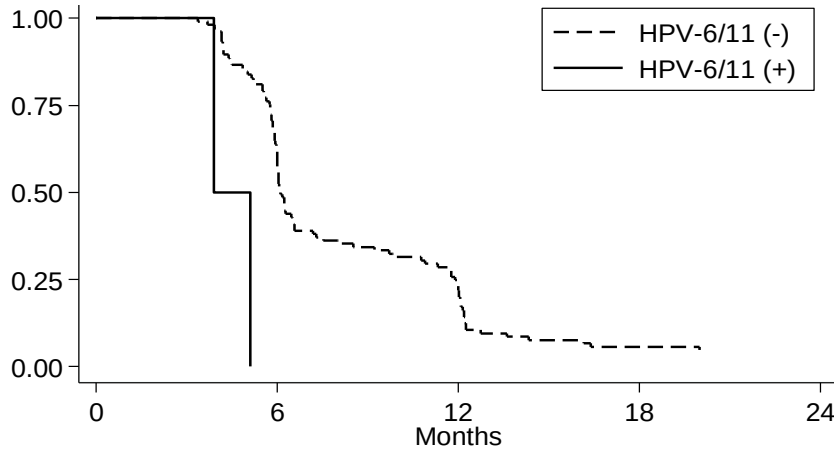
[c] Not statistically significant at 0.01 level of testing.

[d] Not statistically significant at 0.00007 level of testing (Bonferroni corrected p-value threshold).

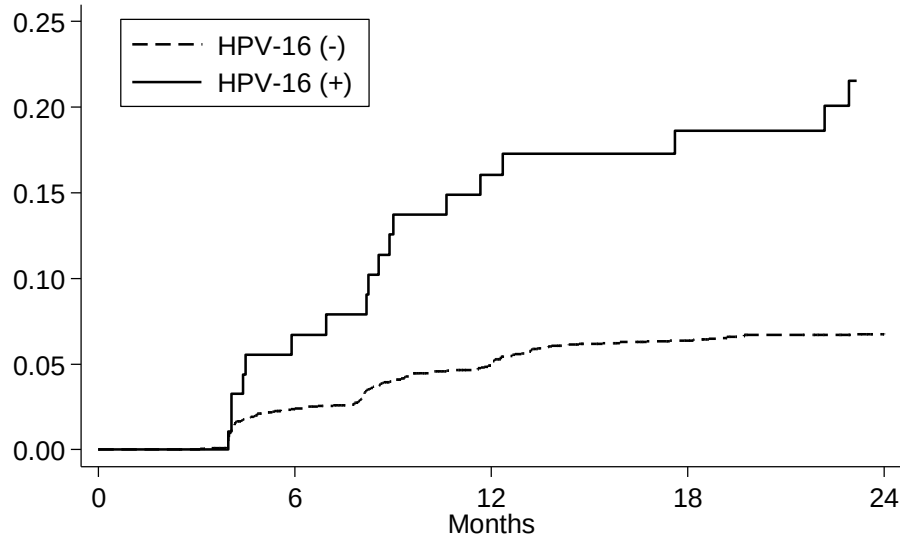
A)


HPV-6/11 (-)
HPV-6/11 (+)
0.25
0.20
0.15
0.10
0.05
0.00
0
6
12
18
24
Months

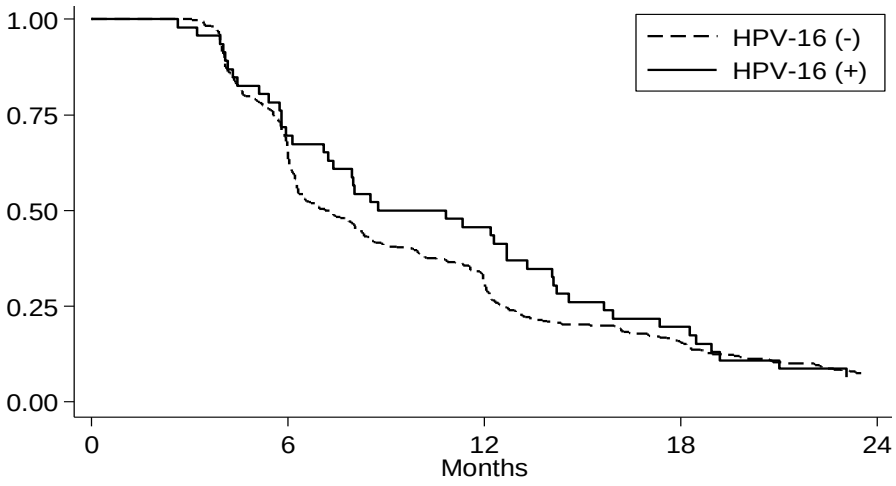
B)


HPV-6/11 (-)
HPV-6/11 (+)
1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
Months

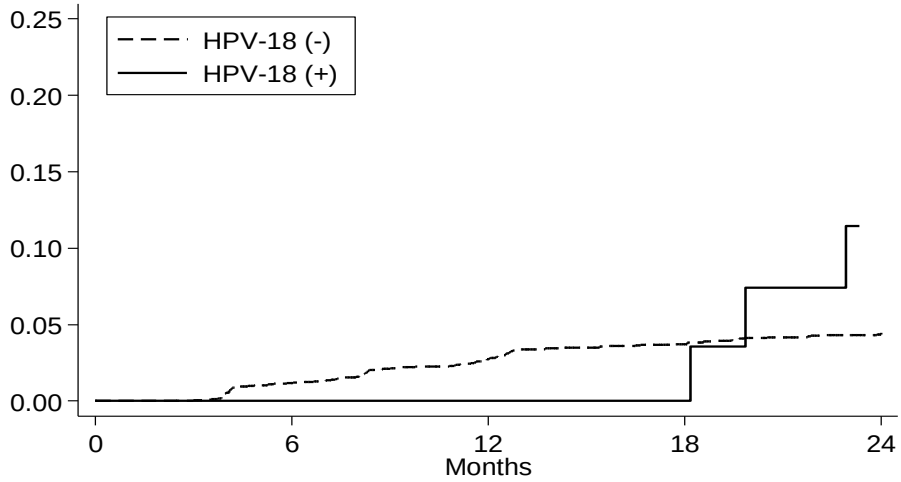
C)


HPV-16 (-)
HPV-16 (+)
0.25
0.20
0.15
0.10
0.05
0.00
0
6
12
18
24
Months

D)


1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
Months
HPV-16 (-)
HPV-16 (+)

E)


0.25
0.20
0.15
0.10
0.05
0.00
0
6
12
18
24
Months
HPV-18 (-)
HPV-18 (+)

F)

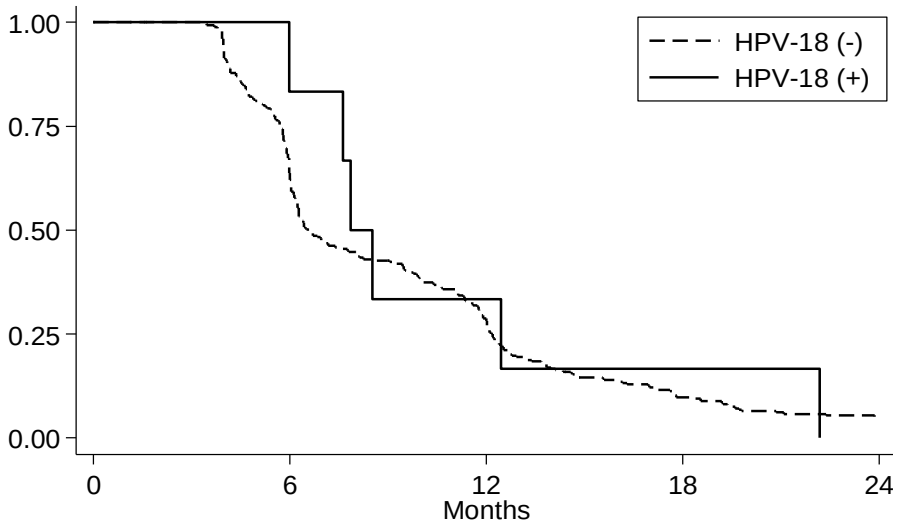

1.00
0.75
0.50
0.25
0.00
0
6
12
18
24
Months
HPV-18 (-)
HPV-18 (+)

Figure 6-1: Kaplan-Meier curves showing time to incidence and clearance of human papillomavirus (HPV) infection with: α-10 types (panels A and B; excluding HPVs 6/11), α-9 types (panels C and D; excluding HPV-16), and α-7 types (panels E and F; excluding HPV18), according to HPV6/11, HPV-16, and HPV-18 status at baseline or time of index infection, respectively. All analyses included pooled results from the Ludwig-McGill, McGill-Concordia, and HITCH cohort studies and were adjusted for age, lifetime number of sexual partners and study. Hazard ratios and associated 95% confidence intervals (CI) for panels A, B, C, D, E and F were 1.1 (95%CI: 0.4-4.1), 2.3 (95%CI: 0.8-6.6), 1.9 (95%CI: 1.2-3.0), 0.9 (95%CI: 0.7-1.3), 1.7 (95%CI: 0.8-3.9) and 1.0 (95%CI: 0.4-2.2), respectively.

6.2 Additional analyses to manuscript IV

Due to space limitations of the journal, Table 6-7 (presented in Appendix 4) was submitted for consideration as online supplementary material only. For each of the three cohort studies, this table presents the number of females that acquired each of the individual HPV types during follow-up, as well as the number of females that cleared each of the individual HPV types (among those who were infected with each of the respective individual types). Females were only included if they had valid HPV DNA results available at baseline and at least one follow-up visit (acquisition analysis), or if they had valid HPV DNA testing results for at least one visit following the visit that they tested positive for HPV (clearance analysis).

CHAPTER 7: MANUSCRIPT V

EVALUATION OF HUMAN PAPILLOMAVIRUS TYPE REPLACEMENT POST-VACCINATION MUST ACCOUNT FOR DIAGNOSTIC ARTIFACTS: MASKING OF HPV52 BY HPV16 IN ANOGENITAL SPECIMENS

7.1 Preamble

The fifth manuscript presented in this thesis deals with an important diagnostic measurement error issue. While it may be true that an observed increase in the prevalence of other HPV types (following a reduction in HPV vaccine-targeted types) could be a result of reduced competition for niche occupation during natural infection (i.e., actual type replacement), there may be another explanation. An apparent post-vaccination increase may be a diagnostic artifact because consensus PCR assays fail to detect HPV types present in low copy numbers in co-infected specimens, such that with a drop in vaccine-preventable types, there may be increased detection of previously masked types. Due to competition for PCR reagents, consensus PCR assays have already shown to be less sensitive in detecting certain HPV types when they are present as part of a co-infection. For example, HPV16, which is the most common HPV type globally and often present in very high viral load concentration, may be “masking” other HPV types, and once the prevalence of this HPV type is reduced in vaccinated populations, unmasking may occur and misinterpreted as type replacement.

The authors of some studies reporting an increase in certain HPV types post-vaccination have alluded to the possibility that this may be caused by diagnostic artifacts. In this study, we reanalyzed 1200 anogenital specimens (all HPV52 negative according to consensus PCR assays; retested using highly sensitive type-specific real-time HPV52 PCR) from six different epidemiologic studies (200 specimens/study; 100 HPV16 positive/study) to explore the potential for unmasking of HPV52 that may be caused by elimination of HPV16.

Evaluation of Human Papillomavirus Type Replacement Post-vaccination Must Account for Diagnostic Artifacts: Masking of HPV52 by HPV16 in Anogenital Specimens

Running title: Diagnostic artifacts in HPV testing

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FOOTNOTES

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Abstract

It has been hypothesized that, following a reduction in human papillomavirus (HPV) vaccine-targeted types, an increase in prevalence of other HPV types may occur due to reduced competition during natural infection. Any apparent post-vaccination increase must be distinguished from diagnostic artifacts consequent to consensus PCR assays failing to detect HPV types present in low copy numbers in co-infected specimens (under the assumption that with a drop in vaccine-preventable types there may be increased detection of previously “masked” types). We reanalyzed anogenital specimens to evaluate unmasking of HPV52 that may be caused by elimination of HPV16. Using highly sensitive type-specific real-time HPV52 PCR, we retested 1,200 anogenital specimens (all HPV52 negative according to consensus PCR assays) from six epidemiologic studies (200 specimens/study; 100 HPV16+/study). Multivariate logistic regression, with adjustment for age and number of sexual partners was used to evaluate the association between HPV16 positivity and detection of HPV52. In our pooled analysis (n=1,196), presence of HPV16 was positively associated with HPV52 detection (adjusted OR=1.47, 95%CI: 0.76-2.82). In our separate (study specific) analyses, a statistically significant association was observed in one study that included HIV infected males (HIPVIRG study; adjusted OR=3.82, 95%CI: 1.19-12.26). We observed a positive association between HPV16 viral load (tertiles) and detection of HPV52 (P for trend=0.003). These results indicate that diagnostic artifacts, resulting from unmasking of HPV52, may occur in some settings in the evaluation of HPV type replacement. Additional studies exploring the extent and severity of unmasking are needed.

Introduction

Infection with oncogenic human papillomavirus (HPV) types is necessary for cervical cancer development. Currently, two commercially available vaccines offer protection against the two major oncogenic HPV types (16 and 18) and associated lesions, but only one of these vaccines also protects against HPV types 6 and 11, which are responsible for the majority of anogenital warts [260].

Vaccination has begun to reduce the prevalence and burden of vaccine-targeted HPV types [193, 194]; however, as this occurs, there is concern that abrogation of selective pressure could lead to an increase in the prevalence of other non-vaccine HPV types. This phenomenon, referred to as “type replacement”, may occur as a result of one or more HPV types becoming unrestricted in their ability to occupy the niche originally taken by vaccine-targeted types during natural infection. However, an apparent rise in non-vaccine HPV types may occur due to diagnostic artifacts if there is competition between vaccine and non-vaccine HPV types for reagents (e.g., primers) in consensus-primer polymerase chain reaction (PCR) assays. In this situation, it is possible that prevalent non-vaccine types may be undetected. For instance, if a co-infected specimen contains a much higher number of HPV16 genome copies, then it may overwhelm the minority type(s) during PCR amplification, and as a result, the specimen may be erroneously labelled as negative for the minority type(s). Therefore, a reduction in the rate of detection of vaccine types post-vaccination in genital specimens may lead to an apparent increase in some HPV types that were previously masked.

Such unmasking effect could be mistaken for type replacement. HPV16 is currently the most common HPV type globally and is often present in high viral load concentrations. Thus, compared with other types targeted by vaccination (HPVs 6, 11, and 18), reductions in HPV16 prevalence post-vaccination will likely be most responsible for unmasking. Recently, unmasking has been cited as a possible explanation for negative vaccine efficacy observed in one trial for some endpoints involving specific HPV types, particularly HPV52 [209]. In addition, two studies evaluating the population effect of vaccination in the United States and Scotland recently revealed slight increases in certain HPV types, including HPV52 [193, 194]. PCR does not always amplify different DNA segments with equal efficiency; reduced sensitivity of consensus primer PCR for detection of HPV52 in co-infected specimens has been reported [163-166]. It is therefore important to explore whether increases in the prevalence of HPV52 and other types observed following vaccination may be the result of true type replacement, or an artifact of unmasking.

Our objective was to explore the potential for unmasking of HPV52 attributable to a reduction in HPV16 post-vaccination. We investigated whether detection of HPV52 using a sensitive type-specific PCR assay varies according to HPV16 positivity and viral load among specimens originally HPV52 negative.

Materials and Methods

Study design and specimen selection

Specimens were available from the following studies: Ludwig-McGill cohort study [202], HPV Infection and Transmission among Couples through Heterosexual Activity (HITCH) study [236], McGill-Concordia cohort study [22], Biomarkers of Cervical Cancer Risk (BCCR) case-control study [238], Canadian Cervical Cancer Screening Trial (CCCaST) [239], and the Human Immunodeficiency and Papilloma Virus Research Group (HIPVIRG) study [244]. Each of these studies was approved by their respective institutional review boards. Informed consent was obtained from all participants prior to enrolment.

In total, 1,200 anogenital specimens from 1,000 women and 200 men were selected for retesting using HPV52 type-specific PCR on the basis of previous testing done using consensus-primer PCR. From each of the aforementioned six studies [22, 202, 236, 238, 239, 244], an equal number of specimens (n=200; all HPV52 negative) were randomly selected based on the following criteria. Half of the specimens (n=100) were positive for HPV16, and the other half were negative for HPV16. Because all anogenital HPV types share a common transmission route, subjects with HPV16 (or any other HPV type) would also be at higher risk of HPV52 infection. Thus, to avoid major confounding we selected for retesting only HPV positive specimens. Among HPV16 negative specimens, half (n=50) were positive for an HPV type phylogenetically related to HPV16 (α-9 species; except HPVs 16 or 52) and the other half were positive for some other non α-9 HPV type.

Laboratory assessments

Self or provider-collected anal, cervical, or cervicovaginal specimens were obtained using swabs, cytobrush or spatula, according to the parent study's protocol. HPV DNA testing and genotyping was performed in the original studies with consensus primer assays (L1 PGMY or MY09/11 and hybridization with oligonucleotide probes and restriction fragment length polymorphism analysis, linear array, or line blot assay), which detect 27 to 40 different HPV types. For the present study, specimens were retested (blinded to HPV16 status) using a type-specific, real-time HPV52 PCR, which is capable of detecting as few as 10 HPV52 copies per assay [167]. HPV16 viral load was quantified according to a well-established real time PCR protocol [245] and expressed as the number of HPV DNA copies per cell.

Statistical analyses

Logistic regression was used to estimate odds ratios (ORs) and associated 95% confidence intervals (CIs) for the effect of HPV16 positivity on HPV52 detection. Separate analyses were performed for each study adjusted for age and lifetime number of sexual partners (multivariate model; covariates based on a priori knowledge), as well as pooled across studies (with adjustment for study in both crude and adjusted models). The CCCaST trial included participants from St. John's (Newfoundland) and Montreal (Quebec). Unfortunately, women from the St. John's site did not provide information on sexual history, which led to the exclusion of some specimens in our fully adjusted

models (n=76). By eliminating adjustment for sexual history as part of our sensitivity analyses, we were then able to include all CCCaST specimens in our pooled analysis. Analyses restricted to specimens from female subjects (i.e., excluding those from HIPVIRG) were also performed.

Logistic regression was also used to evaluate the effect of HPV16 viral load on HPV52 detection. For each study, HPV16 viral load was categorized into study specific tertiles (low, medium, high). We estimated ORs for each tertile with the HPV16 negative group as the reference category. Similar sensitivity analyses as above were performed in our evaluation of the effect of HPV16 viral load on unmasking of HPV52.

Results

Among the 1,200 specimens selected for HPV52 retesting, 1,196 had sufficient beta-globin and were evaluable. In total, 49 specimens tested positive for HPV52 and the majority (30/49) were detected among the HPV16 positive group (Table 1). Focusing on HPV16 negative specimens, detection of HPV52 was similar between the group containing α-9 HPV types and the group that contained other (non α-9) HPV types (11/300 versus 8/298, respectively).

Across all studies, the average number of HPV types detected among HPV16 positive and HPV16 negative specimens was 2.8 and 2.4, respectively. Accounting for age and lifetime number of sexual partners, additional HPV types present within

specimens was associated with an 18% increase in HPV52 detection. Overall, we observed a pooled adjusted OR of 1.47 (95%CI: 0.76-2.82) for the association between HPV16 status and HPV52 detection; however, we also observed substantial heterogeneity across studies (test for heterogeneity: p-value=0.08). A statistically significant positive association was observed in HIPVIRG, but not in the other studies (table 1). A negative association between HPV16 status and HPV52 detection was suggested in the CCCaST study; however, this association was not statistically significant. From the St. John's study site in CCCaST, HPV52 was detected in four of the 76 specimens, all of which were HPV16 negative. Excluding sexual history from our multivariate model, which allowed all CCCaST specimens to be included, had little impact on our results (pooled adjusted OR=1.33, 95%CI: 0.71-2.46). However, in our pooled analysis restricted to female cervicovaginal specimens (HIPVIRG study excluded), a null association between HPV16 status and HPV52 detection was observed (table 1).

We observed a strong positive association between HPV16 viral load (tertiles) and detection of HPV52 (Table 2, P for trend=0.003). HPV16 viral load (measured as number of copies per µl) ranged from 1 to 78,987,500 copies (approximately 8 logs). There was no meaningful change in our viral load results when we restricted our analysis to cervicovaginal specimens only (i.e., females without HIV infection), or when we included all CCCaST specimens (adjustment for age only in our pooled analysis; results not shown).

Discussion

In specimens tested via consensus PCR, HPV16 positivity was associated with masking of HPV52 positivity in the HIPVIRG and BCCR studies. These two studies, unlike the others, included participants with HIV infection or high-grade cervical lesions, respectively. In general, high viral load HPV infections are more common among individuals with low immunity or cervical neoplasia, which may explain why an effect was observed in specimens from these studies, but not the others. Our interpretation is also supported by our results revealing a greater unmasking effect in specimens with higher HPV16 viral load.

To our knowledge, this is the first study designed specifically to evaluate the potential for an HPV type to be masked if in a specimen co-infected with HPV16. Our findings suggest that, all else being equal, elimination of HPV16 via vaccination may lead to some unmasking of previously undetectable infections with a type such as HPV52. Previously, others have compared the performance of different PCR assays and found that in cases of lower viral DNA load and co-infection, consensus primer PCR assays are less accurate than type-specific or multiple primer systems [163-166]. Recently, one study found that in specimens co-infected with HPV16 and either HPV18, 51, 52, or 58, consensus PCR often failed to detect the latter types, particularly at lower viral loads and for HPVs 51 and 52 [165]. Therefore, despite lack of evidence of HPV type competition from most epidemiological studies [12], results from these studies comparing different PCR assays [163-166], as well as the recent report of negative

vaccine efficacy against HPV52 associated cervical neoplasia [209] is what motivated us to focus our evaluation on unmasking of HPV52.

Important strengths of our study were its size and the diverse study populations from which specimens were selected. Had we focused our analysis exclusively on specimens from females or disease free individuals, we would have missed the opportunity to discover an HPV16 induced masking effect in the two aforementioned studies. A possible limitation of our study was that the HPV16 negative group remained positive for other HPV type(s). As a result, masking of HPV52 may have occurred in this group as well, causing our effect estimates to be biased towards zero. But since those with HPV16 are at much higher risk of infection with other types (including HPV52), this decision was intended to avoid confounding by sexual activity and other risk factors common to all HPV types. Despite this conservative approach, we still observed a strong and statistically significant effect in the HIPVIRG study, as well as at higher HPV16 viral loads.

As investigators begin to evaluate HPV type replacement, they will rely on time point comparisons of HPV prevalence from surveys before and after vaccination. However, if an increase in HPV52 (or other HPV types) is observed post-vaccination, unmasking should be suspected. Based on results from this study, correction formulas for adjustment of baseline prevalence of HPV52 infection due to masking may not be necessary in all settings and will likely depend on the risk group being considered. For example, masking of HPV52 may be less common among specimens from low-risk

individuals in North America. Meanwhile, in parts of sub-Saharan Africa or other high-risk regions where there is high prevalence of HIV and HPV co-infection, elimination of vaccine target types could lead to larger increases in the prevalence of HPV52 or other HPV types due to unmasking.

Globally, consensus primer PCR assays are the most common HPV DNA tests used for research and surveillance. To evaluate whether different assays perform similarly in cases of multiple HPV infection, the World Health Organization HPV laboratory network has now assembled blinded “proficiency panels”, and so far results from more than 100 laboratories indicate that masking is a definite problem for some of these assays [166]. In two of our parent studies (Ludwig-McGill and McGill-Concordia), the MY09/11 PCR protocol was used in combination with hybridization using individual oligonucleotide probes/restriction fragment length polymorphism or reverse line blot assay, respectively. In the remaining studies, consensus primer PGMY09/11 PCR was used with either linear array (HITCH and CCCaST) or reverse line blot assay (BCCR and HIPVIRG). Although linear array, which employs a cross-reactive probe to detect HPVs 33,35, 52 and 58, is known to have issues in its ability to accurately detect HPV52 [167, 261], this test was not used in HIPVIRG and therefore issues surrounding this cross-reacting probe cannot be responsible for unmasking that we observed in this study.

To avoid false reports of type replacement, correction formulas to account for unmasking may be useful for comparison of pre- and post-vaccination HPV prevalence

in certain settings. Future studies evaluating the potential for unmasking of HPV52 and other types in low- and high-risk settings will be helpful for determining the extent and severity of unmasking.

Table 7-1: Association between HPV16 status and HPV52 detection based on retesting of selected cervical/anal specimens using HPV52 type-specific PCR*

Study	Years (recruitment and follow-up)/Study Population	HPV52+ specimens/total specimens, N			OR (95% CI) HPV16+ vs. HPV16- (reference)	
		HPV16+	HPV16- (HPV+, α-9 type)	HPV16- (HPV+, not α-9 type)	Crude	Adjusted†
Ludwig-McGill	1993-05; low income females, 18-60 yrs, São Paulo, Brazil	0/98	2/50	1/49	N/E	N/E
McGill-Concordia	1996-02; female students, 17-45 yrs, Montreal, Canada	2/100	2/50	0/50	0.99 (0.14, 7.17)	0.97 (0.13, 7.11)
HITCH	2005-13; female students with a male partner, 18-25 yrs, Montreal, Canada	3/100	0/50	0/50	N/E	N/E
BCCR	2001-09; females with/without precancerous cervical lesions, 18-75 yrs, Montreal, Canada	6/100	2/50	1/49	2.04 (0.50, 8.40)	2.14 (0.47, 9.57)
CCCaST	2002-06; females screened for cervical cancer, 30-69 yrs, Montreal/St. John's, Canada	4/100	2/50	5/50	0.55 (0.16, 1.95)	0.43 (0.11, 1.71)
HIPVIRG	2002-08; MSMs with HIV, 21-67 yrs, Montreal, Canada	15/100	3/50	1/50	4.24 (1.35, 13.25)	3.82 (1.19, 12.26)
All studies‡		30/598	11/300	8/298	1.62 (0.90, 2.92)	1.47 (0.76, 2.82)
All studies‡ (HIPVIRG excluded)		15/498	8/250	7/248	1.00 (0.48, 2.07)	0.82 (0.35, 1.93)

Test for heterogeneity between studies: p-value=0.08.

* All specimens were originally HPV52 negative in the source studies according to consensus primer PCR HPV DNA testing.

† Adjusted for age, lifetime number of sexual partners, and study (pooled analysis); except for CCCaST study (adjusted for age only).

‡ Some specimens from CCCaST study (n=76) were excluded from adjusted pooled analysis because number of sexual partners information was not collected from certain subjects (St. John's study site only).

MSM, men who have sex with men; N/E, not able to estimate

Table 7-2: Association between HPV16 viral-load status and HPV52 detection based on retesting of selected cervical/anal specimens using HPV52 type-specific PCR*

HPV16 viral load (tertiles)	HPV52+ specimens/total specimens, N							OR† (95% CI)
	Ludwig-McGill	McGill-Concordia	HITCH	BCCR	CCCaST	HIPVIRG	Total	All studies‡
HPV16-	3/99	2/100	0/100	3/99	7/100	4/100	19/598	ref
HPV16+								
Low	0/30	0/33	1/33	2/33	0/32	2/33	5/194	0.73 (0.24, 2.21)
Middle	0/30	1/33	1/33	0/33	2/32	7/33	11/194	1.38 (0.55, 3.45)
High	0/30	1/34	1/34	4/34	2/33	6/34	14/199	2.36 (1.08, 5.14)

Test for heterogeneity between studies: p-value=0.52.

* All specimens were HPV52 negative according to consensus primer PCR HPV DNA testing.

† Adjusted for age, lifetime number of sexual partners, and study.

‡ Some specimens from CCCaST study (n=76) were excluded from analysis because number of sexual partners information was not collected from certain subjects (St. John's study site only).

CHAPTER 8: METHODOLOGICAL CONSIDERATIONS

In this chapter, I provide additional discussion concerning the internal and external validity of results from this project. This chapter complements the information provided in each of the individual discussion sections in manuscripts 3, 4 and 5. As with any observational study, there are many possible sources of bias, we therefore decided to focus on what we consider to be the most important sources.

8.1 Internal validity

8.1.1 Information bias

The first possible source of information bias discussed here is misclassification of HPV status. In chapter 2 (section 2.2), I provide details regarding the main PCR protocols employed in each of the parent studies. The MY09/11 and PGMY09/11 protocols are both very sensitive with good overall agreement (kappa range=0.68-0.83) [163, 170, 171]. Modifications to the MY09/11 protocol (leading to the PGMY09/11 protocol) has resulted in even greater sensitivity [163] . As already discussed, both consensus primer PCR protocols are able to amplify and detect a broad spectrum of HPV genotypes, and may detect as few as 10 copies of viral DNA (analytic sensitivity) for most common genital HPV types [163, 262, 263]. Nonetheless, noted deficiencies in these consensus-primer PCR assay's ability to detect certain HPV types (especially when present as part of a co-infection) is what motivated us to conduct our analysis of

unmasking of HPV52 [163-166]. Since specimens were tested for HPV blinded to prior HPV status and questionnaire information, there is no reason for us to suspect that any misclassification of HPV status would be differential between exposure groups (regression or cohort approaches focusing on exposure to other non-vaccine HPV types; manuscript's 3 and 4, respectively); however, in our evaluation of masking of HPV52 (by HPV16; exposure group), our hypothesis was that prior misclassification (based on consensus PCR assay results) would be differential. Results from this study (manuscript 5) suggest that in most low-risk populations, differential masking (under-ascertainment) is unlikely to be a major problem. Nonetheless, we expect that non-differential under-ascertainment (i.e., false-negative HPV results) would produce unbiased risk estimates; whereas non-differential over-ascertainment (i.e., false-positive HPV results) may have led to biased estimates towards the null [264]. Fortunately, false-positives occur so rarely using modern consensus PCR assays [265] that the potential for bias resulting from misclassification of HPV status may be considered low in this project.

The second possible source of information bias in this project may have resulted from misclassification of questionnaire information. In assessing HPV type interactions, it is important that information collected on potential risk factors for multiple HPV infections (e.g., sexual history, condom use) is accurate in order to reduce residual confounding. Since females (in each of the five parent studies) were unaware of their HPV status when filling out questionnaires, it is unlikely that misclassification would be differential. But considering our main concern here is residual confounding, it is less

important whether misclassification of these variables is differential, as any misclassification can result in biased estimates (away or towards the null; depending on the direction of confounding), which becomes especially problematic when the exposure-outcome relation is weak and confounding is strong [264]. Although we should expect there to be some degree of misclassification in self report of lifetime number of sexual partners, condom use and other variables suspected to be associated with multiple HPV infections, self reported condom use has previously been shown to be a valid indicator of STD risk [266, 267], and in our Ludwig-McGill study, 90% of females reported the same number of lifetime sexual partners at their baseline and first follow-up visit, suggesting either consistent inaccurate or accurate report [268] .

8.1.2 Selection bias

In this project, selection bias may have resulted from non-participation, exclusion criteria, and losses to follow-up in the three cohort studies. In order for subjects' refusal to participate (non-participation) to cause bias, refusal must have been associated with exposure and outcome (i.e., different HPV infections), which is extremely unlikely.

Certain exclusion criteria may have led to biased effect estimates, particularly in our evaluation of unmasking. In this study (manuscript 5), we explain that we only selected specimens that were HPV positive in an attempt to reduce residual confounding; however, by doing so, our two comparison groups (HPV16+/HPV16-) may have become too similar. That is, masking of HPV52 in the HPV16 naïve group may

have been caused by some other HPV types (present in higher viral load concentrations than HPV52), therefore biasing our results towards the null.

To explore the potential impact of non-random loss to follow-up in the three cohort studies, I verified whether any of the variables considered potential risk factors of multiple HPV infection (see table 4-3) were associated with number of visits completed among those participants that were included in our cohort analysis. Fortunately, none of these variables were found to be significantly associated with number of visits completed (all p-values were >0.05; data not shown), which provides some reassurance that losses to follow-up did not introduce any serious bias.

8.1.3 Confounding

In our evaluation of HPV type competition using both regression and cohort approaches, it is possible that residual confounding led to biased effect estimates (ORs and HRs) away from zero. Despite the availability of many key variables thought to be associated with acquisition of HPV infection, there were likely some host factors that we did not measure, which may have led to confounding in our assessment of type-type associations. As discussed previously in this section, it is also possible that misclassification of some measured variables may have led to residual confounding.

In planning this project, we decided *a priori* that age and lifetime number of sexual partners were important variables that should be included in our models.

However, in each of the studies, we also explored whether there were any additional variables (measured across all studies) that were significant independent predictors of multiple HPV infection. Additionally, we compared the effect of adding these other measured covariates to our models to determine if any consistently produced a ≥10% change in our effect estimates [243]. Another approach that others have previously adopted to ensure that they reduced opportunity for confounding in their evaluation of HPV type interactions was to restrict their analysis to positive women (i.e., those with ≥1 HPV infection) to ensure that they had focused on a population with sufficient HPV exposure opportunity [101, 109, 210, 212]. However, as we have discussed previously, this approach introduces a form of selection bias (known as collider stratification bias) and was therefore not applied in our main analyses [256].

8.2 External validity

None of the parent studies from which information was available for the current project were population-based. These studies were conducted in Canada and Brazil and included females of different ages. In this project, variation in our effect estimates (focusing on interaction between different HPV types) differed only randomly between studies (i.e., there were no significant predictors of variance across the studies), suggesting that HPV types do not interact differently among healthy individuals from different populations. However, this does not mean that future studies evaluating type replacement will report the same changes in the population prevalence of HPV types not being targeted by vaccination. Recall from our analysis of unmasking of HPV52

(manuscript 5), we suggest that the severity of unmasking will be greater in populations with higher viral load HPV infections, e.g., settings with a high prevalence of immunocompromised individuals. Thus, although the generalizability of our results regarding the tendency for different HPV types to naturally cluster or compete with each other should apply equally to all individuals, differences in diagnostic artifacts may result in greater apparent type replacement in some settings.

CHAPTER 9: DISCUSSION

Current available HPV vaccines have the potential to prevent roughly 70% of invasive cervical cancer cases caused by HPV types 16 and 18 [7]. But there is also the possibility that an even greater proportion of cases (caused by other oncogenic HPV types) may be prevented due to cross-protection against phylogenetically related HPV types [269]. Both vaccines (Cervarix® and Gardasil®) are known to offer some protection against HPV types that are not specifically targeted; however, greater efficacy against HPVs 31, 33 and 45 and associated high-grade pre-invasive cervical lesions has been reported for the bivalent Cervarix® vaccine, which uses a unique adjuvant ("ASO4" that contains aluminum hydroxide and monophosphoryl lipid A) to boost immune response [270]. Alternatively, there is the possibility that vaccination could lead to less than a 70% reduction in cervical cancer cases, due to an increase in the prevalence of other oncogenic HPV types, i.e., type replacement [12]. Therefore, the objective of this project was to explore the potential for type replacement, by evaluating competition between different HPV types (considered a necessary prerequisite for type replacement to occur) using different epidemiological (regression and cohort) approaches [12]. Since it is possible that increases in the observed prevalence may not necessarily be caused by type replacement, but rather a diagnostic artifact referred to as unmasking; a separate objective of this project was to explore the potential for unmasking of HPV52 following a reduction in HPV16 in vaccinated populations. No other study has been published that was designed specifically to evaluate the potential for unmasking caused by diagnostic artifacts.

At the time when this project idea was conceived, few studies had focused on evaluating HPV type competition using cohort or cross-sectional data sets [76-78, 101, 102, 104, 105]. In recent years, other studies designed specifically to evaluate type competition involving vaccine-targeted HPV types (6, 11, 16 and 18) with other HPV types (to inform the possibility of HPV type replacement) have been published [102, 103, 195-199]. Important characteristics of each study, including their main results and conclusions, have been provided previously. In summary, these studies did not provide strong or consistent evidence that negative pairwise interactions exist, which therefore suggests that the likelihood of type replacement is low.

9.1 Summary of findings

The three main studies included as part of this project (manuscripts 3-5) focused on evaluating interactions between different HPV types and masking of HPV52 by HPV16. The mean age of females from the five parent studies from which data were available for our analyses of type competition ranged from 21.0 to 43.7 years [22, 202, 236, 238, 239]; whereas the mean age of males participating in the HIPVIRG study - an additional study from which specimens were selected for retesting along with specimens from our other five studies in our evaluation of unmasking - was 43.0 years [244]. In each of these studies, HPV16 was the most common HPV type detected among all specimens; however, the relative ranking of other common types (after HPV16) did vary. In the majority of studies, HPV infections generally occurred more often as part of a multiple infection than as a single infection.

In our analyses of HPV type competition, using both regression and cohort approaches, we did not find any strong evidence of type competition. In fact, in our regression analyses (manuscript 3), we did not observe a single statistically significant negative association between vaccine-targeted HPV types (6/11 grouped, 6, 11, 16 and 18) with other types. In our pooled analyses (adjusted for age and lifetime number of sexual partners), odds ratios for all pairwise associations ranged from 0.92 (95% CI 0.49-1.52) for HPVs 18 and 89, to 2.53 (95%CI: 1.52-4.53) for HPVs 6/11 and 35, suggestive of either no association or positive association between HPV types, respectively. In our fully adjusted pooled analyses, there were many statistically significant positive associations (95% CI excluded 1.0) involving HPVs 6, 16, and 18; however, not HPV11. The only HPV types that were not involved in a significant positive association (i.e., involving at least one vaccine-targeted HPV type) were HPVs 71, 70, 69, 61, 57, 34 and 31. Among the vaccine-targeted types, HPV16 was involved in the highest number of significant positive associations with other HPV types (n=28).

Results from our prospective analyses (comparing time to acquisition and clearance of different HPV types according to infection with vaccine-targeted HPV types) were in agreement with what we reported in our regression approach, i.e., after adjustment for multiple comparisons, no statistically significant negative or positive associations were observed in our acquisition or clearance analyses, respectively. In general, females infected with vaccine-targeted HPV at baseline or time of the index infection were at greater risk of acquiring other types (HRs>1.0) and at about equal risk of clearing existing infections. In our pooled clearance analyses, there were eight

different HPV types observed to clear more rapidly when present as part of a co-infection with one of the vaccine-targeted HPV types. However, as already mentioned, none of these associations remained statistically significant at the Bonferroni corrected p-value threshold. Furthermore, among the eight HPV types observed to clear more rapidly, not all of them are classified as possible/definite carcinogens [9]; but among those that are, all (except for HPV66) are now being targeted by Merck's nonavalent vaccine, which is expected to be approved and become available shortly [259] .

Manuscript 5 presents results from the first study designed specifically to evaluate the potential for diagnostic artifacts (i.e., unmasking) in future surveillance studies that evaluate vaccine effectiveness. There have already been some reports of increases in certain non-vaccine HPV types (including HPV52) among vaccinated populations [193, 194], which could be attributed to unmasking. In our study, masking by HPV16 appeared to be greater in anal specimens collected from HIV infected males the HIPVIRG study (adjusted OR=3.82, 95%CI: 1.19-12.26) [244], compared with cervical specimens collected from females in the remaining five studies (adjusted pooled OR=0.82, 95%CI 0.35-1.93) [22, 202, 236, 238, 239]. However, in our pooled analysis (including specimens from all studies), we did observe a positive association between HPV16 viral load (divided into tertiles) and detection of HPV52 (P for trend=0.003). Ultimately, these data suggest that masking of HPV52 and perhaps other HPV types may be a greater issue in regions where the prevalence of HIV and associated HPV co-infection and viral load is greater.

9.2 Study strengths and limitations

Strengths and limitations of this project have already been described in each of the respective manuscripts, and are therefore only discussed briefly in this section. For this project, we had access to over 38,000 valid cervical specimens (tested for HPV using similar PCR assays across the five parent studies), making this the largest study ever conducted on this topic. This large sample, combined with our unique (Bayesian) regression approach incorporating shrinkage led to even greater precision and allowed us assess pairwise interactions for rare HPV types. The inclusion of all HPV types and relevant confounders in our regression models also helped reduce spurious associations due to multiple testing, while making possible adjustment for other HPV types. As part of this project, we also conducted the first cohort study among females (n=3200) to evaluate acquisition and clearance of different HPV types according to infection status with each of the vaccine-targeted HPV types (6/11, 16, and 18) separately. Although power in this study was generally sufficient, for some very rare HPV types our precision was poor (reflected by wider confidence intervals), which we previously managed to overcome in our regression analyses using Bayesian methods.

A limitation of some prior studies exploring HPV type interactions is the potential for cross-hybridization between assay probes for HPV types in different PCR protocols. Fortunately, the genotyping methods used in this study (line blot/linear array) generally avoid this problem, as they are more specific compared with other signal detection systems [103, 271]. In this project, a major focus was also to investigate a different

(opposite) measurement error issue, which may have important implications concerning our ability to evaluate type replacement using pre- versus post-vaccination HPV prevalence information. Given our knowledge that consensus primer PCR assays may perform with reduced sensitivity in cases of multiple HPV infections (another potential limitation of our analyses focusing on type interactions) [163-166], we evaluated whether masking of HPV52 occurred more frequently among specimens that were HPV16 positive. To avoid major confounding in this study, all specimens (including those that were HPV16 negative) were positive for an HPV type other than 52, according to original consensus PCR results obtained from the parent studies. However, in our other studies evaluating HPV type interactions (regression/cohort approaches) it is likely that residual confounding occurred, resulting from either misclassification of important covariates and unmeasured behaviour/biological risk factors of multiple infections.

9.3 Implications and future research directions

Despite lack of convincing evidence of negative HPV type interactions in this project, we still cannot rule out the possibility that type competition exists. The safest conclusion we can make is that the factors that create positive associations between different HPV types (e.g., behavioral/biologic risk factors) are greater than those factors leading to negative associations (e.g., natural competition between HPV types). However, it is at least somewhat reassuring that most previous studies (using a variety of different statistical approaches) have come to the same conclusion on this topic.

None of the participants in these studies received prophylactic HPV vaccines, thus we must assume that there is no difference (in type-type interactions) among those naturally infected/uninfected with different HPV types, compared with those that received the vaccines. The next important research step on this topic will be to evaluate type specific changes in HPV prevalence utilizing pre- and post-vaccination survey information from the same populations and using the same type of specimens. Ultimately, these studies will provide us with a definitive answer to question of HPV type replacement, provided that they can be conducted with rigorous attention to population sampling and specimen standardization. To avoid confusion in the event that an increase in certain HPV type(s) is observed, additional studies examining the potential for diagnostic artifacts (unmasking) should be conducted. Specifically, these studies should investigate masking of other HPV types in different low- and high-risk settings. Future studies should also explore other possible sources of HPV type replacement; for example, the potential consequences of reduced excisional procedures for lesions induced by vaccine types (that will no longer be present) on the prevalence of other HPV types often present as a co-infection in these lesions, or the consequences of misattributing HPV16 as the casual type among cervical lesions co-infected with other HPV types [12].

9.4 Future of cervical cancer prevention: Vaccination, screening and other prevention efforts

In addition to type replacement, some policy analysts remain concerned about the duration of protection conferred by HPV vaccines, as well as about the safety of these vaccines in general [176]. Fortunately, the latest vaccine trial results reveal that protection has remained unabated for approximately 10 years (for the licensed vaccines) and even longer for the prototype HPV16 vaccine, without any evidence of waning antibodies [272-274]. Although the duration of protection against phylogenetically related oncogenic types (primarily HPVs 31, 33, and 45) is still being examined, it is expected to be shorter than for the main vaccine-targeted types [3, 209, 275, 276]. Regarding safety, the rate of adverse events reported among recipients in the treatment group in vaccine trials has been comparable to rates among those in the placebo group, and also similar to the rate of events in the general population [277]. Future population based studies that include a much larger number of vaccinated individuals will provide better power to investigate safety of these vaccines for rare adverse events. Other issues/decisions concerning vaccination requiring continuous evaluation/reapproval include: what vaccine to purchase, what age groups to target, number of doses to provide, and if males should be targeted by publically funded programs. As we await the introduction of Merck's nonavalent vaccine (targeting HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58), we expect its arrival will present new issues and debate concerning a wide range of issues. The authors of one study examining the potential cost-effectiveness of this new nonavalent vaccine (compared with the quadrivalent vaccine, and making a number of important assumptions regarding certain parameters) suggest that if the additional cost per dose does not exceed $11, then it will

become even more cost-effective than the current quadrivalent vaccine on the market [259].

Nearly a decade ago, as we were anticipating the arrival of Merck's quadrivalent vaccine, scientists had already begun considering the potential consequences that reduced lesion prevalence (caused by reductions in HPVs 16 and 18) would have on cervical cancer screening programs [278]. Recently, we conducted modeling studies to examine the negative effect that this would have on important Pap test screening parameters, e.g., sensitivity, specificity, negative/positive predictive value [133, 279]. Our main conclusion was that Pap cytology should eventually be abandoned as the primary screening test because its positive predictive value will become far too low to maintain it as a feasible approach. It should be replaced by highly sensitive HPV DNA tests for the detection of pre-invasive cervical cancer lesions, perhaps reserving cytology (a test with excellent specificity) for triaging HPV positive specimens for referral to colposcopy [280]. Now, with the impending arrival of Merck's nonavalent vaccine (that will protect against an even higher percentage of cervical lesions caused by additional oncogenic types) and even stronger evidence that HPV DNA testing is superior to cytology for screening in the prevention of invasive cervical cancer [281, 282], there is added pressure to eliminate Pap (even as a co-test) from primary screening. In the context of screening, commercial HPV DNA tests (such as Hybrid Capture II) have been calibrated to detect approximately 5000 genome copies per test of target DNA [263]. Although the assay is designed to detect high-risk HPV infection(s), which is considered a necessary risk factor for cervical cancer, in screening, the

accuracy (sensitivity/specificity) is defined in terms of the assay's ability to detect high-grade precancerous lesions. Eventually, a risk-assessment strategy that is flexible to the discovery and arrival of new screening tests/multivalent vaccines should be established to keep pace with the rapid technological advancements in this field [283]. It is possible that population vaccine coverage and protection conferred by newer multivalent vaccines may become so high that policy officials eventually decide to eliminate publically funded screening programs altogether, i.e., based on acceptably low levels of cervical cancer risk in the population.

In the last decade, there have also been important discoveries made focusing on microbicides [153]. In 2006, a team of investigators at NIH (led by Dr. John Schiller) initially reported that *carrageenan* (a compound derived naturally from red seaweed) was extremely potent against all HPV types in laboratory (cell culture) tests that they performed, suggesting that it may one day serve as an alternative or complementary primary prevention approach with vaccines [151]. Since then, the team went on to demonstrate carrageenan's ability to inhibit HPV infection in mice [152], as well as in monkeys [88]. Our team of investigators at McGill University (Montreal, Canada) is now conducting the first randomized controlled trial designed to evaluate the effectiveness of carrageenan-gel against cervical HPV infection among heterosexual female individuals [153]. Our group was also recently approved for a grant to conduct a similar randomized controlled trial investigating the same carrageen-gel for prevention against anal HPV infection among MSM individuals.

With many new technologies coming available to combat HPV, cervical cancer and the burden of pre-invasive lesions, it remains an exciting time to be a scientist in this field. The low probability of HPV type replacement (suggested by results from this and other studies) provides reassurance that vaccines will ultimately be effective in reducing the global burden of cervical cancer. With an estimated 530,000 cervical cancer cases and 270,000 deaths occurring globally each year (nearly 90% of these cases occurring in developing countries), greater effort should be placed on translating major discoveries into action, particularly in low resource settings.

CHAPTER 10: CONCLUSIONS

Conclusions that can be drawn from the work in this project include the following:

- HPV16 was the most common type detected among all cervical specimens, in each of the five parent studies included in this project.
- In most studies (all except Ludwig-McGill study), HPV infections occurred more often as part of a multiple infection than as a single infection, and concurrent infection was generally associated with lower age and higher number of lifetime sexual partners.
- No statistically significant negative associations were observed involving vaccine-targeted HPV types with other types; however, many significant positive associations were observed, especially involving HPV16.
- Prior infection with vaccine-targeted HPV types increased the risk of subsequent acquisition of most other HPV types.
- Time to clearance (persistence) of HPV infections was generally not associated with co-infection with vaccine-targeted HPV types.
- A strong masking effect (HPV52 by HPV16) was observed among anal specimens collected from HIV infected individuals in the HIPVIRG study, but not among cervical specimens from the other five studies, which included mainly healthy individuals.
- Across all studies, there was a positive association between HPV16 viral load (divided into tertiles) and detection of HPV52, which suggests that HPV unmasking may ultimately be a greater issue in settings with a high prevalence of immunocompromised individuals

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APPENDIX 1

REPRINT

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Review

Epidemiology and burden of HPV infection and related diseases: Implications for prevention strategies

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ABSTRACT

Human papillomavirus (HPV) infection is a necessary, although not sufficient cause of cervical cancer. Globally, HPV infection accounts for an estimated 530,000 cervical cancer cases (~270,000 deaths) annually, with the majority (86% of cases, 88% of deaths) occurring in developing countries. Approximately 90% of anal cancers and a smaller subset (<50%) of other cancers (oropharyngeal, penile, vaginal, vulvar) are also attributed to HPV. In total, HPV accounts for 5.2% of the worldwide cancer burden. HPVs 16 and 18 are responsible for 70% of cervical cancer cases and, especially HPV 16, for a large proportion of other cancers. Prophylactic vaccination targeting these genotypes is therefore expected to have a major impact on the burden of cervical cancer as well as that of other HPV-related cancers. Over the past 50 years, organized or opportunistic screening with Papanicolaou (Pap) cytology has led to major reductions in cervical cancer in most developed countries. However, due to lack of resources or inadequate infrastructure, many countries have failed to reduce cervical cancer mortality through screening. HPV DNA testing recently emerged as a likely candidate to replace Pap cytology for primary screening. It is less prone to human error and more sensitive than Pap in detecting high-grade cervical lesions. For countries with national vaccination programs, HPV testing may also serve as a low cost strategy to monitor long term vaccine efficacy. Introduction of well organized vaccination and screening programs should be a priority for all countries. Increased support from donors is needed to support this cause.

Contents

Introduction

Human papillomavirus (HPV) is currently one of the most common sexually transmitted infections worldwide (Baseman and Koutsky, 2005; Dunne et al., 2007; Ebrahim et al., 2005). Most individuals (~75%) who engage in sexual activity will become infected with HPV at some point during their lifetime (Baseman and Koutsky, 2005; Koutsky et al., 1988). For the vast majority these infections will be asymptomatic and clear within 1–2 years (Franco et al., 1999; Hildesheim et al., 1994; Ho et al., 1998; Molano et al., 2003; Moscicki et al., 1998; Richardson et al., 2003); however, a substantial increase in risk for cervical cancer exists for women who develop persistent

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infection with high-oncogenic HPV types (HR-HPV) (Ho et al., 1995; Liaw et al., 1999; Remmink et al., 1995; Ylitalo et al., 2000). Infection with low-oncogenic risk HPV types (LR-HPV) is also responsible for considerable morbidity associated with benign lesions known as acuminate condylomata (genital warts) as well as a large proportion of low grade squamous intraepithelial cervical lesions. In this review we discuss the burden of HPV infection and related diseases, mainly focusing on cervical cancer and opportunities for prevention.

Epidemiology of HPV infection

According to a recent meta-analysis that included data from more than 1 million women in 59 countries, the prevalence of genital HPV infection among those with normal cytology ranges from 1.6% to 41.9% (Bruni et al., 2010). Higher HPV prevalence was observed in African and Latin American regions in comparison to European, North American and Asian regions. The estimated average global prevalence of HPV in this particular study was 11.7%, which is similar to previous reports focusing on women (Clifford et al., 2005; de Sanjose et al., 2007). This study, along with others (de Sanjose et al., 2007; Franceschi et al., 2006; Herrero et al., 2005; Lazcano-Ponce et al., 2001; Smith et al., 2008), reported an interesting trend in the female age-specific distribution of HPV whereby there is a first peak at younger ages (<25 years) in all regions; and in the Americas, Africa and Europe, a clear second peak among individuals 45 years or older. The first peak, which comes shortly after sexual debut for most women, is generally attributed to higher levels of sexual activity with multiple partners and low viral immunity. After the first peak, a consistent age-related decline in HPV prevalence has been documented in numerous epidemiological studies. This trend was also observed in one study of female sex workers in Denmark, i.e., a population with high lifelong levels of sexual activity (Kjaer et al., 2000), which underscores the importance of naturally acquired immunity in protecting against HPV infection. Although the reason for the smaller second peak at middle age still remains unclear, possible explanations include immuno-senescence, hormonal changes prior to menopause, changes in male/female sexual behavior, cohort effects, or perhaps higher rates of HPV persistence at older ages (Bruni et al., 2010; Castle et al., 2005; de Sanjose et al., 2007; Gonzalez et al., 2010).

Among sexually active males, genital HPV infection is also very common; however, prevalence varies widely depending on geographic region, risk group, anatomical site (glans/corona, penile shaft, urethra, prepuce, or scrotum), sampling method (cytology brush, wet or dry swab), and HPV testing methodology (general or type specific primer systems) (Dunne et al., 2007; Partridge et al., 2007). In a recent systematic review, Smith and colleagues (Smith et al., 2011) estimated HPV prevalence to be between 1% and 84% in low-risk sexually active men, and between 2% and 93% in high risk men. Unlike the situation among females where genital HPV infection declines substantially after about 30 years of age, prevalence of HPV infection in males generally remains constant or declines only slightly with age after peak prevalence (Smith et al., 2011). One possible explanation for this is that men experience a higher rate of reinfection compared to women. Among circumcised men, the penile shaft and glans are often the most common sites of genital HPV infection (Smith et al., 2010; Weaver et al., 2004) whereas for uncircumcised men, it is the foreskin (Nielson et al., 2007).

In addition to penile and cervical HPV, anal HPV is also very common in both genders. In a recent study of human immunodeficiency virus (HIV) negative men who have sex with men (MSM), anal HPV infection was detected in 57% of participants (Chin-Hong et al., 2004). However, among heterosexual men, prevalence of anal HPV infection is generally less than 10% (Vardas et al., 2011). In studies focusing on women, prevalence and incidence of anal HPV infection are often equal to, or greater than infection with cervical HPV (Goodman et al., 2008; Hernandez et al., 2005; Palefsky et al., 2001). Finally, oral HPV, despite being less common than anogenital infections in adults, should not be overlooked as an important infection site due to its frequent association with many oropharyngeal cancers (D'Souza et al., 2007a, 2007b).

Sexual activity and other risk factors for HPV infection

HPV is highly sexually transmissible in both genders (Burchell et al., 2006; Castellsague et al., 2003; Marrazzo et al., 2001). Epidemiologic studies have consistently reported markers of sexual activity, including number of recent/lifetime sexual partners and age at sexual debut to be among the most important risk factors for HPV infection (Ho et al., 1998; Moscicki et al., 2001; Richardson et al., 2000; Winer et al., 2003). Although age at sexual debut is often strongly associated with other sexual behaviors, it may also be a true causal risk factor for HPV due to greater cervical ectopy during adolescence (Kahn et al., 2002). In addition to peno-vaginal intercourse, HPV is also transmitted by other sexual practices, including peno-anal intercourse, oral sex, and digital-vaginal sex (Hernandez et al., 2008; Sonnex et al., 1999; Winer et al., 2003). There is some evidence that transmissibility may vary by HPV genotype, with HR-HPV types being more strongly associated with sexual behavior than LR-HPV types (Franco et al., 1995; Kjaer et al., 1997; Richardson et al., 2000; Rousseau et al., 2000). HPV may also be transmitted during childbirth from the cervix of infected mothers to the oropharyngeal mucosa of their children, with higher likelihood of transmission occurring for vaginal delivery compared with cesarean section (Tseng et al., 1998).

Independent of sexual activity markers, other factors related to HPV infection or persistence include young age, socioeconomic status, multiparity, circumcision, condom use, oral contraceptive use, smoking, nutrition, immune suppression, viral load, as well as certain genetic polymorphisms in the human leukocyte antigen system (Baldwin et al., 2004; Baseman and Koutsky, 2005; Cotton et al., 2007; Ferguson et al., 2011; Giuliano et al., 2003; Goodman et al., 2008; Kahn et al., 2002; Maciag et al., 2002; Moscicki et al., 2001; Palefsky et al., 2001; Ramanakumar et al., 2010; Richardson et al., 2003; Rousseau et al., 2000; Schlecht et al., 2003; Sellors et al., 2003; Tobian et al., 2009; Winer et al., 2003). Presence of pre-existing HPV infection(s) is also associated with increased risk of acquiring infection with other HPV types (Liaw et al., 2001; Rousseau et al., 2001; Thomas et al., 2000). With regard to condoms, a paradoxical effect has sometimes been reported such that condom use appears to increase risk of HPV infection (Franco, 1997; Manhart and Koutsky, 2002; Richardson et al., 2000), likely a result of higher probability of infection from partners with whom condoms are used and higher probability of transmission per single act of intercourse (Aral and Peterman, 2002; Burchell et al., 2006; Macaluso et al., 2000; Warner et al., 2004). However, when used consistently among partners of newly sexually active women, recent data suggest that condoms may reduce (but not eliminate) the risk of male-to-female genital HPV transmission (Winer et al., 2006). There is also evidence that male circumcision reduces the risk of HPV infection among men, which in turn lowers the risk of subsequent transmission and infection in their partners (Auvert et al., 2009; Castellsague et al., 2002; Tobian et al., 2009; Wawer et al., 2011).

Using an experimental system, investigators at the National Institute of Health (NIH) recently demonstrated that Papanicolaou (Pap) cytology, a common test that is used in screening for cervical cancer, actually increased the number of HPV (pseudovirus) infectious events in a series of female rhesus macaques (Roberts et al., 2011). Despite histological similarities between the macaque and human cervix and strong biologic rationale to support their findings, i.e., physical trauma that occurs during specimen collection leading to exposure of the basal layer of the genital tract to HPV, results from this study should not immediately be interpreted as evidence that Pap tests increase the risk of HPV infection. As the study authors point out, it is possible that the increase may be transient and that the same trauma which leads to an

increase in HPV infection also stimulates the immune system to recognize and combat these infections (Roberts et al., 2011). Ultimately, further studies are needed to adequately address this question and to determine whether frequent Pap testing has contributed to the rise in cervical adenocarcinoma that has been observed in many screened populations (Sherman et al., 2005; Vizcaino et al., 1998).

In recent years, the incidence of oral HPV and related cancers has also increased in the United States (Gillison, 2008). Researchers have identified a strong association between lifetime oral HPV and oral/oropharyngeal cancers (D'Souza et al., 2007b; Heck et al., 2010). Since oral HPV is generally transmitted through oral sex or open mouth kissing (D'Souza et al., 2009), it is common to presume that this increase in disease burden is related to an increase in oral sexual behaviors (e.g., lifetime number of oral sex partners) among adolescents (Chaturvedi et al., 2008; Gillison, 2007). Unfortunately, data on these types of sexual behaviors are largely unavailable, which makes it difficult to empirically verify this time trend assumption.

HPV infection and risk of cervical cancer and other diseases

In 1995, the International Agency for Research on Cancer (IARC) first classified HPV types 16 and 18 as carcinogenic to humans, but based on more recent evidence, the list of carcinogenic HPV types has been expanded to include a total of 13 mucosotropic anogenital HPV types as being definite or probable carcinogens (grade 1 or 2a) based on their frequent association with invasive cervical cancer (ICC) and cervical intraepithelial neoplasia (CIN) (see Table 1 for HR-HPVs) (Schiffman et al., 2009). The oncogenic types (mostly HPV 16) are also causally implicated in other cancers, including penile, anal, vulvar and vaginal cancers (Giuliano et al., 2008; Monk and Tewari, 2007). The remaining genital types (e.g., HPV types 6, 11, 42, 43, 44 and some rarer types) are considered to be of low or no oncogenic risk (Bosch et al., 1995; Lorincz et al., 1992). However, these types may cause subclinical and clinically visible benign lesions known as flat and acuminate condylomata, respectively.

In descending order, the most common HPV types implicated in cervical cancer globally are: 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56 (Li et al., 2011). HPV types 16 and 18 are the most dominant types implicated in cervical cancer in all continents, being responsible for ~70% of ICC cases globally. Substantial variation exists between regions for the other HR-HPV types listed here. In many studies, estimating the fraction of cervical cancer cases attributable to the different HPV types is difficult due to the high prevalence of multiple type infections. For example, a recent meta-analysis based on genotyping information from 30,848 cases of ICC, estimated the prevalence of co-infection (≥2 HPV types) in tumor specimens at 15.7% (Li et al., 2011). Other recent meta-analyses and cross sectional studies evaluating the worldwide distribution of HPV infections consistently reveal the same HPV prevalence patterns (Fig. 1) (Bruni et al., 2010; de Sanjose et al., 2010). This widespread circulation of HR-HPV types strengthens the potential for a phenomenon known as HPV type-replacement, i.e., an increase in other non-vaccine genotypes following HPV vaccination. However, based on evidence that HPVs evolve very slowly and that HPV types do not normally compete with one another during natural infection (Chaturvedi et al., 2005; Kjaer et al., 2005; Lajous et al., 2005; Liaw et al., 2001; Mejlhede et al., 2010; Mendez et al., 2005; Merikukka et al., 2010; Plummer et al., 2007; Rousseau et al., 2001; Rousseau et al., 2003; Thomas et al., 2000; Vaccarella et al., 2010), it is still unlikely that some other HPV type(s) will evolve to fill the niche currently occupied by vaccine target types. Furthermore, phase III trials evaluating both bivalent and quadrivalent vaccines indicate partial cross-type protection (cross-immunity) against many phylogenetically related HPV types (Brown et al., 2009; Jenkins, 2008; Paavonen et al.,

Table 1
HPV types categorized as carcinogenic in representative studies and reviews.

HPV type	Original taxonomic designation	Lorincz et al. (1992)	Bauer et al. (1993)	Hybrid capture 2 (Lorincz, 1996)[a]	GP5/6+ (Walboomers et al., 1999)[b]	Roche's line blot assay (Bosch et al., 1995)[c]	Munoz et al. (2003)	IARC (2005)[d]	IARC (2011)[e]
16		X	X	X	X	X	X	1	1
18		X	X	X	X	X	X	1	1
26						X	Probable		2B
30									2B (analog)
31		X	X	X	X	X	X	1	1
33		X	X	X	X	X	X	1	1
34									2B (analog)
35		X	X	X	X	X	X	1	1
39			X	X	X	X	X	1	1
45		X	X	X	X	X	X	1	1
51		X	X	X	X	X	X	1	1
52		X	X	X	X	X	X	1	1
53							Probable		2B
55						X			
56		X	X	X	X	X	X	1	1
58			X	X	X	X	X	1	1
59				X	X	X	X	1	1
66					X		Probable	1	2B
67									2B
68				X	X	X	X		2A
69									2B (analog)
70									2B
73	Pap238A, MM9					X	X		2B
82	W13B, MM4, IS39 (subtype)					X	X		2B
83	Pap291, MM7					X			
85									2B (analog)
97									2B (analog)
# types		9	11	13	14	17	15–18	13	25

[a] This HR HPV classification is applied in the Hybrid Capture 2 assay (Digene Co.), a validated diagnostic assay widely used in epidemiologic and clinical studies.
[b] This HR HPV classification is the one used in the polymerase chain reaction (PCR) with GP5/6+ primers, which is used in many international studies of cervical cancer etiology and screening.
[c] This HR HPV classification forms the basis of the PGMY line blot PCR protocol.
[d] This HR HPV classification follows the International Agency for Research on Cancer (IARC) Carcinogenicity Evaluation Monograph, vol. 90.
[e] This HR HPV classification follows the IARC Carcinogenicity Evaluation Monograph, vol. 100 (the indices denote the degree of empirical evidence for carcinogenicity: 1, definite carcinogens, 2A, probable carcinogens, 2B, possible carcinogens).

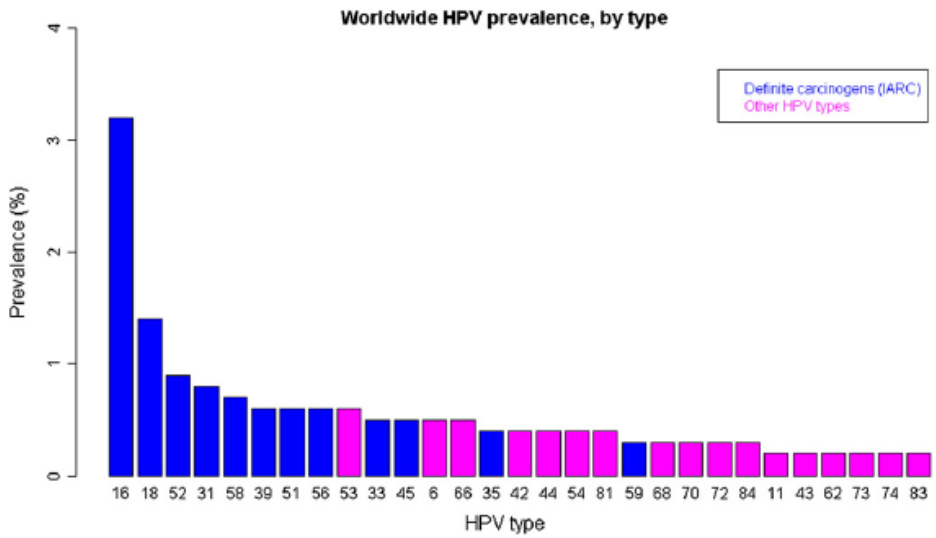

Fig. 1. Worldwide Human Papillomavirus (HPV) prevalence, by type, among females with normal cytological findings. Bars in blue indicate HPV types recognized by the International Agency for Research on Cancer (IARC) as definite carcinogens, whereas the other HPV types are depicted with pink bars.
Data source: Bruni et al. (2010), JID.

2009), suggesting that the benefit from vaccination may be even greater than expected.

Infection with oral HPV is also now recognized as an important cause of oral and oropharyngeal cancers (Gillison, 2008). However, unlike cervical cancer in which 100% of cases are attributable to infection with HPV, only 25–35% of these cancers are attributable to HPV (Kreimer et al., 2005; Parkin, 2006); the major risk factors being alcohol and tobacco use. Among cases of oral/oropharyngeal cancer linked to HPV infection, HPV 16 is by far the most common type detected in tumor specimens (D'Souza et al., 2007b; Herrero et al., 2003). In the largest study conducted on this topic to date (a case–control study that included 1600 cases and 1700 controls from 9 countries), HPV 16 was found in 95% of positive cases (Herrero et al., 2003). Based on evaluation of risk factor profiles for cancers of the head and neck, comparing HPV 16-positive and HPV 16-negative cases, some researchers have decided that these should actually be considered distinct cancers (Gillison et al., 2008a). In their study, sexual behavior (but not alcohol or tobacco use) was an important predictor of head and neck cancers among HPV 16-postive subjects, meanwhile the opposite was observed for HPV 16-negative subjects. In addition to oral and oropharyngeal cancers, HPV is also an important, albeit not a necessary cause of other cancers, e.g., 90% of anal cancers, 40% of penile cancers, and 40% of vaginal or vulvar cancers are attributable to HPV (Kreimer et al., 2005; Parkin, 2006).

Persistent HPV infection and cervical carcinogenesis

Most cervical HPV infections clear spontaneously without ever causing lesions. Only a small proportion of infections (10–30%) will persist beyond 1 or 2 years. Data from cohort studies indicate that the average length of infection is between 4 and 20 months, with HR-HPV types lasting longer than LR-HPV types (Franco et al., 1999; Hildesheim et al., 1994; Ho et al., 1998; Molano et al., 2003; Moscicki et al., 1998; Richardson et al., 2003). Numerous cohort studies have confirmed that risk of CIN and ICC is strongly associated with persistent infection with HR-HPV types (Kjaer et al., 2002; Koutsky et al., 1992; Liaw et al., 1999; Nobbenhuis et al., 1999; Schlecht et al., 2001; Sundstrom et al., 2010). As a result, persistent infection with at least one HR-HPV type is now well established as a key intermediate step in the etiologic pathway to cervical carcinogenesis.

Following persistent infection with HPV, the process of carcinogenesis progresses with disruption of the normal maturation of the transformation zone epithelium of the uterine cervix. These abnormal changes lead to pre-invasive lesions (dysplasia) that are often asymptomatic and discovered only by cytological examination during Pap smear screening. If these low- and high-grade lesions are left untreated they may grow and eventually cross the epithelium to connective tissue border formed by the basement membrane to become invasive. But until invasion occurs, the entire stepwise precancerous lesion process is reversible. In fact, for the majority of women infected with HPV the infection will clear and precancerous lesions will regress; only approximately 1% of low-grade lesions (CIN1) and 12% of high-grade lesions (CIN3) will progress to become invasive if left untreated (Ostor, 1993). However, in the event that precancerous lesions are not detected by screening or do not regress on their own, without effective treatment the invasive cancer will invariably grow to reach blood and lymphatic vessels and become metastatic.

Currently, much less is known about the natural history of other HPV related cancers in comparison to cervical cancer. But when considering the worldwide burden of cervical cancer compared to other HPV related cancers and that HPV infection is often just as common at other sites (e.g., the anus, penis, vulva and vagina), this suggests that the cervix is much more susceptible to HPV-induced carcinogenesis (Schiffman et al., 2007).

Burden of cancer caused by HPV: cervix and other sites

After breast and colorectal cancer, cervical cancer is the 3rd leading cancer site worldwide irrespective of gender and second among women. In 2008, there were an estimated 530,000 cases and 270,000 deaths attributed to ICC, with 86% of cases and 88% of deaths occurring in developing countries (Arbyn et al., in press). In these developing countries, the age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) were 18 and 10 per 100,000 women, respectively; whereas in more developed countries, the ASIR and ASMR were 9 and 3 per 100,000 women, respectively. Globally, incidence of ICC ranges from <3 to >50 cases per 100,000 women for low- and high-burden countries, respectively (Fig. 2) (Arbyn et al., in press). These differences between countries are believed to reflect protection from screening, and variance in exposure to HPV and other

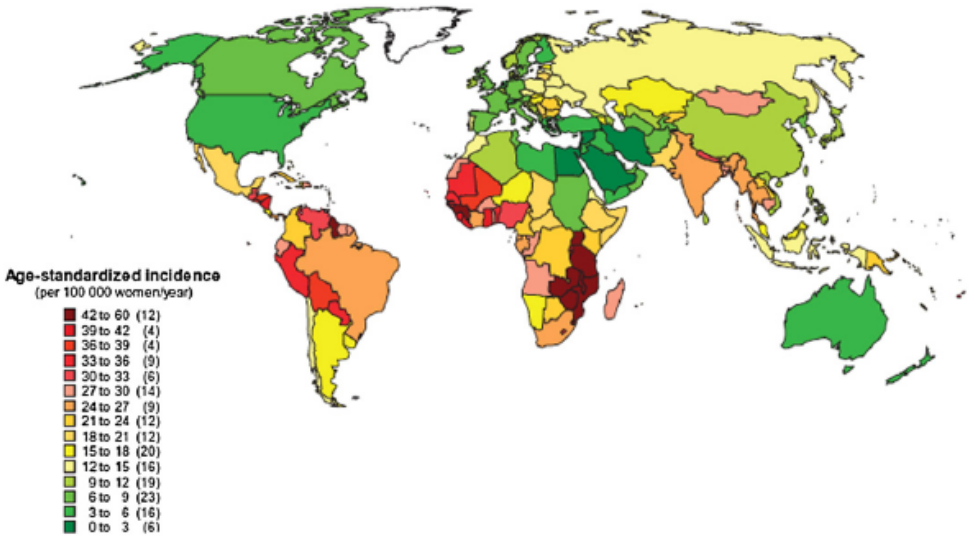

Fig. 2. Global view of the age-standardized incidence rates (ASIRs) of cervical cancer within each country as estimated by GLOBOCAN 2008. In the legend, the numbers in parentheses indicate the number of countries in each range of ASIRs.
Adapted with permission from Arbyn et al. (in press).

cofactors like smoking and oral contraceptive use, and other sexually transmitted infections such as human immunodeficiency virus (Arbyn et al., in press).

The global burden of other HPV related cancers is also substantial. Worldwide, approximately 97,215 cases of non-cervical cancers for which HPV infection may be an etiologic factor are diagnosed annually; roughly 50,780 in men (520 penile, 26,775 oropharyngeal and 13,485 anal cancers) and 46,435 in women (25,600 vaginal/vulvar, 6048 oropharyngeal and 14,787 anal cancers) (Gillison et al., 2008b). However, it is important to recognize that not all of these cases are attributable to HPV and that these estimates represent the upper limit for the annual burden of cancers caused by HPV. Recall that roughly only a quarter of oropharyngeal cancers are attributable to HPV; meanwhile approximately 90% of anal cancers, and 40% of penile, vaginal or vulvar cancers are attributable to the virus. Although there is some evidence implicating HPV with several other cancers (e.g., lung, colon, ovary, breast, prostate, urinary bladder and nasal/sinonasal cancers), current molecular and epidemiological data are sparse and do not yet support a causal role for HPV in the etiology of these cancers (Chaturvedi, 2010; Kreimer et al., 2005; Parkin, 2006).

Globally, HPV accounts for roughly 5.2% of the total cancer burden — the highest among all infectious agents. However, as may be expected, the distribution varies considerably according to country development status, where HPV accounts for approximately 7.7% and 2.2% of all cancer cases in developing and developed countries, respectively (Parkin, 2006). Cervical cancer is the major HPV related cancer contributing to cancer burden in developing countries whereas in more developed countries such as the United States, the burden of non-

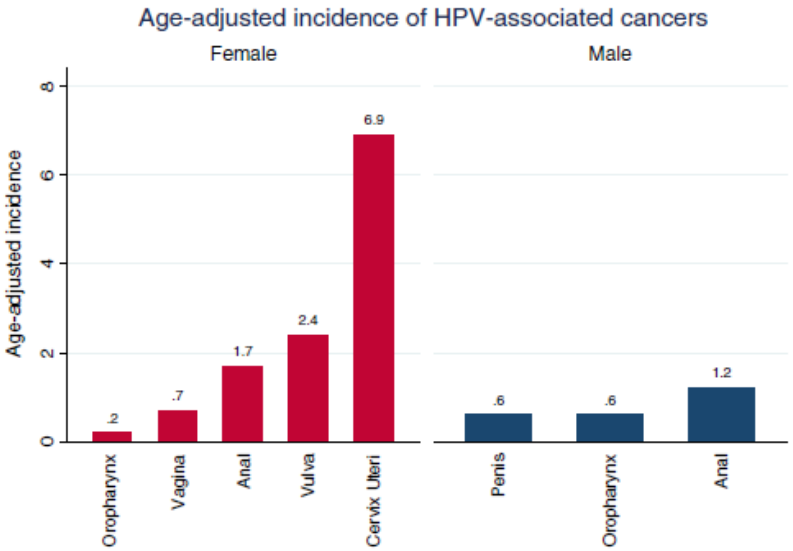

Fig. 3. Age-adjusted incidence rates (rate per 100,000) for malignant HPV-associated cancers in men and women for the year 2006. Rates are standardized to the 2000 United States population and include all ages and races.
Data obtained from the Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence — SEER 9 Regs Research Data, Nov 2010 Sub (1973–2008), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission.

cervical cancers now approximates that of cervical cancer only (Fig. 3) (Gillison et al., 2008b).

Current and future opportunities for prevention

The discovery of HPV infection as a necessary cause of cervical cancer has created many new paths for prevention. The most promising strategies include screening for infection with HR-HPV types and immunization to prevent infection with HR-HPV types.

Pap cytology screening, which has over 50 years of history in medicine, is considered the primary reason we have witnessed a major reduction in cervical cancer mortality in most high-income countries (Arbyn et al., 2009; Jemal et al., 2010). But in spite of its successes, the Pap test is far from perfect. The average sensitivity of cytology to detect CIN is 51% and its average specificity is 98% (Cuzick et al., 2006; Nanda et al., 2000). The Pap test's low sensitivity is a reflection of its highly subjective nature, as it is based on interpretation of morphologic alterations present in cervical samples. The high false-negative rate is a severe limitation which has important medical, financial, and legal implications. In the United States, false-negative Pap smears are one of the most common reasons for medical malpractice litigation (Franco et al., 2003). Liquid based cytology (LBC) has improved the efficiency of smear processing, but does not improve sensitivity of the test (Ronco et al., 2007). To bring screening program sensitivity to an acceptable level, Pap tests in Canada and the United States tend to be done annually, which is a costly endeavor. For nations with opportunistic or organized cervical cancer screening programs, management and follow-up of patients with detected abnormalities places a substantial burden to the health care system. At this time, roughly one in ten Pap smears processed by cytotechnicians in the United States is positive for abnormalities, which require either additional follow-up or treatment (Franco et al., 2003). Unfortunately, many developing countries that have invested into screening programs have yet to witness a substantial reduction in cervical cancer. Poor education of healthcare workers, and a lack of costly safeguards to ensure high coverage, compliance and quality are often cited as the cause for this failure (Denny et al., 2006).

Recently, HPV DNA testing has been suggested as an alternative to primary screening using Pap cytology, perhaps reserving the latter for the triage of HPV positive cases (Tota et al., 2010). Compared to Pap, HPV DNA testing is less dependent on quality of personnel training and is much more objective. Individual randomized controlled trials (RCTs) (Bulkmans et al., 2004, 2007; Elfgren et al., 2005; Kitchener et al., 2009; Kotaniemi-Talonen et al., 2005; Leinonen et al., 2009; Mayrand et al., 2007; Naucler et al., 2007, 2009; Ronco et al., 2006, 2008; Sankaranarayanan et al., 2009) and two recent pooled analyses (Arbyn et al., 2006; Cuzick et al., 2006) comparing the accuracy of HPV testing against Pap cytology found the former to be much more sensitive and only slightly less specific in detecting high grade cervical precancerous lesions (CIN grade 2 or higher). Although results from these and other ongoing RCTs provide the information that is necessary to compare accuracy, it will probably not be sufficient at this point in time to convince most policymakers to adopt HPV testing as the primary screening test. Eventually, results from demonstration projects evaluating the safety of extending screening intervals using HPV testing, as well as lowered HPV test costs resulting from high volume testing and market expansion, may eventually be enough to persuade policymakers to make the change. Also, with the added cost of vaccination to cervical cancer prevention programs there will be added pressure to recommend HPV testing in order to maintain cost-effective cervical cancer screening in the era of HPV vaccination (Tota et al., 2010).

In developing countries, where Pap screening has had little success, there is renewed hope that less frequent screening using HPV DNA testing may finally help reduce mortality from ICC (Schiffman et al., 2011). In a large RCT conducted in rural India, investigators found that a single round of screening using HPV DNA testing was sufficient to reduce the incidence of advanced cervical cancer and mortality by about

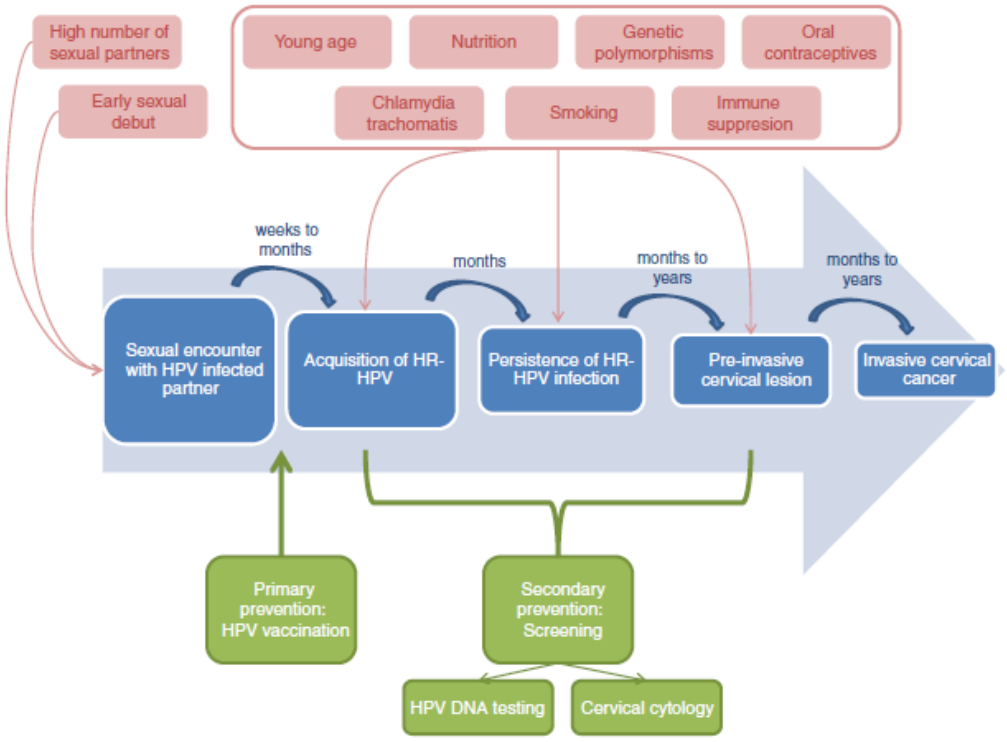

Fig. 4. The natural history, risk factors and opportunities for prevention of cervical cancer (HPV: Human Papillomavirus; HR-HPV: high oncogenic risk HPV). The blue boxes depict the natural history of cervical cancer carcinogenesis, from exposure to HPV, to acquisition and persistence of the infection, to pre-invasive lesions that may progress to invasive cervical cancer (ICC). For each step leading to ICC, only a fraction of cases progresses to the next step, whereas the majority will regress. The salmon boxes above highlight some of the major risk factors for the initial contact with HPV and for the progression to subsequent steps. The green boxes below indicate where opportunities for prevention lie.

half, providing a solid evidence base to support its implementation in other low resource settings. Ultimately, integration of screening with prophylactic HPV vaccination, which currently protects against the most common LR- (HPVs 6 and 11) and HR-HPV types (HPVs 16 and 18), offers the greatest potential to reduce the burden of ICC and other HPV related diseases (Fig. 4) (Brown et al., 2009; Franco et al., 2008; Giuliano et al., 2011; Harper et al., 2006; Paavonen et al., 2009). Unfortunately, for many of these nations where the burden of HPV and cervical cancer is the highest, vaccination and HPV testing remain too expensive. Vaccination uptake has also been slow in some developed countries where cost is not as much of a barrier. According to data from the National Health Interview Survey, less than one quarter of preadolescent and adolescent girls (aged 9–17) in the United States had initiated the HPV vaccination series by the end of 2008 (Wong et al., in press).

A novel approach to prevention also lies in the potential to inhibit prevalent HPV infection. Recently, investigators from the NIH identified that the compound *carrageenan*, a safe and inexpensive gelling agent derived naturally from seaweed, serves as a potent HPV infection inhibitor (Buck et al., 2006; Roberts et al., 2007, 2011). There has been interest in carrageenan as a vaginal microbicide targeting HIV and herpes viruses, but cell culture tests have found that it is a thousand times more effective against HPV than against HIV (Buck et al., 2006). Carrageenan has already been shown to inhibit HPV infection in mice (Roberts et al., 2007) and monkeys (Roberts et al., 2011). In the same set of experiments described above in which Pap was shown to increase HPV infection in rhesus monkeys, lubrication with carrageenan gel during an internal digital exam following specimen collection greatly reduced risk of infection (Roberts et al., 2011). RCTs are currently planned in Canada and the United States to evaluate the efficacy of carrageenan against HPV in human populations (deliverable as a topical microbicide prior to sex) (Peres, 2011).

Prevention strategies in developed and developing countries

Despite breakthroughs in screening and prevention, cervical cancer remains an important cause of cancer death globally, especially in developing countries where the majority of the burden lies. Although prophylactic vaccination is expected to substantially reduce HPV-associated morbidity and mortality, it currently remains too expensive for introduction in most resource-poor countries (Goldie et al., 2008; Anon, 2011). Rwanda recently became the first African country to introduce a national prevention program that includes both HPV vaccination and testing, made possible by donations from the vaccine and HPV test manufacturers, i.e., Merck and Qiagen, respectively (Anon, 2011). If good vaccine and screening coverage is attainable in this setting, then it would serve as a useful model for other parts of sub-Saharan Africa. However, unless other financing mechanisms become available, it may take many years before similar prevention strategies are introduced in other countries that could benefit the most.

In high-resource nations that already have successful cervical cancer screening programs in place, the addition of vaccination programs is expected to have a major impact. Pap screening currently leads to the detection and treatment of a large number of low- and high-grade cervical lesions, especially in young women, for whom ablative treatment carries substantial risk of adverse reproductive outcomes including preterm delivery and miscarriage. Prophylactic vaccination of females prior to their sexual debut would prevent a large proportion of these precancerous lesions, cervical cancer, and some other non-cervical HPV-related cancers. Australia recently became the first country to witness a significant decline in the rate of high-grade cervical lesions following implementation of HPV vaccination (Brotherton et al., 2011). However, this reduction may simply be a reflection of lowered screening uptake among young vaccinated women. Future studies that involve linkage between registries are expected to provide us with a better estimate of the benefits of vaccination. Australia is also one of the few countries with a community based catch-up program targeting women up to the age of 26 for vaccination. Most other government programs are exclusively targeting females 12 years of age at this time, and in these settings it is expected to take longer before there is a noticeable decline in cervical abnormalities and HPV related cancers.

In the post-vaccination era, HPV DNA testing will also serve an important second purpose by providing a low cost surveillance approach to monitor vaccine efficacy, protection duration, and cross protection or type replacement. Integration of primary and secondary cervical cancer prevention strategies via record linkage and shared resources inherently lends itself to being treated best as a single prevention strategy. Linkage of registries also provides the necessary data to evaluate the success of prevention strategies and to inform international policies, hopefully putting pressure on high-income countries, non-governmental organizations, pharmaceutical companies and other donors to provide their support.

Conflict of interest statement

ELF has served as consultant to Merck, Roche and Gen-Probe, and received unconditional grants from Merck in support of one of his studies. The other authors have no specific associations with industry to report.

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APPENDIX 2

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Practice of Epidemiology

Epidemiologic Approaches to Evaluating the Potential for Human Papillomavirus Type Replacement Postvaccination

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Currently, 2 vaccines exist that prevent infection by the genotypes of human papillomavirus (HPV) responsible for approximately 70% of cervical cancer cases worldwide. Although vaccination is expected to reduce the prevalence of these HPV types, there is concern about the effect this could have on the distribution of other oncogenic types. According to basic ecological principles, if competition exists between ≥2 different HPV types for niche occupation during natural infection, elimination of 1 type may lead to an increase in other type(s). Here, we discuss this issue of "type replacement" and present different epidemiologic approaches for evaluation of HPV type competition. Briefly, these approaches involve: 1) calculation of the expected frequency of coinfection under independence between HPV types for comparison with observed frequency; 2) construction of hierarchical logistic regression models for each vaccine-targeted type; and 3) construction of Kaplan-Meier curves and Cox models to evaluate sequential acquisition and clearance of HPV types according to baseline HPV status. We also discuss a related issue concerning diagnostic artifacts arising when multiple HPV types are present in specific samples (due to the inability of broad-spectrum assays to detect certain types present in lower concentrations). This may result in an apparent increase in previously undetected types postvaccination.

cervical cancer; human papillomavirus; HPV type replacement; vaccination

Abbreviations: CI, confidence interval; E, expected; HPV, human papillomavirus; O, observed; PCR, polymerase chain reaction.

The discovery of human papillomavirus (HPV) as a necessary cause of cervical cancer (1) has enormous public health implications and has already led to the establishment of 2 highly effective HPV vaccines (2, 3). Both Gardasil (Merck & Company, Whitehouse Station, New Jersey) and Cervarix (GlaxoSmithKline, London, United Kingdom) prevent infection by the 2 genotypes of HPV that cause the majority (approximately 70%) of cervical cancer cases (HPV types 16 and 18), but only Gardasil protects against additional types (HPV types 6 and 11) that are responsible for most cases (approximately 90%) of genital warts (4–6). Countries that have implemented HPV vaccination will eventually experience major reductions in the incidence of cervical cancer and other HPV-related diseases. However, the existence of other oncogenic HPV types not targeted by the vaccine raises a concern that one or more of these other types may eventually take over the ecological niches vacated by the eradication of vaccine types; this is a concept referred to as "type replacement" (7–10). The important question that remains is: Is it possible to obtain epidemiologic insights concerning the likelihood that HPV type replacement may or may not occur?

In this article, we present different epidemiologic approaches to evaluating the potential for HPV type replacement, with examples taken from the Brazilian Ludwig-McGill cohort study (11). We also discuss another important issue related to assessing type replacement: namely, the accuracy of detecting type-specific prevalence when there is coinfection with multiple types of HPV.

HPV TYPE REPLACEMENT IN THE POSTVACCINATION ERA

Concern about type replacement is an argument against HPV vaccination that is used by some policy analysts (12),

who often cite the pneumococcal vaccine experience as evidence (13–16). However, unlike pneumococcal infection, in which the pathogen (*Streptococcus pneumoniae*) has a high rate of genetic mutation and recombination, HPVs are DNA viruses that are extremely stable genetically. In fact, the mutation rate for this virus has been estimated at only 1 base pair every 10,000 years (17). Therefore, the emergence of escape mutants that avoid vaccine immunity or entirely new HPV types is unlikely. Emergence of an existing type is also unlikely because of the relatively slower sexual infection dynamics and because the majority of the population is unexposed to specific HPV types (e.g., HPV-16 or -18), implying that any possible natural competition cannot have greatly affected the pool of susceptible persons who may acquire other types. Nonetheless, if it can be demonstrated that HPV types compete with one another during natural infection, there is still the theoretical possibility that type replacement may occur. The existence of natural type competition is a necessary condition for replacement, the other being that such natural type competition needs to be stronger than the cross-protection afforded by vaccines if type replacement is going to be possible (10).

To date, over 150 HPV genotypes have been identified, including more than 40 anogenital types (18–20). Based on the nucleotide sequence of the *L1* (late) capsid gene, papillomaviruses have been classified into high- and low-order clusters, referred to as genus and species, respectively. Most genital HPV types occupy a single genus, α, within which there exist 15 species (19–21). Genotypes from the same species share at least 60% of their nucleotide sequence identity, and as a result they often exhibit similar biological and pathological properties (19, 20). Among the 13 HPV types classified by the International Agency for Research on Cancer as definite or probable carcinogens, most belong to 2 species (α-7 or α-9) (22, 23). After HPVs 16 and 18, the 10 most common types implicated in cervical cancer globally (in order of decreasing prevalence) are 58, 33, 45, 31, 52, 35, 59, 39, 51, and 56 (5, 6).

According to Gause's ecological competitive exclusion principle (24), 2 species cannot stably coexist when competing for the same ecological niche. If niches overlap and one of the competing species is removed, the remaining one would then take over the available niche space and increase in prevalence. Alternatively, if a symbiotic species is removed, we would expect both species to decrease in prevalence (24). Type replacement after vaccination strongly depends on whether different HPV types interact during natural infection. Plausible competition mechanisms include generation of cross-reactive systemic or local immunity. However, it is well established that if vaccination provides cross-immunity that is at least equivalent to that of natural infection, available niche space will not be increased (25). Thus far, phase III trials of HPV vaccines have found that vaccination induces antibodies at much higher levels than natural infection. Therefore, the vaccine-induced partial cross-type protection against certain HPV types, mainly types 31, 33 (α-9), and 45 (α-7), is likely to be well above natural cross-type immunity (3, 26), implying that type replacement is unlikely to occur for these types. Although negative vaccine efficacy (which could be misconstrued as type replacement) was reported in one of these trials for HPVs 52 and 58 (both from the α-9 species) (26), the finding could not have been due to type replacement, because this is a viral dynamics phenomenon that implies within-group transmission. Clinical trial populations do not replicate the transmission conditions seen in entire populations. As we discuss below, a diagnostic artifact is a likely explanation.

EPIDEMIOLOGIC APPROACHES TO EVALUATING HPV TYPE COMPETITION

Probabilistic approach

To gain insight into the possibility of type replacement, it is useful to evaluate competition between HPV types during natural infection. Competition of this sort may be reflected by a low probability of coinfection between 2 specific HPV types. For each pair combination involving a vaccine type and a nonvaccine type, we may calculate the expected frequency (E) of coinfection under a model of statistical independence and compare this with the observed frequency (O). This approach was first used in the late 1980s to evaluate multiple HPV infections in a Brazilian population (27) and has since been used by other investigators (7, 8, 28–35).

Table 1 presents a hypothetical example of how one can gain insights from epidemiologic studies as to whether or not any given HPV type, say type "X," could occupy the niche vacated by HPV-16. The two halves of the table show what would be a good clue if one assumes that this type competes with HPV-16 and thus would normally be observed less often than expected by chance alone. In the upper half of the table, HPV-X was found in 20 women who were also infected with HPV-16 (out of the total proportion (7%) of HPV-16-positive women among the 10,000 included in the study). Assuming independence between infections, one can calculate what the expected frequency of co-occurrence would be from the product of the two prevalences. The result is 35. In other words, the ratio of the observed number to the expected number (O/E = 20/35 = 0.57) is less than 1, and the 95% statistical confidence bounds indicate that this O/E ratio is statistically significant. The conclusion would be that type X tends to occur less frequently than expected in women who are infected with HPV-16. This would be cause for concern, because it suggests that HPV-X is suppressed by HPV-16 and thus its frequency could increase in the future post-HPV vaccination. Most epidemiologic studies that have examined the O/E relationship for different pairs of HPV types (30–34) have seldom found the situation in the upper portion of the table; rather, these studies have found a scenario that is comparable to the one in the lower portion of the table. The marginal distributions of HPV types are the same for type X and for type 16, but the observed frequency is now 40, indicating that HPV-X is actually detected more frequently when HPV-16 is present. However, since there are shared risk factors for HPV infection, O/E ratios greater than 1.0 do not necessarily rule out the possibility of competition between genotypes.

Table 1. Hypothetical Example of Analysis of Co-occurrence of Different Types of Human Papillomavirus in Epidemiologic Studies[a]

HPV-X Status	HPV-16 Status			O[b]	E[c]	O/E Ratio	95% CI
	No. of HPV-16+ Women	No. of HPV-16– Women	Total No. of Women				
Type X Co-occurs With HPV-16 Less Frequently Than Expected							
No. of HPV-X+ women	20	480	500	20	35	0.57[d]	0.35, 0.88
No. of HPV-X– women	680	8,820	9,500				
Total no. of women	700	9,300	10,000				
Type X Co-occurs With HPV-16 More Frequently Than Expected							
No. of HPV-X+ women	40	460	500	40	35	1.14[e]	0.82, 1.56
No. of HPV-X– women	660	8,840	9,500				
Total no. of women	700	9,300	10,000				

Abbreviations: CI, confidence interval; E, expected; HPV, human papillomavirus virus; O, observed.
[a] Concomitant (cross-sectional) or sequential (cohort) acquisition.
[b] Observed frequency of coinfection with HPV-16 and HPV-X.
[c] Expected frequency of coinfection with HPV-16 and HPV-X.
[d] Interpretation: HPV type X is under "suspicion" for replacement.
[e] Interpretation: HPV type X is not "suspected" for replacement.

Using period prevalence data for the first year of subject follow-up from the Ludwig-McGill cohort study ($n = 2,462$ women), we compared the observed and expected numbers of coinfections, focusing on HPV-16 for this example. The Ludwig-McGill study has been described in detail elsewhere (11). Briefly, it included an average of 10 follow-up visits per woman (every 4 months during the first year and twice annually in subsequent years), with questionnaire administration, Papanicolaou cytology, and HPV testing performed at each visit. In Figure 1, the majority of log(O/E) ratios were above the null. The average weighted log(O/E) ratio was 0.87 (95% confidence interval (CI): 0.67, 1.06). For some types, the O/E ratios were zero because those types were not observed in coinfection with HPV-16. These types were included in our calculation of the average weighted O/E ratio. Previously, other investigators who have evaluated HPV type interactions have restricted their analysis to positive women (i.e., women with ≥1 HPV infection) to ensure that they have focused on a population with sufficient HPV exposure opportunity (27, 29, 30, 36). This approach leads to higher expected frequencies (reduced O/E ratios) for all pairwise combinations, making results difficult to compare.

Considering that mucosotropic HPV infections share a common route of transmission and many risk factors (37, 38), it is not surprising that infection with multiple HPV types occurs often, in up to 50% of infected women (38, 39) and more frequently than expected by chance (7, 8, 28, 30, 32–34, 40). Thus, in calculating the expected frequency of coinfection, our assumption that infections occur independently is a major limitation, leading to biased estimates of the O/E ratio away from zero. Therefore, to account for correlation between HPV infections, we should attempt to adjust for common risk factors in evaluating pairwise interactions (41), which would reduce most positive associations, thus improving our ability to detect competition between HPV types.

Regression approach

Another approach to evaluating type competition is to construct logistic regression models for each vaccine type separately and calculate the odds ratio for each pairwise association involving nonvaccine types. Conceptually, the interpretation of odds ratios is the same as for O/E ratios; that is, odds ratios less than 1.0 would indicate that the odds of being infected with a particular nonvaccine HPV type are lower among persons with a vaccine type than among those without a vaccine type, and vice versa for odds ratios greater than 1.0. A benefit of this approach is that confounding, as described above, may be addressed by the addition of relevant covariates to the model. In particular, factors such as age and number of sexual partners, which are normally predictive of multiple HPV infections, should be included (29, 38, 42–45). Positive associations that persist after adjustment may indicate synergistic effects between specific HPV types, but more likely indicate either residual confounding or polymerase chain reaction (PCR) cross-reactivity.

In a recent pooled analysis of International Agency for Research on Cancer HPV prevalence surveys, Vaccarella et al. (32) evaluated clustering patterns between all HPV types via hierarchical regression models with woman-level random effects, which presumably should have accounted for any residual variation in HPV infection risk not captured by covariates in their model. Although only a single statistically significant negative association was observed (between HPV-16 and HPV-81), multiple positive associations were observed (between HPV types 33 and 35, 33 and 58, 33 and 39, 18 and 45, and 31 and 35). Because results from this study differed by genotyping method, the authors attributed clustering of these HPV types to a diagnostic artifact and not true biological interaction.

Chaturvedi et al. (33) also examined HPV coinfection patterns among women from a vaccine study in Costa Rica. To

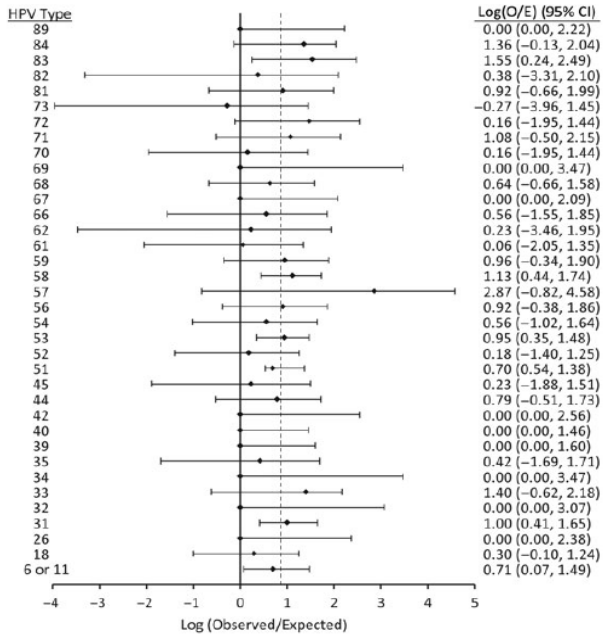

Figure 1. Log(observed/expected) ratios (log(O/E)) and 95% confidence intervals (CI) for coinfections involving human papillomavirus (HPV) type 16 and other HPV types. Ratios were calculated using 1-year period prevalence information. The dashed line represents the average weighted log(O/E) of 0.87 (95% CI: 0.67, 1.06). HPV types belonging to the same species as HPV-16 (α-9) include types 31, 33, 35, 52, 58, and 67. For HPV types with an O/E ratio of 0 (types 26, 32, 34, 39, 40 42, 67, 69, and 89), 0 was listed for the log and lower range of the 95% CI.

account for positive correlation between HPV infections, they adjusted for predictors of multiple infection but also calculated a pooled odds ratio by averaging across all pair-specific odds ratios (separately for HPV types 6, 11, 16, and 18) and used this to represent the underlying affinity of each of these vaccine types for being involved in coinfection. They calculated the difference between the pair-specific odds ratio and the pooled odds ratio (log scale) to assess whether any particular pair of genotypes deviated from the pooled odds ratio. This procedure was repeated for a total of 300 type-type combinations (25 HPV types), and statistically significant negative associations were observed between HPVs 44 and 68, 44 and 73, and 18 and 33, whereas HPVs 11 and 53, 31 and 33, 34 and 42, 45 and 68, and 45 and 73 were found to be positively associated. In general, HPV genotypes occurred independently, and phylogenetic relatedness had no influence.

The 2 studies described above did not account for the presence of other HPV types in evaluation of pairwise interactions, which according to Rositch et al. (36) may lead to confounding. Another issue is that for rare HPV types, few or no coinfections may be observed, which could lead to nonpositivity or wide confidence intervals and extremely limited power to detect competition with these types. Rositch et al. (36) addressed some of these issues using data from a randomized controlled trial of Kenyan males through a semi-Bayesian regression approach. Multivariate hierarchical logistic models for 4 outcome types (HPVs 6, 11, 16, and 18) included variables identified a priori as predictors of multiple HPV infection, as well as all other HPV types. The hierarchical component was introduced through prior means for type-specific estimates, obtained by calculating the crude average log odds of coinfection for each type. By intentionally introducing some bias using priors, this produces a shrinkage effect that reduces the overall error across estimates and improves the precision of each estimate (46). A mix of null and positive associations, but no negative associations, was reported in this study.

Using Ludwig-McGill data, we illustrate the effects of shrinkage and adjustment for confounding in Figure 2. Panel A presents results from a multivariate logistic regression model with HPV-16 as the outcome and all other types as predictor variables. Woman-level clustering was accounted for with woman-specific intercepts. The odds ratio estimates for some rare HPV types were highly unstable, and these types were excluded from the model. Panel B presents the results from a similar model, with the addition of age and lifetime number of sexual partners at baseline as covariates. The average weighted log odds ratio appeared to be only slightly reduced by adjustment, from 0.38 (95% CI: 0.10, 0.62) to 0.37 (95% CI: 0.12, 0.58), possibly because these variables were not strong predictors of coinfection in the Ludwig-McGill data set. Panel C results are from a model similar to that in panel B, with the addition of a fully Bayesian approach to shrinkage, where the prior distribution for type-specific odds ratio estimates was centered around the pooled estimate. Shrinkage reduced the problem of nonpositivity, since unstable estimates were pulled (shrunk) more closely towards the overall mean, which enabled us to include rare types in the model. The confidence intervals were also narrower in comparison with panels A and B. The pooled log odds ratio from the shrinkage model was 0.53 (95% CI: 0.21, 0.77).

By addressing issues of sparse data and confounding by a common route of transmission, regression approaches that employ shrinkage to stabilize estimates and include adjustment for confounders may be useful in this context for evaluation of HPV type competition (47).

Cohort approach

When cohort information is available, comparison of sequential acquisition and clearance of HPV types according to infection with vaccine types is another useful approach to evaluating type competition. For acquisition, time to incident HPV infection(s) may be assessed for each of the non-vaccine types separately (or grouped together by species) according to baseline infection with one of the vaccine types. For evaluation of clearance, the approach is similar except that eligible women must be positive for the specific type(s) under study at baseline. Using Cox regression with adjustment for important confounding factors, we may calculate hazard ratios and associated confidence intervals. If

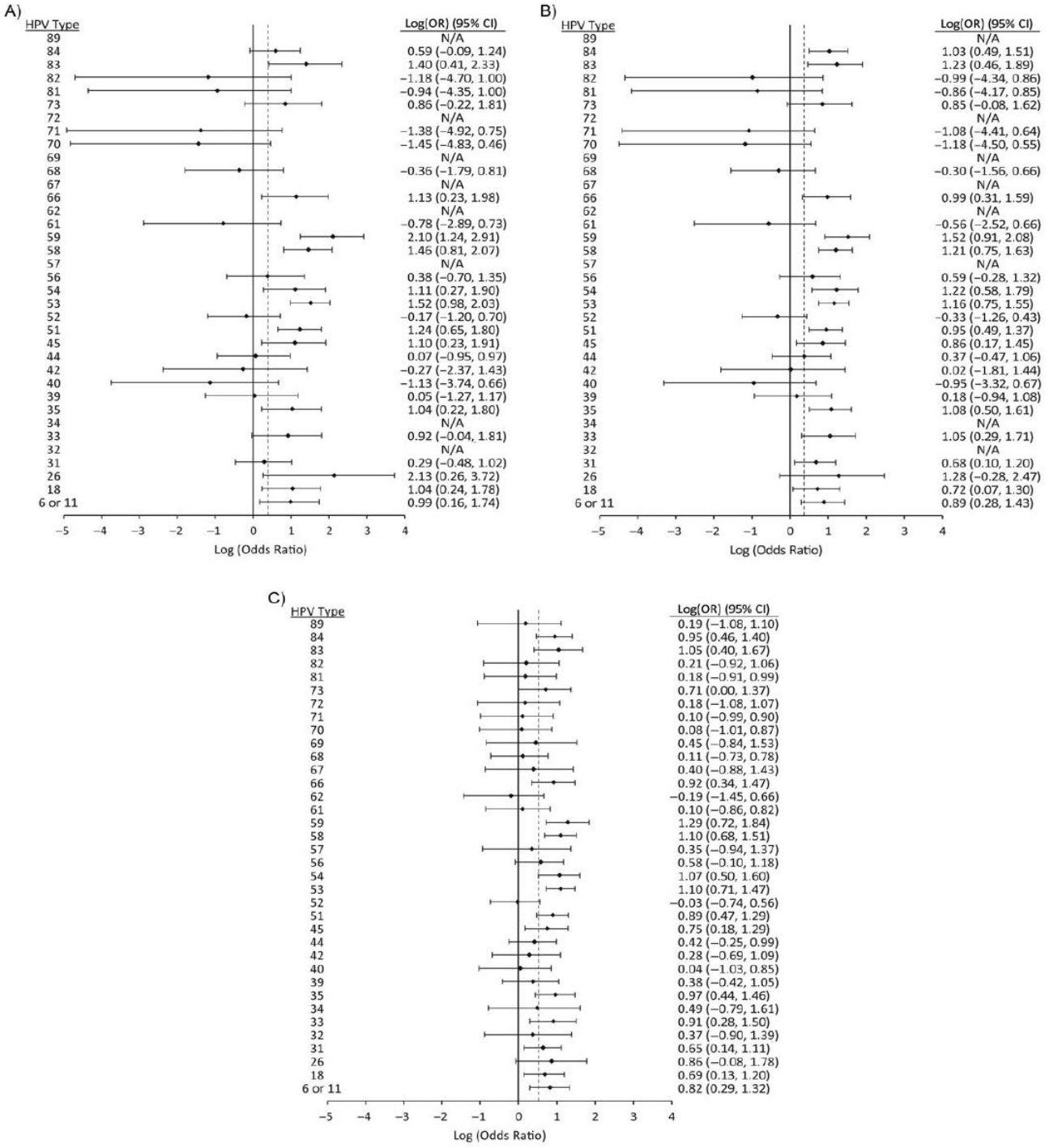

Figure 2. Log(odds ratios) (log(OR)) and 95% confidence intervals (CI) for human papillomavirus (HPV) type 16 for coinfection with other HPV types. Estimates were obtained from logistic regression models adjusting for all other HPV types (A); adjusted for all other types, age, and lifetime number of sexual partners at baseline (B); and adjusted for all other types, age, lifetime number of sexual partners, and shrinkage (C). Dashed lines represent the average weighted log(OR) in panels A and B, which were 0.38 (95% CI: 0.10, 0.62) and 0.37 (95% CI: 0.12, 0.58), respectively, and the pooled log(OR) from hierarchical logistic regression in panel C, which was 0.53 (95% CI: 0.21, 0.77). HPV types belonging to the same species as HPV-16 (α-9) include types 31, 33, 35, 52, 58, and 67. In panels A and B, rare HPV types (types 32, 34, 57, 62, 67, 69, 72, and 89) were excluded from the model because they caused model instability. These types were included in the model shown in panel C because the hierarchical model is able to stabilize estimates. N/A, not applicable.

we categorize women with a vaccine type as the exposed group, hazard ratios less than 1.0 would indicate that the risk of becoming infected with a particular nonvaccine HPV type was lower among those infected with a vaccine type than among those without a vaccine type, and vice versa for hazard ratios greater than 1.0. Our interpretation is similar to what we described for O/E ratios and odds ratios, except that for clearance it is the opposite; that is, hazard ratios greater than 1.0 indicate accelerated clearance of certain HPV types among persons with a vaccine genotype and thus potential type competition.

Previous studies examining the natural history of HPV did not suggest that prior infection with one or more HPV types inhibits acquisition of other types or facilitates clearance of prevalent types in women (7, 8, 28, 29, 40, 48). Rather, the majority of studies found that the presence of preexisting HPV infection actually increased a woman's risk of acquiring other types, including those from the same species (8, 28, 40). Although these studies did not focus specifically on vaccine target types, they still provided valuable insights concerning type competition in general.

Using Ludwig-McGill cohort data, we prepared Kaplan-Meier curves to compare acquisition and clearance of HPV infection with α-9 genotypes (excluding HPV-16) between women with and without HPV-16 infection at baseline (Figure 3). Despite adjustment for important risk factors for multiple infection (e.g., age, lifetime number of sexual partners), women infected with HPV-16 still appeared more likely to acquire other phylogenetically related HPV types and less likely to clear infections with these genotypes.

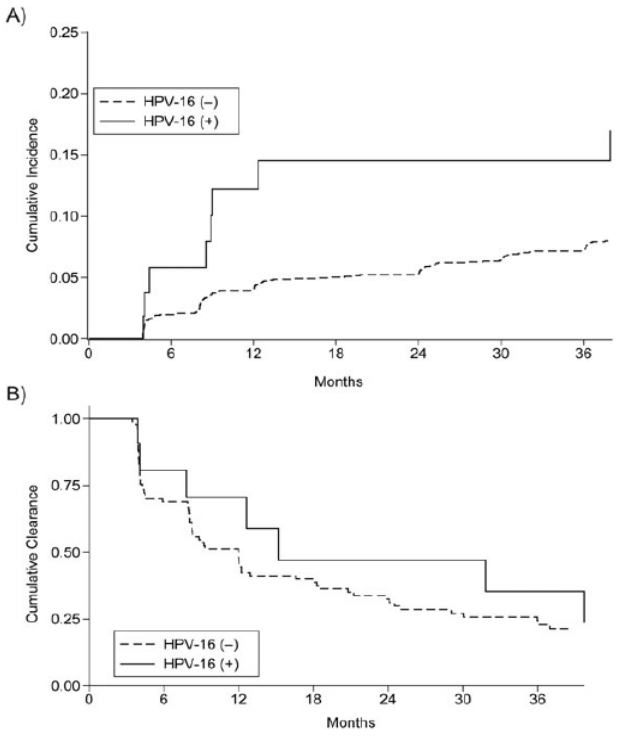

Figure 3. Kaplan-Meier curves showing time to incident human papillomavirus (HPV) infection (A) and clearance of existing HPV infection (α-9 genotypes, excluding HPV-16) (B) according to HPV-16 status at baseline, adjusted for age and lifetime number of sexual partners. Hazard ratios and associated 95% confidence intervals (CI) for panels A and B were 1.49 (95% CI: 0.82, 2.73) and 0.79 (95% CI: 0.38, 1.64), respectively.

Comparing approaches

Based on results presented here from the Ludwig-McGill study, type competition does not appear to exist between HPV-16 and other genotypes; that is, estimates less than 1.0 (O/E ratios, odds ratios, and hazard ratios for incidence) or greater than 1.0 (hazard ratios for clearance) were not statistically significant. Although the probabilistic approach is arguably the most intuitive, it does not permit adjustment for confounding and is more likely to produce biased estimates, making it more difficult to reliably assess type competition. We therefore recommend using regression and cohort approaches. Evidence of type competition that is consistently reported across approaches and studies should be a strong signal to investigators that type replacement is more likely to occur for the flagged HPV type(s).

DIAGNOSTIC ARTIFACTS

An additional concern related to HPV type replacement postvaccination is the possibility of diagnostic artifacts. Currently, the most common HPV DNA tests being used for research and surveillance are consensus (or general) primer PCR assays with MY09/11 or GP5+/6+ primer sets. By targeting sequences in the *L1* gene of HPV, these assays amplify and detect a broad spectrum of mucosotropic HPV types (49). However, there may be competition for reagents (e.g., primers) between at least 1 of the current HPV vaccine types and other prevalent types in consensus PCR assays. The impact of this may be that in the presence of vaccine types, other prevalent HPV types are being missed (50). For instance, if a specimen contains 1,000,000 HPV-16 genome copies but only 1,000 HPV-31 genome copies, then during amplification the HPV-16 sequences will overwhelm the minority type during the exponential phase of replication, and the resulting signal for HPV-16 will be revealed at the expense of HPV-31. Hence, this specimen may be erroneously labeled as an HPV-16 monoinfection. However, if HPV-16 is removed, the existing 1,000 molecules will have the entire reagent mixture for their amplification to proceed unhindered, and the specimen will be HPV-31-positive.

In the postvaccination era, surveillance will be necessary to monitor trends in the distribution of HPV types. If an increase in nonvaccine types is observed, it will be important to distinguish whether this results from true type replacement or represents a diagnostic artifact. For example, if we observe an increase in the prevalence of HPV-31 postvaccination, an alternative explanation to type replacement is that HPV-31 had always been present but was underestimated in the presence of vaccine types that were eliminated. In HPV vaccine trials, a differential increase in prevalence may

occur in the intervention arm, since this group would be protected against future infection by vaccine types, whereas the placebo arm would not. By ignoring this possibility, one may arrive at erroneous conclusions when interpreting vaccine efficacy against nonvaccine HPV types.

Numerous studies that have compared PCR methods have noted deficiencies in the sensitivity of consensus PCR versus type-specific or multiple-primer PCR systems (e.g., PGMY09/11 and modified GP5+/6+), particularly in cases of multiple infection and low viral DNA load (51–59). Recently, Mori et al. (59) found that in samples containing HPV-16 and either HPV-18, -51, -52, or -58, these latter types were not sufficiently amplified by consensus PCR at lower viral loads. Consistent with previous reports (51, 53, 57), sensitivity was most severely affected for types 51 and 52. Therefore, negative vaccine efficacy against certain HPV types (26) may simply be a consequence of inadequate test performance, and just as it is important to identify types that should be monitored for replacement, it is equally important to evaluate the test used and ensure that it performs adequately. The World Health Organization HPV LabNet provides blinded "proficiency panels" designed to evaluate whether the assays used can detect a monoinfection equally well in the presence of other HPV types. Comparison of results from more than 100 laboratories worldwide that have used a variety of HPV assays has shown that underestimation of some HPV types when other types are present in the same sample is a definite problem for some assays, but not for others (60, 61). In this regard, continued monitoring for adequate performance of assays used for surveillance will be of critical importance.

OTHER ISSUES TO CONSIDER IN THE EVALUATION OF HPV TYPE REPLACEMENT

The term *unmasking* has previously been used in the pneumococcal vaccine literature to describe detection of apparent type replacement resulting from misattribution of a strain of microorganism causing disease when multiple strains are present (62, 63). Because multiple infection with oncogenic HPV types is also common in evaluating cases of cervical cancer, assigning causality to a particular HPV type is often difficult and may also lead to misclassification in this scenario (64). When investigators are faced with this situation, they often will apply an oncogenic hierarchy in which the lesion is attributed to the HPV type present that usually progresses most rapidly to cause cancer. Often, this will either be HPV-16 or HPV-18, which may or may not be present in the actual lesion (65). When multiple HPVs are present, there could also be different lesions individually caused by different types. Cervical excisional treatment may remove multiple lesions and HPV types simultaneously. However, when excisional procedures for vaccine types detectable by screening are no longer performed in the future, the number of women at risk for disease caused by nonvaccine types may seem to increase. van der Marel et al. (66) used genotyping and laser-capture microdissection PCR analysis to evaluate high-grade cervical lesions with multiple HPV infections (including HPV-16) and found that HPV-16 was the causal type in all cases. We therefore expect that type replacement observed as a consequence of errors in assigning causality or reduced rates of excisional treatment will be low.

The possibility that HPV vaccination could lead to an increase in risky sexual behavior (i.e., "risk compensation") (67) due to a perceived lower risk of sexually transmitted infections among young vaccinees also has important implications for HPV type replacement. To investigate this, Liddon et al. (68) recently evaluated data from a large national US survey and found no association between HPV vaccination and reported risky sexual behaviors. Although these results may provide comfort to concerned parents and health officials, only prospective follow-up studies can provide a definitive answer to this question.

So far, there are no indications that the biological prerequisites for type replacement are present in the HPV field. Diagnostic laboratory artifacts may explain some deviations from random effects. Furthermore, the significant cross-protection seen after vaccination is likely to dwarf possible tendencies for replacement that may not have been possible to detect because of insufficient statistical power. Moreover, even if type replacement is observed, unless it leads to disease, it may not have important public health implications. Because HPV-16 and HPV-18 pose much higher cancer risks than any other HPV type, replacement by a nononcogenic type or an oncogenic type that entails much lower risk of cancer may not have any major consequences. Results from long-term surveillance studies comparing the prevalences of different HPV types implicated in cervical cancer or high-grade lesions (pre- vs. postvaccination) will eventually provide a clearer estimate of the population-level impact of current vaccines. Until then, we may gain valuable insight through evaluation of type competition to identify HPV types considered suspicious for replacement. In the unlikely event that such signals were to be found, types that were flagged could then be included in the new generation of multivalent vaccines (69, 70).

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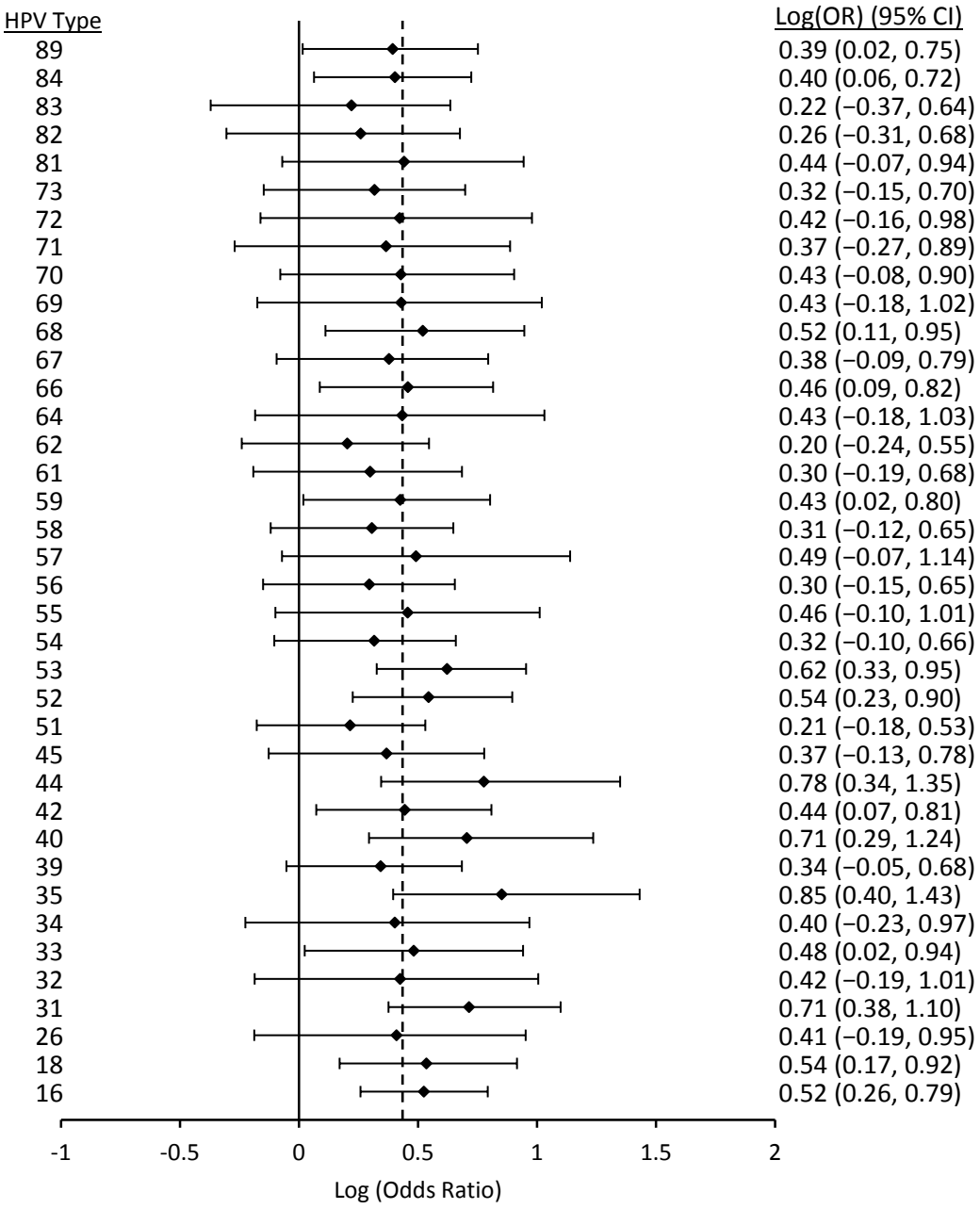
APPENDIX 3

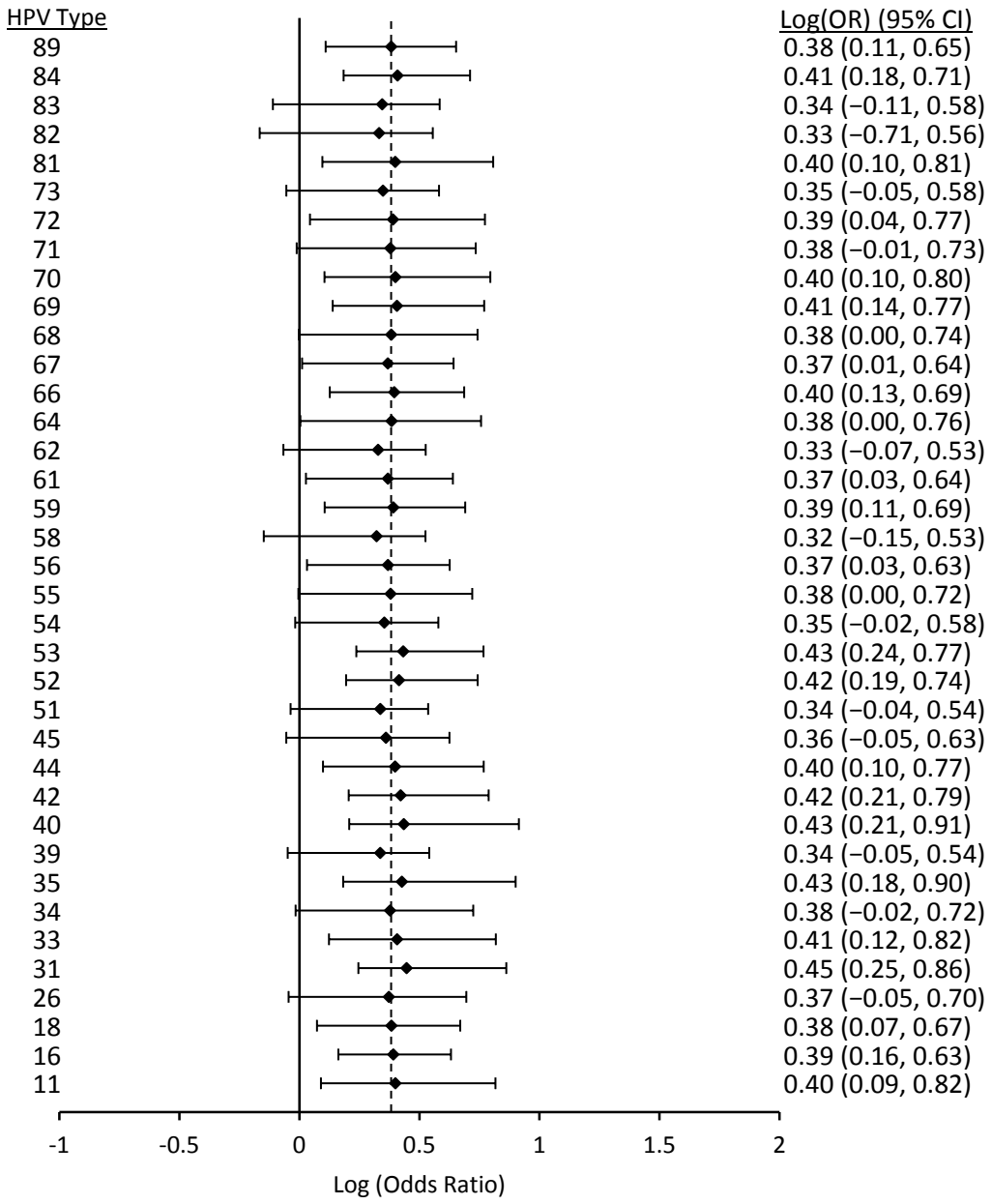
ADDITIONAL ANALYSES

TO MANUSCRIPT III

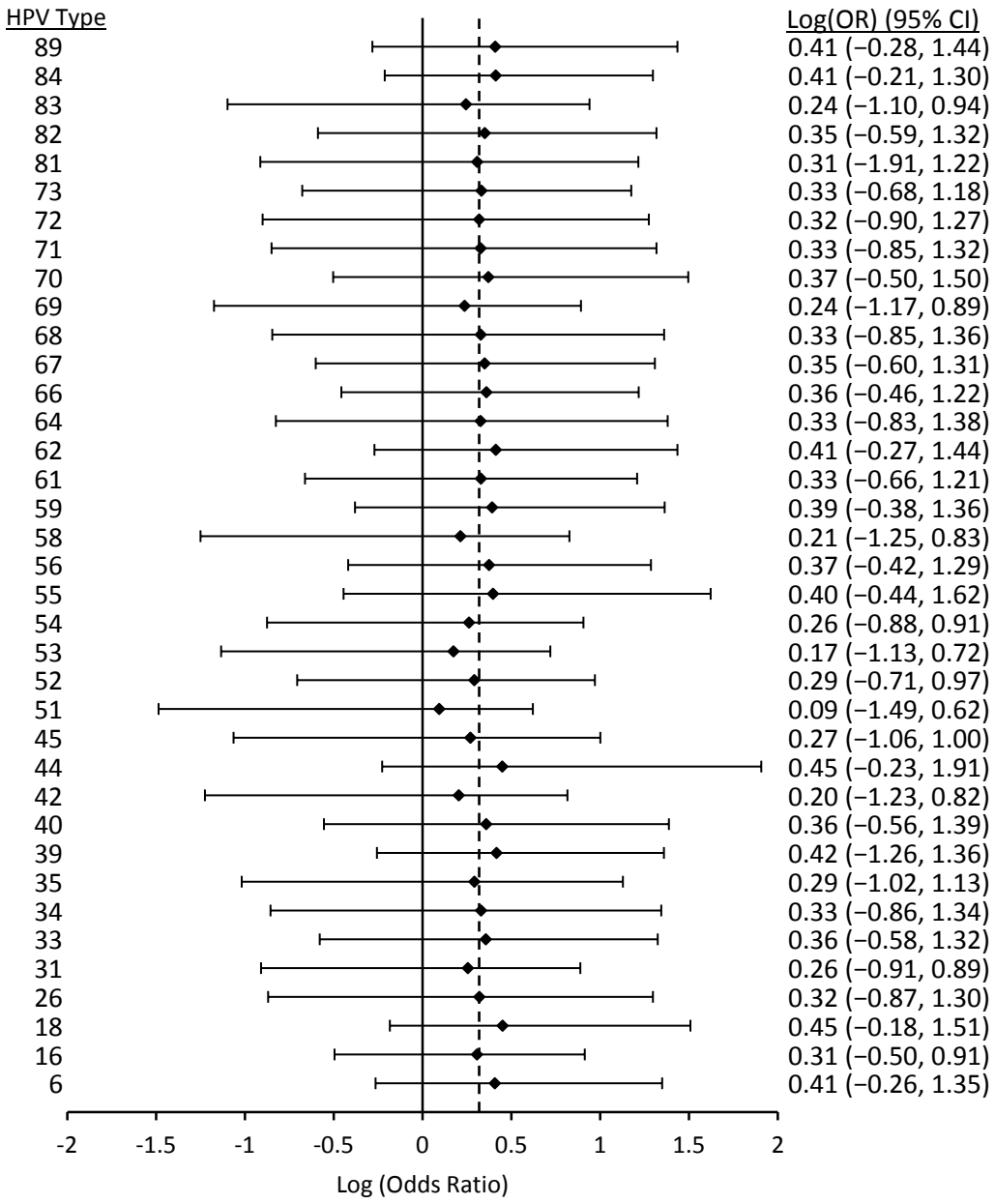
A)


HPV Type
Log(OR) (95% CI)
89 0.39 (0.02, 0.75)
84 0.40 (0.06, 0.72)
83 0.22 (−0.37, 0.64)
82 0.26 (−0.31, 0.68)
81 0.44 (−0.07, 0.94)
73 0.32 (−0.15, 0.70)
72 0.42 (−0.16, 0.98)
71 0.37 (−0.27, 0.89)
70 0.43 (−0.08, 0.90)
69 0.43 (−0.18, 1.02)
68 0.52 (0.11, 0.95)
67 0.38 (−0.09, 0.79)
66 0.46 (0.09, 0.82)
64 0.43 (−0.18, 1.03)
62 0.20 (−0.24, 0.55)
61 0.30 (−0.19, 0.68)
59 0.43 (0.02, 0.80)
58 0.31 (−0.12, 0.65)
57 0.49 (−0.07, 1.14)
56 0.30 (−0.15, 0.65)
55 0.46 (−0.10, 1.01)
54 0.32 (−0.10, 0.66)
53 0.62 (0.33, 0.95)
52 0.54 (0.23, 0.90)
51 0.21 (−0.18, 0.53)
45 0.37 (−0.13, 0.78)
44 0.78 (0.34, 1.35)
42 0.44 (0.07, 0.81)
40 0.71 (0.29, 1.24)
39 0.34 (−0.05, 0.68)
35 0.85 (0.40, 1.43)
34 0.40 (−0.23, 0.97)
33 0.48 (0.02, 0.94)
32 0.42 (−0.19, 1.01)
31 0.71 (0.38, 1.10)
26 0.41 (−0.19, 0.95)
18 0.54 (0.17, 0.92)
16 0.52 (0.26, 0.79)
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

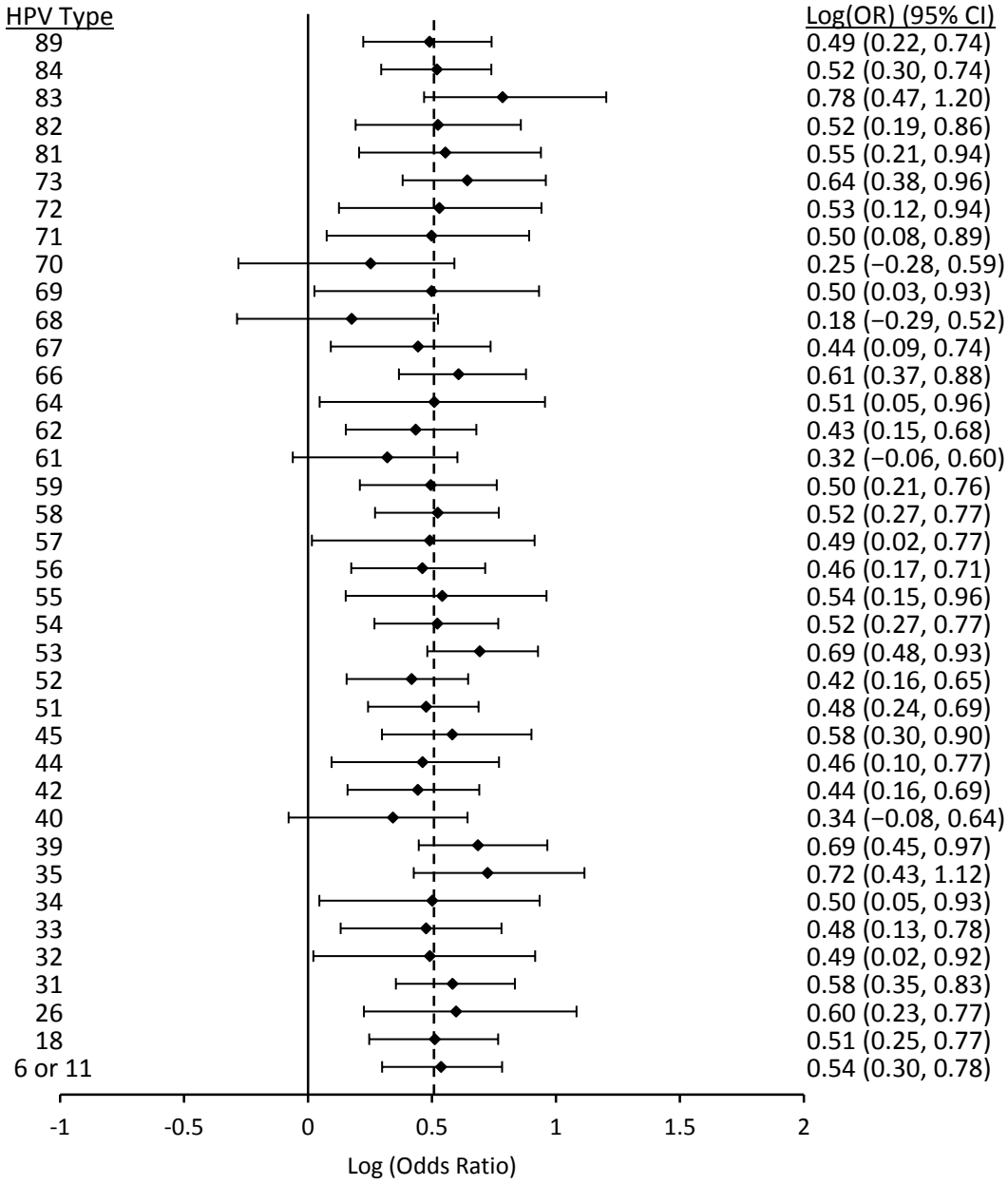
B)


HPV Type
Log(OR) (95% CI)
89 0.38 (0.11, 0.65)
84 0.41 (0.18, 0.71)
83 0.34 (−0.11, 0.58)
82 0.33 (−0.71, 0.56)
81 0.40 (0.10, 0.81)
73 0.35 (−0.05, 0.58)
72 0.39 (0.04, 0.77)
71 0.38 (−0.01, 0.73)
70 0.40 (0.10, 0.80)
69 0.41 (0.14, 0.77)
68 0.38 (0.00, 0.74)
67 0.37 (0.01, 0.64)
66 0.40 (0.13, 0.69)
64 0.38 (0.00, 0.76)
62 0.33 (−0.07, 0.53)
61 0.37 (0.03, 0.64)
59 0.39 (0.11, 0.69)
58 0.32 (−0.15, 0.53)
56 0.37 (0.03, 0.63)
55 0.38 (0.00, 0.72)
54 0.35 (−0.02, 0.58)
53 0.43 (0.24, 0.77)
52 0.42 (0.19, 0.74)
51 0.34 (−0.04, 0.54)
45 0.36 (−0.05, 0.63)
44 0.40 (0.10, 0.77)
42 0.42 (0.21, 0.79)
40 0.43 (0.21, 0.91)
39 0.34 (−0.05, 0.54)
35 0.43 (0.18, 0.90)
34 0.38 (−0.02, 0.72)
33 0.41 (0.12, 0.82)
31 0.45 (0.25, 0.86)
26 0.37 (−0.05, 0.70)
18 0.38 (0.07, 0.67)
16 0.39 (0.16, 0.63)
11 0.40 (0.09, 0.82)
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

C)


HPV Type
Log(OR) (95% CI)
89 0.41 (−0.28, 1.44)
84 0.41 (−0.21, 1.30)
83 0.24 (−1.10, 0.94)
82 0.35 (−0.59, 1.32)
81 0.31 (−1.91, 1.22)
73 0.33 (−0.68, 1.18)
72 0.32 (−0.90, 1.27)
71 0.33 (−0.85, 1.32)
70 0.37 (−0.50, 1.50)
69 0.24 (−1.17, 0.89)
68 0.33 (−0.85, 1.36)
67 0.35 (−0.60, 1.31)
66 0.36 (−0.46, 1.22)
64 0.33 (−0.83, 1.38)
62 0.41 (−0.27, 1.44)
61 0.33 (−0.66, 1.21)
59 0.39 (−0.38, 1.36)
58 0.21 (−1.25, 0.83)
56 0.37 (−0.42, 1.29)
55 0.40 (−0.44, 1.62)
54 0.26 (−0.88, 0.91)
53 0.17 (−1.13, 0.72)
52 0.29 (−0.71, 0.97)
51 0.09 (−1.49, 0.62)
45 0.27 (−1.06, 1.00)
44 0.45 (−0.23, 1.91)
42 0.20 (−1.23, 0.82)
40 0.36 (−0.56, 1.39)
39 0.42 (−1.26, 1.36)
35 0.29 (−1.02, 1.13)
34 0.33 (−0.86, 1.34)
33 0.36 (−0.58, 1.32)
31 0.26 (−0.91, 0.89)
26 0.32 (−0.87, 1.30)
18 0.45 (−0.18, 1.51)
16 0.31 (−0.50, 0.91)
6 0.41 (−0.26, 1.35)
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

D)


HPV Type
Log(OR) (95% CI)
89
0.49 (0.22, 0.74)
84
0.52 (0.30, 0.74)
83
0.78 (0.47, 1.20)
82
0.52 (0.19, 0.86)
81
0.55 (0.21, 0.94)
73
0.64 (0.38, 0.96)
72
0.53 (0.12, 0.94)
71
0.50 (0.08, 0.89)
70
0.25 (−0.28, 0.59)
69
0.50 (0.03, 0.93)
68
0.18 (−0.29, 0.52)
67
0.44 (0.09, 0.74)
66
0.61 (0.37, 0.88)
64
0.51 (0.05, 0.96)
62
0.43 (0.15, 0.68)
61
0.32 (−0.06, 0.60)
59
0.50 (0.21, 0.76)
58
0.52 (0.27, 0.77)
57
0.49 (0.02, 0.77)
56
0.46 (0.17, 0.71)
55
0.54 (0.15, 0.96)
54
0.52 (0.27, 0.77)
53
0.69 (0.48, 0.93)
52
0.42 (0.16, 0.65)
51
0.48 (0.24, 0.69)
45
0.58 (0.30, 0.90)
44
0.46 (0.10, 0.77)
42
0.44 (0.16, 0.69)
40
0.34 (−0.08, 0.64)
39
0.69 (0.45, 0.97)
35
0.72 (0.43, 1.12)
34
0.50 (0.05, 0.93)
33
0.48 (0.13, 0.78)
32
0.49 (0.02, 0.92)
31
0.58 (0.35, 0.83)
26
0.60 (0.23, 0.77)
18
0.51 (0.25, 0.77)
6 or 11
0.54 (0.30, 0.78)
-1
-0.5
0
0.5
1
1.5
2
Log (Odds Ratio)

E)

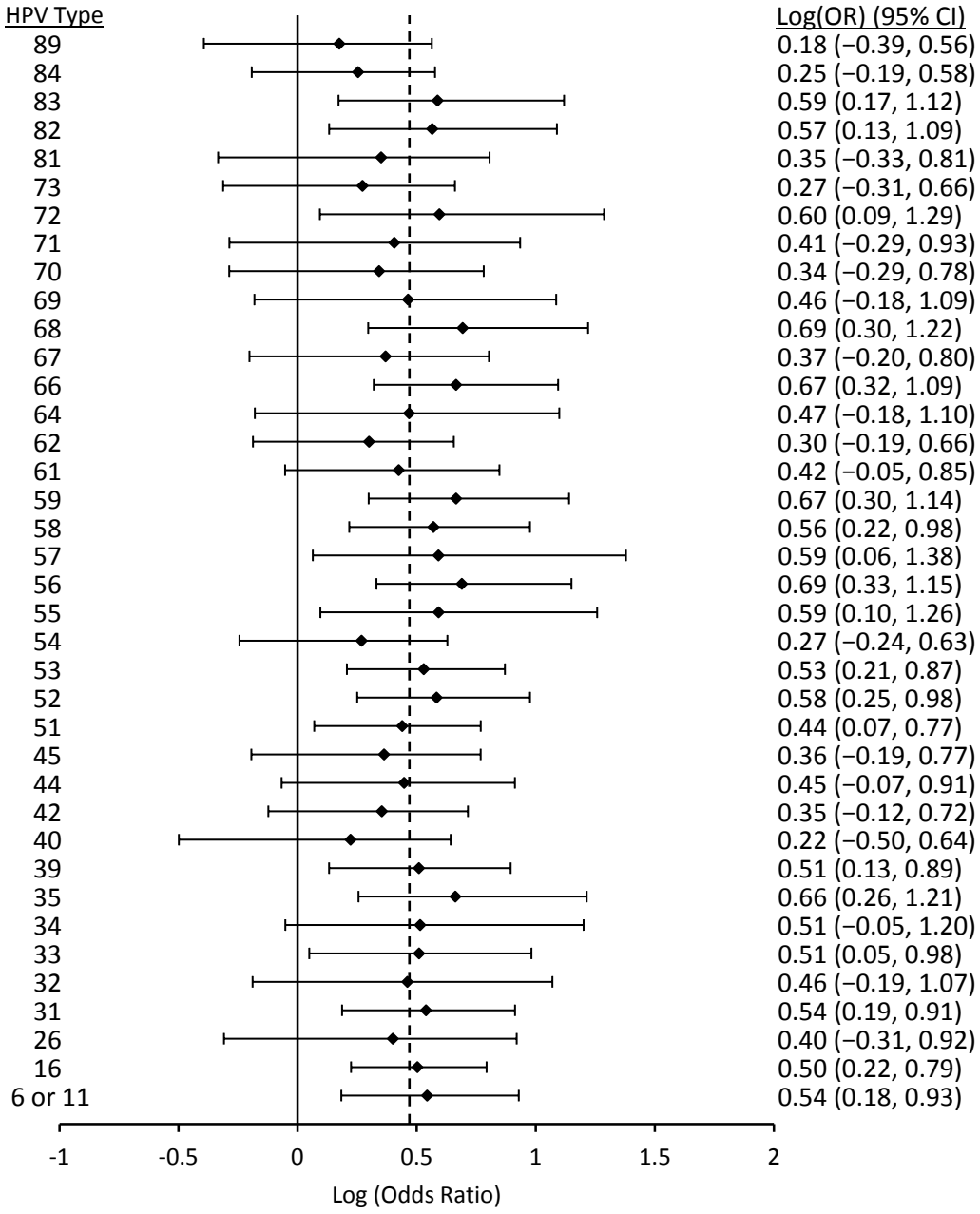
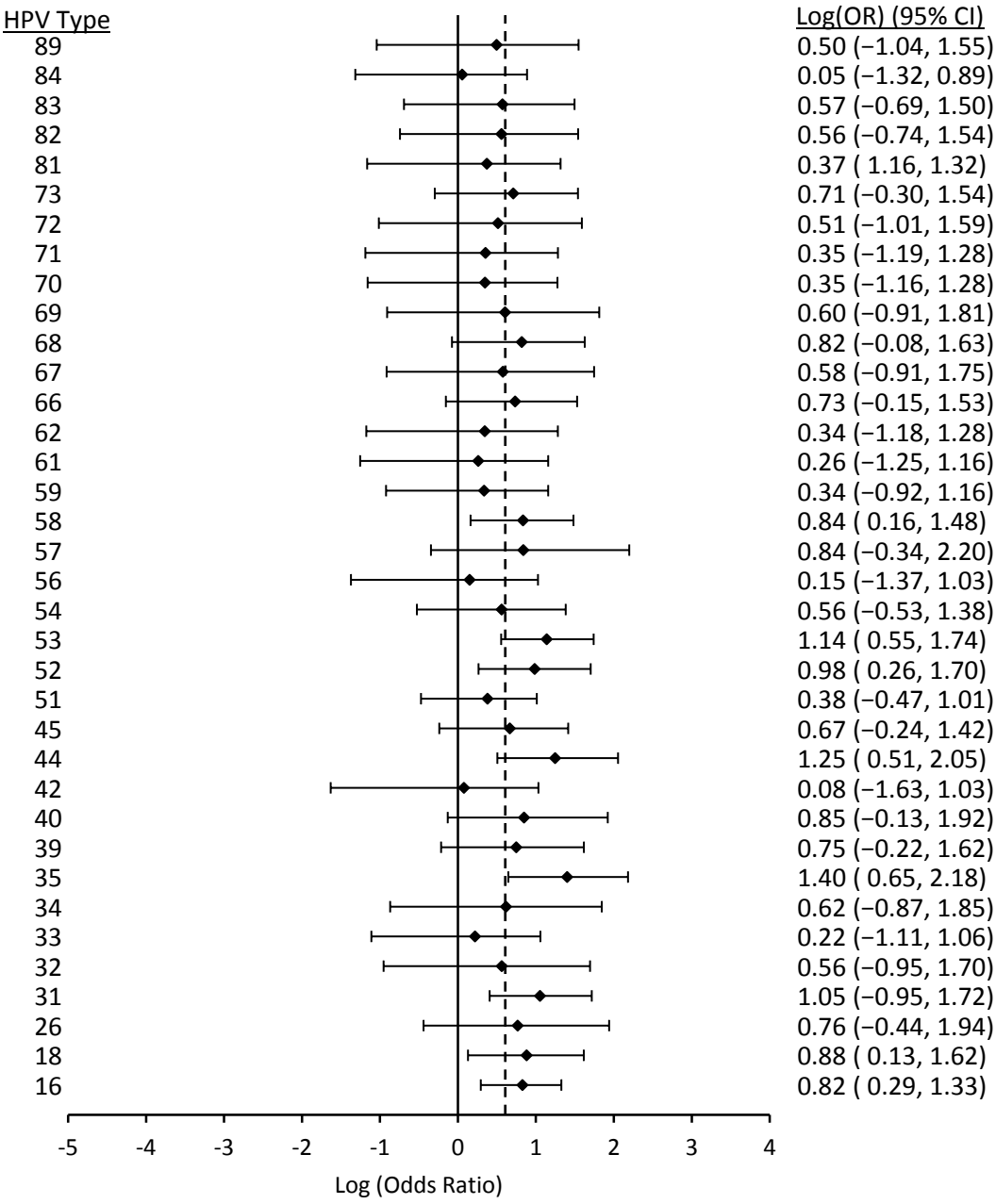


Figure 5-3:

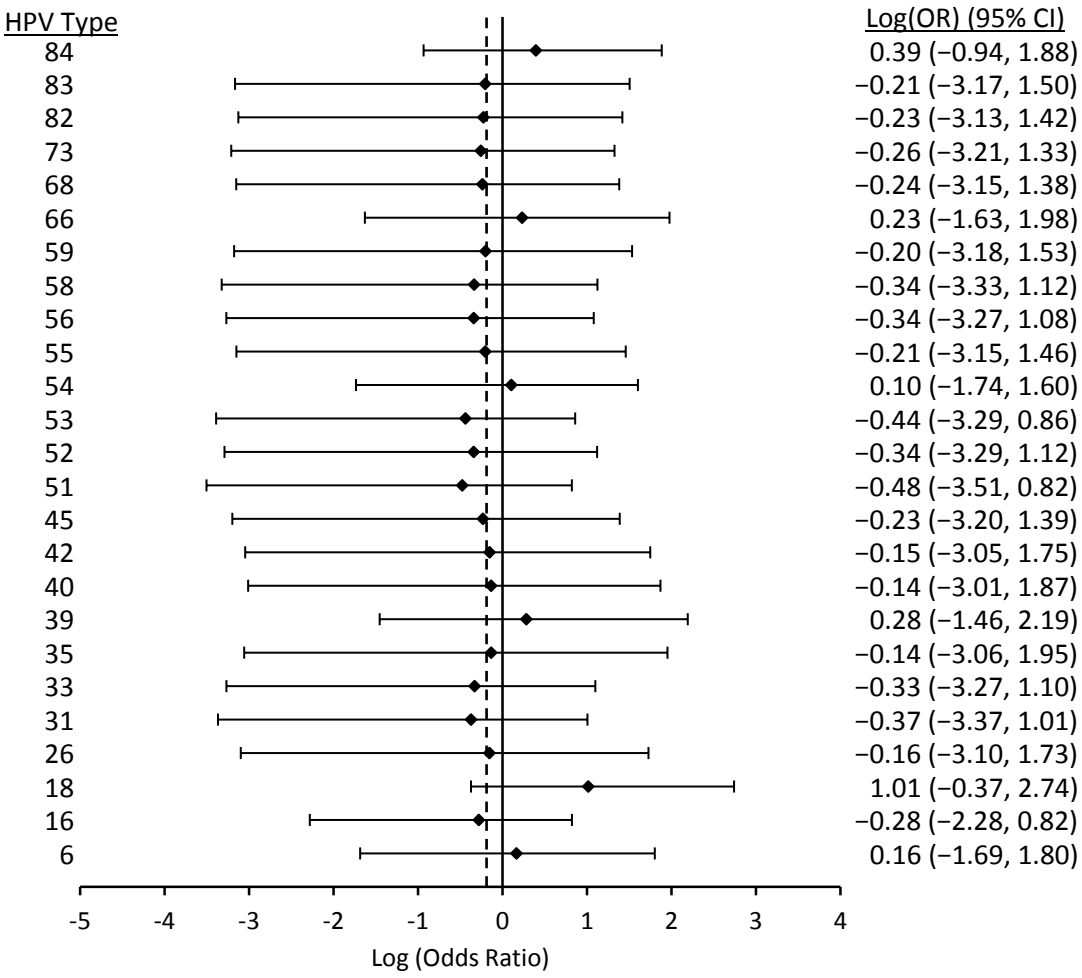
Log (odds ratios) and 95% confidence intervals for HPVs 6/11, 6, 11, 16 and 18 for co-infection with other HPV types (panels A-E, respecively). Estimates were obtained from logistic regression models adjusted for all other HPV types, and age only. In panels A-E, the dashed lines represent the average

pooled log(OR) from hierarchical logistic regression, which were 0.43 (95%CI: 0.30-0.56), 0.38 (95%CI: 0.28-0.48), 0.26 (95%CI: -0.02-0.56), 0.51 (95%CI: 0.41-0.60), and 0.47 (95%CI: 0.33-0.60), respectively. All analyses included pooled results from Ludwig-McGill (except for panels B and C; due to our inability to distinguish between HPVs 6 and11), McGill-Concordia, HITCH, BCCR, and CCCaST studies.

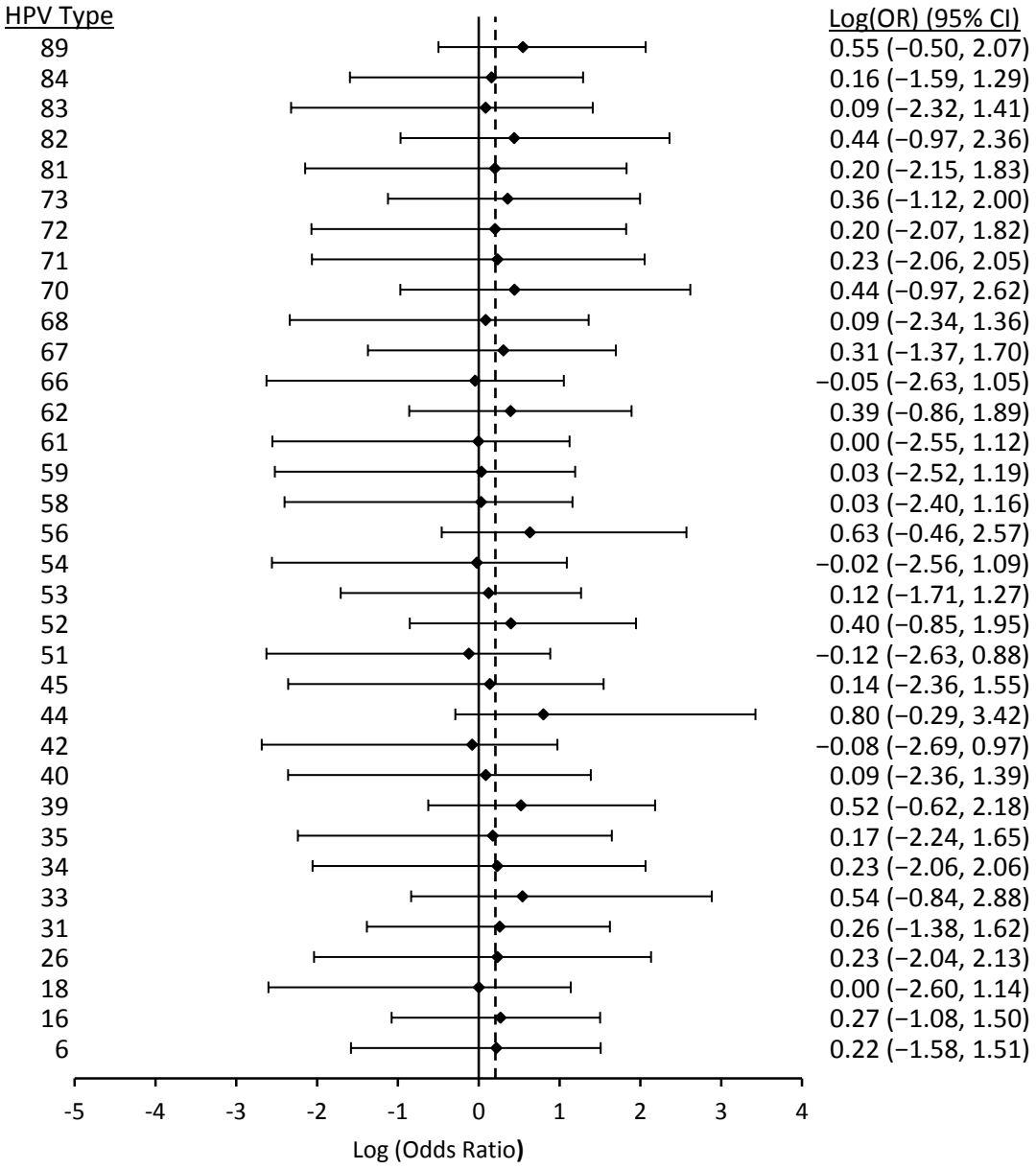
A)


HPV Type
Log(OR) (95% CI)
89 0.50 (−1.04, 1.55)
84 0.05 (−1.32, 0.89)
83 0.57 (−0.69, 1.50)
82 0.56 (−0.74, 1.54)
81 0.37 (1.16, 1.32)
73 0.71 (−0.30, 1.54)
72 0.51 (−1.01, 1.59)
71 0.35 (−1.19, 1.28)
70 0.35 (−1.16, 1.28)
69 0.60 (−0.91, 1.81)
68 0.82 (−0.08, 1.63)
67 0.58 (−0.91, 1.75)
66 0.73 (−0.15, 1.53)
62 0.34 (−1.18, 1.28)
61 0.26 (−1.25, 1.16)
59 0.34 (−0.92, 1.16)
58 0.84 (0.16, 1.48)
57 0.84 (−0.34, 2.20)
56 0.15 (−1.37, 1.03)
54 0.56 (−0.53, 1.38)
53 1.14 (0.55, 1.74)
52 0.98 (0.26, 1.70)
51 0.38 (−0.47, 1.01)
45 0.67 (−0.24, 1.42)
44 1.25 (0.51, 2.05)
42 0.08 (−1.63, 1.03)
40 0.85 (−0.13, 1.92)
39 0.75 (−0.22, 1.62)
35 1.40 (0.65, 2.18)
34 0.62 (−0.87, 1.85)
33 0.22 (−1.11, 1.06)
32 0.56 (−0.95, 1.70)
31 1.05 (−0.95, 1.72)
26 0.76 (−0.44, 1.94)
18 0.88 (0.13, 1.62)
16 0.82 (0.29, 1.33)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

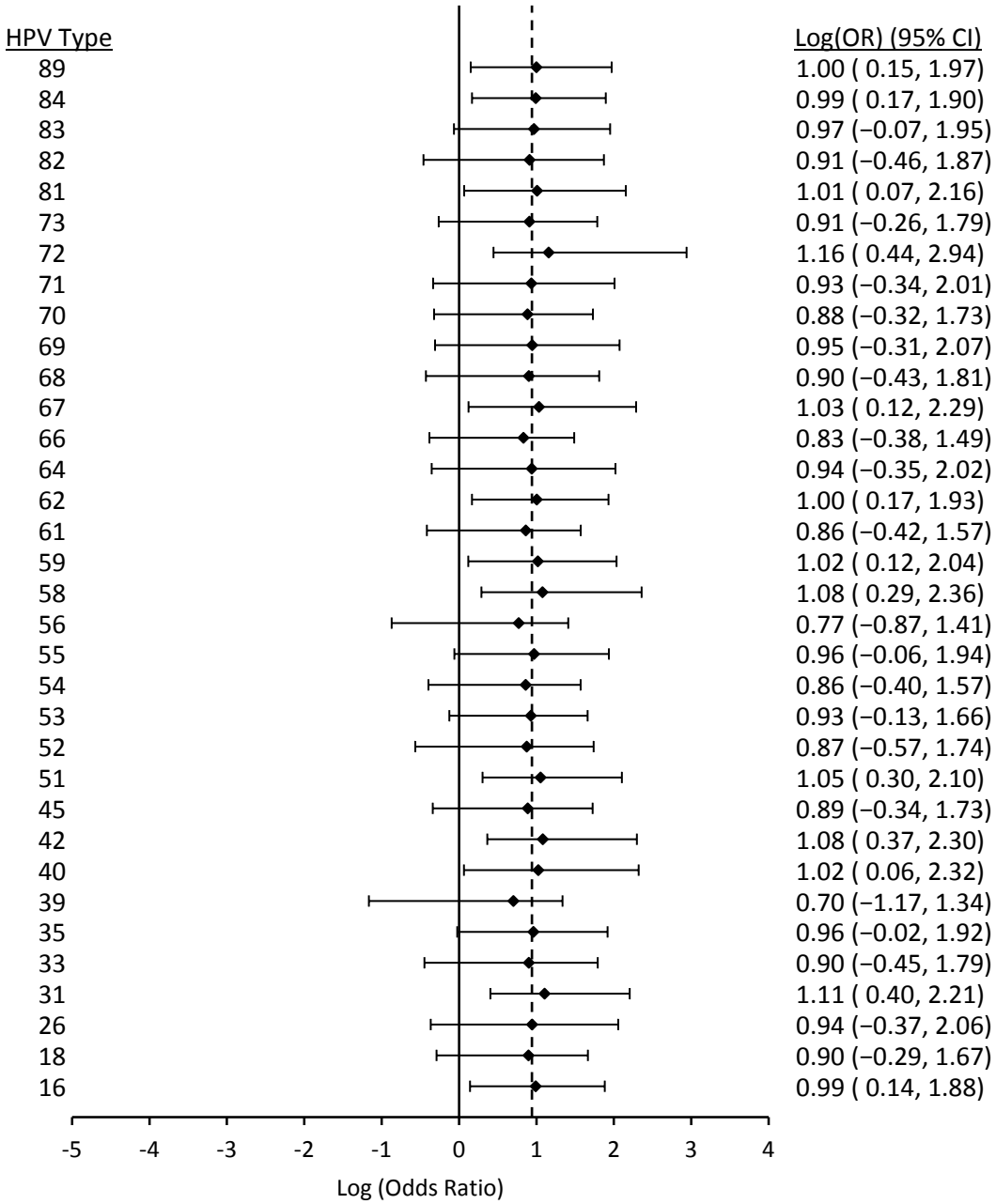
B)


HPV Type
Log(OR) (95% CI)
84 0.39 (−0.94, 1.88)
83 −0.21 (−3.17, 1.50)
82 −0.23 (−3.13, 1.42)
73 −0.26 (−3.21, 1.33)
68 −0.24 (−3.15, 1.38)
66 0.23 (−1.63, 1.98)
59 −0.20 (−3.18, 1.53)
58 −0.34 (−3.33, 1.12)
56 −0.34 (−3.27, 1.08)
55 −0.21 (−3.15, 1.46)
54 0.10 (−1.74, 1.60)
53 −0.44 (−3.29, 0.86)
52 −0.34 (−3.29, 1.12)
51 −0.48 (−3.51, 0.82)
45 −0.23 (−3.20, 1.39)
42 −0.15 (−3.05, 1.75)
40 −0.14 (−3.01, 1.87)
39 0.28 (−1.46, 2.19)
35 −0.14 (−3.06, 1.95)
33 −0.33 (−3.27, 1.10)
31 −0.37 (−3.37, 1.01)
26 −0.16 (−3.10, 1.73)
18 1.01 (−0.37, 2.74)
16 −0.28 (−2.28, 0.82)
6 0.16 (−1.69, 1.80)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

C)


HPV Type
Log(OR) (95% CI)
89 0.55 (−0.50, 2.07)
84 0.16 (−1.59, 1.29)
83 0.09 (−2.32, 1.41)
82 0.44 (−0.97, 2.36)
81 0.20 (−2.15, 1.83)
73 0.36 (−1.12, 2.00)
72 0.20 (−2.07, 1.82)
71 0.23 (−2.06, 2.05)
70 0.44 (−0.97, 2.62)
68 0.09 (−2.34, 1.36)
67 0.31 (−1.37, 1.70)
66 −0.05 (−2.63, 1.05)
62 0.39 (−0.86, 1.89)
61 0.00 (−2.55, 1.12)
59 0.03 (−2.52, 1.19)
58 0.03 (−2.40, 1.16)
56 0.63 (−0.46, 2.57)
54 −0.02 (−2.56, 1.09)
53 0.12 (−1.71, 1.27)
52 0.40 (−0.85, 1.95)
51 −0.12 (−2.63, 0.88)
45 0.14 (−2.36, 1.55)
44 0.80 (−0.29, 3.42)
42 −0.08 (−2.69, 0.97)
40 0.09 (−2.36, 1.39)
39 0.52 (−0.62, 2.18)
35 0.17 (−2.24, 1.65)
34 0.23 (−2.06, 2.06)
33 0.54 (−0.84, 2.88)
31 0.26 (−1.38, 1.62)
26 0.23 (−2.04, 2.13)
18 0.00 (−2.60, 1.14)
16 0.27 (−1.08, 1.50)
6 0.22 (−1.58, 1.51)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

D)


HPV Type
Log(OR) (95% CI)
89 1.00 (0.15, 1.97)
84 0.99 (0.17, 1.90)
83 0.97 (−0.07, 1.95)
82 0.91 (−0.46, 1.87)
81 1.01 (0.07, 2.16)
73 0.91 (−0.26, 1.79)
72 1.16 (0.44, 2.94)
71 0.93 (−0.34, 2.01)
70 0.88 (−0.32, 1.73)
69 0.95 (−0.31, 2.07)
68 0.90 (−0.43, 1.81)
67 1.03 (0.12, 2.29)
66 0.83 (−0.38, 1.49)
64 0.94 (−0.35, 2.02)
62 1.00 (0.17, 1.93)
61 0.86 (−0.42, 1.57)
59 1.02 (0.12, 2.04)
58 1.08 (0.29, 2.36)
56 0.77 (−0.87, 1.41)
55 0.96 (−0.06, 1.94)
54 0.86 (−0.40, 1.57)
53 0.93 (−0.13, 1.66)
52 0.87 (−0.57, 1.74)
51 1.05 (0.30, 2.10)
45 0.89 (−0.34, 1.73)
42 1.08 (0.37, 2.30)
40 1.02 (0.06, 2.32)
39 0.70 (−1.17, 1.34)
35 0.96 (−0.02, 1.92)
33 0.90 (−0.45, 1.79)
31 1.11 (0.40, 2.21)
26 0.94 (−0.37, 2.06)
18 0.90 (−0.29, 1.67)
16 0.99 (0.14, 1.88)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

E)

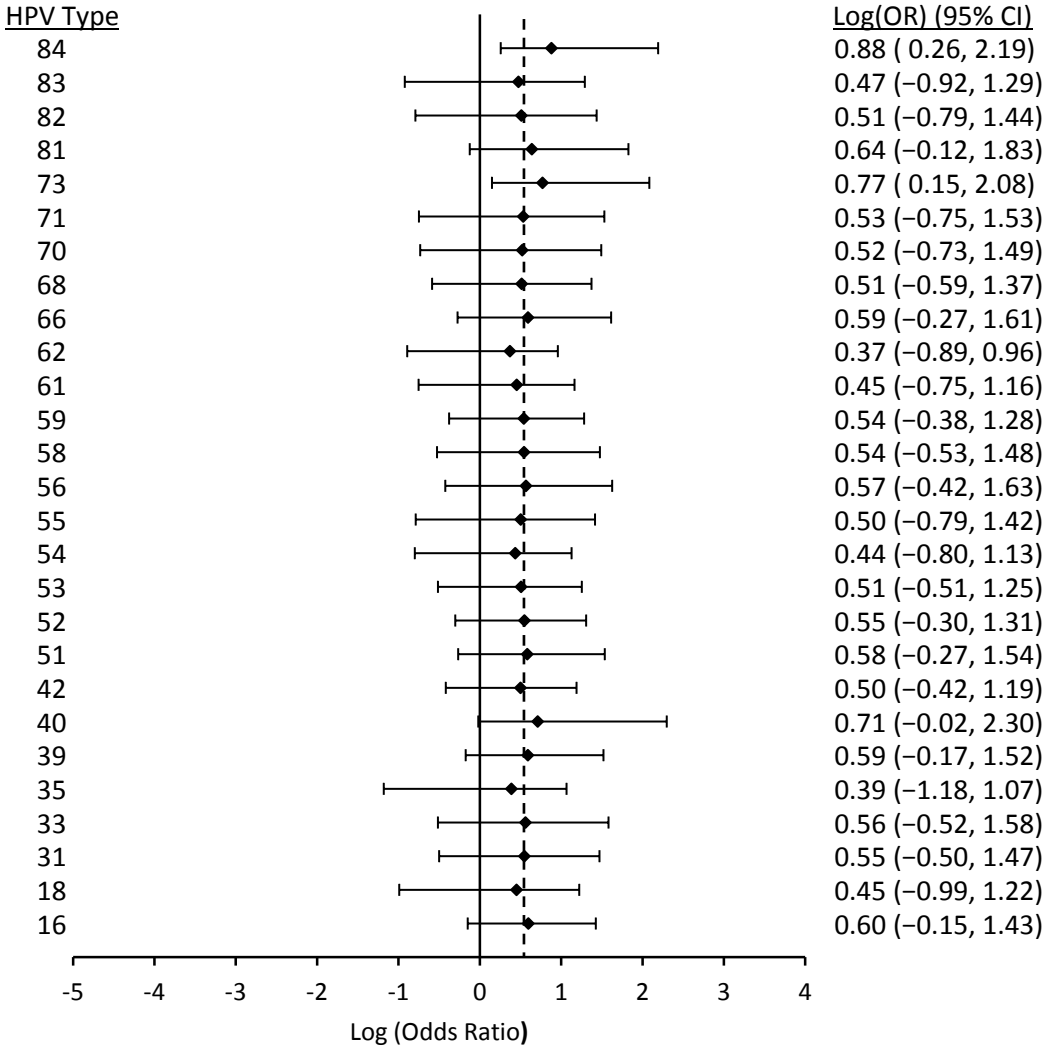
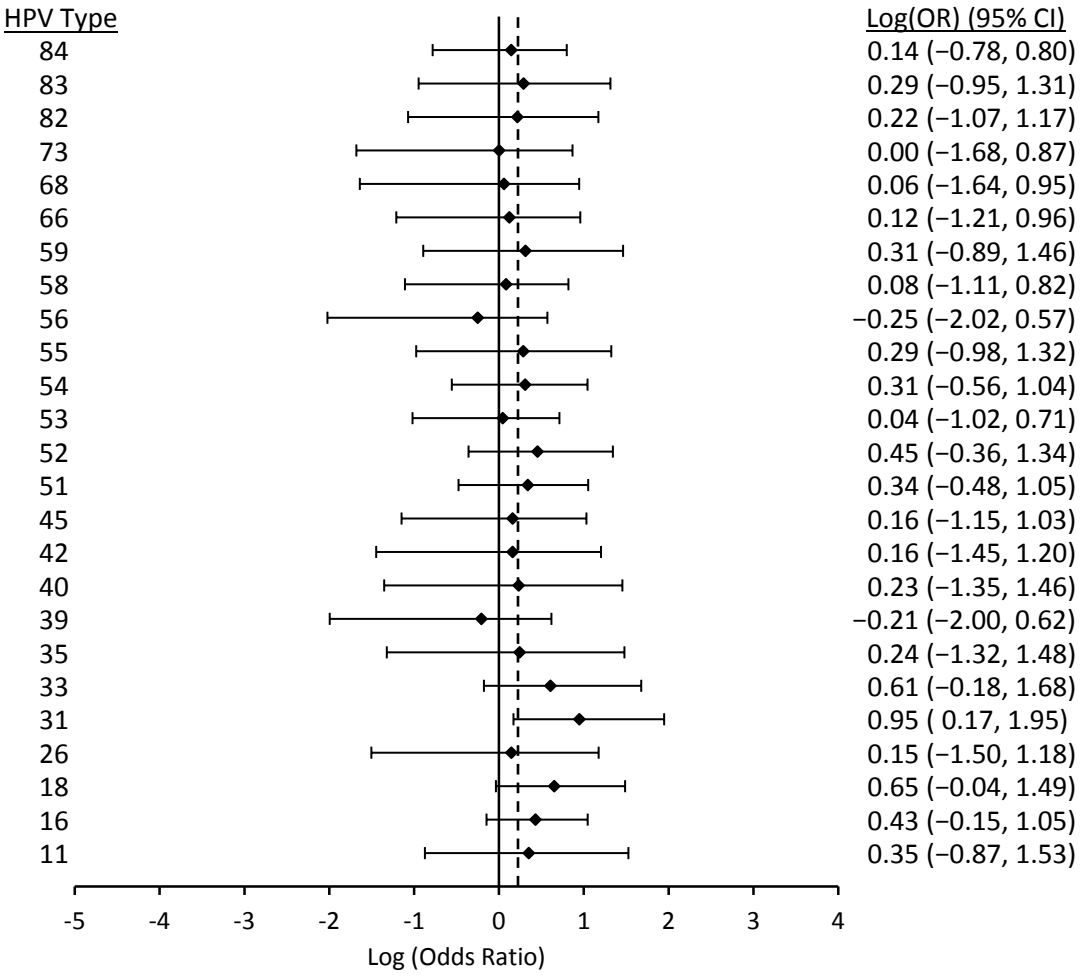
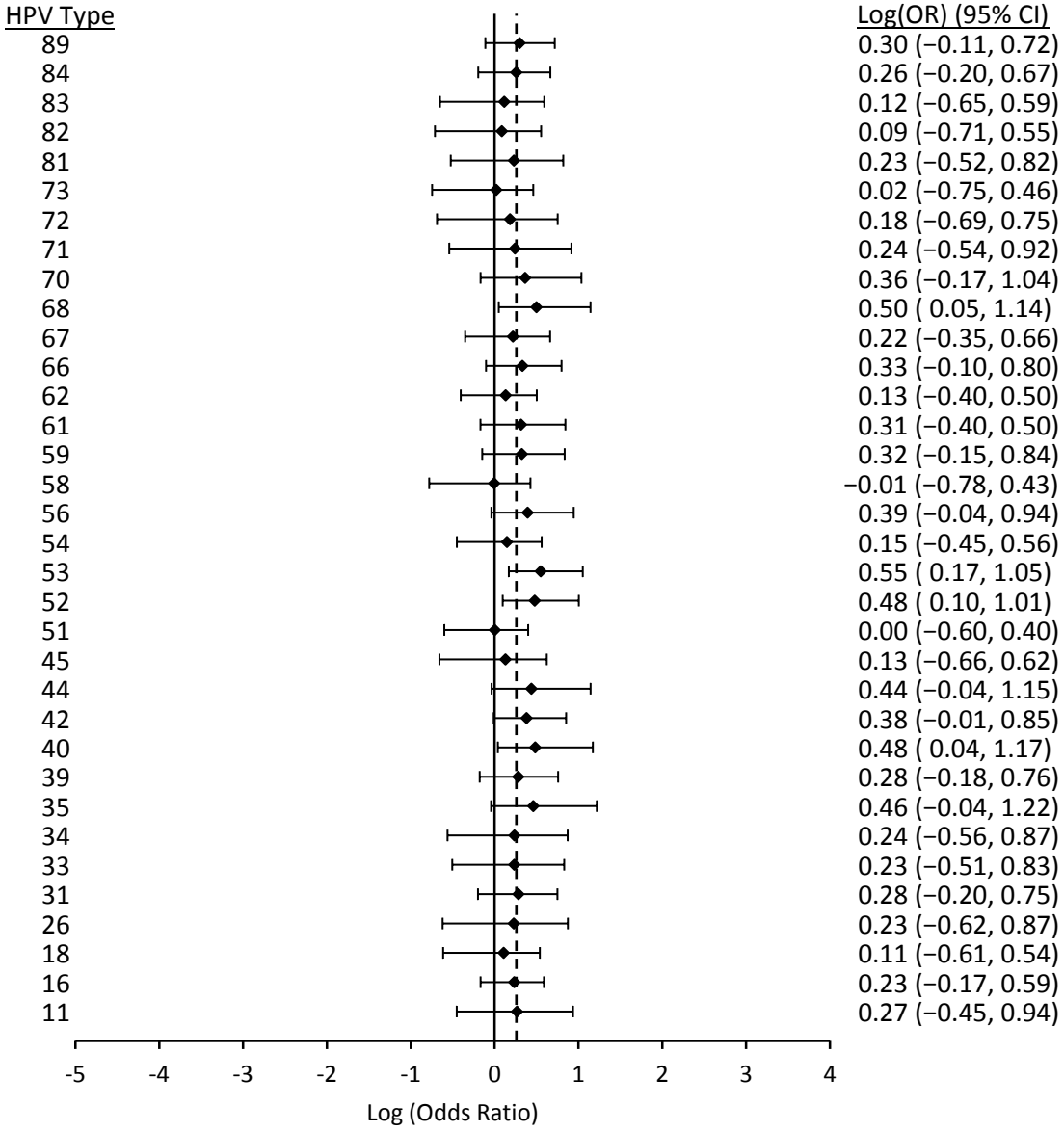


Figure 5-4: Log (odds ratios) and 95% confidence intervals for HPV6/11 with other HPV types from the Ludwig-McGill, McGill-Concordia, HITCH, BCCR, and CCCaST studies (panels A-E, respecively). Estimates were obtained from logistic regression models adjusted for all other HPV types, age, and lifetime number of sexual partners (except CCCaST; adjusted for other HPV types and age only). In panels A-E, the dashed lines represent the average pooled log(OR) from hierarchical logistic regression, which were 0.61 (95%CI: 0.18-0.88), 0.19 (95%CI: -0.31-0.51), 0.27 (95%CI: 0.08-0.45), 0.96 (95%CI: 0.54-1.39), and 0.50 (95%CI: -0.30-1.088), respectively.

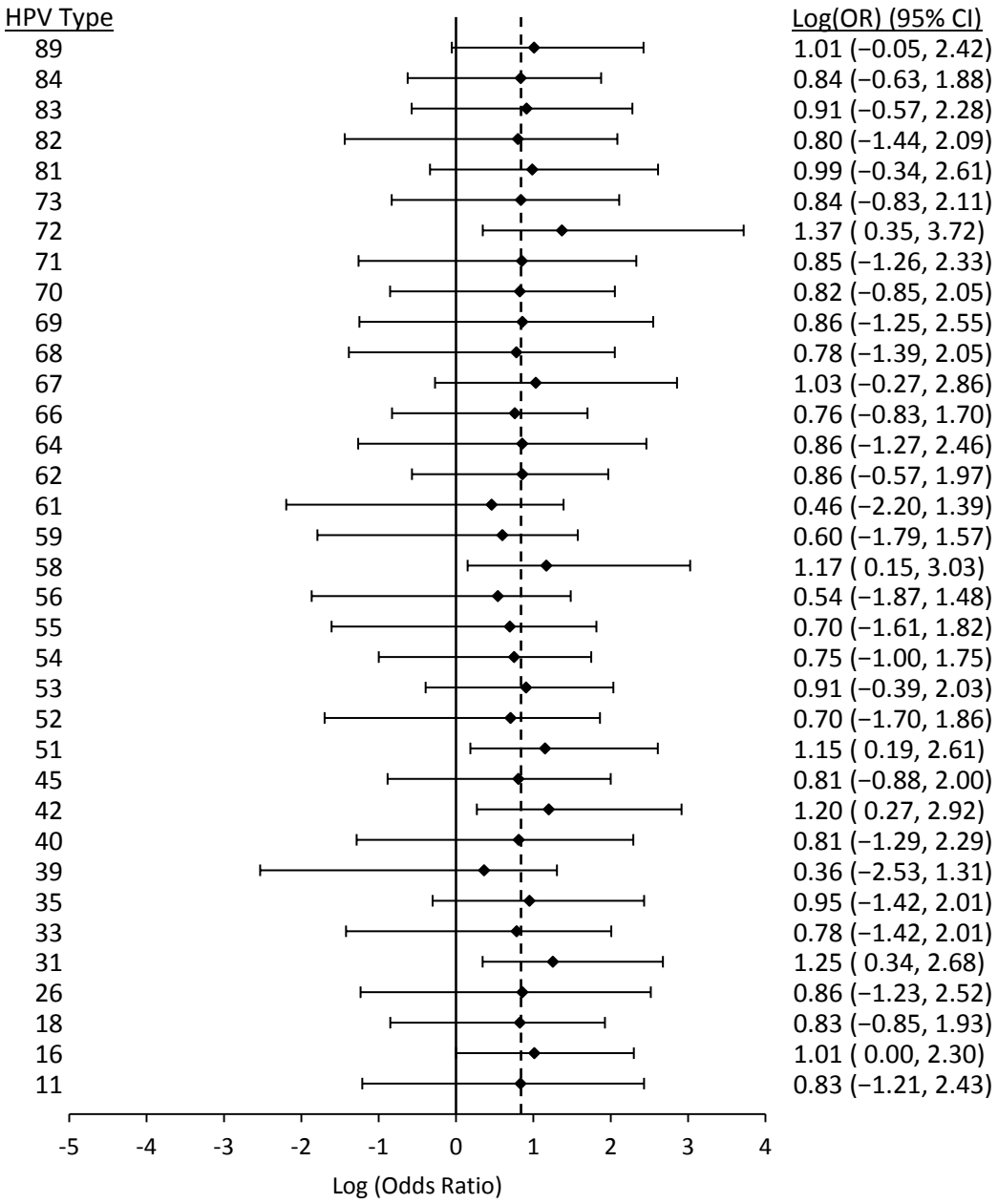
A)


HPV Type
Log(OR) (95% CI)
84
0.14 (−0.78, 0.80)
83
0.29 (−0.95, 1.31)
82
0.22 (−1.07, 1.17)
73
0.00 (−1.68, 0.87)
68
0.06 (−1.64, 0.95)
66
0.12 (−1.21, 0.96)
59
0.31 (−0.89, 1.46)
58
0.08 (−1.11, 0.82)
56
−0.25 (−2.02, 0.57)
55
0.29 (−0.98, 1.32)
54
0.31 (−0.56, 1.04)
53
0.04 (−1.02, 0.71)
52
0.45 (−0.36, 1.34)
51
0.34 (−0.48, 1.05)
45
0.16 (−1.15, 1.03)
42
0.16 (−1.45, 1.20)
40
0.23 (−1.35, 1.46)
39
−0.21 (−2.00, 0.62)
35
0.24 (−1.32, 1.48)
33
0.61 (−0.18, 1.68)
31
0.95 (0.17, 1.95)
26
0.15 (−1.50, 1.18)
18
0.65 (−0.04, 1.49)
16
0.43 (−0.15, 1.05)
11
0.35 (−0.87, 1.53)
-5
-4
-3
-2
-1
0
1
2
3
4
Log (Odds Ratio)

B)


HPV Type
Log(OR) (95% CI)
89 0.30 (−0.11, 0.72)
84 0.26 (−0.20, 0.67)
83 0.12 (−0.65, 0.59)
82 0.09 (−0.71, 0.55)
81 0.23 (−0.52, 0.82)
73 0.02 (−0.75, 0.46)
72 0.18 (−0.69, 0.75)
71 0.24 (−0.54, 0.92)
70 0.36 (−0.17, 1.04)
68 0.50 (0.05, 1.14)
67 0.22 (−0.35, 0.66)
66 0.33 (−0.10, 0.80)
62 0.13 (−0.40, 0.50)
61 0.31 (−0.40, 0.50)
59 0.32 (−0.15, 0.84)
58 −0.01 (−0.78, 0.43)
56 0.39 (−0.04, 0.94)
54 0.15 (−0.45, 0.56)
53 0.55 (0.17, 1.05)
52 0.48 (0.10, 1.01)
51 0.00 (−0.60, 0.40)
45 0.13 (−0.66, 0.62)
44 0.44 (−0.04, 1.15)
42 0.38 (−0.01, 0.85)
40 0.48 (0.04, 1.17)
39 0.28 (−0.18, 0.76)
35 0.46 (−0.04, 1.22)
34 0.24 (−0.56, 0.87)
33 0.23 (−0.51, 0.83)
31 0.28 (−0.20, 0.75)
26 0.23 (−0.62, 0.87)
18 0.11 (−0.61, 0.54)
16 0.23 (−0.17, 0.59)
11 0.27 (−0.45, 0.94)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

C)


HPV Type
Log(OR) (95% CI)
89 1.01 (−0.05, 2.42)
84 0.84 (−0.63, 1.88)
83 0.91 (−0.57, 2.28)
82 0.80 (−1.44, 2.09)
81 0.99 (−0.34, 2.61)
73 0.84 (−0.83, 2.11)
72 1.37 (0.35, 3.72)
71 0.85 (−1.26, 2.33)
70 0.82 (−0.85, 2.05)
69 0.86 (−1.25, 2.55)
68 0.78 (−1.39, 2.05)
67 1.03 (−0.27, 2.86)
66 0.76 (−0.83, 1.70)
64 0.86 (−1.27, 2.46)
62 0.86 (−0.57, 1.97)
61 0.46 (−2.20, 1.39)
59 0.60 (−1.79, 1.57)
58 1.17 (0.15, 3.03)
56 0.54 (−1.87, 1.48)
55 0.70 (−1.61, 1.82)
54 0.75 (−1.00, 1.75)
53 0.91 (−0.39, 2.03)
52 0.70 (−1.70, 1.86)
51 1.15 (0.19, 2.61)
45 0.81 (−0.88, 2.00)
42 1.20 (0.27, 2.92)
40 0.81 (−1.29, 2.29)
39 0.36 (−2.53, 1.31)
35 0.95 (−1.42, 2.01)
33 0.78 (−1.42, 2.01)
31 1.25 (0.34, 2.68)
26 0.86 (−1.23, 2.52)
18 0.83 (−0.85, 1.93)
16 1.01 (0.00, 2.30)
11 0.83 (−1.21, 2.43)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

D)

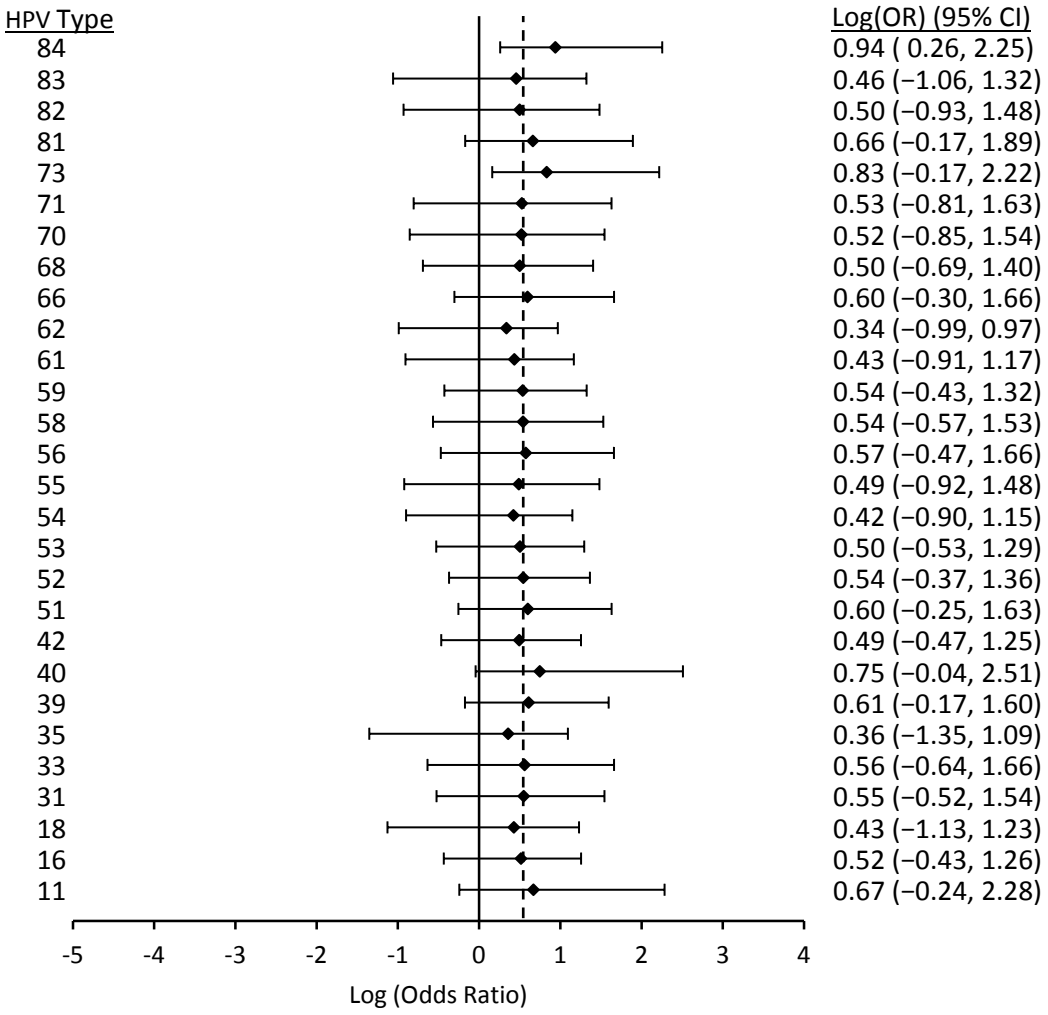
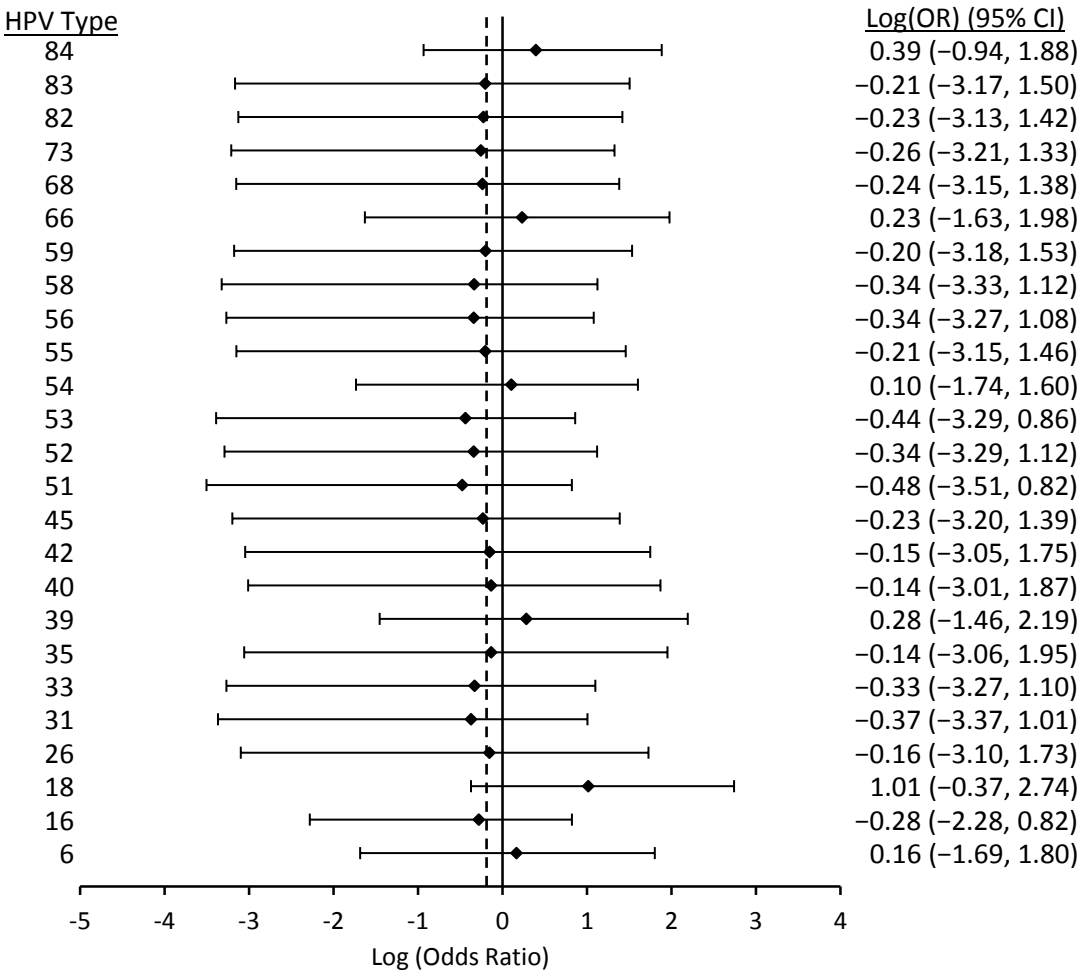


Figure 5-5: Log (odds ratios) and 95% confidence intervals for HPV6 with other HPV types from the McGill-Concordia, HITCH, BCCR, and CCCaST studies (panels A-D, respecively). Estimates were obtained from logistic regression models adjusted for all other HPV types, age, and lifetime number of sexual partners (except CCCaST; adjusted for other HPV types and age only). In panels A-D, the dashed lines represent the average pooled log(OR) from hierarchical logistic regression, which were 0.22 (95%CI: -0.30-0.56), 0.26 (95%CI: 0.07-0.41), 0.26 (95%CI: -0.02-0.56), 0.54 (95%CI: 0.14-0.91), and 0.84 (95%CI: 0.25-1.18), respectively.

A)


HPV Type
Log(OR) (95% CI)
84 0.39 (−0.94, 1.88)
83 −0.21 (−3.17, 1.50)
82 −0.23 (−3.13, 1.42)
73 −0.26 (−3.21, 1.33)
68 −0.24 (−3.15, 1.38)
66 0.23 (−1.63, 1.98)
59 −0.20 (−3.18, 1.53)
58 −0.34 (−3.33, 1.12)
56 −0.34 (−3.27, 1.08)
55 −0.21 (−3.15, 1.46)
54 0.10 (−1.74, 1.60)
53 −0.44 (−3.29, 0.86)
52 −0.34 (−3.29, 1.12)
51 −0.48 (−3.51, 0.82)
45 −0.23 (−3.20, 1.39)
42 −0.15 (−3.05, 1.75)
40 −0.14 (−3.01, 1.87)
39 0.28 (−1.46, 2.19)
35 −0.14 (−3.06, 1.95)
33 −0.33 (−3.27, 1.10)
31 −0.37 (−3.37, 1.01)
26 −0.16 (−3.10, 1.73)
18 1.01 (−0.37, 2.74)
16 −0.28 (−2.28, 0.82)
6 0.16 (−1.69, 1.80)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

B)

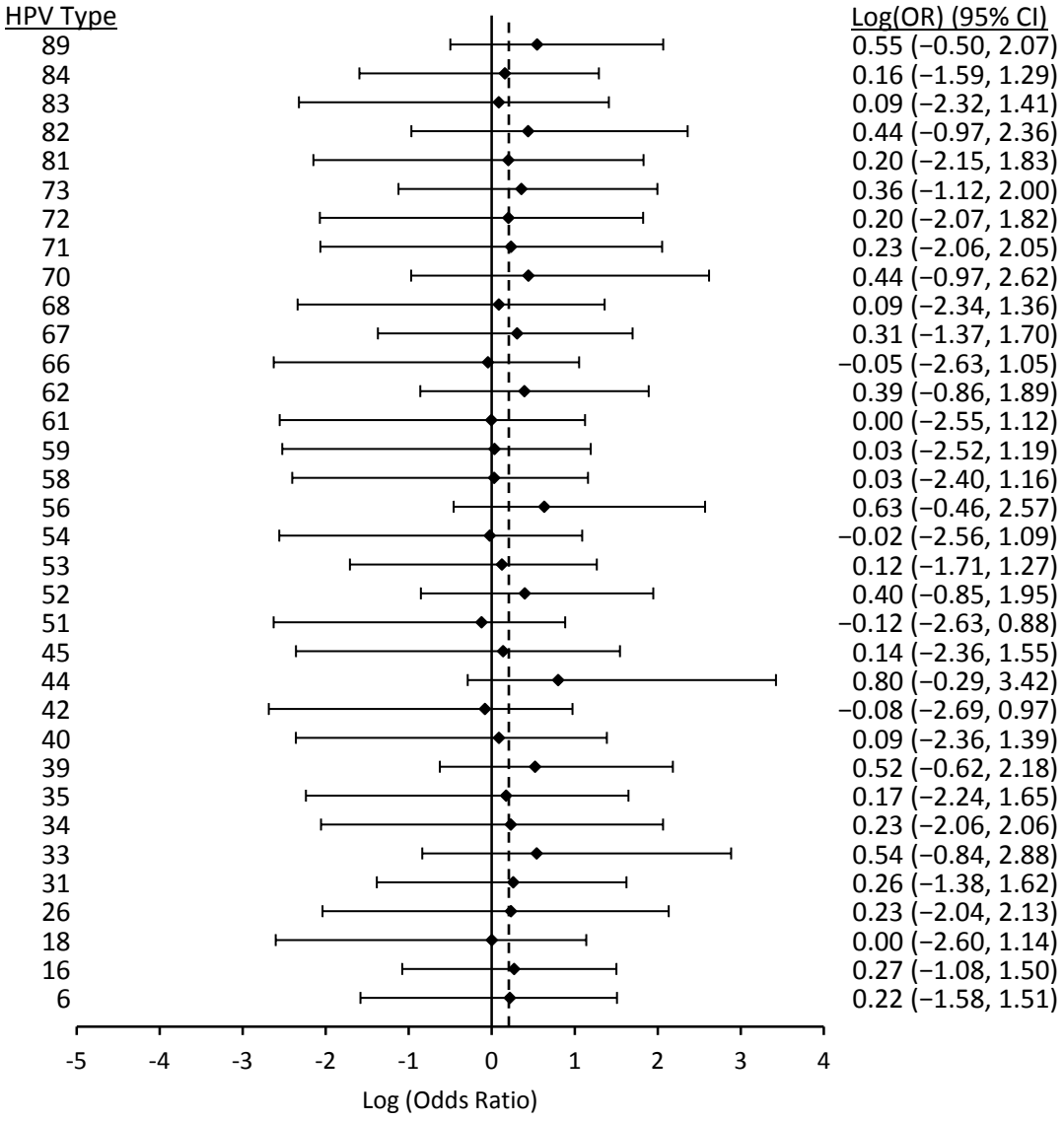
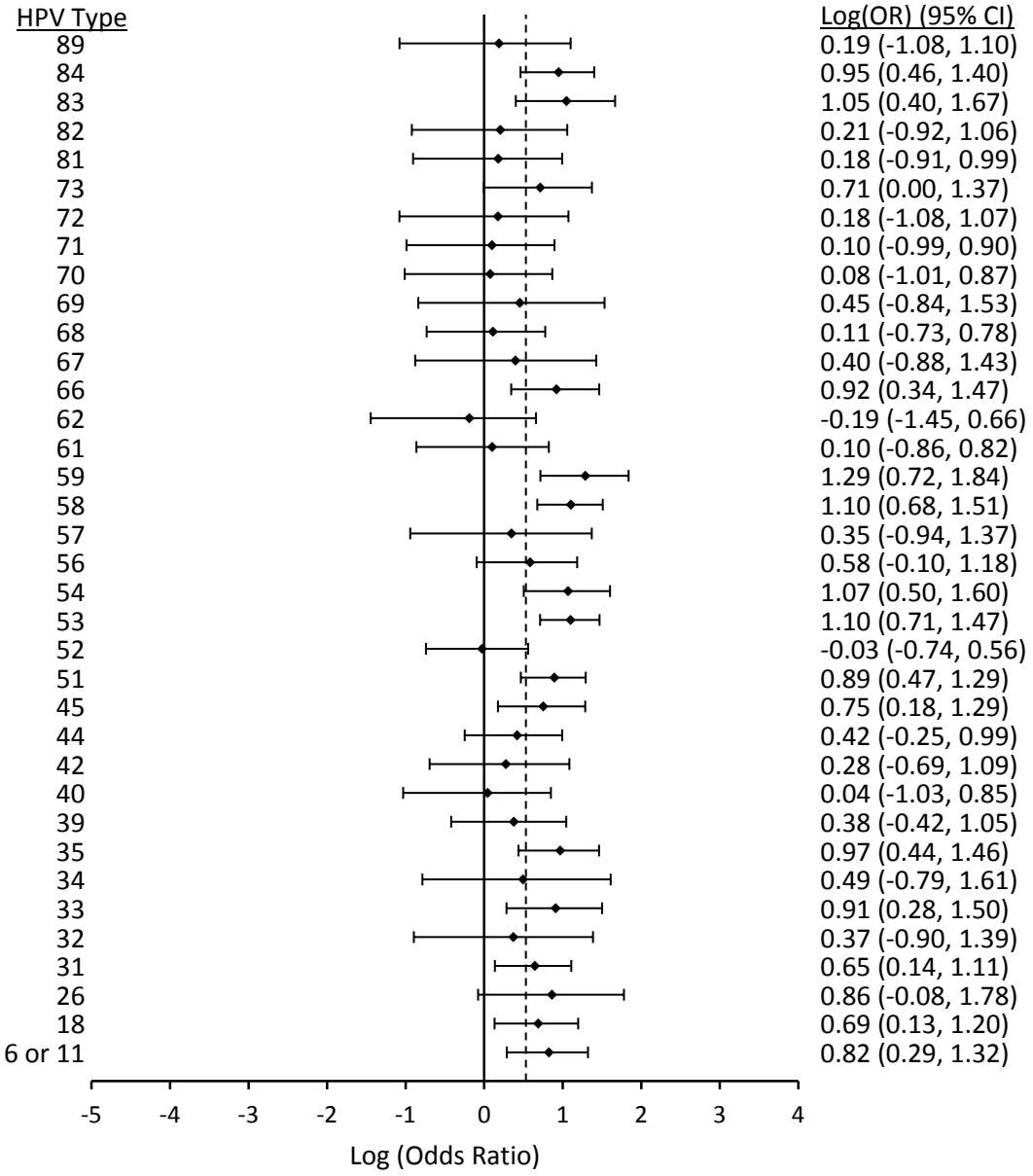
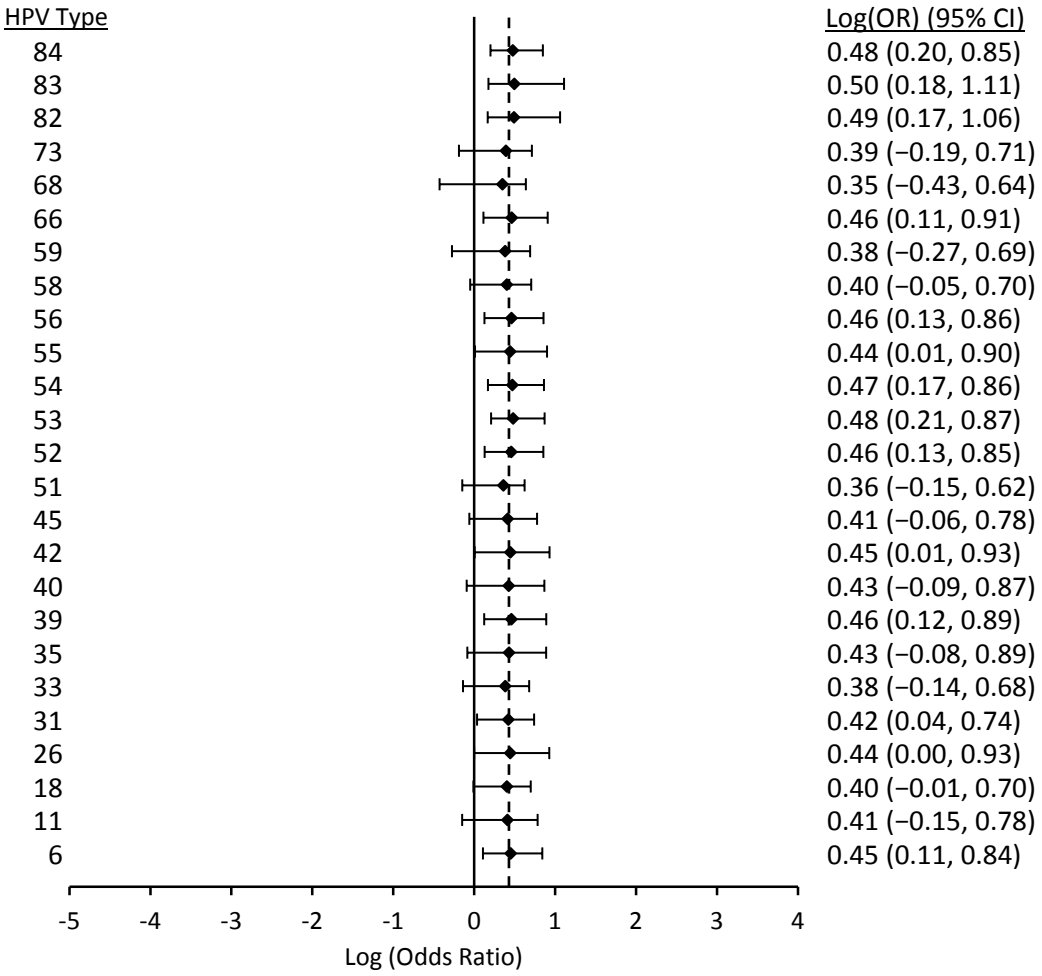


Figure 5-6: Log (odds ratios) and 95% confidence intervals for HPV11 with other HPV types from the McGill-Concordia and HITCH studies (panels A and B, respecively). Estimates were obtained from logistic regression models adjusted for all other HPV types, age, and lifetime number of sexual partners. In panels A and B, the dashed lines represent the average pooled log(OR) from hierarchical logistic regression, which were -0.19 (95%CI:-1.42-0.52) and 0.21 (95%CI: -0.48-0.60), respectively.

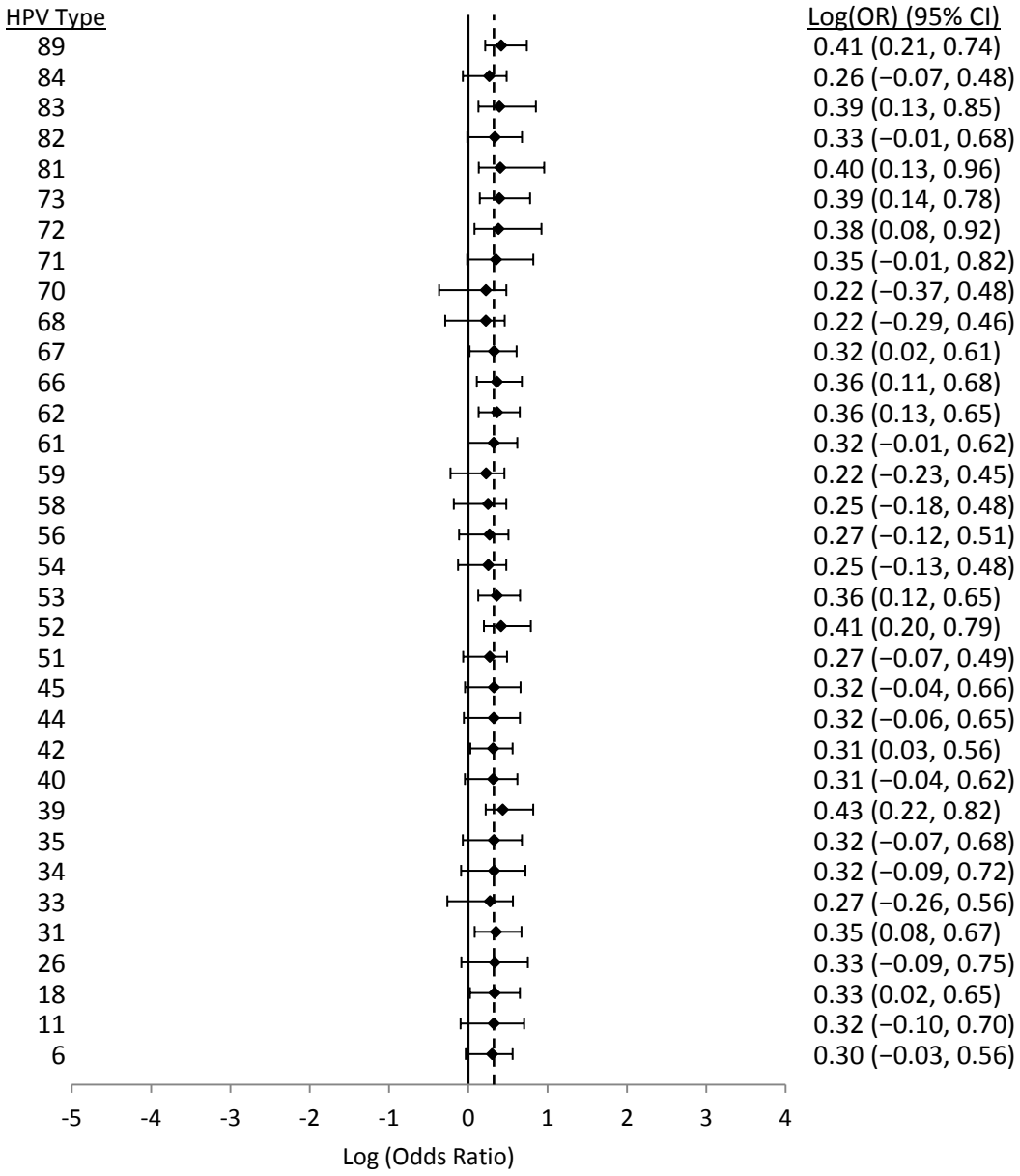
A)


HPV Type
Log(OR) (95% CI)
89 0.19 (-1.08, 1.10)
84 0.95 (0.46, 1.40)
83 1.05 (0.40, 1.67)
82 0.21 (-0.92, 1.06)
81 0.18 (-0.91, 0.99)
73 0.71 (0.00, 1.37)
72 0.18 (-1.08, 1.07)
71 0.10 (-0.99, 0.90)
70 0.08 (-1.01, 0.87)
69 0.45 (-0.84, 1.53)
68 0.11 (-0.73, 0.78)
67 0.40 (-0.88, 1.43)
66 0.92 (0.34, 1.47)
62 -0.19 (-1.45, 0.66)
61 0.10 (-0.86, 0.82)
59 1.29 (0.72, 1.84)
58 1.10 (0.68, 1.51)
57 0.35 (-0.94, 1.37)
56 0.58 (-0.10, 1.18)
54 1.07 (0.50, 1.60)
53 1.10 (0.71, 1.47)
52 -0.03 (-0.74, 0.56)
51 0.89 (0.47, 1.29)
45 0.75 (0.18, 1.29)
44 0.42 (-0.25, 0.99)
42 0.28 (-0.69, 1.09)
40 0.04 (-1.03, 0.85)
39 0.38 (-0.42, 1.05)
35 0.97 (0.44, 1.46)
34 0.49 (-0.79, 1.61)
33 0.91 (0.28, 1.50)
32 0.37 (-0.90, 1.39)
31 0.65 (0.14, 1.11)
26 0.86 (-0.08, 1.78)
18 0.69 (0.13, 1.20)
6 or 11 0.82 (0.29, 1.32)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

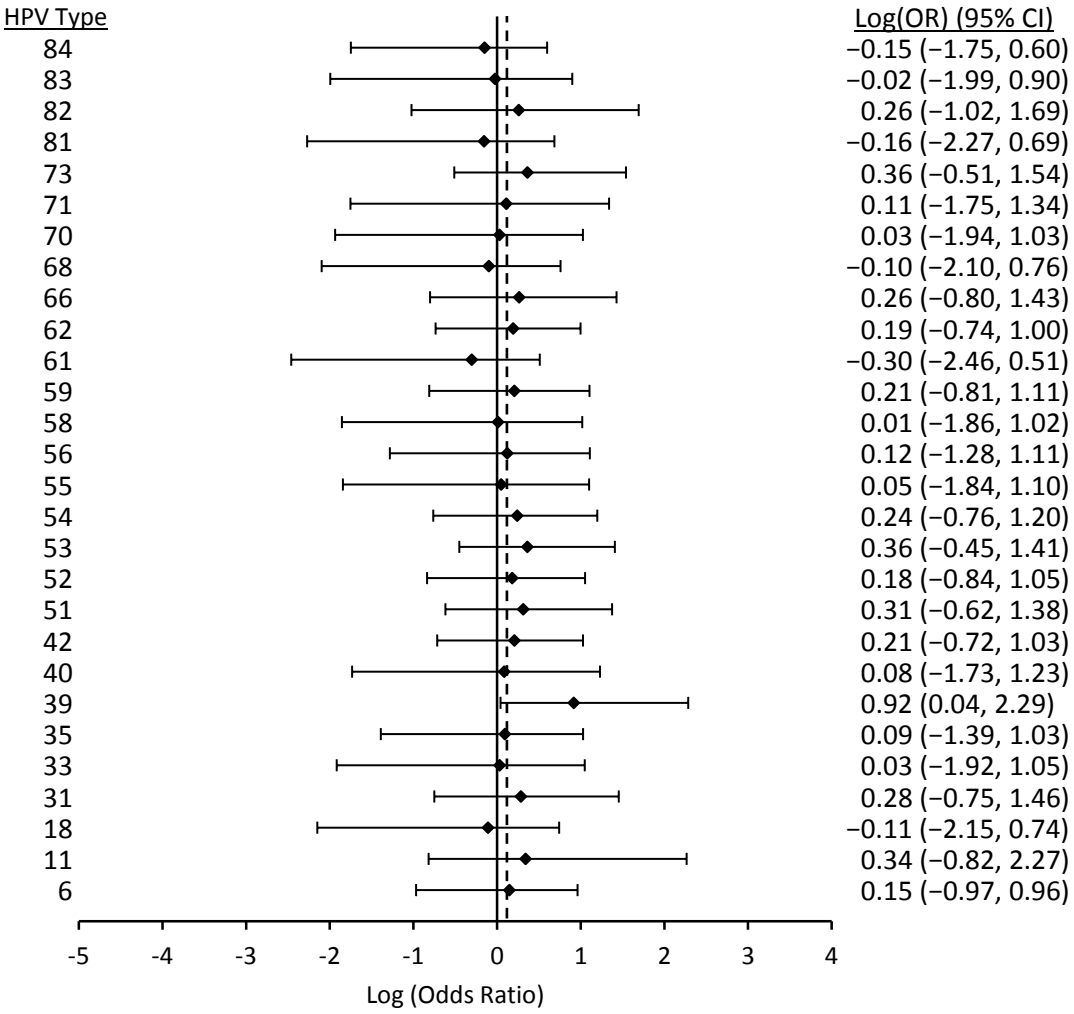
B)


HPV Type
Log(OR) (95% CI)
84
0.48 (0.20, 0.85)
83
0.50 (0.18, 1.11)
82
0.49 (0.17, 1.06)
73
0.39 (−0.19, 0.71)
68
0.35 (−0.43, 0.64)
66
0.46 (0.11, 0.91)
59
0.38 (−0.27, 0.69)
58
0.40 (−0.05, 0.70)
56
0.46 (0.13, 0.86)
55
0.44 (0.01, 0.90)
54
0.47 (0.17, 0.86)
53
0.48 (0.21, 0.87)
52
0.46 (0.13, 0.85)
51
0.36 (−0.15, 0.62)
45
0.41 (−0.06, 0.78)
42
0.45 (0.01, 0.93)
40
0.43 (−0.09, 0.87)
39
0.46 (0.12, 0.89)
35
0.43 (−0.08, 0.89)
33
0.38 (−0.14, 0.68)
31
0.42 (0.04, 0.74)
26
0.44 (0.00, 0.93)
18
0.40 (−0.01, 0.70)
11
0.41 (−0.15, 0.78)
6
0.45 (0.11, 0.84)
-5
-4
-3
-2
-1
0
1
2
3
4
Log (Odds Ratio)

C)


HPV Type
Log(OR) (95% CI)
89 0.41 (0.21, 0.74)
84 0.26 (−0.07, 0.48)
83 0.39 (0.13, 0.85)
82 0.33 (−0.01, 0.68)
81 0.40 (0.13, 0.96)
73 0.39 (0.14, 0.78)
72 0.38 (0.08, 0.92)
71 0.35 (−0.01, 0.82)
70 0.22 (−0.37, 0.48)
68 0.22 (−0.29, 0.46)
67 0.32 (0.02, 0.61)
66 0.36 (0.11, 0.68)
62 0.36 (0.13, 0.65)
61 0.32 (−0.01, 0.62)
59 0.22 (−0.23, 0.45)
58 0.25 (−0.18, 0.48)
56 0.27 (−0.12, 0.51)
54 0.25 (−0.13, 0.48)
53 0.36 (0.12, 0.65)
52 0.41 (0.20, 0.79)
51 0.27 (−0.07, 0.49)
45 0.32 (−0.04, 0.66)
44 0.32 (−0.06, 0.65)
42 0.31 (0.03, 0.56)
40 0.31 (−0.04, 0.62)
39 0.43 (0.22, 0.82)
35 0.32 (−0.07, 0.68)
34 0.32 (−0.09, 0.72)
33 0.27 (−0.26, 0.56)
31 0.35 (0.08, 0.67)
26 0.33 (−0.09, 0.75)
18 0.33 (0.02, 0.65)
11 0.32 (−0.10, 0.70)
6 0.30 (−0.03, 0.56)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

D)


HPV Type
Log(OR) (95% CI)
84 −0.15 (−1.75, 0.60)
83 −0.02 (−1.99, 0.90)
82 0.26 (−1.02, 1.69)
81 −0.16 (−2.27, 0.69)
73 0.36 (−0.51, 1.54)
71 0.11 (−1.75, 1.34)
70 0.03 (−1.94, 1.03)
68 −0.10 (−2.10, 0.76)
66 0.26 (−0.80, 1.43)
62 0.19 (−0.74, 1.00)
61 −0.30 (−2.46, 0.51)
59 0.21 (−0.81, 1.11)
58 0.01 (−1.86, 1.02)
56 0.12 (−1.28, 1.11)
55 0.05 (−1.84, 1.10)
54 0.24 (−0.76, 1.20)
53 0.36 (−0.45, 1.41)
52 0.18 (−0.84, 1.05)
51 0.31 (−0.62, 1.38)
42 0.21 (−0.72, 1.03)
40 0.08 (−1.73, 1.23)
39 0.92 (0.04, 2.29)
35 0.09 (−1.39, 1.03)
33 0.03 (−1.92, 1.05)
31 0.28 (−0.75, 1.46)
18 −0.11 (−2.15, 0.74)
11 0.34 (−0.82, 2.27)
6 0.15 (−0.97, 0.96)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

E)

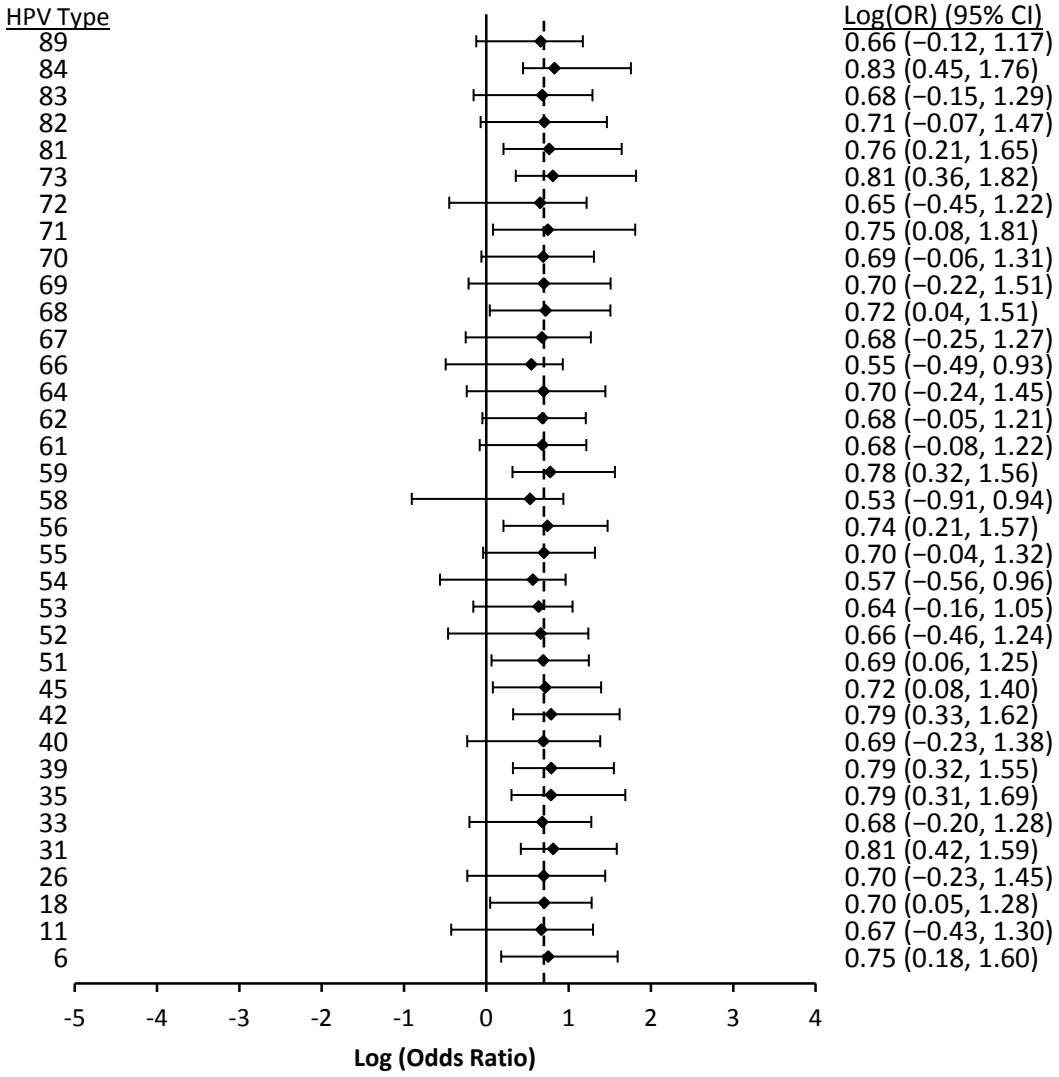
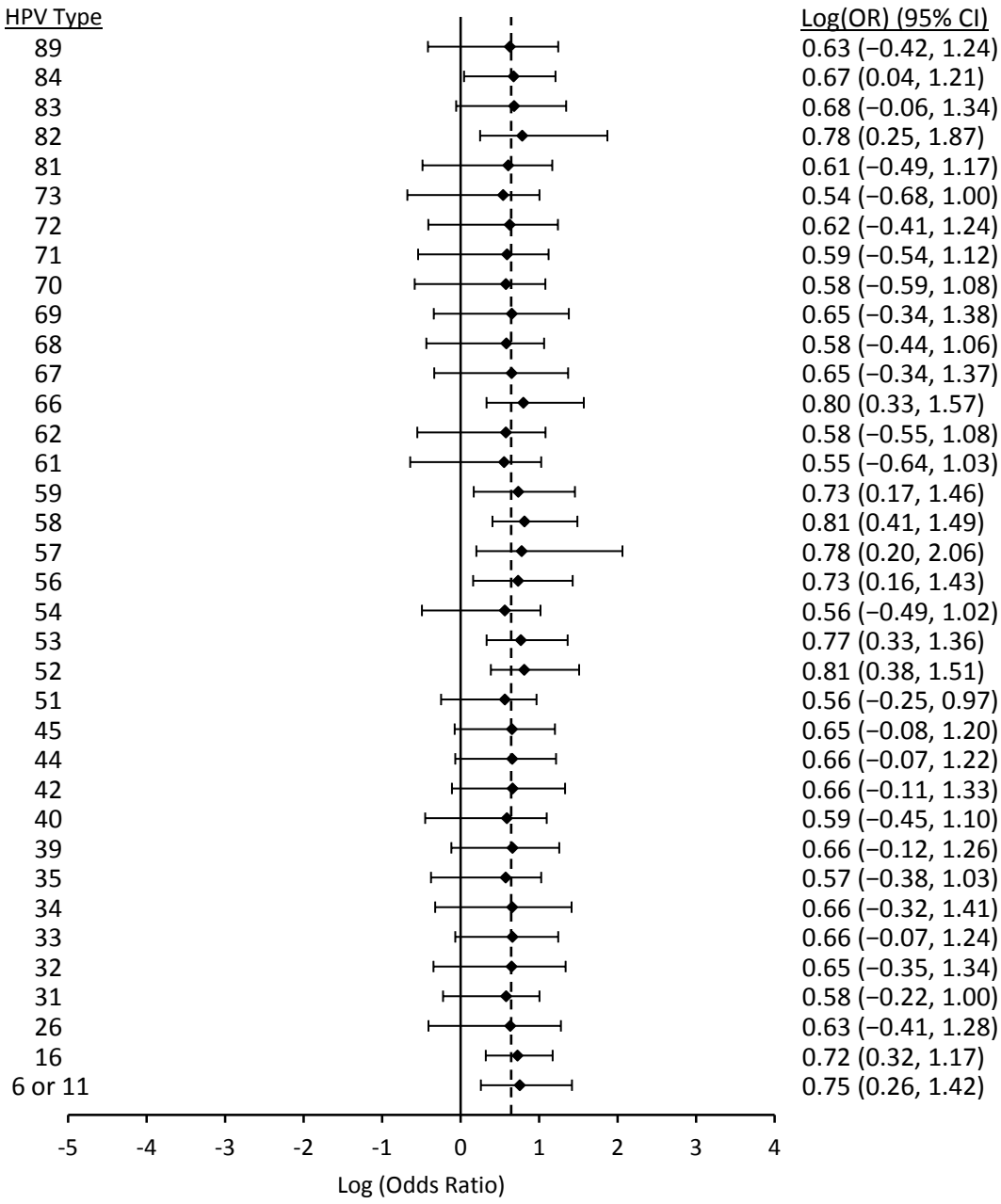
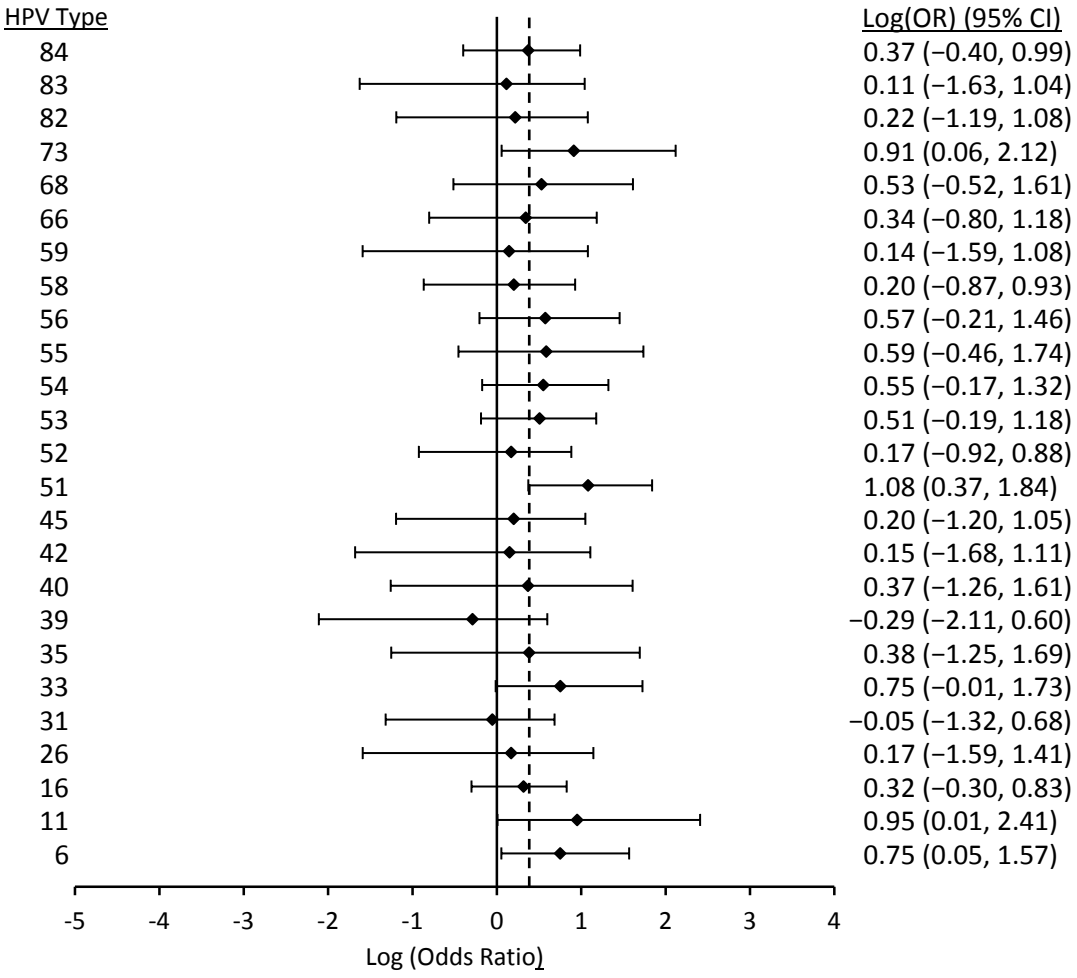


Figure 5-7: Log (odds ratios) and 95% confidence intervals for HPV16 with other HPV types from the Ludwig-McGill, McGill-Concordia, HITCH, BCCR, and CCCaST studies (panels A-E, respecively). Estimates were obtained from logistic regression models adjusted for all other HPV types, age, and lifetime number of sexual partners (except CCCaST; adjusted for other HPV types and age only). In panels A-E, the dashed lines represent the average pooled log(OR) from hierarchical logistic regression, which were 0.53 (95%CI: 0.21-0.77), 0.43 (95%CI: 0.25-0.60), 0.32 (95%CI: 0.22-0.42), 0.12 (95%CI: -0.47-0.46), and 0.70 (95%CI: 0.47-0.88), respectively.

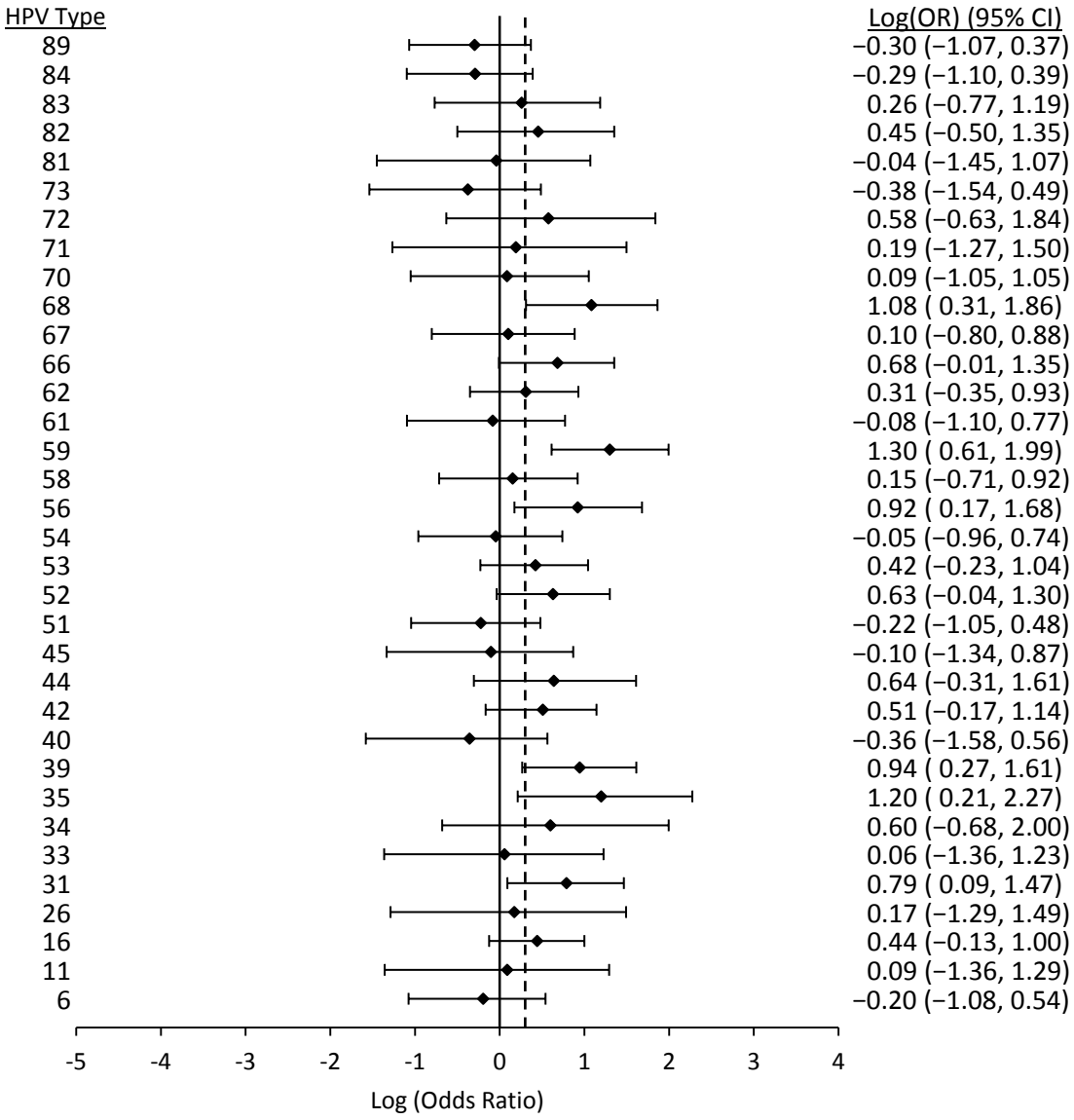
A)


HPV Type
89
84
83
82
81
73
72
71
70
69
68
67
66
62
61
59
58
57
56
54
53
52
51
45
44
42
40
39
35
34
33
32
31
26
16
6 or 11
Log(OR) (95% CI)
0.63 (−0.42, 1.24)
0.67 (0.04, 1.21)
0.68 (−0.06, 1.34)
0.78 (0.25, 1.87)
0.61 (−0.49, 1.17)
0.54 (−0.68, 1.00)
0.62 (−0.41, 1.24)
0.59 (−0.54, 1.12)
0.58 (−0.59, 1.08)
0.65 (−0.34, 1.38)
0.58 (−0.44, 1.06)
0.65 (−0.34, 1.37)
0.80 (0.33, 1.57)
0.58 (−0.55, 1.08)
0.55 (−0.64, 1.03)
0.73 (0.17, 1.46)
0.81 (0.41, 1.49)
0.78 (0.20, 2.06)
0.73 (0.16, 1.43)
0.56 (−0.49, 1.02)
0.77 (0.33, 1.36)
0.81 (0.38, 1.51)
0.56 (−0.25, 0.97)
0.65 (−0.08, 1.20)
0.66 (−0.07, 1.22)
0.66 (−0.11, 1.33)
0.59 (−0.45, 1.10)
0.66 (−0.12, 1.26)
0.57 (−0.38, 1.03)
0.66 (−0.32, 1.41)
0.66 (−0.07, 1.24)
0.65 (−0.35, 1.34)
0.58 (−0.22, 1.00)
0.63 (−0.41, 1.28)
0.72 (0.32, 1.17)
0.75 (0.26, 1.42)
-5
-4
-3
-2
-1
0
1
2
3
4
Log (Odds Ratio)

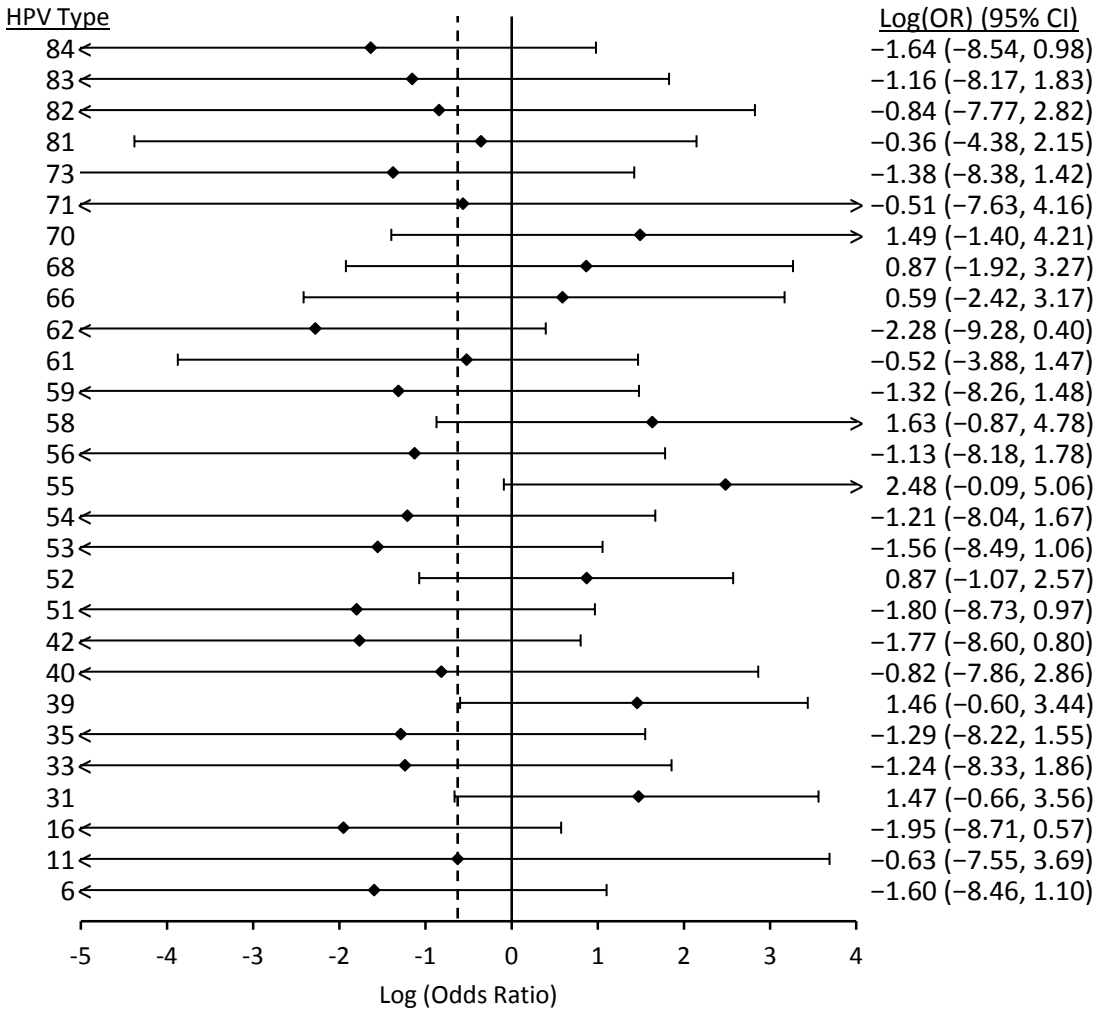
B)


HPV Type
Log(OR) (95% CI)
84 0.37 (−0.40, 0.99)
83 0.11 (−1.63, 1.04)
82 0.22 (−1.19, 1.08)
73 0.91 (0.06, 2.12)
68 0.53 (−0.52, 1.61)
66 0.34 (−0.80, 1.18)
59 0.14 (−1.59, 1.08)
58 0.20 (−0.87, 0.93)
56 0.57 (−0.21, 1.46)
55 0.59 (−0.46, 1.74)
54 0.55 (−0.17, 1.32)
53 0.51 (−0.19, 1.18)
52 0.17 (−0.92, 0.88)
51 1.08 (0.37, 1.84)
45 0.20 (−1.20, 1.05)
42 0.15 (−1.68, 1.11)
40 0.37 (−1.26, 1.61)
39 −0.29 (−2.11, 0.60)
35 0.38 (−1.25, 1.69)
33 0.75 (−0.01, 1.73)
31 −0.05 (−1.32, 0.68)
26 0.17 (−1.59, 1.41)
16 0.32 (−0.30, 0.83)
11 0.95 (0.01, 2.41)
6 0.75 (0.05, 1.57)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

C)


HPV Type
Log(OR) (95% CI)
89 −0.30 (−1.07, 0.37)
84 −0.29 (−1.10, 0.39)
83 0.26 (−0.77, 1.19)
82 0.45 (−0.50, 1.35)
81 −0.04 (−1.45, 1.07)
73 −0.38 (−1.54, 0.49)
72 0.58 (−0.63, 1.84)
71 0.19 (−1.27, 1.50)
70 0.09 (−1.05, 1.05)
68 1.08 (0.31, 1.86)
67 0.10 (−0.80, 0.88)
66 0.68 (−0.01, 1.35)
62 0.31 (−0.35, 0.93)
61 −0.08 (−1.10, 0.77)
59 1.30 (0.61, 1.99)
58 0.15 (−0.71, 0.92)
56 0.92 (0.17, 1.68)
54 −0.05 (−0.96, 0.74)
53 0.42 (−0.23, 1.04)
52 0.63 (−0.04, 1.30)
51 −0.22 (−1.05, 0.48)
45 −0.10 (−1.34, 0.87)
44 0.64 (−0.31, 1.61)
42 0.51 (−0.17, 1.14)
40 −0.36 (−1.58, 0.56)
39 0.94 (0.27, 1.61)
35 1.20 (0.21, 2.27)
34 0.60 (−0.68, 2.00)
33 0.06 (−1.36, 1.23)
31 0.79 (0.09, 1.47)
26 0.17 (−1.29, 1.49)
16 0.44 (−0.13, 1.00)
11 0.09 (−1.36, 1.29)
6 −0.20 (−1.08, 0.54)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

D)


HPV Type
Log(OR) (95% CI)
84 −1.64 (−8.54, 0.98)
83 −1.16 (−8.17, 1.83)
82 −0.84 (−7.77, 2.82)
81 −0.36 (−4.38, 2.15)
73 −1.38 (−8.38, 1.42)
71 −0.51 (−7.63, 4.16)
70 1.49 (−1.40, 4.21)
68 0.87 (−1.92, 3.27)
66 0.59 (−2.42, 3.17)
62 −2.28 (−9.28, 0.40)
61 −0.52 (−3.88, 1.47)
59 −1.32 (−8.26, 1.48)
58 1.63 (−0.87, 4.78)
56 −1.13 (−8.18, 1.78)
55 2.48 (−0.09, 5.06)
54 −1.21 (−8.04, 1.67)
53 −1.56 (−8.49, 1.06)
52 0.87 (−1.07, 2.57)
51 −1.80 (−8.73, 0.97)
42 −1.77 (−8.60, 0.80)
40 −0.82 (−7.86, 2.86)
39 1.46 (−0.60, 3.44)
35 −1.29 (−8.22, 1.55)
33 −1.24 (−8.33, 1.86)
31 1.47 (−0.66, 3.56)
16 −1.95 (−8.71, 0.57)
11 −0.63 (−7.55, 3.69)
6 −1.60 (−8.46, 1.10)
-5 -4 -3 -2 -1 0 1 2 3 4
Log (Odds Ratio)

E)

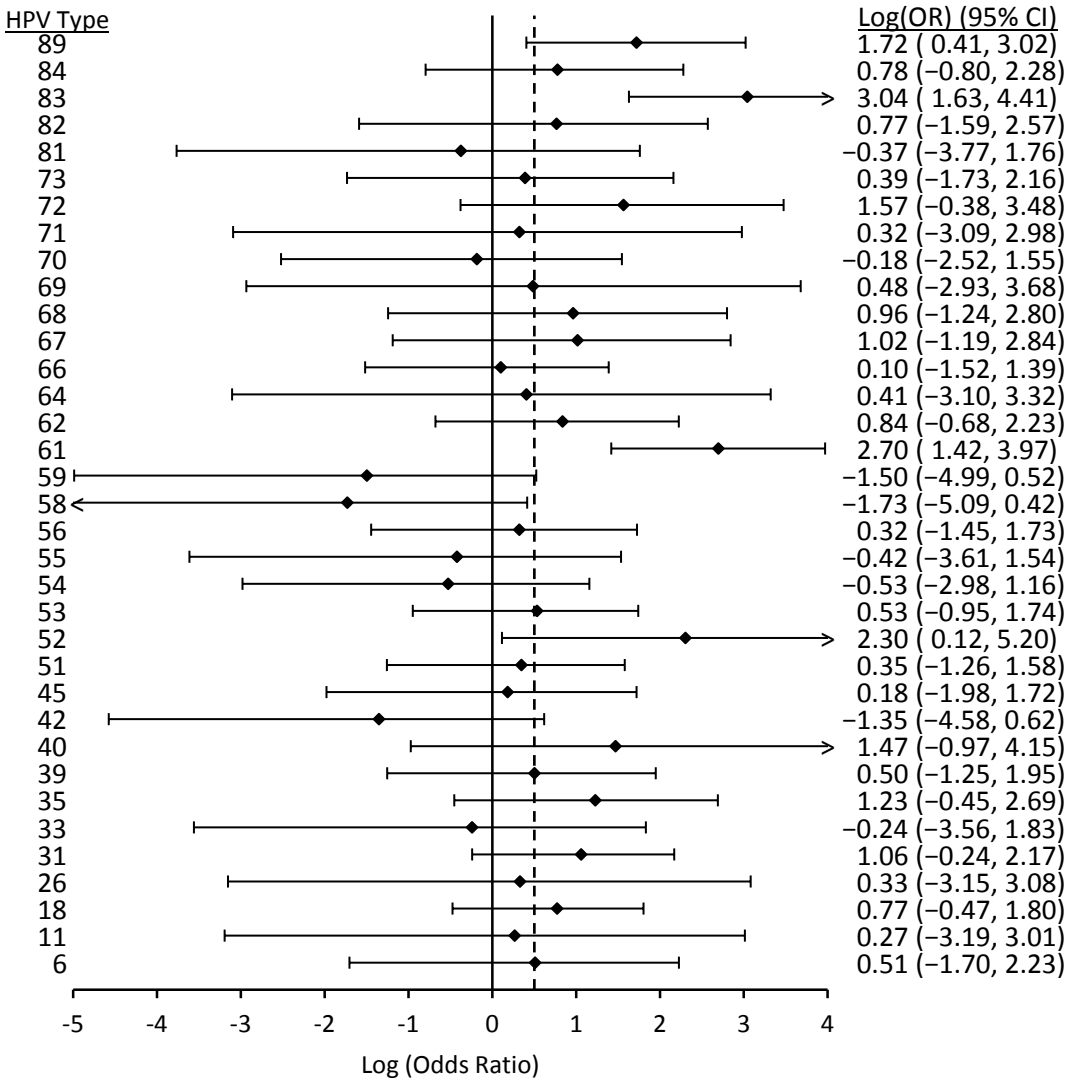


Figure 5-8: Log (odds ratios) and 95% confidence intervals for HPV18 with other HPV types from the Ludwig-McGill, McGill-Concordia, HITCH, BCCR, and CCCaST studies (panels A-E, respecively). Estimates were obtained from logistic regression models adjusted for all other HPV types, age, and lifetime number of sexual partners (except CCCaST; adjusted for other HPV types and age only). In panels A-E, the dashed lines represent the average pooled log(OR) from hierarchical logistic regression, which were 0.64 (95%CI: 0.33-0.84), 0.38 (95%CI: -0.10-0.71), 0.30 (95%CI: -0.01-0.59), -0.63 (95%CI: -3.41-0.47), and 0.50 (95%CI: -0.30-1.088), respectively.

APPENDIX 4

ADDITIONAL ANALYSES

TO MANUSCRIPT IV

Table 6-7: Number of women who acquired (baseline cases excluded) or cleared (baseline cases included) specific HPV infections in three epidemiologic cohort studies

	Ludwig-McGill[a]		McGill-Concordia		HITCH	
HPV Acquisition/ Clearance	Acquisition (n=2185)[b] No.	Clearance (n=1124)[c] No. (Total)[d]	Acquisition (n=578)[b] No.	Clearance (n=279)[c] No. (Total)[d]	Acquisition (n=437)[b] No.	Clearance (n=249)[c] No. (Total)[d]
α-10 species	63	178 (187)	32	35 (42)	43	34 (55)
HPV-6/11	35	95 (103)	32	35 (42)	40	31 (49)
HPV-44	29	87 (95)	N/D	N/D	10	9 (12)
HPV-55	N/D	N/D	11	10 (10)	N/D	N/D
α-9 species	209	497 (556)	90	82 (148)	66	82 (137)
HPV-16	108	243 (276)	61	47 (81)	26	46 (96)
HPV-31	35	79 (95)	21	18 (29)	24	27 (40)
HPV-33	17	47 (50)	12	6 (14)	6	6 (8)
HPV-35	22	75 (82)	1	2 (2)	5	5 (7)
HPV-52	34	98 (107)	19	23 (32)	26	38 (53)
HPV-58	35	94 (110)	12	16 (23)	15	17 (35)
HPV-67	7	83 (87)	N/D	N/D	29	43 (48)
α-7 species	123	275 (286)	64	53 (84)	42	64 (98)
HPV-18	32	83 (87)	25	26 (39)	11	17 (25)
HPV-39	13	39 (41)	24	13 (19)	25	34 (50)
HPV-45	21	70 (80)	7	13 (18)	13	12 (16)
HPV-59	24	61 (65)	12	8 (11)	18	33 (42)
HPV-68	33	67 (71)	9	6 (11)	6	11 (19)
HPV-70	14	45 (48)	N/D	N/D	6	6 (8)
Other types						
HPV-26	5	19 (19)	3	4 (5)	1	0 (1)
HPV-32	2	8 (8)	N/D	N/D	N/D	N/D
HPV-34	0	4 (4)	N/D	N/D	1	3 (4)
HPV-40	13	43 (43)	2	0 (2)	20	18 (24)
HPV-42	5	30 (32)	5	3 (4)	44	38 (62)
HPV-51	57	142 (154)	44	41 (51)	31	42 (66)
HPV-53	66	158 (170)	31	37 (47)	33	29 (55)
HPV-54	30	69 (73)	34	23 (31)	25	29 (43)
HPV-56	19	60 (65)	19	23 (28)	21	24 (36)
HPV-57	2	6 (6)	0	0 (0)	N/D	N/D
HPV-61	19	60 (63)	N/D	N/D	21	11 (24)
HPV-62	21	54 (58)	N/D	N/D	31	34 (58)
HPV-66	11	51 (59)	24	18 (22)	44	43 (59)
HPV-69	2	5 (5)	N/D	N/D	0	0 (0)
HPV-71	13	35 (43)	N/D	N/D	0	2 (2)
HPV-72	6	13 (16)	N/D	N/D	0	0 (2)
HPV-73	22	56 (58)	17	13 (15)	25	30 (36)
HPV-81	14	34 (36)	N/D	N/D	4	5 (9)
HPV-82	10	25 (25)	12	13 (15)	10	19 (21)
HPV-83	14	44 (51)	10	11 (11)	7	9 (14)
HPV-84	28	96 (107)	47	44 (54)	45	54 (72)
HPV-89	9	23 (24)	N/D	N/D	53	65 (88)

Abbreviations: HPV, human papillomavirus; N/D, presence of HPV type was not determined.

[a] Subject follow-up was truncated after two years from baseline (acquisition) or from time of index HPV infection (clearance) in the Ludwig-McGill study.

[b] Total number of females with valid HPV DNA results available at baseline and at least one follow-up visit that were included in our analysis of acquisition.

[c] Total number of females that tested positive for HPV at any visit, followed by a valid HPV DNA testing result in at least one visit that were included in our analysis of loss of HPV infection (clearance).

[d] Total number of women with particular HPV infection, and at least one follow-up visit following index infection.

APPENDIX 5

ETHICS APPROVAL FORMS

McGill

Faculty of Medicine
McGill University
McIntyre Medical Sciences Building

Postal address:
3655 Drummond Street
Montreal, PQ, Canada H3G 1Y6

July 14, 1995

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec
H3A 2T5

Dear Dr. Franco:

Thank you for the correspondence of June 29, 1995, providing revised study documentation in support of the research proposal entitled *"Molecular Epidemiology of Persistent Cervical HPV Infection" [Brazil].*

I am pleased to inform you that final approval for the clinical protocol and revised consent form was granted on July 4, 1995. Enclosed you will find the original certification of ethical approval.

Please note that a review of all research involving human subjects is required on an annual basis in conformity with the date of the initial approval. Should there be any modifications to the study, over the next twelve months, please advise the IRB accordingly.

We trust this meets with your complete satisfaction.

Sincerely,

Elisabeth Clark
Coordinator
Institutional Review Board

cc: IRB Files

McGill

Faculty of Medicine
McGill University
McIntyre Medical Sciences Building

Postal address:
3655 Drummond Street
Montreal, PQ, Canada H3G 1Y6

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

DR. A. FUKS	DR. C. WEIJER
MS. S. BATT	MS. S. WOLFE
MR. A. CANDIB	
DR. B. FREEDMAN	
DR. L. HUTCHISON	
DR. P. RENE	

has examined your protocol entitled: "Molecular Epidemiology of Persistent Cervical HPV Infection"

as proposed by: Dr. Eduardo L. Franco (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

July 4, 1995
Date

Chairman, IRB

Dean of Faculty

Institutional Review Board Assurance Number: M-1458/01XB

McGill

Faculty of Medicine
3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

Faculté de médecine
3655, Promenade Sir William Osler
Montréal, QC. H3G 1Y6

Fax/Télécopieur: (514) 398-3595

APR 16 2003

March 25, 2003

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec H3A 2T5

Dear Dr. Franco,

The research proposal AØØ-M52-02B entitled "Persistent Human Papillomavirus Infection and Cervical Intraepithelial Neoplasia – New Objectives" was re-submitted for review by the Institutional Review Board, Faculty of Medicine at its meeting of March 24, 2003.

The Committee has re-examined the proposal as well as the proposed anonymization procedure and we are pleased to inform you that full Board approval for the study (April 2002) and your correspondence (April 22, 2002) was provided on March 24, 2003, valid until **March 2004**. The certification document *(executed)* is enclosed.

We ask you to take note that review of all research involving human subjects is required on an annual basis in accord with the date of initial approval (March 24, 2003). Should any modification to the study or unanticipated development occur prior to the next review, please advise IRB promptly.

Yours sincerely,

Serge Gauthier, M.D.
Co-Chair,
Institutional Review Board

cc: A03-M52-02B

McGill

Faculty of Medicine
3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

Faculté de médecine
3655, Promenade Sir William Osler
Montréal, QC, H3G 1Y6

Fax/Télécopieur: (514) 398-3595

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

SERGE GAUTHIER, MD	GEOFFREY BLAKE, MD
MARK S. GOLDBERG, PHD	VINCENT GRACCO, PHD
MARIGOLD HYDE, BSC	ABBY LIPPMAN, PHD
HARVEY SIGMAN, MD	SALLY TINGLEY, BCOM

has examined the research project **A03-M52-03B** entitled **"Persistent Human Papillomavirus Infection and Cervical Intraepithelial Neoplasia – New Objectives"**

as proposed by: Eduardo Franco (Applicant) to CIHR (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

March 25, 2003
Date

Chair, IRB

Dean of Faculty

Institutional Review Board Assurance Number: M-1458

McGill

Faculty of Medicine
3655 Drummond Street
Montreal, QC H3G 1Y6
Fax: (514) 398-3595

Faculté de médecine
3655, rue Drummond
Montréal, QC, H3G 1Y6
Télécopieur: (514) 398-3595

October 6, 2000

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec H3A 2T5

Dear Dr. Franco,

We have received your response to the issues raised regarding recruitment methods for the research proposal A00-M38-00 entitled **"Biomarkers of Cervical Cancer Risk"**.

The responses and revisions (your letters dated June 27, 2000 and September 12, 2000) to the Board's questions were found to be acceptable and we are pleased to inform you that final approval for the study (February 24, 2000), revised letter of introduction and consent form (June 27, 2000) and revised letter of introduction and consent form for control participants (June 27, 2000) was provided on October 6, 2000 valid until October 2001. The certification of approval (executed) is enclosed.

It is the responsibility of the investigator to assure that the approved research protocol and consent form is deposited with the Research Ethics Boards of each hospital where patient treatment and data analysis will take place.

We ask you to take note that review of all research involving human subjects is required on an annual basis in accord with the date of initial approval. Should any modification to the study (including the consent form) or unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

J. Lawrence Hutchison, M.D.
Chair
Institutional Review Board

cc: Ms. F. Cantini
Ms. L. Fateen
REB files : JGH/RVH
A10-M38-00

McGill

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CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

LAWRENCE HUTCHISON, MD

SHARI BAUM, PHD — PATRICIA DOBKIN, PHD

HAROLD FRANK, MD — WILSON MILLER, MD

HARVEY SIGMAN, MD — SALLY TINGLEY, BCOM

has examined the research project **A10-M38-00** entitled **"Biomarkers of Cervical Cancer Risk"**

as proposed by: Dr. Eduardo Franco (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

October 6, 2000
Date

Chair, IRB

Dean of Faculty

Institutional Review Board Assurance Number: M-1458

McGill

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October 8, 2004

JAN 24 2005

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec H3A 2T5

Dear Dr. Franco,

We have received correspondence in support of the research proposal AØØ-M77-04A entitled "HITCH Cohort – HPV Infection and Transmission among Couples Through Heterosexual Activity" which was reviewed by the Institutional Review Board, Faculty of Medicine at its meeting of September 13, 2004.

The responses and revisions were found to be acceptable and we are pleased to inform you that final approval for the clinical protocol (September 2004) and revised consent forms for men and women (October 1, 2004), was provided on October 8, 2004, valid until **September 2005**. The certification document *(executed)* is enclosed.

We ask you to take note that review of all research involving human subjects is required on an annual basis in accord with the date of initial review and approval (September 14 2004). Should any modification to the study or unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

C. Celeste Johnston

Celeste Johnston, DEd, RN
Co-Chair,
Institutional Review Board

cc: AØ9-M77-04A

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JAN 2 4 2005

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

CELESTE JOHNSTON, DED	ARTHUR CANDIB, MED
CATHERINE GARDNER, BSC	RICHARD HAMILTON, MD
LAWRENCE HUTCHISON, MD	NEIL MACDONALD, MD
WILSON MILLER, MD, PHD	ROBERT L. MUNRO, BCL
ROBERTA PALMOUR, PHD	MARGARET SWAINE, BA

has examined the research project **A09-M77-04A** entitled **"HITCH Cohort – HPV Infection and Transmission among Couples Through Heterosexual Activity"**

as proposed by: Eduardo Franco (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

October 8, 2004
Date

Co-Chair, IRB

Dean of Faculty

Institutional Review Board Assurance Number: FWA 00004545

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April 29, 2002

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec H3A 2T5

Dear Dr. Franco,

The research proposal A00-M42-02B entitled "Efficacy Trial of HPV Versus Pap Testing for Cervical Cancer Precursors" was reviewed by the Institutional Review Board, Faculty of Medicine at its meeting of April 22, 2002.

The Committee raised the following issues for your consideration:

Scientific Protocol

a) No mention is made in the protocol of the psychological risks and the counseling that will be made available, as indicated in the consent form.

Consent Form

b) There is no section on confidentiality
c) Page 3, the actual consent section should be in the 1st person (please refer to the McGill Guidelines)
d) Further details are required regarding what a PAP smear is and how it is done, as well as how the HPV testing is done.
e) Risks section should also include risks related to having PAP smears.
f) There is no mention of the number of women that will be studied.

The study was approved pending appropriate responses and revisions.

Yours sincerely,

Harvey Sigman, M.D.
Co-Chair,
Institutional Review Board

cc: A00-M42-02B

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May 16, 2002

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec H3A 2T5

Dear Dr. Franco,

We have received correspondence in support of the research proposal AØØ-M42-02B entitled "Efficacy Trial of HPV Versus Pap Testing for Cervical Cancer Precursors", which was reviewed by the Institutional Review Board, Faculty of Medicine at its meeting of April 22, 2002.

The responses and revisions were found to be acceptable and we are pleased to inform you that final approval for the protocol (March 2002) and revised consent form (May 13, 2002) was provided on May 16, 2002, valid until **April 2003**. The certification of approval (executed) is enclosed.

It is the responsibility of the investigator to ensure that the approved research protocol and consent form is deposited with the Research Ethics Board of each hospital where subject recruitment or study data will be collected.

We ask you to take note that review of all research involving human subjects is required on an annual basis in accord with the date of initial approval. Should any modification to the study or unanticipated development occur prior to the next review, please advise IRB promptly.

Yours sincerely,

Harvey Sigman, M.D.
Co-Chair,
Institutional Review Board

cc: Ms. F. Cantini – JGH
Ms. E. Boyle – MUHC/MGH
Ms. L. Fateen – MUHC/RVH
Ms. A. Collins - SMH
A04-M42-02B

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CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

HARVEY SIGMAN, MD

FRANCES ABOUD, PHD — GEOFFREY BLAKE, MD

PIERRE DESCHAMPS, BCL, LSCR — VINCENT GRACCO, PHD

ABBY LIPPMAN, PHD — MICHAEL THIRLWELL, MD

SALLY TINGLEY, BCOM

has examined the research project **A04-M42-02B** entitled **"Efficacy Trial of HPV Versus Pap Testing for Cervical Cancer Precursors"**

as proposed by: Dr. Eduardo Franco (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

May 16, 2002
Date

Chair, IRB

Dean of Faculty

Institutional Review Board Assurance Number: M-1458