HUMAN PAPILLOMAVIRUS INFECTION AND TRANSMISSION AMONG COUPLES THROUGH HETEROSEXUAL ACTIVITY

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STATEMENT OF ORIGINALITY

The body of work described in this thesis represents original research by the PhD student, Ann Burchell. I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material, which to a substantial extent, has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

Under the supervision and guidance of the research supervisor and thesis committee, I played a major role in the conception and design of the HITCH Cohort Study; carried out extensive literature review; wrote a paper describing the state of knowledge regarding HPV transmission dynamics in collaboration with coauthors (Manuscript I); conducted the feasibility study; drafted the research proposal submitted to the Canadian Institutes of Health Research for funding; and conceived and wrote the simulation model to estimate transmissibility (Manuscript II). I was also the Project Coordinator of the HITCH Cohort Study and as such was responsible for hiring, training and supervising staff, directing recruitment, and data collection and management. Therefore, the design of the entire cohort study is described in this thesis. I conducted all statistical analysis of HITCH data reported in this thesis, including Manuscripts III & IV. Statistical analysis for the PhD thesis utilized data obtained at the enrolment visit to ensure timely completion of the degree.

DESCRIPTION OF THESIS AND LIST OF ORIGINAL PAPERS

This is a manuscript-based thesis. Manuscripts I and II provide the rationale, context and preliminary work conducted to justify a couple-based study of HPV infection and transmission. Manuscripts III and IV report the results of analyses conducted to address thesis objectives 1, 2, and 3. Remaining analyses to address thesis objectives 4 and 5 are reported in Sections 8 and 9.

Manuscript I: Ann N. Burchell, Rachel L. Winer, Silvia de Sanjosé, Eduardo L. Franco. Epidemiology and transmission dynamics of genital human papillomavirus infection. *Vaccine* 2006, 24(S3):S52-S61

Manuscript II: Ann N. Burchell, Harriet Richardson, Salaheddin M. Mahmud, Helen Trottier, Pierre-Paul Tellier, James Hanley, François Coutlée, Eduardo L. Franco. Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada. *American Journal of Epidemiology* 2006; 163:534-543

Manuscript III: Ann N. Burchell, Pierre-Paul Tellier, James Hanley, François Coutlée, Eduardo L. Franco. Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner. Submitted, March 2009.

Manuscript IV: Ann N. Burchell, Pierre-Paul Tellier, James Hanley, François Coutlée, Eduardo L. Franco. Distribution and genotype concordance of human papillomavirus infections among couples in new sexual relationships. Submitted, March 2009.

CONTRIBUTIONS OF AUTHORS

Manuscript I

The PhD student, **Ann Burchell**, was the lead author. She was responsible for the development of the outline, coordination of contributions to each section, and overall coherence of the paper. She prepared the initial drafts for the sections on duration of infection, routes of infection, sexual behaviour leading to exposure to HPV, and HPV transmissibility. She compiled the individual contributions from S. de Sanjosé and R.L. Winer, drafted the overall document, and finalized all revisions.

Silvia de Sanjosé prepared the initial draft of prevalence among women and men. Importantly, she was the lead researcher for the meta-analysis whose preliminary results are described in this paper. S. de Sanjosé conducted the meta-analysis which was later published in more detail in Lancet Infectious Diseases [1]. **Rachel L. Winer** prepared the initial drafts of the sections on HPV incidence and risk factors for infection. Both provided extensive feedback on the working drafts of the manuscript.

E.L. Franco, the PhD research supervisor and one of the editors of the monograph provided feedback and guidance on all manuscript versions.

Manuscript II

The PhD student, **Ann N. Burchell**, conceived the idea to conduct the simulation, analyzed the original empirical data from the McGill-Concordia Cohort Study to provide fixed parameter estimates for the simulation, wrote the simulation program and interpreted the results, and drafted the manuscript.

Harriet Richardson, a graduate of the Department of Epidemiology, McGill University, coordinated the McGill-Concordia Cohort Study and provided assistance in the interpretation of data from that study.

Salaheddin M. Mahmud and **Helen Trottier** provided statistical advice on the simulation model.

Pierre-Paul Tellier was a coinvestigator of the McGill-Concordia Cohort Study and Director of the McGill Student Health Service Clinic where women were recruited and attended study visits. **Gail Kelsall** was a research nurse for that former study. Both were involved in data collection.

James Hanley provided statistical advice on the simulation modeling as part of his role as a member of the thesis committee.

François Coutlée was a coinvestigator of the McGill-Concordia Cohort Study and was responsible for laboratory analysis of cervical specimens for HPV testing in that study.

Eduardo L. Franco is the PhD Supervisor and was the Principal Investigator of the McGill-Concordia Cohort Study. He provided access to the data from the McGill-Concordia Cohort Study and advice on the simulation modeling and its interpretation.

All authors read an earlier version of the paper, provided feedback, and approved the final paper.

Manuscripts III and IV

The PhD student, **Ann N. Burchell**, drafted the original research protocol for the HITCH Cohort Study, was Project Coordinator of this study, analysed and interpreted the data, and drafted the manuscripts.

Pierre-Paul Tellier is a coinvestigator of the HITCH Cohort Study and Director of the McGill Student Health Service Clinic where women were recruited and attended study visits. He provided guidance and expertise on sexual health issues among adolescents and young adults.

James Hanley is a coinvestigator of the HITCH Cohort Study and a member of the thesis committee. He provided statistical advice on the original research design and analysis conducted for these papers.

François Coutlée is a coinvestigator of the HITCH Cohort Study and was responsible for laboratory analysis of genital specimens for HPV testing. He provided guidance and expertise on the biological interpretation of these results.

Eduardo L. Franco is the PhD Supervisor and was the Principal Investigator of the HITCH Cohort Study. He secured funding for this study and provided extensive guidance and expertise regarding the epidemiology of HPV infection, cervical cancer, and and HPV-related disease.

All authors read an earlier version of the papers, provided feedback, and approved the final papers.

LIST OF ABBREVIATIONS

CI, confidence interval

DNA, deoxyribonucleic acid

GEE, generalized estimating equations

HITCH, HPV Infection and Transmission among Couples through Heterosexual activity

HPV, human papillomavirus

HR-HPV, high oncogenic risk HPV types

IARC, International Agency for Research on Cancer

ICC, intra-class correlation coefficient

IQR, inter-quartile range

LR-HPV, low oncogenic risk HPV types

LA-HPV, Linear Array HPV genotyping test

NS, not statistically significant

OR, odds ratio

PCR, polymerase chain reaction

PRR, prevalence rate ratio

SD, standard deviation

STI, sexually transmitted infection

GLOSSARY OF TERMS

Definitions of sexual terms in HITCH questionnaires

Partners or sexual partners: People who have engaged in sexual activity together—whether once, or just a few times, or as regular partners, or as married partners

Sexual activity: Mutual masturbation, oral sex, vaginal sex or anal sex

Mutual masturbation: Hand stimulation of a person's genital area by his/her partner, not involving intercourse (vaginal, oral or anal)

Oral sex: A man's or woman's mouth on a partner's genital area

Vaginal sex or vaginal intercourse: A man's penis in a woman's vagina. This is what most people usually think of as "having sex" or "sexual intercourse".

Anal sex or anal intercourse: A man's penis in a sexual partner's anus/ rectum

Coitarche: A person's first experience of coitus, or vaginal intercourse. Commonly used in the phrase "age at coitarche". Alternative phrase is "age at first intercourse".

Sexual orientation: How a person self-identifies in terms of their sexuality and preference for the gender of their sex partners. It may not necessarily be consistent with their actual behaviour. For example, a person may self-identify as heterosexual but report having had same-sex partners. In the questionnaire,

participants were asked, “Do you consider yourself to be heterosexual/straight, bisexual, homosexual, or other (specify)?”

HITCH partner: The sexual partner with whom a participant enrolled in the HITCH Cohort Study

Concurrent/extra-dyadic partner: A sexual partner other than the HITCH partner since the start of that sexual relationship. Also called an extra-dyadic partner in social network terminology because it is a partner external to the couple or dyad

Monogamous couple: A couple for which neither partner reported concurrent or extra-dyadic partners

Beta-globin: A marker of human cells/DNA. The detection of a beta-globin DNA sequence in a biological specimen ensures that it contains human DNA and thus it serves to check for the integrity of the specimen.

Exposed couple: A couple for whom at least one partner had detectable HPV infection. That is, there was exposure to an infected partner within the couple and therefore an opportunity for HPV transmission was present.

HPV transmission probability per partnership (β_p): The probability that an infected partner transmits HPV to a susceptible partner, irrespective of the duration of that partnership or the quantity or nature of sexual encounters

HPV transmission probability per coital act (β_a): The probability that an infected partner transmits HPV to a susceptible partner in a single vaginal sex encounter or act of coitus

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ABSTRACT

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI). The vast majority of these infections clear spontaneously. The small proportion that persists may result in substantial morbidity and treatment costs. High oncogenic risk HPV (HR-HPV) types, including HPV-16 and 18, are recognised unequivocally as the main causal factor for cervical cancer, and may also cause other anogenital neoplasms and head and neck cancers. Infections with types that have low oncogenic risk (LR-HPV), such as HPV-6 and -11, are associated with benign lesions including genital warts. Many projections of the impact of the new HPV vaccines and screening technologies use dynamic transmission models which require sound estimates of the probability of transmission upon exposure. Furthermore, biological and practical limitations of the current vaccines require that we explore as many prevention options as possible. A critical research question is whether condoms provide protection.

The main aims of the thesis were to characterize patterns of HPV infection among heterosexual couples in a new relationship, to identify risk factors for HPV infection, and to estimate HPV transmission probabilities per partnership and per coital act.

I carried out a preliminary Monte Carlo simulation to estimate the probability of HPV transmission, then designed and conducted a study of heterosexual couples. The thesis objectives were addressed using baseline data from the ongoing HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity). The study population consists of young (aged 18-24) women attending a university or junior college (CEGEP) in Montreal and their male partners.

Results from the simulation analysis suggested that HPV is highly transmissible, which was confirmed by the cross-sectional analysis of the HITCH study. Among the 263 couples enrolled between 05/2005 and 08/2008, HPV prevalence was 56% among women and men. In nearly two thirds (169) of couples, at least one partner was infected with one or more types. The current partner's status was the most important risk factor for prevalent infection. Analysis of the patterns of type-specific concordance and discordance revealed that the extent of concordance was far greater than expected, and was consistent with rapid transmission between partners. There was evidence for a protective effect of condoms, but protection was incomplete and was stronger among men than among women.

There are two unique features of the study that are novel for HPV research. It is the first large-scale study of HPV acquisition that involves the male partner. Secondly, it is the only study to restrict enrolment to couples in a new sexual relationship, a time at which considerable transmission is believed to occur. Results are likely to influence prevention efforts for cervical cancer and other HPV-related disease, including behavioural strategies to reduce risk. Results will also provide improved estimates of HPV transmission parameters to be used in models of the population health impact and cost effectiveness of vaccination strategies.

RESUME

Le virus du papillome humain (VPH) est l'infection transmissible sexuellement (ITS) la plus répandue. Une grande majorité des infections à VPH se résorbent spontanément. Cependant, la petite proportion d'infections à VPH persistantes peut avoir des coûts substantiels de traitement et de morbidité comme conséquence. Des génotypes de VPH à haut risque oncologique (HR-HPV), y compris HPV-16 et 18, sont identifiés sans équivoque comme facteur causal principal pour le cancer cervical, et peuvent également causer d'autres cancers anogénitaux, de la tête et du cou. Les infections avec des génotypes de VPH à bas risque oncologique (LR-HPV), comme HPV-6 et -11, sont associées aux lésions bénignes comprenant les verrues génitales. Plusieurs projections de l'impact des nouveaux vaccins de VPH et des techniques de dépistage utilisent des modèles de transmission dynamiques qui exigent des évaluations précises de la probabilité de transmission lors de l'exposition. En outre, les limitations biologiques et pratiques des vaccins courants exigent que nous explorions autant d'options de prévention que possibles. Une question critique de recherches est de savoir si les condoms assurent une protection.

Les objectifs principaux de la thèse consistaient à caractériser des modèles typiques d'infection au VPH parmi les couples hétérosexuels dans une nouvelle relation, identifier des facteurs de risque pour l'infection au VPH, et estimer les probabilités de transmission du VPH par relation de couple et par acte coïtal.

J'ai effectué une simulation préliminaire de Monte Carlo pour estimer la probabilité de transmission de VPH, puis j'ai conçu et entrepris une étude des couples hétérosexuels. Les objectifs de thèse ont été élaborés en utilisant les données de base de l'étude de cohorte HITCH (HPV Infection and Transmission among Couples through Heterosexual activity) qui se poursuit toujours. La population de l'étude est composée de jeunes femmes âgées de 18 à 24 ans, étudiantes à l'université ou au collège (CEGEP) à Montréal et leurs partenaires masculins.

Les résultats de l'analyse de simulation ont suggéré que le VPH a un taux de transmission élevé, ce qui a été confirmé par l'analyse des coupes de l'étude HITCH. Parmi les 263 couples inscrits entre 05/2005 et 08/2008, la prévalence de VPH était de 56% parmi les femmes et les hommes. Dans approximativement deux tiers (169) des couples, au moins un des deux partenaires était infecté par un ou plusieurs types. Le statut actuel du partenaire était le facteur de risque le plus important pour la propagation de l'infection. L'analyse des modèles de concordance et de discordance des génotypes-spécifiques a indiqué que l'ampleur de la concordance était beaucoup plus importante que prévue, et était cohérant avec une transmission rapide entre les partenaires. Il y avait d'évidence d'un effet protecteur des condoms, mais la protection était incomplète et était plus forte parmi les hommes que parmi des femmes.

Il y a deux dispositifs uniques dans l'étude qui sont nouveaux dans la recherche sur le VPH. C'est la première étude à grande échelle sur l'acquisition de VPH qui fait participer les partenaires masculins aussi. Deuxièmement, c'est la seule étude qui limite son recrutement qu'aux couples dans une relation sexuelle récente, période pendant laquelle on croit qu'une transmission considérable de VPH se produit. Les résultats sont susceptibles d'influencer les efforts de prévention du cancer du col de l'utérus et toute autre maladie liée à une infection de VPH, mais aussi les stratégies comportementales pour réduire le risque. Les résultats fourniront également des meilleures estimations des paramètres de transmission de VPH à employer dans les modèles de santé des populations et de rentabilité des stratégies de vaccination.

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI), and most sexually-active persons acquire HPV over a lifetime [2]. The vast majority of these infections will clear spontaneously, but a small proportion will persist. Persistent HPV infections result in substantial morbidity and invoke high costs associated with the treatment of clinically-relevant lesions. Some 13-18 mucosal HPV types are considered to be of high oncogenic risk (HR-HPV). HR-HPV is recognised unequivocally as the main causal factor for cervical cancer, and is further responsible for a substantial proportion of many other anogenital neoplasms and head and neck cancers. Infections with HPV types that have low oncogenic risk (LR-HPV), such as HPV-6 and -11, are associated with benign lesions of the anogenital areas known as condylomata acuminata (genital warts), oral and laryngeal papillomas, conjunctival papillomas, as well as low-grade squamous intraepithelial lesions of the cervix.

The acknowledgement that cervical cancer is caused by an STI has produced a change from an oncological to an infectious disease approach for prevention strategies. Currently, there are two vaccines available to prevent HPV infection with the two HPV types that cause 70% of cervical cancers (Gardasil®, Merck & Co., Inc., NJ, USA, currently approved in Canada; Cervarix®, GlaxoSmithKline Biologicals, Rixensart, Belgium, pending approval in Canada) and two additional HPV types that cause 90% of genital warts (Gardasil®). Yet at the initiation of my PhD studies in September 2003, vaccination was not yet a reality. At that time, HPV vaccines were being tested in clinical trials and their success was only anticipated based on the first trial report [3]. During the course of the PhD thesis, evidence for vaccine efficacy accumulated from Phase 2 and 3 trials. [4-9] The quadrivalent vaccine Gardasil® was authorized for marketing in Canada on July 10, 2006 by Health Canada [10]. The National Advisory Committee on Immunization (NACI) issued a statement on guidelines for HPV vaccine use in February 2007 [11]. This was followed by the gradual implementation of government-funded vaccination programmes for adolescent girls by Ontario,

Newfoundland and Labrador, Nova Scotia, and Prince Edward Island in 2007/08 and the remaining provinces, including Quebec, in 2008/09 [12].

The development of efficacious HPV vaccines led to a consideration of the most appropriate public health policy for implementation. Modelling work to project the public health and economic impact of HPV vaccines under various implementation strategies has occurred at a rapid pace [13-19]. Many of these projections use dynamic transmission models which require sound knowledge of the natural history of transmission and HPV acquisition, including the probability of transmission upon exposure. Results from transmission studies are eagerly awaited by modellers to improve their forecasting estimates.

Transmission studies are also important in the post-vaccine era to inform all possible prevention strategies. The quadrivalent HPV vaccine licensed in Canada, Gardasil®, is designed to prevent infection with types HPV-6 and HPV-11 (which cause 90% of genital warts) and HPV-16 and HPV-18 (which cause 70% of cervical cancer and 90% of anal cancers). Yet there are still some cancers caused by the other HR-HPV types, and duration of immunity is unknown. Vaccination of young female adolescent cohorts (grades 4-9) has been initiated in all ten provinces [12]. Nevertheless, benefits will take years to be realized. Many teenage girls and women are still susceptible to infection since vaccination outside of the government-funded programme is only available privately. The current high cost for private purchase of the complete dose of three injections is prohibitive to many women (prices range from $300 to $500 USD). Even for girls who may now receive vaccine free-of-charge, the implementation of these programmes has not occurred without debate [20,21,21,22]. Province-wide vaccine coverage in the first year of roll-out in Ontario was 53%, which was considerably lower than the goal of 85% [12]. If high vaccine coverage cannot be achieved, there will be much continued transmission of the vaccine-preventable HPV types within these adolescent cohorts.

Therefore, there is a need to better understand the conditions under which HPV transmission is facilitated or hindered to provide as many prevention options as possible. A critical research question is whether condoms provide protection. Previous work in this area frequently produced null findings, or even the paradoxical result that condom use was associated with infection, probably due to the fact that condoms tend to be used with new or casual partners who are more likely to be infected [23,24]. Measurement error was also a limitation of much previous investigation of condom use.

The HITCH Cohort Study

The overall aim of the HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity) is to further our understanding of HPV transmission to better inform prevention strategies. The design was based on the state of HPV research as it was in 2003/04 (the topic is still relevant now), and the team's extensive experience conducting epidemiologic studies of HPV, including two studies of female university students attending the McGill University Student Health Services Clinic and the Concordia University Health Clinic [25,25,26], my own experience conducting epidemiological studies of other STI [27-29], a feasibility study, and a simulation study to estimate the transmissibility of HPV [30].

HITCH is a cohort investigation of HPV transmission among young, heterosexual couples. It was initiated in 2005 and is ongoing. The study population consists of young (aged 18-24) women attending a university or CEGEP/college in Montreal and their male partners. HITCH will ultimately enrol 600 couples of which 368 were already accrued and being followed as of March 2009. Women are followed for 24 months and men for four months. Computerized self-completed questionnaires are used to collect sexual behaviour information. Men provide clinician-obtained samples of the epithelium of the penis and scrotum. Women self-collect vaginal swabs. Participants also provide blood, oral, and hand

specimens. HPV-DNA testing and high-resolution typing is done by polymerase chain reaction, which identifies all relevant mucosa-associated genotypes of HPV.

There are two unique features of the HITCH Cohort Study that are novel for HPV research. It is the first large-scale study of HPV acquisition that involves the male partner. Secondly, it is the only study to restrict enrolment to couples in a new sexual relationship, a time at which considerable transmission is believed to occur. This restriction has an important implication. It allows for the analysis and interpretation of cross-sectional enrolment data as if they were from a retrospective cohort for rapid HPV transmissions that occur within the first months of sexual activity with a partner.

Results from the HITCH Cohort Study and the specific analyses for the PhD thesis are likely to influence prevention efforts for cervical cancer and other HPV-related disease, including behavioural strategies to reduce risk. Results will also provide improved estimates of HPV transmission parameters to be used in models of the population health impact and cost effectiveness of various HPV vaccination strategies.

Objectives

The overall aim of the HITCH Cohort Study is to further our understanding of HPV transmission between heterosexual couples to better inform prevention strategies. For the PhD thesis itself, the objectives focused on research questions that could be addressed with data obtained at the enrolment visit. These were:

1. To describe male and female type-specific HPV prevalence;

2. To describe gender-specific risk factors for HPV infection;

3. To describe type-specific HPV concordance/discordance in newly-formed relationships;

4. To identify risk factors for type-specific HPV concordance versus discordance among newly-formed couples in whom at least one partner is positive for HPV; and

5. To estimate HPV transmission probabilities per partnership and per coital act, using type-specific HPV concordance at enrolment as a proxy measure of rapid transmission among couples in whom at least one partner is positive for that HPV-type.

It was hypothesized that the HPV transmission probability would average 60% per partnership, and between 5-40% per coital act. These *a priori* estimates were based on three independent sources of information. The first was the only HPV-related transmission study that had been conducted by 2003/04, which was a study of the transmission of genital warts published in 1971 [31]. The second was the preliminary simulation analysis conducted for Manuscript Two [30]. The third was an estimate used for mathematical modeling of the impact of HPV vaccination by Barnabas and colleagues [32].

SECTION 2: LITERATURE REVIEW AND MANUSCRIPT I

Several infectious agents have been established as carcinogenic or probably carcinogenic to humans by the International Agency for Research on Cancer (IARC). Among those for which the evidence is compelling are hepatitis B and C virus (HBV and HCV) (liver cancer), certain genotypes of human papillomavirus (HPV) (cervical, anogenital, and oral cancers), Epstein-Barr virus (EBV) (certain types of lymphomas and nasopharyngeal carcinoma), human T cell lymphotropic virus I (some forms of leukemias), human immunodeficiency virus (HIV) (AIDS-associated malignancies), human herpes virus 8 (HHV-8) (Kaposi's sarcoma), *Helicobacter pylori* (stomach cancer and mucosa-associated lymphoid tissue lymphomas), *Schistosoma haematobium* (bladder cancer), and some forms of liver flukes (e.g., genus *Opistorchis*) (liver cholangiocarcinoma) [33]. Altogether, it has been estimated that these agents cause 18 percent of incident cancers worldwide (12 percent, 6 percent, and 0.1 percent for viral, bacterial and parasitic infections, respectively) including as much as 5 percent for HPV alone [33].

Epidemiology of cervical cancer

HPV infection is recognized today as the necessary causal factor of all cervical cancer cases in the world [34,35]. Cervical cancer is the second most common malignant neoplasm of women globally, accounting for nearly 10% of all cancers (non-melanoma skin cancers excluded). It is estimated that 493,000 new cases of invasive cervical cancer were diagnosed in 2002, 83% of which were in developing countries [36]. Cervical cancer can be characterized as a disease of poorer nations, with a disproportionate number of cases and the greatest proportion of deaths occurring in such regions. The highest risk areas for cervical cancer are in sub-Saharan Africa, Melanesia, the Caribbean, and Latin America, with average annual incidence rates above 30 per 100,000 women (rates standardized according to the world population of 1960). Not surprisingly, in view of the substandard healthcare conditions, these areas also bear a disproportionately high mortality burden due to cervical cancer. Every year, an

estimated 273,000 deaths from cervical cancer occur worldwide, with over three-fourths of them in developing countries [36].

One of the main reasons for the global heterogeneity in cervical cancer incidence and mortality is the implementation of Pap cytology screening in high-income countries over the past 50 years. In those countries where universal screening was adopted, there was a 50%-80% reduction in cervical cancer rates [37]. Cervical cancer rates are now substantially lower in Western Europe and North America at less than 10 new cases annually per 100,000 women [38]. In 2008 in Canada, 1,300 new diagnoses and 380 deaths were estimated, resulting in age-standardized incidence and mortality rates of 7.1 and 1.8 per 100,000 women per year, respectively [39]. Most cases occur among women aged 30 to 59 years. Cervical cancer takes a particularly heavy toll among Aboriginal women and Latin American immigrants. These groups experience cervical cancer rates that are comparable to those in high-risk developing countries [37].

Evidence for HPV as the cause of cervical cancer

Over 130 types of HPV have been catalogued thus far [40]. These are classified according to their tissue tropism (mucosal or cutaneous) and oncogenic potential (high-oncogenic risk [HR] or low-oncogenic risk [LR]). There are about 40 HPV types that infect the mucosal areas of the body, such as the epithelial lining of the anogenital or oral tract. Between 13–18 types have been identified as HR-HPV according to their degree of association with malignancy [34,41]. The latest conservative classification published by the WHO International Agency for Research on Cancer (IARC) referred to HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, and 66 as having high oncogenic potential [34].

The evidence for oncogenicity resulted from years of multidisciplinary research by molecular biologists, virologists, immunologists, clinicians and epidemiologists. Clues that cervical cancer was somehow related to sexual behaviour were present as long ago as the mid-1800s when Dr. Domenico Rigoni-Stern reviewed Italian mortality records and observed that cervical cancer

predominantly occurred among married women and almost never among nuns [42,43]. It was these and subsequent observations of the strong and consistent associations between women's number of sexual partners and cervical cancer risk that eventually lead to the identification of the causal agent, HPV.

During much of the 1960's and 1970's, the consistency of epidemiologic findings pointing to a sexually-transmitted infection model propelled research efforts to identify the putative causal microbial agent or agents. Many sexually-transmitted agents were considered, and the herpes simplex virus (HSV-2), syphilis, gonorrhea and *Chlamydia trachomatis* were suspected. The evidence available at the time indicated that genital infection with HSV-2 was the most likely culprit. Although HSV was proven carcinogenic in vitro and in vivo, the evidentiary link to cervical cancer was mostly indirect [44]. In the 1980's the attention gradually turned to a new candidate, HPV, with the emergence of a consistent evidence base from molecular biology. Harold Zur Hausen was the primary leader behind the long-standing hypothesis that proliferation of HPV in the cervical epithelium leads to disruption of cell maturation that develops as cervical intraepithelial neoplasia (CIN), the precancerous lesion [45]. He and others subsequently conducted groundbreaking research that led to an understanding of how the early viral oncogenes E6 and E7 interfere with key regulators of the cell cycle, thus immortalizing cervical cells and preventing them from undergoing senescence and being lost by the normal exfoliation that regenerates the epithelium.

The molecular basis for plausibility was essential for the scientific community to accept that HPV infection was the likely cause of cervical cancer. This acceptance did not come easily. There was much skepticism concerning the role of HPV infection. Reasons included observations that HPV infection was quite ubiquitous and, as such, it could not plausibly be a cause of disease. Contributing to the controversy were the weak associations that were observed in early molecular epidemiologic studies, unlike that which one would expect from a key intermediate endpoint in cervical carcinogenesis. Later it was learned that measurement error in detecting cervical HPV DNA (thus leading to

misclassification of the exposure) in these initial case-control studies had produced considerable underestimation of the relative risk for the effect of HPV infection on cervical cancer (reviewed in [46]).

As the experience with HPV DNA testing methodology led to the adoption of modern assays, such as polymerase chain reaction, the true magnitude of the relative risks were revealed. It was a series of large and well-conducted case-control studies by the IARC using modern laboratory techniques that demonstrated that infection with certain HPV types is unequivocally one the strongest cancer risk factors ever found [47,48]. For example, the relative risk between tobacco and lung cancer is estimated between 7 and 15 [49], whereas the relative risk between HPV-16 and squamous-cell cervical cancer is 435 [47]. These studies also produced precise HPV type-specific estimates of relative risks, allowing for identification of specific types for prevention strategies such as HPV vaccination [47,50].

The role of HPV for other cancers and benign disease

HPV has been implicated in the development of malignancies of other anogenital sites besides the cervix including vagina, vulva, penis and anus [51]. Unlike cervical cancer, in which 100% of cancers are caused by HPV, cancers of other anogenital sites show lower but still substantial risk attributions for HPV. It is estimated that 90% of anal cancers and 40% of vaginal, vulvar and penile cancers are attributable to HPV [33]. These cancers are rare but there is some evidence that they may be rising. For example, in Quebec the incidence of anal cancer among women rose from 0.4 per 100,000 in 1984-86 to 0.7 per 100,000 in 1999-2001 [52]. It is also thought that HPV may cause a substantial proportion of malignancies of the upper aero-digestive tract [51].

Benign HPV-related tumours include low-grade squamous intraepithelial lesions of the cervix and anogenital condylomata acuminata (anogenital warts). The term “benign” is used here to reflect low to no risk of progression to cancer. Although these diseases are not cancerous they still contribute a substantial burden of

disease. Anogenital warts include vulvo-vaginal, perianal and penile warts. An estimated 90% are caused by HPV-6 and 11 [53]. These infections are responsible for substantial morbidity among adults and invoke high costs associated with the treatment of clinically relevant lesions. Canadian population-based data are unavailable, but notably the incidence of anogenital warts has dramatically increased in both the United States and the United Kingdom over the past three decades [54].

Other benign diseases associated with HPV include laryngeal papillomas (laryngeal warts), oral warts or oral focal epithelial hyperplasia, and papillomas in the conjunctiva. Oral HPV can cause the rare disease called Recurrent Respiratory Papillomatosis (RRP) which might occur in children as well as in adults. Juvenile-Onset Recurrent Respiratory Papillomatosis (JORRP) is mostly caused by HPV-6 or 11 perinatally acquired from an infected mother [55]. Although JORRP is a rare event with an incidence of 4.3 cases per 100,000 children-years in United States, it has a devastating impact on the quality of life of the child and its family, and accounts for significant health care expenditures and mortality [55].

Preamble to Manuscript I

The burden of HPV-associated cancers and benign conditions is substantial. The success of Pap test screening for precancerous cervical lesions is a major achievement, but it occurs at great cost and is not 100% effective. The sensitivity of Pap cytology to detect high-grade CIN or invasive cervical cancer is relatively low at 55 percent, whereas its specificity is considered high, at 97 percent [56]. Most developing countries have yet to derive the same benefit from Pap test screening, either because programmes were not implemented or were instituted with incomplete quality assurance and follow-up procedures that are necessary for effective screening [57]. As a result, incidence of cervical cancer has not declined in many developing countries, possibly due also to secular changes in sexual behaviour [57]. Furthermore, screening for other HPV-associated cancers and

benign conditions does not exist. There is considerable interest in exploring other opportunities for prevention of all HPV-related disease.

Now that these diseases are known to have an infectious cause, the paradigm for prevention has reoriented to an infectious disease approach. As such, an understanding of the epidemiology of HPV and its transmission dynamics is essential to inform prevention strategies, including behavioural approaches such as the promotion of condom use, and to accurately project the population impact of the newly-available HPV vaccines.

As the HPV vaccines were approaching licensure in 2006, a group of over 100 international experts came together to produce a monograph summarizing the state of knowledge regarding cervical and other HPV-related cancers, HPV epidemiology, screening, and vaccine introduction and implementation needs. I was given the responsibility of writing a chapter in this monograph which focused on the current knowledge of the epidemiology of HPV infection and transmission dynamics in the pre-vaccine era. This monograph chapter is presented in the thesis as Manuscript I.

MANUSCRIPT I:

Epidemiology and Transmission Dynamics of Genital Human Papillomavirus Infection

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ABSTRACT

This chapter provides an overview of the epidemiology of HPV infection, with a focus on the dynamics of sexual transmission. We explore concepts related to the spread of sexually transmitted infections, including population prevalence, duration of infectivity, patterns of sexual contacts, and transmissibility, including modifiers of susceptibility and infectivity[58]. HPV prevalence and incidence are high in most studies, particularly of young women. There is strong evidence that transmission occurs primarily via sexual activity, most commonly vaginal and anal intercourse. Although the duration of infectivity may be short, current evidence suggests HPV is highly transmissible. The implications of transmission dynamics for the success of future HPV vaccines are discussed.

INTRODUCTION

This chapter provides an overview of the epidemiology of HPV infection, with a focus on the dynamics of sexual transmission. We explore concepts related to the spread of sexually transmitted infections (STI), including population prevalence (an indicator of the burden of disease and of the probability of encountering an infected partner), duration of infectivity, patterns of sexual contacts, and transmissibility, including determinants of susceptibility and infectivity [58,59]. The implications for future HPV vaccine are also discussed.

PREVALENCE

Genital HPV infection is the most common STI among women [58]. HPV infects the mucosal areas of the cervix, vagina, vulva, and anus. Detection of HPV types by polymerase chain reaction (PCR) assays varies greatly by age and by geography as shown in a pooled analysis conducted by the International Agency for Research on Cancer (IARC) [60] and in a meta-analysis of published studies (S. de Sanjose, unpublished data, 2006).

Age-specific prevalence and geographic variation of HPV infection in women

Among asymptomatic women in the general population, the prevalence of HPV infection ranges from 2%-44% [61]. A recent meta-analysis estimated HPV prevalence among women with normal cytology using data from 78 published studies [62]. As shown in **Table 2.1,** the adjusted global prevalence was 10.41% (95% confidence interval 10.2-10.7%), with considerable variation by region. No data were available for Oceania. The number of women harboring HPV DNA worldwide is estimated to be 291 million. Around 105 million women worldwide would have an HPV 16 or 18 infection, the most common oncogenic types in cervical carcinomas, at least once in their lifetime. The IARC pooled analysis used the same PCR method to evaluate specimens systematically collected throughout the world and largely corroborate these observations [60].

The meta-analysis also indicated that prevalence is highest for young women and decreases in the middle age groups (see **Figure 2.1**). At age 65 and older an increase of the HPV prevalence is observed in the crude analysis. However, the adjustment for potential confounding factors (such as study design, sampling collection device, HPV assay, etc.) results in a flattening of the age-specific shape in these age groups, although the estimates are not statistically significantly different. This pattern is observed in many studies all over the world, with the exception of Asia, where the age-specific curves decrease smoothly with increasing age and no second peak is observed [61,63]. The reasons for the second peak and its geographic variation are unclear, but may be influenced by one or more non-mutually exclusive mechanisms, as follows [61]. Reactivation of previously undetectable infections acquired earlier in life could occur due to a gradual loss of type-specific immunity (or sudden, via hormonal influences during the post-menopausal years). The second peak could also originate from acquisition of new infections due to sexual contacts with new partners later in life. Also plausible is a cohort effect, i.e., the varying prevalence at different ages may reflect the changing experience of successive birth cohorts in being exposed to HPV in different eras. Because the changes in sexual mores over the last several

decades affected some cultural groups more than others, this explanation cannot be ruled out. Further, birth cohort differences in cofactors that may affect HPV progression or clearance (e.g., smoking, parity, oral contraceptives) and competing risks (e.g., mortality due to other causes) could also be involved. Finally, in populations without routine screening, a dip in prevalence in middle-aged women may not occur because underlying lesions remain undiagnosed and untreated.

Geographic and cultural variations in sexual behaviour of women and their male partners may result in differential rates of new HPV acquisition. Older men's behaviour may be more critical than women's. Data from 29 countries indicate with considerable regional homogeneity that 80% of men and 65% of women aged 40 to 80 years were sexually active in the past year, with the exception of Asia, where both men and women reported lower sexual activity [64]. In this same study, 5-11% of men compared to 1-6% of women reported more than one current partner (E.O. Laumann, personal communication, 2006). It will be difficult to elucidate the causes of age-related changes without frequent and long-term follow up of cohorts in multiple settings [61].

HPV prevalence in men

HPV DNA has been clearly identified in the male genitalia, anal mucosa and oral cavity, but compared to women, fewer prevalence data exist. Sampling methods in men are more variable and have not been thoroughly validated. There are also difficulties associated with collecting cell specimens via exfoliation of cornified epithelium, which further contributes to the heterogeneity in methods. Partridge and Koustky [65] reviewed 13 studies, and observed an HPV prevalence ranging from 3.5% to 45% for all types, and 2.3% to 34.8% for high-risk (HR) HPV. In all but one study, the most common type was HPV 16. The prevalence of low-risk (LR) HPV ranged from 2.3% to 23.9%. Penile HPV prevalence increased with increasing number of sex partners and with the number of sex worker partners [65,66]. Men who have sex with men have been observed to have a particularly high

prevalence of HPV [67]. Few HPV serological studies have been conducted among males. The largest one reported lower seropositivity than among women and a peak prevalence among men aged 30-39 [68]. Overall, the HPV data in men suggest that HPV prevalence is lower than in women and that penile tissues may be less receptive to HR-HPV types [65].

DURATION

The duration of infectivity is an important component of the rate of spread of an STI in a population, with infections of longer duration having a potentially greater impact [58]. Longitudinal research has consistently shown that most HPV infections detected via molecular hybridization techniques are transient, and are no longer detectable within one to two years [61,69]. HR infections seem to persist longer than LR ones [61]. Among HR types, there is some evidence that HPV 16 may persist longer than other types. This suggests that the rate of spread of HR-HPV in populations, including HPV 16, would be greater than for LR-HPV, assuming equivalent sexual contact patterns and transmissibility.

HPV infection among men seems also to be of short duration, with most infections no longer detectable after one year [70,71]. There is some evidence that more HR than LR male infections persist [71,72].

It is unknown whether HPV is sufficiently infectious to result in transmission for the entire duration of detectable infection. Infectiousness may vary with viral load, since HPV positivity has been shown to correlate with viral load in the partner [73], but little data are available.

INCIDENCE

The key measure to determine the spread of an STI is incidence, the number of new HPV infections in a susceptible population over time. Other demographic influences notwithstanding, young women have high rates of HPV acquisition, although the influence of age is not so clear for men. Several studies have

reported cumulative incidences of 40% or greater after three years of follow-up [61]. Rates of HPV infection in young women are high following sexual debut, and remain high with acquisition of each new sex partner [74,75]. As with prevalence, incidence in women tends to decline with age, although second peaks are sometimes observed in older women [76,77]. Incidence rates are generally higher for HR-HPV types than for LR types, with varying estimates according to the population studied and number of HPV types tested [61]. Incidence rates for HPV 16 tend to be higher than those observed for other HPV types [61]. Co-infection with multiple HPV types and sequential infection with new types are common, and the risk of acquiring new HPV types appears to be independent of prior infection with other types [61].

Few studies have evaluated HPV acquisition in men. Nevertheless, the evidence suggests that incidence is similarly high among men, with cumulative incidences ranging from 14% to 21% within 3-8 months [65].

ROUTES OF INFECTION

Data supporting sexual intercourse as the primary route of genital HPV infection include documented transmission of genital warts between sex partners [31], concordance in sex partners for type-specific and HPV-16 variant-specific HPV DNA (see **Table 2.2**), the rarity of genital HPV infection in women who have not had vaginal intercourse [78], the strong and consistent associations between lifetime numbers of sex partners and HPV prevalence in women [78] and men (albeit less consistently) [65], and increased risk of HPV acquisition following new and recent sex partners [79]. Sexual intercourse includes both vaginal and anal intercourse. Receptive anal sex is strongly associated with HPV detection in the anal canal in men who have sex with men [65], and to a lesser degree for women [80]. One explanation for the latter is that some anal HPV infections in women may occur via viral shedding of cervical or vaginal HPV infections in vaginal discharge [80].

Although plausible, mechanisms other than sexual intercourse are less common routes of genital HPV infection (see **Table 2.3**). While oral and digital infection with genital HPV types clearly occurs, the risk of transmission via digital-genital or oral-genital contact appears to be minimal. Similarly, HPV infection via perinatal transmission or in children does occur, as both HPV DNA and serum antibodies have been detected in infants and children. Data suggest that this is rare and unlikely to result in persistent infection, however. Nonetheless, the possibility of infection through mechanisms other than intercourse suggests that transmission via sexual intercourse between two virgins is theoretically possible.

SEXUAL BEHAVIOUR LEADING TO EXPOSURE TO HPV

Knowledge of patterns of sexual behaviour and sexual networking in populations is fundamental for the understanding of HPV transmission dynamics [81]. Generally, the trend in many western countries is that sex behaviours and attitudes have become more permissive over time [58]. Many aspects of sexual behaviour affect the likelihood of encountering an HPV-infected partner (**Table 2.4**).

Sexual debut

Several cross-sectional studies have reported that earlier age at first intercourse ("sexual debut") or shorter intervals between menarche and age at first intercourse were risk factors for prevalent HPV infection [82]. However, the reasons for this relationship are unclear. Earlier intercourse may be a marker for other risky sexual behaviours, such as greater lifetime numbers of partners and concurrent partnerships [58]. Indeed one study reported that the association with age at first intercourse was mediated by other sexual behaviour variables [83]. Conversely, in a recent longitudinal study of 15-19 year old women sampled within one year since sexual debut the risk of infection *increased* with the interval between menarche and age at first intercourse, likely due to the tendency of older women to form partnerships with older, more sexually experienced partners [75]. Biological mechanisms, including cervical immaturity, inadequate production of protective

cervical mucus, and increased cervical ectopy, may make younger women and adolescents more susceptible to HPV infection [82].

In developed countries, the age at sexual debut appears to be decreasing over time [58], although some recent data suggest a reversal of this trend in the United States [84]. In developing countries, there is considerable variability in the prevalence of virginity, age at sexual debut, and premarital sex among women aged 15-24 [85]. In 10 countries from sub-Saharan Africa, Latin America and the Caribbean, the prevalence of premarital sex was greater in countries in sub-Saharan Africa (see **Figure 2.2a**) [85]. However, in Latin America there is evidence that the prevalence of virginity is declining over time and premarital sex is increasing (see **Figure 2.2b**) [85]. The trend of increased exposure to HPV at younger ages has important implications for vaccination programs.

Number of partners and acquisition of new partners

The associations between numbers of new and recent sex partners and likelihood of detecting HPV DNA in female genital tract specimens are strong and consistent [78,79]. The rate of acquisition of partners, or contact rate, plays a key role in STI transmission dynamics [59]. Population surveys show heterogeneity in the number of lifetime and recent sex partners, with a majority having no or one partner, and a minority having multiple partners [58]. More sex partners and non-spousal/non-cohabitating partners are consistently reported among men than women, and among the young than the old [58,85]. Gender differences could be explained by a small proportion of women having sex with many partners (e.g., sex workers), or by underreporting of sexual activity by women, or men's overreporting [85].

Characteristics of partners and sexual networks

Characteristics of male partners are critical for female HPV acquisition. In case-control studies of cervical cancer, male partners of cases report higher numbers of partners than those of controls [79]. Female HPV prevalence and acquisition have been positively associated with women's estimates of their male partners' lifetime

number of partners [74] or not knowing a male partner's prior sexual experience [74,75].

Patterns of sexual networking are critical for transmission dynamics [81]. Sexual networks are made up of individuals who are sexually connected, either directly or indirectly. Important network features that increase the chances of transmission are larger network size, higher contact rates, and the patterns of sexual mixing or partner choice [81]. Random mixing occurs when an individual is equally as likely to have sex with any other individual [59]. Assortative mixing occurs when similar individuals tend to form contacts, whereas dissortative mixing occurs when individuals tend to form contacts with individuals who are different from them. Dissortative mixing tends to increase the risk for STI transmission [81]. Most surveys show that mixing tends to be moderately assortative with respect to age, race/ethnicity, or number of sex partners [59], but not always [58,81]. For example, in many cultures women tend to form partnerships with older men [59,81]; this could explain in part the high HPV prevalence among younger women, and its geographical variation.

"Core groups" or groups of highly sexually active individuals with many partners are believed to contribute disproportionately to the spread of most STIs [59,81]. HPV infection is not restricted to core groups, however, as it is also relatively common among moderately sexually active individuals [58,78]. This may be due to inherent biological properties of HPV as a virus that is well adapted to be transmitted via skin-to-skin contact and to infect only the epithelial lining of susceptible body areas without the need to invade connective tissue or to be disseminated regionally or systemically. Should HPV vaccines reduce HPV transmission in the general population, HPV could then become concentrated in core groups, and the behaviours of these highly sexually active individuals will be of greater importance for research and prevention [86]. Direct targeting of vaccines to core groups would not be expected to reduce HPV population prevalence, however, given the lessons learned from the hepatitis B vaccine [87].

Bridging occurs when sexual linkages are formed between members of high and low prevalence subpopulations, and provide a conduit for infection between them [58]. For example, STI transmission between the homosexual and heterosexual populations is possible through bisexual activity [86], and could have implications for female-only vaccination strategies.

Concurrency and serial monogamy

The timing of sexual partnerships plays a role in determining STI spread. An example is sex partner concurrency, in which sexual partnerships overlap each other in time [81]. Concurrent partnerships are not uncommon. They are reported by 32% to 54% of adolescents and 12% to 40% of adults in the United States [88]. Because awareness that one's partner has other partners has been shown to be poor [88], this implies that long-term monogamy on the part of one partner may not necessarily reduce risk of infection.

The timing of non-overlapping partnerships, or serial monogamy, may also be important. A United States survey of sexual behaviour found that, among serially monogamous women, the mean gap between partners was 8 months for women aged 15-19, 11 months for women in their twenties, and 18 months for women aged 30-44 [89]. Given the average duration of HPV infection among women, serial monogamy must contribute to HPV transmission. Knowing a partner for more than 8 months has been associated with lower risk of HPV acquisition among women [74], which could be explained by clearance or waning infectivity in the male. Likewise, intercourse with a partner who had no other recent partners would be expected to reduce infection risk [74].

TRANSMISSIBILITY AND FACTORS AFFECTING TRANSMISSION

Probability of transmission upon exposure

To our knowledge, there have been no published reports of the transmissibility of HPV based on empirical data [78]. A study of the transmissibility of genital warts,

conducted before HPV was identified as the causal agent, observed that 60% of sexual partners of patients with warts subsequently acquired them [31]. This suggests high transmissibility, at least for HPV types that cause genital warts.

To date, research of HPV in couples has consisted of cross-sectional assessment of prevalent HPV infection in both partners, rather than transmission per se (**Table 2.2**). Most, but not all, of these studies found relatively poor concordance for type-specific HPV positivity. In two studies, HPV-type-specific positive concordance was greater than expected by chance [73,90]. Concordance was associated with more recent sexual intercourse [90] and higher viral load [73]. Methods for HPV testing among men are in the process of being refined, and it is possible that some of these previous studies had limited ability to detect HPV infections. Nevertheless, HPV status in couples where the woman has cervical lesions is likely not reflective of those in couples where the female is lesion-free. Further, couples in these studies tended to be older, with relationships of long duration. The transmission event likely occurred years prior to enrolment, and many infections would have resolved. To study HPV transmission, one would ideally recruit relatively young couples that are newly forming.

A stochastic computer simulation study investigated values of HPV transmissibility that were consistent with observed incidence among female university students [30]. The probability of HPV transmission per coital act ranged from 5% to 100%, with a median of 40%. Similarly, Barnabas et al. [32] recently estimated the per-partner male-to-female transmission probability as 60% for HPV 16 using Finnish data on seroprevalence. This is identical to the observed per-partner transmission probability for genital warts [31].

These results suggest that HPV is more transmissible than other viral STIs, but is comparable to bacterial STIs. Studies of HIV- or HSV-2-discordant couples indicate that the probability of transmission is 1 per 1,000 acts of intercourse [59,91]. Per-partnership transmission probabilities for bacterial STIs range from 20% for chlamydia, 50% for gonorrhea, 60% for syphilis, to 80% for *Haemophilus*

ducreyi, the causal agent of genital ulcers [59]. With high transmissibility, vaccines would need to reduce infectivity several-fold in breakthrough infections to stop the chain of transmission. This could happen via a reduction in viral load.

Factors affecting the probability of transmission

A number of factors may influence the probability of transmission of an STI such as viral load, other STIs, circumcision, condoms, immune mediators of susceptibility or infectivity, and nutrition (**Table 2.4**). Cervical infection with other STIs, such as *C. trachomatis*, may increase susceptibility to HPV infection via cervical inflammation or microabrasions, or facilitate persistence of HPV infection through immunologic mechanisms [92]. The similar sexual behaviour risk factor profiles for HPV and other STIs, however, make it difficult to discern whether other STIs are simply markers for exposure to HPV, or act as true cofactors by increasing susceptibility or infectivity [61].

Evidence for male circumcision as a risk factor for genital HPV infection in both men and women is conflicting [65]. One study reported a protective association against prevalent HPV infections and repeat detection of prevalent infections at a one-year follow-up visit, but not against detection of new infections [71]. Male circumcision has not been linked to female HPV acquisition, although some but not all case-control studies have reported that male partners of women with cervical cancer are less likely to be circumcised than male partners of control women [79]. If male circumcision does contribute to the spread of HPV infection, it is unclear whether it affects men's susceptibility to infection, and/or infectivity and persistence upon infection.

Condoms are an effective barrier against genital HIV transmission; however, data for other STIs, including HPV, are equivocal [23]. Condoms appear to offer some protection against developing high-grade cervical neoplasia and invasive cervical cancer [23], and have been shown to promote regression of cervical neoplasia and penile lesions, and clearance of infection in men and women [69]. Nonetheless, most

studies evaluating the relationship between condom use and HPV infection have failed to demonstrate a protective effect of condoms [23]. This may in part be due to a tendency for condoms to be used more often in casual relationships, where the probability of encountering an infected partner is higher [61]. Data from a recent prospective cohort study of female university students enrolled prior to or within 2 weeks of their first intercourse, however, did show an over three-fold protective effect of condoms on HPV acquisition [93]. Even with consistent condom use, HPV infections can still be transmitted through contact with areas of unprotected genital skin. Furthermore, a protective effect of condoms, even if one exists, may diminish over multiple sex acts in ongoing relationships due to high infectivity [30].

Increased genital HPV prevalence has been observed in men and women with immunodeficiencies, regardless of the cause. High HPV prevalence has been consistently observed among HIV-seropositive populations of women and men [67]. Some HLA class II polymorphisms have also been shown to influence risk of acquisition and clearance of HPV infections [61].

While there is evidence to suggest that hormonal factors may influence susceptibility to HPV infection [78], associations between hormonal contraceptive use and HPV infection have been inconsistent [94]. Hormonal contraception may increase susceptibility to infection (e.g., via increased ectopy [94]) or it may also be confounded by unmeasured sexual behaviours. Most studies have not reported associations between hormonal contraceptive use and HPV infection independent of sexual behaviour [94]. Risk of persistent HPV infection seems to be negatively associated with consumption of fruits and vegetables and dietary or circulating levels of vitamin C and E, ands several carotenoids [61].

Finally, the effect of smoking on HPV acquisition is unclear. Most studies in both men and women have failed to associate smoking with HPV detection, or positive associations were attenuated after controlling for sexual behaviour [78,79]. One study did report a significant positive association between current smoking and incident HPV infection, even after controlling for measured sexual behaviour variables [74].

While one explanation for this finding is that smoking increases susceptibility to infection, smoking may also be a proxy measure of unmeasured sexual behaviours.

IMPLICATIONS FOR VACCINES AND FUTURE RESEARCH

There is strong evidence that transmission occurs primarily via sexual activity, most commonly vaginal and anal intercourse, although perinatal and non-sexual transmission does occur. The common tools for STI prevention, namely the promotion of abstinence or delay in sexual activity, monogamy, condoms, and treatment of existing infections, are not all equally applicable to HPV. Delay in coitarche and monogamy should reduce risk, but will not eliminate it, since HPV is highly prevalent and any sexual activity poses a risk. Condoms may provide some protection, but transmission may still occur via unprotected areas of genital skin. Currently, no treatment of existing infections is available to reduce the duration of infectiousness.

The features of transmission dynamics have important implications for future HPV vaccines. With longer duration of infectivity, more frequent formation of sexual partnerships that facilitate exposure between infected and susceptible individuals, and/or higher transmissibility, the extent of vaccine coverage necessary to reduce population HPV prevalence increases. Many of these issues vary across populations, suggesting that the potential vaccine impact will be population specific even with equivalent coverage. Furthermore, the nature of transmission dynamics will reduce the impact of vaccines in the face of vaccine failure. This would include scenarios where the vaccine has no effect in some individuals (“take”), if the vaccine does not fully eliminate susceptibility (“degree”), or if there is loss of protective immunity over time (“duration”).

To further our understanding of HPV transmission dynamics, data on acquisition and persistence among heterosexual men and men who have sex with men are urgently needed. The natural history of HPV infection and patterns of viral load

and how this impacts on infectiousness, remains to be understood in both men and women. Frequent and long-term follow-up of women is necessary to determine the causes of age-related changes in HPV positivity. In particular, longitudinal studies of older women are needed to evaluate whether new partner acquisition is associated with HPV detection at all ages, and patterns of viral load by age. Ideally studies of HPV acquisition would also determine the HPV status of sexual partners.

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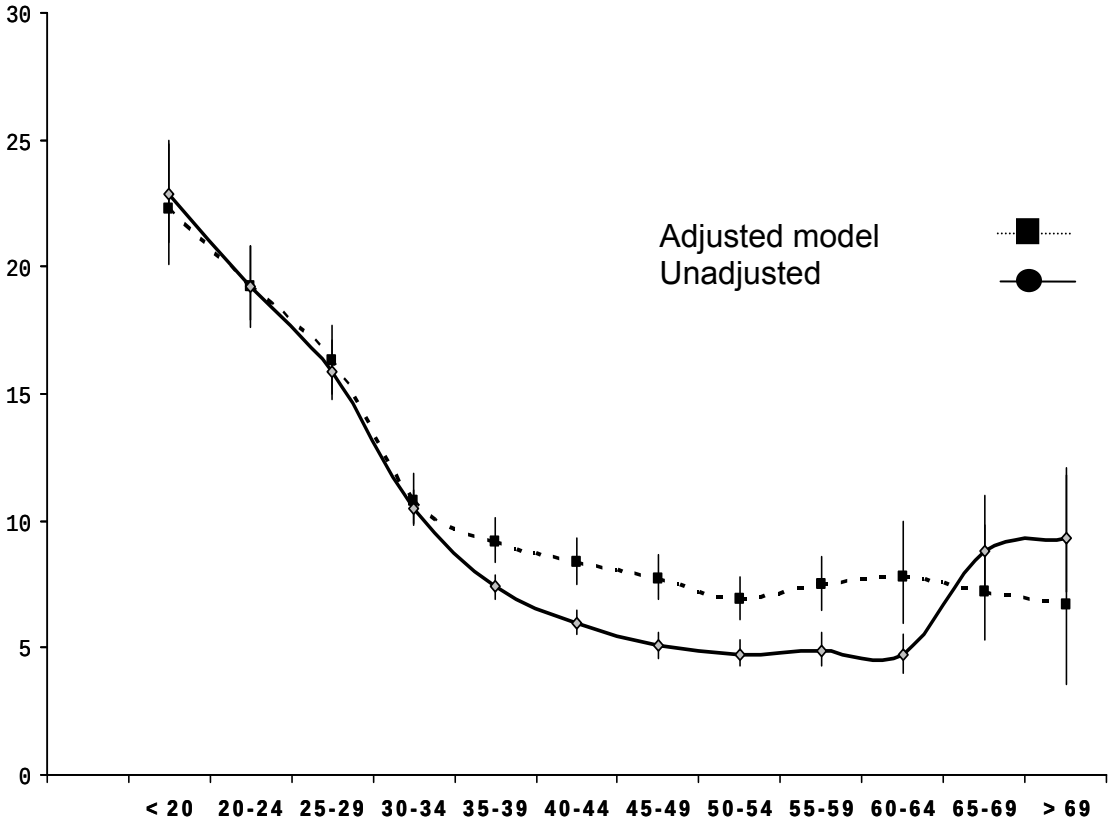
Figure 2.1. Age-specific HPV prevalence among women with normal cytology. Crude and adjusted estimates are presented based on the meta-analysis of 78 studies.



Adapted from de Sanjose et al. [62]

Age-specific prevalence estimates were calculated by means of logistic models based on a discriminatory analysis that included geographical area, study type, study design, youngest and oldest age values of each study, publication year, sampling collection device, cell storage medium, HPV assay, primer used and HPV type-specific assay.

Figure 2.2a. Percentage of never-married women aged 15-24 years who reported premarital sex in the past 12 months, selected countries in Africa. Adapted from Curtis & Sutherland, 2004 [85].

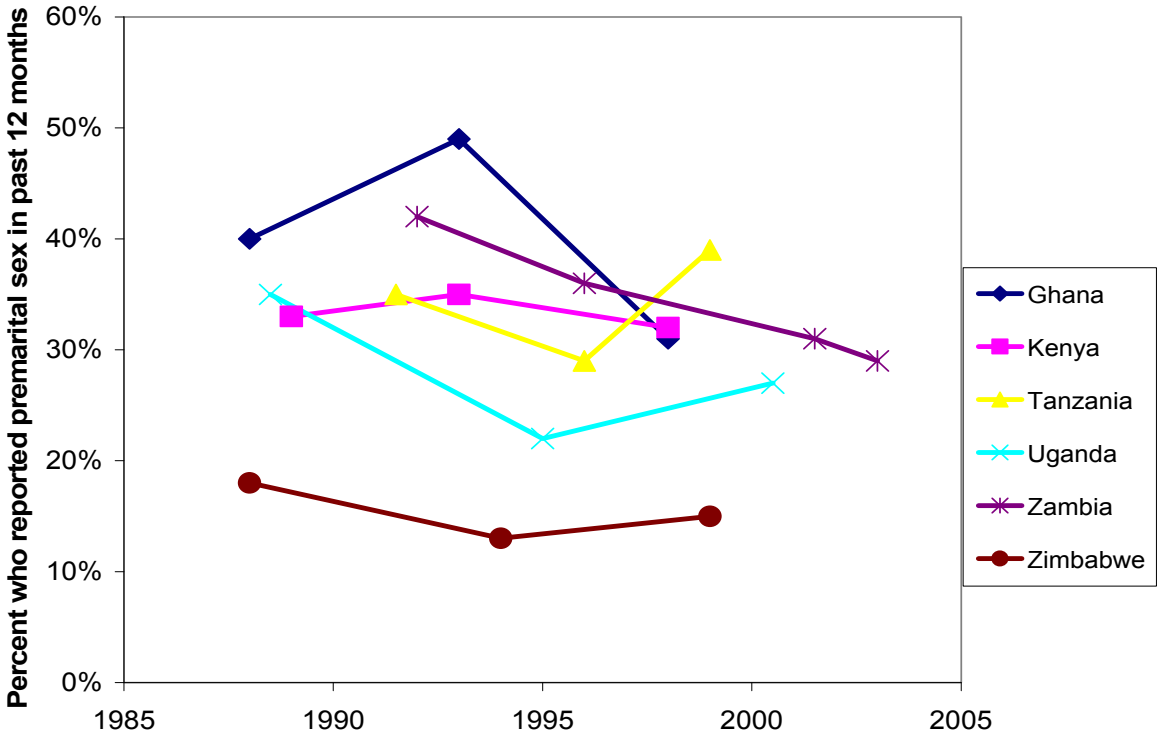


Figure 2.2b. Percentage of never-married women aged 15-24 years who reported premarital sex in the past 12 months, selected countries in Latin America and the Carribean. Adapted from Curtis & Sutherland, 2004 [85].

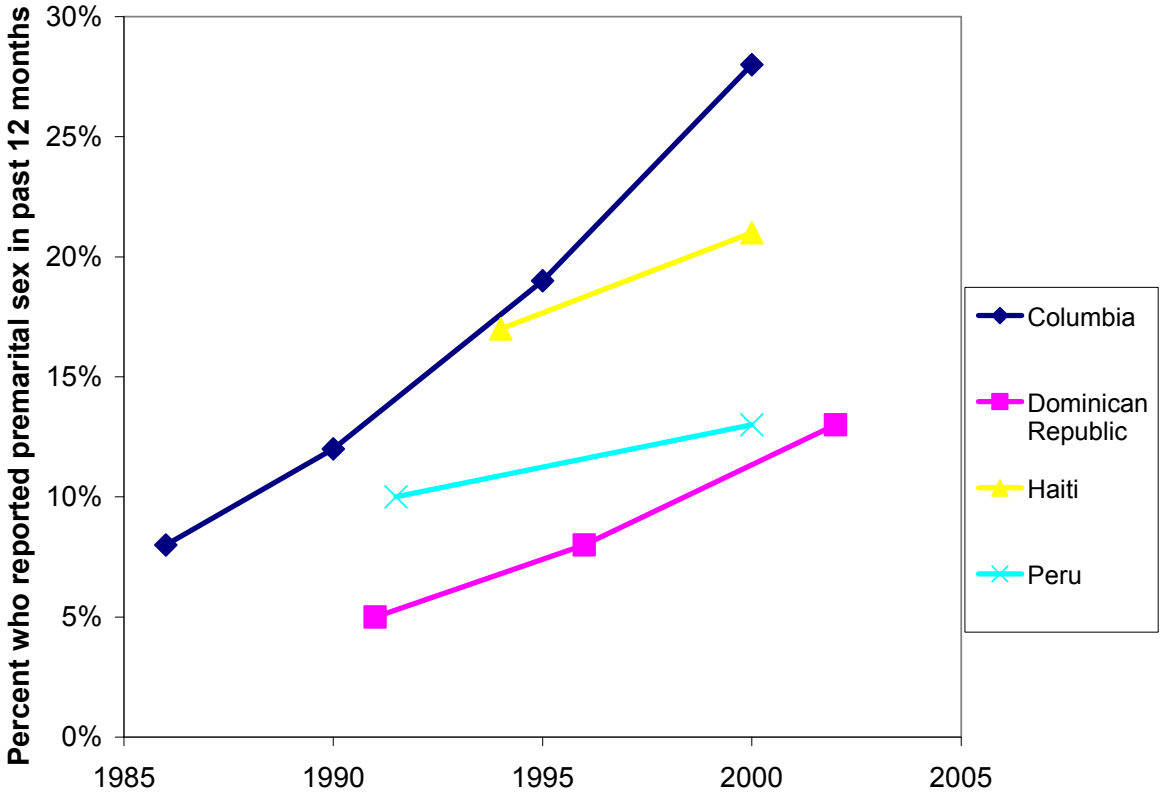


Table 2.1. HPV prevalence estimated for women with no cervical cancer, based on a meta-analysis of 78 studies of women with normal cytology, by world regions.

	No STUDIES	*No WOMEN TESTED*	*No WOMEN HPV +*	*ADJUSTED HPV PREVALENCE [95% CI]*
GLOBAL ESTIMATE	**78**	**157,879**	**15,764**	**10.41 [10.16 - 10.67]**
AFRICA	**8**	**6,226**	**1,429**	**22.12 [20.87 – 23.43]**
AMERICA	**24**	**40,399**	**6,291**	**12.95 [12.41 - 13.51]**
EUROPE	**27**	**70,129**	**4,649**	**8.08 [7.77 - 8.41]**
ASIA	**19**	**41,125**	**3,395**	**7.95 [7.53 - 8.40]**

Adjusted for region, study type, study design, publication year, sampling collection device, cell storage medium, HPV assay, primer used and youngest and oldest age of each included study. Adapted from de Sanjosé et al. [62]

Table 2.2. Review of studies of HPV-type-concordance among couples.

Reference	Population	Sample	Age	Relationship duration	Finding
Hippeläinen et al. *Sex Transm Dis* 1994; 21:76-82.	Women with abnormal Pap smear and their male partners (Finland)	270 couples	♀: mean 27 (range 15-62); ♂: mean 32 (range 17-74)	Median 18 months, mean 41 months, range 1-300	6% (15/270) of couples were HPV-positive concordant for the same type.
Kyo et al. *J Infect Dis* 1994; 170:682-5.	Women evaluated for infertility or who had cervical intraepithelial neoplasia (CIN) or cervical cancer, and their male partners (Japan)	53 couples	Not reported	All married for 2+ years	17% (9/53) of couples were HPV-16 positive concordant. In couples where at least one partner had HPV (n=26), 35% were concordant. Discordancy was more likely to be female positive and male negative than female negative and male positive.
Baken et al. *J Infect Dis* 1995; 171:429-32.	Heterosexual partners attending STD clinic (Seattle)	50 couples, 45 with HPV result	♀: mean 26; ♂: mean 29	Unspecified	29% (13/45) of couples were concordant for the same HPV type. In couples where at least one partner had HPV (n=41), 32% were concordant. Concordance decreased with time since last intercourse.
Castellsagué et al. *J Infect Dis* 1997; 176:353-61.	Women enrolled in case-control studies for cervical neoplasia and their husbands (Spain and Columbia)	816 couples, 431 with HPV result	♂: mean 45	Excluded relationships <6 months duration.	HPV observed in 286/431 couples. Of these, 2% (7/286) were HPV-positive-type-concordant.

Reference	Population	Sample	Age	Relationship duration	Finding
Franceschi et al. *Br J Cancer* 2002; 86:705-11.	Women enrolled in case-control studies for invasive cervical carcinoma (ICC) and in situ cervical cancer (CIS) and their husbands (Spain, Columbia, Brazil, Thailand, and the Philippines)	964 couples	♂: median 45, 50, and 38 for husbands of control women, women with ICC, and women with CIS, respectively	Excluded relationships <6 months duration.	HPV-16 positive concordance observed in 0.02% (1/465), 4% (17/383) and 3% (4/116) of couples where the wife was a control, an ICC case, or a CIS case, respectively.
Bleeker et al. *Clin Infect Dis* 2005; 41:612-20.	Women with CIN lesion and their male partners (The Netherlands)	238 couples, 181 with HPV result	♀: mean 34.7 (range 19-55); ♂: mean 37.6 (range 22-58)	Mean 10.6 years, range 0.6-35 years	Type-specific HPV-positive concordance in 37% (67/181). In couples where HPV was present in at least one partner, 38% (67/176) were type-positive concordant. Increasing association between viral load in one partner and HPV positivity in the other.

Table 2.3. Review of selected studies evaluating HPV transmission via non-sexual intercourse contact.

Reference	Population	Findings
Genital HPV infection associated with sexual contact other than intercourse		
Marrazzo et al. *J Infect Dis* 1998; 178:1604-9.	Cross-sectional study of women who have sex with women, including 21 women reporting only female sex partners (United States)	HPV DNA detected in genital tract specimens from 19% of women reporting only female sex partners
Sonnex et al. *Sex Transm Dis* 1999; 75:317-9.	Cross-sectional study of 14 men and 8 women with genital warts (United Kingdom)	27 percent of subjects tested positive for the same HPV DNA type in both finger brush and genital samples.
Winer et al. *Am J Epidemiol* 2003; 157:218-26.	Longitudinal study of female university students, including 148 women reporting no history of vaginal intercourse at enrollment (United States)	The 24-month cumulative incidence of HPV DNA infection in virgin women was 7.9% (95% CI: 3.5-17.1); any type of non-intercourse sexual contact (finger-vulvar, penile-vulvar, or oral-penile) reported by virgin women was associated with an increased risk of HPV infection.
Oral HPV infection associated with oral sex		
Coutlée et al. *Sex Transm Dis* 1997; 24:23-31.	Cross-sectional study of 178 (158 ♂ , 20 ♀) HIV+ and 109 HIV- (73 ♂ , 36 ♀) individuals (Canada)	32 of 287 (11.2%) oral samples tested positive for HPV DNA; a univariate association between unprotected oral sex and oral HPV (OR=5.5; 95% CI: 1.6–18.4) was no longer apparent after adjustment for other sexual behaviour variables and genital infections.
Winer et al. *Am J Epidemiol* 2003; 157:218-26.	Longitudinal study of 603 female university students (United States)	Only 5 of 2,619 (0.02%) oral samples tested positive for HPV DNA; there was no association between oral HPV and report of oral-penile contact in the past 12 months (HR=0.5, 95% CI: 0.07-3.5).

Reference	Population	Findings
Kreimer et al. *J Infect Dis* 2004; 189:686-98.	Cross-sectional study of 190 (108 ♂ , 82 ♀) HIV+ and 396 HIV- (231 ♂ , 165 ♀) individuals (United States)	18 of 583 (3.1%) oral samples tested positive for HPV DNA; associations between oral sex and oral HPV were inconsistent and varied according to HIV serostatus and reports of oral sex with same-sex versus opposite-sex partners; ORs for ≥2 vs 0-1 recent oral sex partners: HIV- 0.2 (95%CI: 0.0-1.2), HIV+ 12.8 (95% CI: 3.1-52.7).
Rintala et al. *J Clin Virol* 2006; 35:89-94.	Longitudinal study of 131 heterosexual married couples (Finland)	The 24-month cumulative incidence of oral HPV DNA in both men and women was around 10%; oral HPV was not associated with oral sex habits.
HPV infection in children and infants		
Smith et al. *Sex Transm Dis* 2004; 31: 57-62.	Longitudinal study with type-specific HPV DNA testing in 574 mother-infant pairs (United States)	1.6% of oral and genital samples taken from infants a median of 65 hours post delivery were positive for HPV DNA. Type-specific concordance between mother and infant pairs was less than 1%. At 3-month follow-up, no HPV DNA was detected in any of the infants tested
Dunne et al. *J Infect Dis* 2005; 191:1817-9.	Cross-sectional HPV-16 seroprevalence survey of 1,316 children aged 6-11 (United States)	2.4% of children were seropositive, with higher prevalence in boys than girls (3.5% vs 1.2%) and in children >7 years than in children ≤ 7 years (3.3% vs 0.4%)

Table 2.4. Summary of proposed risk factors for HPV acquisition and transmission, according to hypothesized mechanism of action

	Hypothesized to affect likelihood of exposure to HPV-infected partner	Hypothesized to affect likelihood of transmission upon exposure through effects on… Infectivity / Duration	Susceptibility
Early age at sexual debut	↑		↑
Greater number of partners	↑		
Similarity or dissimilarity between individuals and their sex partner(s)	↑ / ↓		
Acquisition of new partner	↑		
Concurrent/extra-dyadic partners	↑		
Short intervals between partners	↑		
Concomitant infection with other STI	↑	↑	↑
Male circumcision	↓	↓	↓
Condoms	↑ / ↓	↓	
Immune suppression (e.g., HIV infection, transplantation)			↑
Certain human leukocyte antigen (HLA) complex alleles and haplotypes		↑	↑
Hormonal contraceptives		↑	↑
Diet deficient in certain micronutrients		↓	
Smoking		↑	↑

Refer to text for details and strength of evidence.

SECTION 3: STUDY DEVELOPMENT AND PRELIMINARY ESTIMATE OF HPV TRANSMISSION PROBABILITY (MANUSCRIPT II)

There is a gap in knowledge regarding the transmissibility of HPV and the conditions under which transmission is facilitated or hindered. In response to this research need, I designed the HITCH Cohort Study, in collaboration with the research team. The design was informed by two preliminary research works that are described in this section: (1) a feasibility study and (2) an estimation of the probability of HPV transmission per coital act using a simulation study.

Feasibility study

In addition to scientific validity, a study must be considered acceptable and relevant by the women and men who would be targeted for participation. In 2004, I conducted semi-structured interviews with 32 undergraduate students who would be candidates for the HITCH Cohort Study. I asked interviewees for their opinions on the possible procedures, specifically whether they found them acceptable or objectionable.

Feasibility Study Design The feasibility study aimed to recruit 30 men and women, aged 18-24, to participate in a voluntary, confidential interview, with no obligation to participate in the future study. A notice regarding the study was posted at the McGill University Student Heath Service Clinic and circulated by email to students on a mailing list for health promotion and McGill athletics. I interviewed students who consented in a private room at the McGill University Student Heath Service Clinic. Respondents were given a $20 honourarium.

Feasibility study respondents 18 women and 14 men were interviewed (n=32). The median age was 21 years (range 19-27) and 91% were currently McGill students. Current students had a median of 1 year remaining in their program of study (range 0.25-4).

Opinions regarding the measurement of sexual behaviour

Respondents were told that the future study would need to obtain detailed

information about sexual histories and current sexual behaviour. When asked how a computerized, self-completed questionnaire would affect their willingness to participate, 81% (26/32) said it probably or definitely would make them more willing, and 6/32 said it would not affect their decision.

Two formats for computerized self-completed questionnaires were described. The first was through an Internet site from a location of their choice. The Internet site would be completely confidential, with security safeguards including a login identifier and password. Alternatively, it could be done in a private room at the clinic where someone would be available to provide assistance, if needed. More respondents preferred the clinic (62%, 20/32) over the Internet option (34%, 11/32), at least for the initial questionnaire. (One respondent had no preference.) Reasons for preferring the clinic option were primarily for the availability of assistance, but also because there would be fewer distractions at the clinic or because it was more "official". Reasons for preferring the Internet option included convenience and privacy. The vast majority (30/32) had access to a computer they could use privately.

Women participating in the HITCH cohort would need to complete questionnaires on an ongoing basis. When female respondents were asked how the frequency of these questionnaires would affect their willingness to participate, most (16/18) preferred a schedule of once per month to once every 3 months.

Female and male respondents were read the following list of potential "tokens of appreciation" for the completion of questionnaires, and asked how each would affect their willingness to participate: a thank-you letter, $2, a lottery ticket, $5, $10, an electronic gift certificate worth $10, or $25. Few reported that a thank-you letter (8/32), $2 (11/32) or lottery ticket (13/32) would affect their willingness to participate. However, respondents were probably or definitely more willing to participate with relatively small monetary amounts ($5, 20/32; $10, 28/32). An electronic gift certificate worth $10 did make some respondents more willing

(19/32), but not as much as $10 in cash. Virtually all were more willing to participate if offered $25 per completed questionnaire (29/32).

Opinions regarding female specimen collection Two options for collection of specimens were described to the female feasibility study respondents. The first was a pelvic exam and Pap smear. Most (15/18) said this procedure would not adversely affect their willingness to participate. When asked how frequently they were willing to undergo a pelvic exam, 2/18 said once per month, 10/18 said once every 3 months, 4/18 said once every 4 months, 2/18 said once every 6 months, and 1/18 said annually.

The second option explained to female respondents was self-collected specimens using a swab "similar to a large Q-tip". Respondents were asked to read the instructions that would be given to women. When asked how this procedure would affect their willingness to participate, 13/18 said it would make them less willing, four said it would not affect their decision to participate, and one said it would make them more willing to participate. Women whose willingness to participate was unaffected made comments such as "it sounds no more difficult than inserting a tampon" or acknowledged that once they learned the procedure, they were sure they could get used to it. Among women whose willingness to participate was adversely affected, the most common reason given was concern about making a mistake during self-collection (e.g. "I'm unqualified", "sounds easy to mess up") and a preference that a trained professional did the collection. A minority of women said that they were not comfortable enough with their bodies or genitals to do the self-collection procedure. When prompted, some women said they would be more willing to self-collect specimens if the research nurse collected the first specimen, at which time the nurse could provide instructions on how to self-collect future specimens. Despite their uncertainty about self-collected specimens, respondents were willing to do so more frequently than pelvic exams. 7/18 said they would self-collect once a month, 8/18 said every three months, 2/18 said every six months, and 1/18 said annually.

Regarding possible "tokens of appreciation" for the provision of specimens for HPV testing, few women (4/18) said a thank-you letter would make them more willing to participate. Most were probably or definitely more willing to participate with the provision of $10 (11/18), $20 (16/18), or $25 (16/18). Some said an electronic gift certificate worth $10 would make them more willing (10/18), but less so than $10 cash.

Opinions regarding male specimen collection Two options for collection of specimens from male HITCH participants were described to the male feasibility study respondents. The first was a medical examination of their genitals, at which time a specimen would be collected by painless skin swabbing. The nurse would apply a moistened swab ("similar to a large Q-tip") to the outside of the penis only. 9/14 men said this procedure would not adversely affect their willingness to participate, and acknowledged the scientific need to conduct such a procedure. Among men who found the procedure unappealing, the reasons were that it was too personal and that they would feel uncomfortable disrobing for a study.

The second option explained to male respondents was the provision of a urine specimen. None said this procedure would adversely affect their willingness to participate.

Few males (3/14) said a thank-you letter would make them more willing to provide a genital specimen. Most were probably or definitely more willing to do so with the provision of $10 (10/14), $20 (14/14), or $25 (14/14). Some said an electronic gift certificate worth $10 would make them more willing (6/14), but less so than $10 cash.

Opinions regarding enrolment of couples It was explained that researchers would like to enrol couples for the HITCH cohort to better understand the transmission of HPV between men and women. Respondents were told that information collected from either partner (either from the questionnaire or the

HPV result) would be completely confidential, and would not be released to the other partner.

Women were asked, “In general, how likely is it that you would ask a sexual partner to participate in a study of HPV infection?” 7/18 said they definitely would ask, 3/18 said they probably would, 7/18 said “it depends, I would need to know more”, and 1/18 said she probably would not ask. Among the women who had a current male partner, 2/13 thought he definitely would participate if asked, 6/13 though he probably would, and 2/13 said “it depends”. Of the 3/13 women who said their partner probably would not participate, one woman said it was because her partner lived in the United States, and another said it was because she was about to break up with her partner.

Men were asked, “In general, how likely is it that you would participate in a study of HPV infection if you learned about it from a sexual partner?” 5/14 said they definitely would participate, 5/14 said they probably would, 3/14 said “it depends, I would need to know more”, and 1/14 said he definitely would not participate. Several men commented that their willingness to participate would be increased if their partner encouraged them to enrol, particularly if it was a study in which she believed and if she was concerned about HPV and cervical cancer.

Female and male participants were asked about the ideal characteristics of a relationship in which they would be willing to enrol in an HPV study as a couple. The minimal relationship duration was on average 5 months (range 1-12). Respondents repeatedly stressed that this duration was strongly dependent on the couple involved. Some reported that, for them, a relationship would have to be very close, exclusive, committed, trusting and loving before they would consider enrolling in HITCH. Others reported that having a relationship with open communication about sensitive matters, including sexuality, was all that was required.

Respondents were asked if they had concerns about asking a partner, or being asked by their partner, to enrol. Among women, the most common concern was the reaction of the male partner (e.g., he might get upset, suspicious). In fact, several men did say that they would want to know the female partner's motives for participation (e.g. was the study an excuse for STI screening or a way to find out his previous sexual history?). Respondents said these concerns would not be an issue if each partner understood that the reason for participating was for the advancement of research. Other concerns were confidentiality (e.g. disclosing personal information about their sexual relationship) and learning of HPV results. For the latter, respondents expressed some anxiety at the thought of learning that they or their partner had HPV.

Women were asked how a "token of appreciation" for the enrolment of a partner would affect their willingness to talk to a partner about HITCH. Some women (13/18) thought that a token of appreciation that could be shared by the couple (e.g. a gift certificate for a night out for 2 at the movies) would increase their willingness to enrol a male partner, but others (5/18) indicated that their decision to enrol a partner would be independent of an incentive.

Other concerns and suggestions offered by feasibility study respondents Some suggested that the study should include an educational component, as they acknowledged that they knew little about HPV. Many advised on the best approach to provide HPV results. Some thought results should be provided, as long as it was done in a gentle, informative, reassuring way. One male participant, however, though the results should be blinded. This man had learned much about HPV, as his partner had been diagnosed with cervical lesions. He was concerned that the provision of results would create a lot of unnecessary anxiety when there is no cure and HPV is so common.

A number of respondents offered suggestions on recruitment for the proposed study, or ways to ensure continued participation. These included methods of study promotion, ensuring the study procedures were convenient, flexible, and not

overly burdensome, and timely communication between the study and the participants, including the provision of the research results.

Willingness to enrol in HITCH At the end of the interview, all respondents were asked "Given all that we have talked about today, please tell me how you now feel about participating in such a study?" Among women, 5/18 said they definitely would, 10/18 said they probably would, and 3/18 said "it depends, I would need to know more". Among men, 6/14 said they definitely would, 7/14 said they probably would, and 1/14 said he probably would not. Overall, 88% (28/32) said they definitely or probably would participate in HITCH.

Preamble to Manuscript II

Study feasibility is important but so too is a reasonable estimate of the number of study outcomes that could be expected. This is needed to project the desired sample size and to determine whether the proposed study would be sufficiently powered to identify risk factors for HPV infection and transmission. An estimate of the rate of HPV incidence among women attending McGill or Concordia University in 1996-2001 was available from the previous cohort study conducted in this population [26]. However, there was no estimate of male HPV prevalence or incidence among heterosexual men in Montreal. Nor was there an estimate of the transmissibility of HPV itself.

To deal with the uncertainty surrounding such parameters, I conducted a simulation study that utilized the female incidence data from the previous McGill-Concordia Study [26]. In brief, this simulation estimated the extent of male prevalence and HPV transmissibility that would be consistent with the incidence actually observed among women. The information gleaned from what was initially a sample size estimation proved to be informative for our understanding of HPV epidemiology as a whole. The results were presented at the International Papillomavirus Conference in Vancouver, 2005 [95] and then published in the American Journal of Epidemiology in 2006 [30].

The simulation study was a novel approach. To my knowledge, it was the first published estimate of the HPV transmission probability based in part on empirical data. At the time of the simulation, no longitudinal study of HPV transmission had been done anywhere in the world. The only transmission data that was available was a study of the transmission of genital warts, published in 1971 before the causal agent, HPV was recognized [31].

MANUSCRIPT II:

Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada

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For reprint and publishers' waiver, please see **Appendix B**.

ABSTRACT

Plausible ranges of the probability of human papillomavirus (HPV) transmission per coital act among newly-forming couples were estimated using stochastic computer simulation. Comparative empirical data were from a cohort study of female university students in Montreal. Female type-specific prevalence and frequencies of sexual intercourse and condom use were set equal to those in the cohort. Simulations included 240 combinations of varying male type-specific prevalence, the relative risk for protected versus unprotected sex, and per-act transmission probabilities. Those that produced expected HPV incidence within the 95 percent confidence interval (CI) observed in the cohort were selected. The observed 6-month cumulative incidence of any new HPV type following acquisition of a new partner was 17.0 percent (95 percent CI: 11.4, 23.0). Expected incidences consistent with cohort findings occurred in 54/240 (22.5 percent) simulations. The range of per-act transmission probabilities was 5-100 percent (median 40 percent). Male HPV prevalence was the same as or greater than that for women in all consistent simulations. Varying condom effectiveness did not produce better fitting data. This simulation suggests that HPV transmissibility is several-fold higher than other viral STI, such as HIV or HSV-2. With high transmissibility, any potential protective effect of condoms would disappear over multiple intercourse acts, underlining the need for an effective HPV vaccine.

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI). Cervical HPV infection is found in 5 to 40 percent of asymptomatic women of reproductive age [96], and as many as 75 percent of adults may eventually be infected with at least one HPV type in their lifetime [58]. Risk rises with increasing number of sexual partners, younger age of sexual debut, and with recent acquisition of new partners [25,74,97-99]. The vast majority of these infections will be transient [76,97,100-103]. However, a substantial increase in risk of cervical neoplasia exists for women who develop persistent, long-term infections with oncogenic HPV types [97,101,104-106]. It is now well established that HPV infection is the central, probably necessary cause of cervical cancer [107].

The acknowledgement that cervical cancer is caused by an STI has produced a change from a noninfectious to an infectious disease paradigm, with corresponding changes to prevention strategies. There is currently great enthusiasm concerning the possible application of HPV testing as an adjunct to Pap cytology screening for cervical cancer [108] and widespread interest in the development of HPV vaccines [109]. Yet assessments of the potential impacts of these proposed strategies are hampered by limited information on the sexual transmissibility of HPV. To date, most natural history models that can predict the impact of prevention strategies based on HPV testing and HPV vaccination have been based on empirical data that has come exclusively from epidemiologic studies in women [110-114]. A better understanding of the sexual transmission dynamics of HPV would lead to more informed decision-making when comparing different prevention strategies through more valid mathematical prediction models (e.g. vaccine policy [115]).

In the absence of empirical data on HPV transmissibility, computer simulation may be a useful tool for estimation. The objective of this study was to simulate probabilities of HPV transmission per coital act in a hypothetical population to estimate plausible ranges for this parameter that would be coherent with observed rates of HPV incidence among young, sexually active women enrolled in a cohort study we previously conducted in Montreal.

MATERIALS AND METHODS

To estimate probabilities of HPV transmission per coital act, hypothetical populations of newly-forming heterosexual couples were simulated. Acquiring a new partner has been shown to be a key determinant of HPV acquisition [74]. Therefore, newly-forming rather than long-standing couples were the object of analysis.

McGill-Concordia Cohort Study

The source of empirical data for HPV incidence was the McGill-Concordia Cohort Study, a prospective cohort study of young, female university students in Montreal, Canada. Female students attending either the McGill or Concordia university health services clinics were recruited for a study of the natural history of HPV infection and cervical neoplasia. The study methodology is described in detail elsewhere [26]. In brief, 621 female participants were followed for 24 months at 6-month intervals in 1996-2001. At each visit, a cervical specimen was collected and tested for 27 HPV types using L1 consensus primers MY09/MY11 and HMB01 and the line blot assay (Roche Molecular Systems) [116]. Women also self-completed a questionnaire at each clinic visit, which collected information on sexual history and behavior since the last visit. In the overall cohort, baseline cervical HPV prevalence was 29 percent for any type, 22 percent for high-risk oncogenic types, 15 percent for low-risk types, and 7 percent for HPV-16 [26].

Of 2,058 follow-up study visits by the 621 cohort study participants, there were 238 visits where a woman reported a new sexual partner since her last visit, and no other partners. These are termed “new partner visits” for the remainder of this paper. They represent 182 women, as some women reported more than one instance (36 women reported 2 instances, 7 reported 3 instances, and 2 reported 4 instances). Empirical estimates of simulation parameters and 6-month cumulative incidence of a new HPV type were based on data from these 238 new partner

visits. Each new partner visit was assigned two time points: time *t* was the date of the visit when a new sexual partner was reported and time *t – 1* was the date of the visit just prior to time *t*. The median duration of the interval between *t – 1* and *t* was 6 months (range 3-28 months). Cumulative incidence of any new type of HPV was calculated using the Kaplan-Meier method. To account for repeated event times, the 95 percent confidence interval (CI) was estimated using bootstrap sampling of the 182 women who reported at least one new partner visit [117].

Simulation approach

A stochastic Monte Carlo computer simulation was used to produce hypothetical cohort data for a population of 10,000 newly-forming heterosexual couples. The assumed values for fixed and variable parameters used in the simulations are summarized in Table 3.1.

The first step was to assign the initial type-specific HPV positivity for each hypothetical female. This involved the drawing of a random variable from the uniform probability distribution, which was compared with the type-specific prevalence at time *t – 1* among the observed 238 new partner visits in the McGill Concordia Cohort Study (Table 3.1). For example, the observed prevalence of HPV-16 was 4.37 percent at time *t – 1* for the 238 new partner visits. Then if the drawn random variable ≤ 0.0437, the hypothetical female was assigned to be HPV-16 positive at time *t – 1* (i.e., female HPV-16 positivity ~ Bernoulli (0.0437)).

The type-specific HPV prevalences of male partners of McGill-Concordia cohort women are unknown since they were not the object of study in that cohort. Given this uncertainty, the male-to-female prevalence rate ratio (PRR) at time *t – 1* was varied from 0.5 to 2.0 (Table 3.1). Random mixing of males and females, with respect to HPV status, was assumed. That is, HPV positivity in one partner was considered to be independent of that in the other when the couple was initially formed.

Simulated data on the frequency of intercourse over a 6-month interval was then generated for each couple. Intercourse frequencies per month were set to be the same as those reported for the 238 observed new partner visits in the cohort (Table 3.1). This distribution was highly skewed to the right (median=7, mean=9.48, standard deviation=9.95, skewness=1.91, range 0.5-54). Therefore, for each hypothetical couple, intercourse frequency was a randomly-drawn number from the gamma distribution (shape=1, scale=10), rounded to the nearest integer. Each couple was also randomly assigned condom use frequency. Because definitions of "sometimes" and "regular" use of condoms were not provided in the McGill-Concordia Cohort Study questionnaire, it was assumed that women interpreted "sometimes" as condom use 50 percent of the time, and "regularly" as condom use 75 percent of time.

Given the uncertainty regarding the efficacy of condom use, the relative risk (RR) of HPV transmission for a single act of protected versus unprotected intercourse was varied from 0.1 to 1.0 (Table 3.1). A lower bound of 0.1 was selected as it most closely approximates that for another viral STI, human immunodeficiency virus (HIV), for which considerable data on condom effectiveness has been accumulated [118].

To simulate incidence of HPV in the female partner, per-act transmission probabilities were assigned in the range from 0.001 to 1.0 (the latter indicating 100 percent probability of transmission in a single act of intercourse) (Table 3.1). A lower bound of 0.001 was selected as it is the lower-bound estimate for HIV in conditions of low viral load and long-standing partnerships [119,120]. In the simulation, transmission probabilities were only applied in the discordant couples, where a male had an HPV type not present in the female, and transmission was theoretically possible. For each act of intercourse, a variable was randomly drawn from the uniform distribution; if it was less than or equal to the assumed value of the per-act transmission probability, then a transmission event was assigned. The assignment of events took into account condom use for that act.

The simulation creates a dataset with complete information for 10,000 couples, with data on each act of intercourse, including the specific act for which HPV transmission occurred. In an actual cohort study, only summary information is known, and the date of HPV infection is interval-censored between two clinic visits. Therefore, the simulated dataset was modified to most closely match the real-world environment. McGill-Concordia cohort study participants reported a new partner in the 6-month period since their last visit, but the date of first sexual encounter with that partner was not reported. In the simulated data, this date was randomly assigned with uniform probability over the 6-month interval. Type-specific status of simulated women was output for time $t - 1$ and time t, and Kaplan-Meier analysis was used to calculate the expected cumulative incidence of any new HPV type at 6 months, as it would have been observed in a hypothetical cohort.

For each of the 240 possible combinations of the male-to-female PRR, RR for condoms, and per-act transmission probability value, 100 simulations of 10,000 couples were run. Resulting cumulative incidences were averaged over the 100 simulations to provide the best estimate of what would be expected under those conditions. These were then compared to the 95 percent CI for the observed cumulative incidence in the 238 new partner visits in the McGill-Concordia Cohort Study. Any simulated conditions which produced expected cumulative incidences within this range were considered compatible.

RESULTS

Cumulative incidence of HPV infection of all types at 6 months for women in the 238 new partner visits in the McGill-Concordia Cohort Study was 17.0 percent (95 percent CI: 11.4, 23.0). Of 240 simulations, 54 (22.5 percent) produced modeled cumulative incidences that fell within the empirically observed range of 11.4 to 22.3 percent. Frequency distributions of these 54 consistent simulations with respect to the male-to-female PRR, values of transmission probabilities per act, and the protective effect of condoms are shown in **Figure 3.1**.

All of the simulations that gave modeled incidences consistent with the observed data assumed that men's prevalence was the same as women's prevalence, or greater (**Figure 3.1a**). The highest proportion (54 percent) of consistent simulations assumed men's prevalence was 1.5 times that of women.

Figure 3.1b shows that values of per-act transmission probabilities ranged from 0.05 to 1.00, with a median of 0.40. Per-act transmission probabilities between 0.001 and 0.025 were not consistent with the observed cohort data. The distribution appears bimodal, which reflects the relationship between per-act transmissibility and the assumed prevalence among men. The median per-act probability value was 0.625 when the male-to-female PRR was assumed to equal 1.0, 0.30 when the PRR was assumed to equal 1.5, and 0.10 when the PRR was assumed to equal 2.0.

The number of acts, n, required for certain transmission can be estimated with the following equation: Probability (Infection) = $1 - (1 - \lambda)^n$, where λ is the per-act transmission probability [119]. At the median value of the per-act transmission probability for all consistent simulations (0.40), a woman would have a 100% probability of becoming infected within 11 acts of intercourse. At the lowest estimated value of the per-act transmission probability (0.05), this increases to 104 acts of intercourse.

Figure 3.1c shows that no single estimate of per-act effectiveness of condoms produced better fitting data; simulations ranging from RR=0.1 to RR=1 gave modeled cumulative incidences that fit the observed data.

Figure 3.2 shows how the relationship between the expected 6-month cumulative incidence of any new HPV type and the per-act transmission probability varies with the assumed male-to-female PRR, under the assumption that condoms offer no protection (i.e., RR=1.0) (**Figure 3.2a**) and that they offer a four-fold protection (i.e., RR=0.25) (**Figure 3.2b**). The observed 95 percent CI for cumulative incidence in the McGill-Concordia Cohort Study is shown for

comparison (shaded area in the graph). If one assumes HPV prevalence is equivalent in men and women, then the per-act transmission probability most consistent with the observed data is 0.20 or greater. However, if one assumes HPV is more prevalent among women than men, then the per-act transmission probability may be as low as 5 percent. Further, this graph shows that per-act transmission probabilities could be no more than about 0.20 if one assumes that HPV prevalence is twice as high among men as in women.

Similarly, **Figure 3.3** shows how the relationship between the expected 6-month cumulative incidence of any new HPV type and the per-act transmission probability varies with the assumed protective effects of condoms, if male HPV prevalence is 1.5 times that of females (PRR=1.5). Again, the observed 95 percent CI for cumulative incidence in the McGill-Concordia Cohort Study is shown for comparison. All simulated RR values for condom effectiveness were compatible with the observed data, but higher condom effectiveness implies higher transmissibility. That is, if RR=1 then the plausible range for transmissibility is about 5 to 70 percent, whereas if RR=0.1 then this range shifts to 10 to 100 percent.

Further analysis was carried out to determine the influence of specific assumptions in the simulation results. Results were similar when incidence density per 100 woman-months was used as the comparative outcome, rather than the Kaplan-Meier cumulative incidence at 6 months (data not shown). The simulations presented above assumed that regular condom use reported by women in the McGill-Concordia Cohort Study indicated use 75 percent of the time; results were similar when regular use was assumed to be condom use 95 percent of the time (data not shown). Finally, random assignment of female HPV positivity at time $t - 1$, sexual frequency, and condom use frequency in 10,000 hypothetical couples assumes that these are uncorrelated. To test this assumption, simulations of HPV incidence among the observed 238 new partner visits, using reported data on female HPV positivity at time $t - 1$ and sexual and condom use frequency, were repeated 1,000 times for each of the 240 combinations of per-act

transmissibility, male-to-female PRR, and the RR for condom use. Again, results were consistent with the results reported above (data not shown).

DISCUSSION

The modeled HPV per-act transmission probabilities that were consistent with observed cumulative incidence among young female university students ranged from a lower limit of 5 percent per act to an upper limit of 100 percent per act. At the median, 40 percent per act, the probability of male-to-female transmission would reach 100 percent with only 11 acts of intercourse. Per-act transmissibility values of less than 5 percent were inconsistent with the observed data.

The results suggest that HPV prevalence among male partners of this university student population in Montreal were equal to or greater than that among women. Other research of HPV prevalence in both sexes of the same university student population has reported slightly less to equivalent prevalence among males compared to females [74,121,122]. In STD clinic populations, higher prevalence was observed among males compared to females in Denmark and Greenland [123]. However, comparison of sex-specific HPV prevalence within the same population (e.g. university students) assumes that sexual networks are confined to that population. This may not be the case if female students have male partners outside the student population. Sexual network and partnership studies would be needed to verify the true HPV infection status of women's partners.

STI transmission dynamics involve three distinct components: (i) transmissibility from an infected to an uninfected partner upon exposure, (ii) the likelihood of sexual exposures between infected and uninfected persons, and (iii) the duration of the infection [58]. The first, transmissibility, can only be measured empirically in studies of discordant couples [124,125]. One such study, conducted by Oriel [31], examined the transmission of genital warts before HPV was identified as the causal agent. Participants were patients at a hospital's venereology department in London, England. Sexual partners of the index patient in the 9 month period

before and after the appearance of warts were recorded for 97 patients. Sixty percent (53/88) of the sexual partners of the index patients subsequently developed warts, suggesting high transmissibility.

To our knowledge, there have been no published reports of the transmissibility of HPV itself based on empirical data, but it is thought to be high [78,115]. Unlike most STI, HPV is not concentrated in "core groups"—small groups of highly sexual active individuals [58,78]. An epidemiologic pattern of high prevalence among moderately sexually active individuals may result from either a long duration of infectivity and/or high infectivity [78]. Considerable evidence suggests that most HPV infection among women is short-lived [26,76,97,126]. Although less studied, HPV infection among men seems also to be of short duration [70], which suggests that high transmissibility may explain the observed prevalence in most populations.

The estimated per-act transmission probabilities for HPV in this simulation study were high in comparison with other viral STI, but were comparable to those presumed for bacterial STI. Studies of HIV-discordant couples indicate that the probability of HIV transmission is 1 per 1,000 acts of intercourse [119]. This is believed to increase as much as 10-fold with high seminal viral load, which may occur during acute primary infection or when either partner is co-infected with other STI [120,127]. Even in such circumstances, the range of plausible HPV per-act transmission probabilities indicates that HPV would still be considerably more infectious than HIV. Similarly, the probability of transmission of herpes simplex virus type 2 (HSV-2) is estimated to be 1 per 1,000 acts among stable, long-standing couples [91]. Transmission probabilities for other STI are available in the literature; however, these are typically reported as the probability of transmission *per partnership*, not per coital act, and are considered an average across partnerships of varying duration. These range from 20 percent for *Chlamydia* and 50 percent for gonorrhea [59] to 60 percent for syphilis [128] and 80 percent for *Haemophilus ducreyi*, the infectious agent for genital ulcers [59]. The higher rate of transmission of the latter two agents is related at least in part to the presence of genital ulcers that increase transmission of STI.

The high per-act transmission probability estimated in this simulation study suggests that women exposed to an infected partner would acquire HPV within the first acts of intercourse. Consistent with high transmissibility, neither the frequency of sex or the number sex acts was associated with incident HPV infections among women in the McGill-Concordia cohort (data not shown).

The estimation of per-act transmission probabilities in this simulation study relied on the accuracy of the measured cumulative incidence of HPV in the Montreal cohort. In any given 6-month period where women reported a single new partner, and no other partners, the cumulative incidence was 17.0 percent (95 percent CI 11.4, 23.0). This rate is consistent with that among women starting their first sexual relationship, as reported by Collins and colleagues [129]. In that study, women were censored when a second sexual partner was acquired; cumulative incidence of any type of HPV was 20 percent at 6 months following the first act of intercourse.

Assumptions must be made in any simulation exercise, and this study was no exception. The simulation of HPV–concordant and –discordant couples assumed random mixing of men and women, at least with respect to HPV status. Surveys of sexual behavior show that mixing may not be random, rather it may tend to be moderately assortative, such that "like" mix with "like" [58,130]. High rates of HPV even among moderately sexually active populations [78], suggest that an assumption of random mixing with respect to HPV status may not be untenable. Nevertheless, if substantial assortative mixing was present, our simulation would have resulted in an underestimation of per-act transmissibility.

This simulation assumed that couples remained together, and that no dissolution of partnerships occurred. This assumption, if violated, would have lead to an underestimate of transmissibility, but this bias was minimized by the short time interval for simulation (6 months). Further, per-act transmission probabilities were presumed constant. It is possible that the risk of transmission varies over time, and future efforts to study transmissibility should examine this issue. The

random assignment of female HPV positivity at time *t – 1*, sexual frequency, and condom use frequency in the hypothetical couples presumes that these are uncorrelated. Such correlations were not influential when they were simulated, nor did analysis of the cohort itself reveal correlation among these variables. It was also assumed that women who reported "regular" condom use had in fact used them 75 percent of the time. Regular use was not assumed to indicate 100 percent use of condoms because even among those who always use them, partial condom use can occur (i.e., not applying the condom before insertion, removing the condom sometime during intercourse, and condom breakage or slippage). As many as 38 percent of young heterosexual condom users report delaying the application of the condom at least occasionally [131-133]. Nevertheless, when the simulations were repeated assuming regular use indicated use 95% of the time, the results were nearly equivalent.

Whether or not condoms provide any level of protection against HPV transmission remains a subject of debate [23]. In vitro studies demonstrate that latex condoms are impermeable to all known sexually transmitted pathogens [134]. Although a substantial body of research indicates that condoms significantly reduce the risk of HIV infection [118], research of HPV has found equivocal results [23 118]. A paradoxical effect is occasionally reported, such that condom use appears to increase risk of HPV infection [23-25]. Methodological issues that have limited the evaluation of condom effectiveness include imprecise measurement and the inability to distinguish with whom participants use condoms or the infection status of that partner [23,135].

A critical implication of a high per-act transmission probability for HPV found in this simulation is that condoms may not offer effective protection over multiple acts of intercourse, and this could explain an absence of observed effects in many research studies. A protective effect of condoms, even if one exists, is virtually lost with high infectivity [136]. Simulated conditions in this study showed that high per-act transmission probabilities result in significant transmission, even with a 10-fold protective effect of condoms. Although condoms may offer protection in

relatively brief encounters involving only one or just a few acts of intercourse, they would be ineffective in partnerships where multiple sex acts occur in an ongoing relationship. For example, if the true per-act transmission probability is 40 percent certain transmission occurs within 11 acts of intercourse. If condoms reduce risk of transmission by half to 20 percent, then certain transmission would occur within 24 acts, which is within about 10 weeks according to the intercourse frequency reported by women in the McGill-Concordia Cohort Study.

Fortunately, HPV vaccines are a promising alternative to condoms. Preliminary evidence from proof-of-principle trials shows great promise for vaccines against HPV-16 alone [3], HPV-16 and -18 [4], and HPV-6, -11, -16 and -18 [6]. The findings of this simulation study provide a strong rationale for maximizing coverage of an HPV vaccine upon licensure and considering the benefits of extending vaccination to young men before they engage in sexual activity. A second implication is that high transmissibility will magnify the impacts of poor vaccine coverage, poor "take", or waning of immunity over time. Close monitoring of population coverage and the vaccine effectiveness over time will be necessary. A first generation of validated natural history models has been used to assess the potential impact of changes in these parameters on long-term vaccine efficacy [112-114]. However, these Markov models have been built exclusively on the basis of probabilistic assumptions that are consistent with findings from epidemiologic studies that focused on the natural history of HPV and cervical neoplasia in women. We believe that the approach described here may provide the HPV transmissibility framework that could be incorporated into these models to enhance their ability to make projections of vaccine efficacy under a wider range of scenarios than has been possible with the first-generation models.

Table 3.1. Fixed and varied parameters used in simulations of male-to-female HPV transmission *

Fixed parameters	Value
Type-specific HPV prevalence among women at time *t – 1*	
Type 6	1.75%
11	0.44%
16	4.37%
18	2.62%
26	0.00%
31	3.06%
33	2.18%
35	0.00%
39	2.62%
40	0.00%
42	0.44%
45	3.06%
51	1.75%
52	2.18%
53	2.62%
54	2.18%
55	0.44%
56	1.75%
57	0.00%
58	2.18%
59	0.87%
66	2.18%
68	0.87%
73	0.00%
82	0.87%
83	0.87%
84	4.37%
Monthly intercourse frequency	
Mean	9.48
SD	9.95
Gamma shape parameter	1
Gamma scale parameter	10
Condom use frequency	
Never	13%
Sometimes	26%
Regularly	61%

Varied parameters	Value
Male-to-female HPV prevalence rate ratio (PRR)	
5 levels	0.50
	0.67
	1.00
	1.50
	2.00
Relative risk (RR) for condom use per act	
4 levels	0.10
	0.25
	0.50
	1.00
Probability of HPV transmission per act	
12 levels	0.001
	0.010
	0.025
	0.050
	0.100
	0.150
	0.200
	0.300
	0.400
	0.500
	0.750
	1.000

* Fixed parameters were based on observed data from the McGill-Concordia cohort study (see text for details)

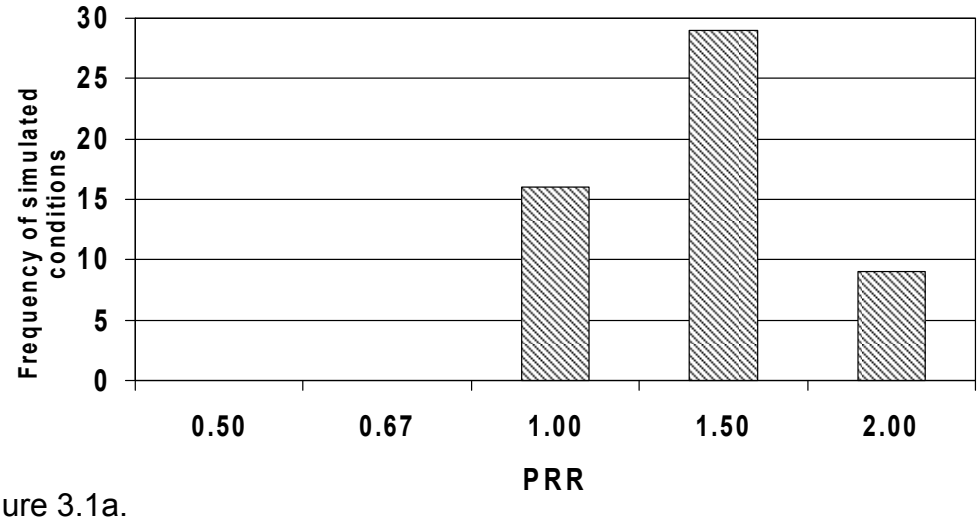


Figure 3.1a.

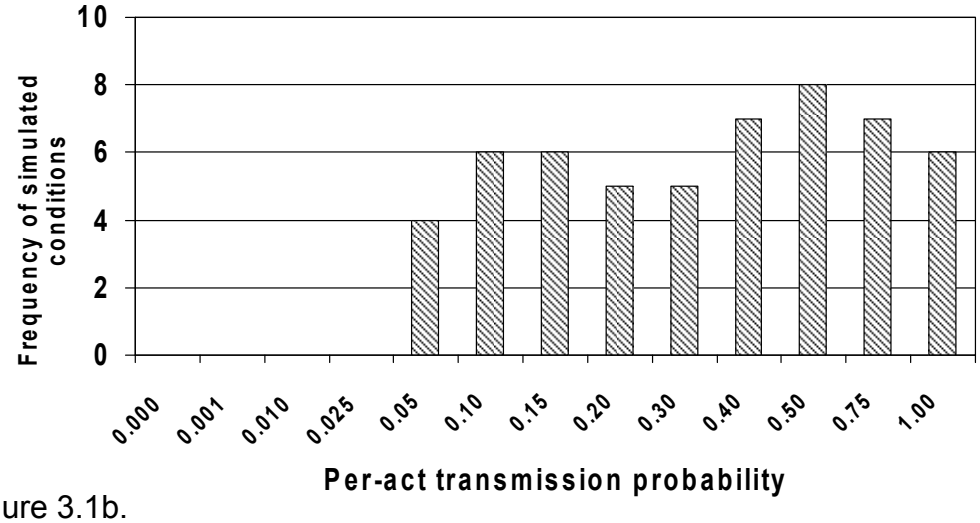


Figure 3.1b.

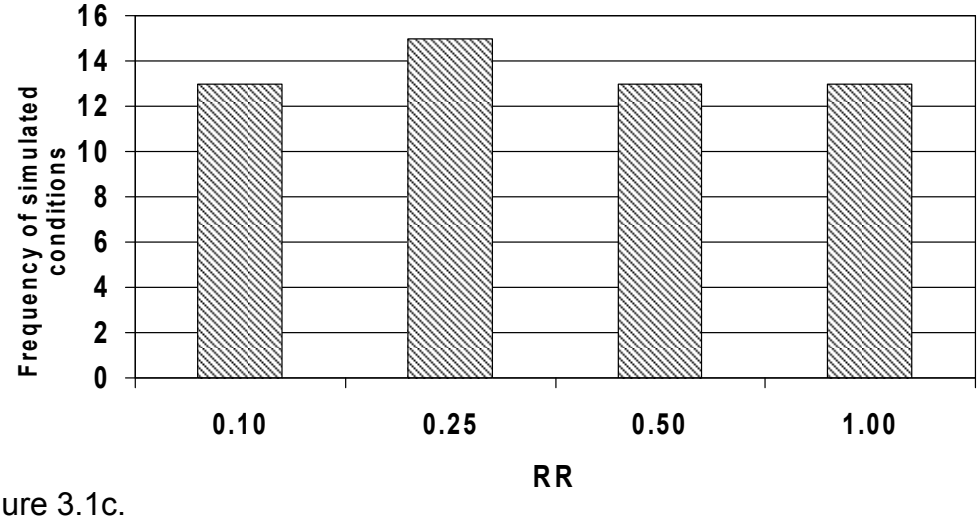


Figure 3.1c.

Figure 3.1. Frequency distributions of 54 simulated conditions for a hypothetical cohort of 10,000 newly-forming heterosexual couples that were consistent with empirically-observed cumulative incidence of any new HPV type among women in the McGill-Concordia Cohort Study (out of 240 simulated conditions). (a) By male-to-female HPV prevalence rate ratio (PRR). (b) By HPV transmission probability per coital act. (c) By relative risk (RR) of condoms for a protected versus an unprotected act.

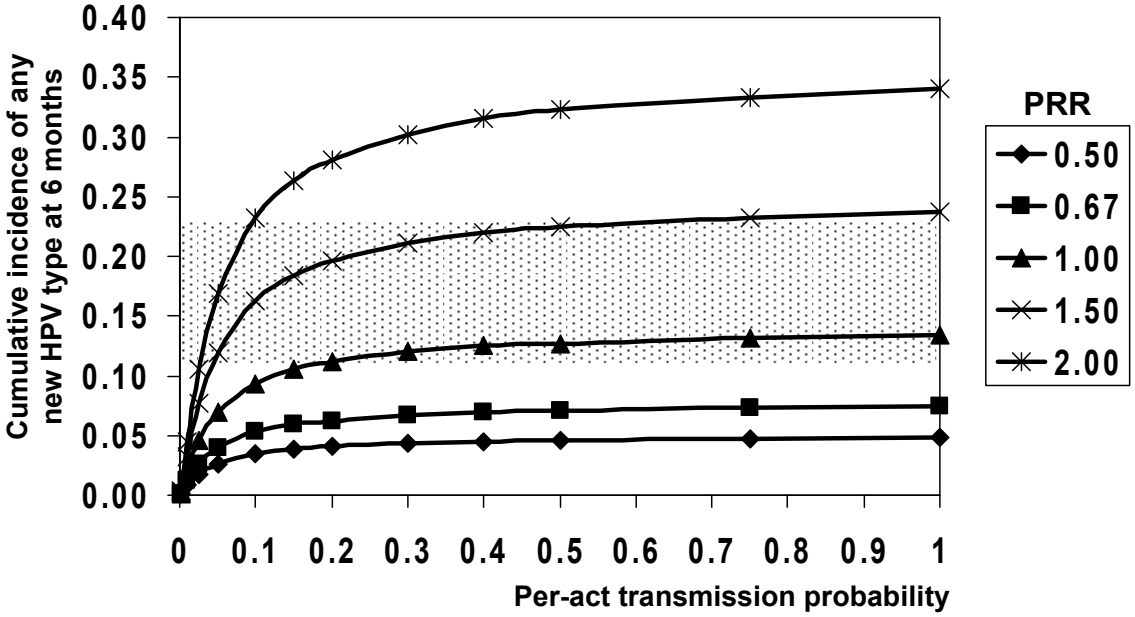


Figure 3.2a. RR=1

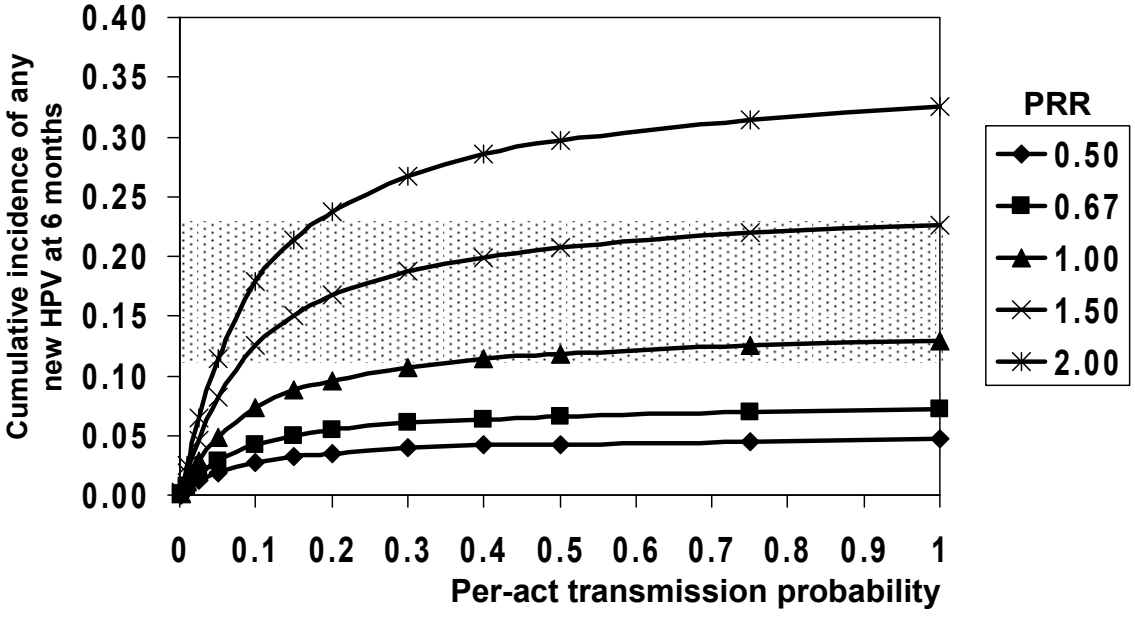


Figure 3.2b. RR=0.25

Figure 3.2. Expected cumulative incidence of HPV in a simulated cohort of 10,000 women by transmission probability per coital act and male-to-female prevalence rate ratio (PRR). The empirically-observed 95 percent CI for incidence among women in the McGill-Concordia Cohort Study (0.114, 0.230) is shaded. (a) Results shown assuming no protective effect of condoms (relative risk for a single protected versus unprotected act = 1.0). (b) Results shown assuming that condoms offer four-fold protection (relative risk for a single protected versus unprotected act = 0.25).

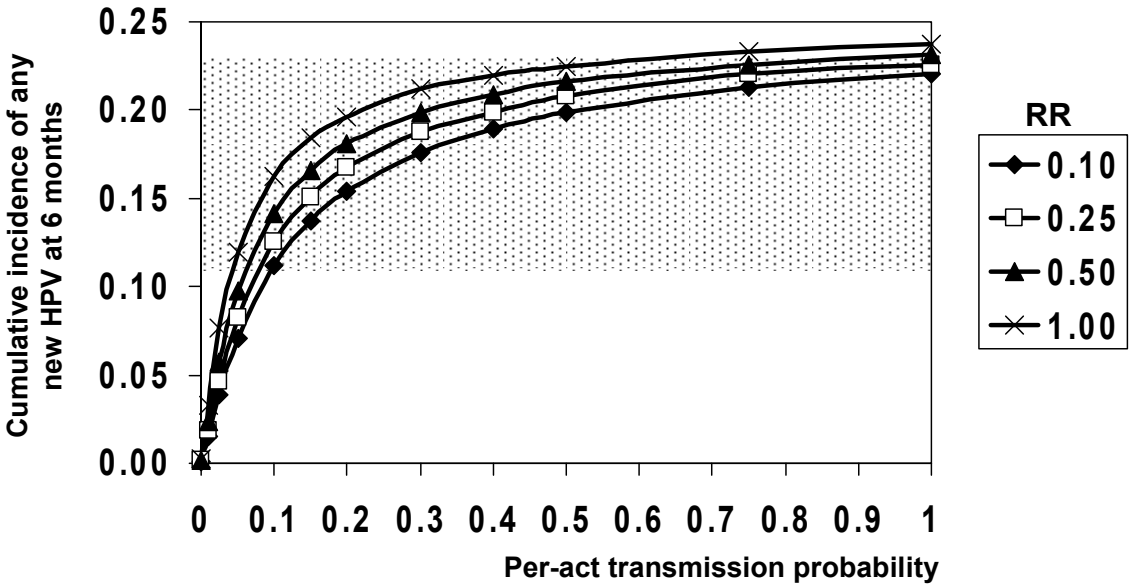


Figure 3.3. Expected cumulative incidence of HPV in a simulated cohort of 10,000 women by transmission probability per coital act and relative risk (RR) of transmission for a protected versus an unprotected act. The empirically-observed 95 percent CI for incidence among women in the McGill-Concordia Cohort Study (0.114, 0.230) is shaded. Results shown assuming male prevalence is 1.5 times that of women (male-to-female prevalence rate ratio = 1.5).

Epilogue to Manuscript II

Following the simulation study reported in Manuscript Two, I used an alternative, maximum likelihood method to estimate HPV transmissibility, overall and by type [137,138]. The probability of male-to-female HPV transmission per coital act and male HPV prevalence were estimated under the assumption that transmission is a binomial process, following the equation: Probability (Infection) = $1 - \{1 - p[1 - (1 - \beta_a)^{Ai}]\}^{Ni}$, where p is the prevalence of HPV among male partners, β_a is the per-act transmission probability, A_i is the number of acts of intercourse per male partner, and N_i is the number of male partners [139]. As for the simulation analysis, data were from the previous cohort study of female students at McGill and Concordia universities [26,140]. The analysis was restricted to the 164 women who reported a new partner since her last clinic visit, and no other partners. HPV outcomes included acquisition of any new HPV type, any new HR-HPV, or any new LR-HPV. PROC NLIN in SAS was used to derive maximum likelihood estimates of male HPV prevalence and β_a, the per-act transmission probability. 95%CIs were calculated by bootstrapping at the level of the woman. Consistent with the simulation approach, the point estimate of the per-act transmission probability for any HPV type was 48% (95%CI 0.14-0.78). Point estimates suggested that LR types are more transmissible than HR types, but confidence intervals overlapped (HR-HPV: β_a=0.31, 95%CI 0.078-0.66; LR-HPV: β_a=0.65, 95%CI 0.21-0.95).

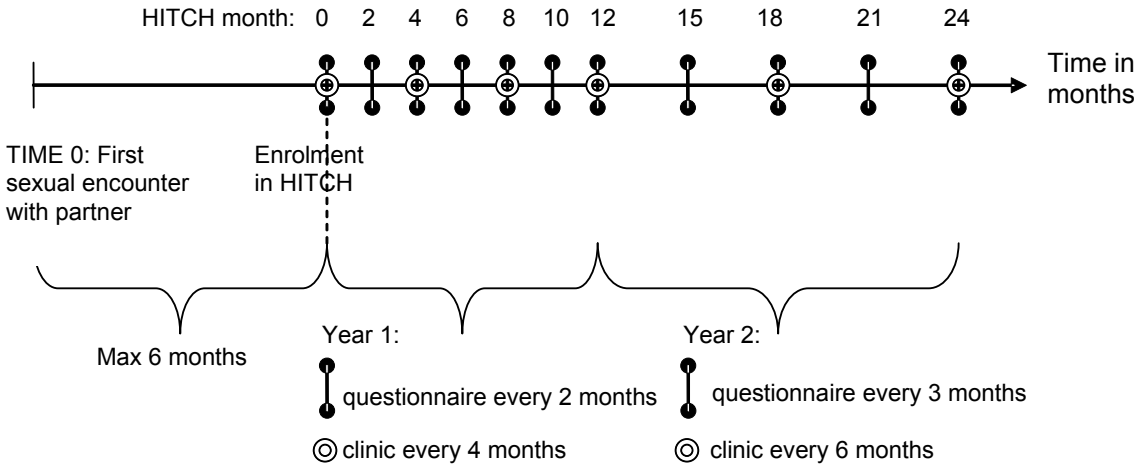
The results from both the simulation and maximum likelihood methods using empirical data from women suggest that HPV is highly transmissible. Nevertheless, the transmission probability estimates from these methods lacked precision. Much uncertainty remains regarding type differences. The prevalence of HPV among women's male partners was unknown, and the need to estimate that parameter obscures the interpretation of transmission to women. It is only in studies of sexual partners that questions can be answered regarding the transmissibility of HPV, how this may vary by HPV type, and which conditions and characteristics may modify transmission risk upon exposure.

SECTION 4: HITCH COHORT STUDY METHODS

Study design

HITCH is an ongoing cohort investigation of HPV transmission in young, heterosexual couples that was initiated in May 2005. The study aims to recruit 600 couples. Women are then followed for 24 months. Men return for a single follow-up visit at month 4. Women are encouraged to enrol new partners acquired during follow-up. Computerized self-completed questionnaires are used to collect sexual behavioural information. Among men, HPV infection status is obtained through sampling of the genital epithelium at months 0 and 4. Among women, genital HPV infection status is measured at months 0, 4, 8, 12, 18, and 24 using self-collected vaginal swabs. HPV testing and typing is done by a consensus primer polymerase chain reaction protocol that permits distinguishing among 36 genotypes of HPV. Men and women provide a blood sample for HPV-antibody testing at each clinic visit. Beginning in summer 2008, men and women also provide an oral specimen and a sample of epithelial cells from the fingers at the baseline and 4 month follow-up visit. See **Figure 4.1** for time points and time frames.

Figure 4.1. Timeline for follow-up of female HITCH participants



Study population and eligibility criteria

The study population consists of young (aged 18-24) women attending university or CEGEP/college in Montreal and their male partners. A self-selected volunteer sample of heterosexual couples is recruited. Because of the complexity of study logistics and the need for subject retention, a representative, population-based sample is not feasible. A population-based study would undoubtedly suffer from low response and poor compliance rates. Willingness to return for follow-up will be greatest among committed volunteers.

Eligible women are:

- aged 18-24 years;
- enrolled at a university or CEGEP/college in Montreal;
- intend to remain in Montreal for at least the next two years;
- are currently heterosexually active with a male partner within whom she initiated sexual activity within the previous 6 months;
- willing to comply with follow-up for at least 24 months;
- have an intact uterus and no history of cervical lesions/cancer;
- not currently pregnant nor planning to become pregnant in the next 24 months.

Eligible men are aged 18 and older and willing to comply with follow-up for at least four months.

HITCH does not exclude women who have a detectable HPV type upon enrolment. This strategy was used in the previous study of this population [26]. In fact, it was observed that Brazilian women in whom any type of HPV was detected at baseline were more likely to acquire a new type at follow-up [141]. Receipt of HPV vaccine has no bearing on women's eligibility.

One of the most important determinants of HPV infection among women is age (**Section 3, Manuscript I**). Most studies indicate a sharp decrease in prevalence after age 30 [142-145]. The rate of acquisition of HPV is high among young adolescent and adult women, with an estimated 46% acquiring at least one HPV

type within 3 years of sexual debut [129]. For these reasons, HITCH restricts participation to young women. No upper age restriction is placed on the male partner, however.

Previous prospective studies suggest that most HPV transmission occurs within 0-12 months of acquisition of a new partner [74,129]. It is critical to recruit couples that are newly forming. Ideally, couples would be recruited before their first episode of sexual activity. However, this scientific need must be balanced with the conditions under which most couples would be willing to enrol. In the feasibility study (**Section 3**), the minimal relationship duration before couples would be willing to enrol was on average 5 months (range 1-12). For these reasons, HITCH limits enrolment to couples that have formed within six months. This allows sufficient time for potential participants to feel comfortable, but maximizes the number of couples in whom transmission will not yet have occurred at the time of enrolment, particularly because condoms are used more frequently in the early stages of a relationship [124,135,146].

Recruitment

Recruitment is achieved through study promotion such as posters, pamphlets, and print and electronic advertisement on university and college campuses and at venues commonly frequented by students. Part-time "peer recruiters" assist with and advise on study promotion. These are undergraduate students attending McGill University, Concordia University, and Université de Montréal. Because peer recruiters are members of the study target population, they are well positioned to identify the most appropriate locations and venues for promotion, and can provide feedback on the appeal and suitability of promotional materials and messages.

Promotional materials and advertisements invite interested couples to visit the study website (www.mcgill.ca/hitchcohort) and to contact the study nurses. Posters have been most successful, with 83% of all study inquiries reporting having learned of HITCH in this manner. A further 8% of study inquiries report

having learned about HITCH through advertisements, and 5% through in-person promotional activities such as classroom announcements and study booths at campus events. Only 3% report learning about HITCH by word-of-mouth.

Of those who contacted the study as of September 2008, 37% were eligible, 40% were ineligible, and for 23% eligibility status could not be confirmed. Fifty-eight percent of those whose eligibility status was confirmed were enrolled. Common reasons for refusals included inability or unwillingness to make the time commitment to attend multiple clinic visits; partner's unwillingness to participate; concern regarding the provision of genital or blood specimens or information on sexual behaviour; or break-up of the couple before enrolment could take place.

It is plausible to assume that a considerable number of female participants may terminate relationships with their enrolled HITCH partner. Many of these women will acquire new partners over the course of follow-up. Among women who had a current partner at enrolment in the previous McGill-Concordia Cohort Study, 48% acquired at least one new partner, with an average rate of new partner acquisition of 1.44 new partners over the full 24-month period. An American national survey of sexual behaviour found that, among serially monogamous young women, the average gap between partners was less than 6 months among 47% of women [89]. Female HITCH participants who acquire new male partners are encouraged but not obliged to enrol them. Such secondary male partners will be ideal for study because female HPV status will be known prior to acquisition of the new partner[74].

Enrolment

Eligible couples have an appointment scheduled for their enrolment visit at either the McGill or Concordia student health services clinics. The clinics provide medical care year-round to full-time students. Two previous HPV studies among university women were successfully conducted at these clinics [25,26].

At the enrolment visit, informed written consent is obtained by the research nurse. See **Appendix A** for a copy of the consent forms. Participation is completely voluntary and confidential. Participants are given ample privacy and time to review the consent form. They are given the opportunity to ask the research nurse questions without their partner present to ensure that no one feels coerced by their partner to enrol. The research nurse verifies that both partners have understood the study procedures and their rights as research participants. Subjects indicate that they consent by signing the consent form, which is witnessed by the research nurse. Participants are given a copy for their own records. The consent forms and all study procedures have been approved by the institutional ethical review boards at McGill University, Concordia University, and Université de Montréal.

Following provision of consent, women are instructed in the self-collection of vaginal specimens, and their first specimen is collected. Men undergo an examination of their genitals and a specimen is obtained by the research nurse. Participants complete their first questionnaire at the clinic. Female participants are given an access code and password for subsequent questionnaires to be done between clinic visits. Referrals for an STI assessment are made when appropriate. Men and women each provide a blood specimen. Beginning in 2008, oral and hand specimens are also collected. Finally, the research nurse provides information about HPV and safer sex.

Follow-up among women

Because male-to-female transmission is the scientific focus of the study, women are most actively followed-up (**Figure 4.1**). After enrolment, women return to the clinic at months 4, 8, 12, 18, and 24. At these visits they meet with the research nurse, provide a blood specimen, self-collect a vaginal sample for HPV-DNA testing, and self-complete a computerized questionnaire. Beginning in 2008, female follow-up visits that coincide with a visit by her male partner also involve the collection of oral and hand specimens.

Each female follow-up visit is also an opportunity to check in with the participant, thank them for their participation, and motivate them to continue participating. The nurse reminds women about safer sex guidelines, encourages timely Pap testing as recommended by their university health clinic, and addresses any health concerns. The nurse inquires about changes in relationship status, and encourages the enrolment of new partners.

Follow-up computerized questionnaires are completed by women every two months in year one and every three months in year two. Scheduled follow-up questionnaires that coincide with clinic visits are completed at a computer at the clinic. For follow-up questionnaires scheduled to take place between clinic visits, women login from a computer of their choice, typically from their home or university residence.

Follow-up among men

One male follow-up visit at month 4 was added to the HITCH study design in 2006 so that female-to-male transmission may be assessed. It also provides information on duration of infection among men who were infected at enrolment. Month 4 was selected for three reasons: 1) because transmission is believed to occur rapidly; 2) because dissolution of relationships is anticipated to be common and so follow-up is preferable earlier rather than later; and 3) to enhance comparability of male follow-up data with women's. At this follow-up visit, men meet with the research nurse, provide a blood and genital specimen, and complete a web-based questionnaire using a computer at the clinic. Beginning in 2008, men also provide oral and hand specimens. The nurse reminds men about safer sex guidelines and addresses any health concerns.

Enrolment of new male partners

Women who acquire a new male partner over their two years of follow-up will be encouraged (but not obliged) to enrol him. When this occurs, the female participant and her new partner will be scheduled for a clinic visit, to be attended on the same day. The male will provide informed consent, genital, blood, oral and

hand specimens, and complete a computerized questionnaire. Women will provide genital, blood, oral and hand specimens, and complete a web-based questionnaire, as she would at a typical follow-up clinic visit.

Incentives

An important challenge for longitudinal studies is minimizing loss to follow-up. The suitability of incentives was carefully considered in the feasibility study (**Section 3**). Most interviewees reported increased willingness with relatively small monetary amounts. Nevertheless, respondents were not asked about blood specimens in the feasibility study. Given the importance of properly collected genital specimens, willingness to provide blood specimens, and long-term retention of subjects, HITCH offers participants $50 for each clinic visit and $10 for each computerized questionnaire completed between clinic visits.

Computerized self-completed questionnaire

Considerable research over the past two decades has greatly improved our understanding of the optimal approaches to maximize respondents' willingness to report sexual behaviour while minimizing measurement error [125,147,148]. A secure, confidential study-designated Internet site is used to provide participants with protected access to the computerized questionnaire by assigned login names and passwords. Subjects complete a questionnaire during clinic visits using computers with access to the Internet. Female participants also complete questionnaires between clinic visits from a computer of their choice, such that they complete 10 questionnaires in total (**Figure 4.1**).

Self-completed questionnaires were chosen to increase response rates through reduced participant burden and to enhance the validity of reported responses. People tend to be more comfortable disclosing personal, sensitive information in telephone interviews and self-completed questionnaires than in face-to-face interviews [125,147]. Self-completed questionnaires also reduce social desirability bias, which in face-to-face interviews can result in over-reporting of sexual activity by men and underreporting by women [125]. Although face-to-face methods

are superior to explain complicated questions and ensure fewer missing responses [125,149], this is a minor concern for university students who are accustomed to frequent examination and are well experienced with computers and the Internet. All should have access to Internet as this is the official mechanism of communication between these universities with their students. A further advantage of computerized over paper-and-pencil self-completed questionnaires is that this format may reduce non-response for sensitive questions [150]. Computerized questionnaires also reduce labour and costs for data input and management because information is entered into the database "live". This does not eliminate the need for careful data review and cleaning, but greatly reduces the time required to obtain data for analysis.

Reliability of sexual behaviour reporting is enhanced for short periods of recall, and drops for periods of six months or longer [147,151-154]. Internet web-based diaries were successfully used in a study of HPV infection among sexually inexperienced, young, female university students [155]. Compared with diary methods, people tend to recall fewer partners, more sexual encounters, and more frequent condom use than they actually had when retrospectively recalling behaviour over long intervals (e.g. 6-12 months) [152]. Nevertheless, frequent diaries might compromise response rates and subject retention if used over a long period of time. Baer and colleagues [155] did not observe this. However, their study was conducted with a considerably less sexually experienced population than HITCH, which by design enrols sexually active adults. HITCH uses a compromise to maximize measurement accuracy and minimize attrition. Retrospective reporting is used for relatively short time frames (between 2-4 months), a strategy that was favoured by the vast majority of participants in the feasibility study.

Survey instruments were based on validated instruments successfully used in previous HPV and STI research by our team and others [26,28,146] and principles of questionnaire design [149]. Unique design considerations for the computerized format were also considered [156,157]. Draft versions were critically reviewed for

content validity by members of the research team and external experts in the fields of cervical cancer, HPV and STI. Before computerization, paper-based questionnaires were pre-tested with 7 couples at the McGill clinic (n=14). Detailed sexual exposures are measured including specific sexual acts, the frequency and nature of condom use, and characteristics of sexual partners. Information is collected for each partner separately. This prevents problems in interpretation when new, regular, and casual partners are combined [146]. Respondents provide the initials of their partner(s), or an alias, so that continued sexual activity with partners can be tracked over time. Information on smoking, the use of hormonal contraceptives, and concurrent genital infections are also captured to verify their roles as co-factors. The first months of the study were a run-in phase to adapt and pre-test the computerized questionnaire interface. This run-in phase further evaluated ease of self-completion, the user-friendliness of the web-based format, suitability and appropriateness of measures, and length.

Four questionnaire versions were developed: a male induction, a female induction, a female follow-up, and a male follow-up version. (See **Appendix C** for the female and male enrolment questionnaires. This is provided in its paper format as an example. All are available upon request.) English and French versions are available. Respondents may toggle back-and-forth between languages in the computerized format. Each version contains a main questionnaire and two modules.

Main Questionnaire: All complete the main questionnaire, although not all questions will be applicable to all respondents. Questionnaire sections are general information, smoking history, reproductive history, sexual history, sexual activity with enrolled HITCH partner, sexual activity with other partners (including same-sex partners), contraceptive history, and medical history including HPV vaccination. At enrolment, there are also sections about knowledge of HPV and self-perceived risk of infection, cervical and penile cancer.

Other Partner (OP) Module: This is completed for each reported sexual partner other than the HITCH partner. For rare cases where respondents report >5 other partners, this module is completed only for each ongoing partner. At enrolment, respondents who do not report concurrent partners are asked to complete the OP Module for their last partner.

Aggregated Partner (AP) Module: Respondents may report more than five other partners in any questionnaire, although this is anticipated to occur rarely given our experience with the previous McGill-Concordia Cohort Study [26]. The AP module accommodates this scenario while minimizing respondent burden. Respondents complete this module for sexual behaviour aggregated over all partners who were not ongoing (e.g., casual partners, sex trade).

Upon enrolment, participants are given their HITCH ID number and password so that they may login to complete a computerized questionnaire. It is accessed via a link on the study website (www.mcgill.ca/hitchcohort). The computerized layout is similar to the paper version. The computerized questionnaires make use of customized text to refer to specific dates or partners to personalize the questionnaire and improve recall. Skip patterns are programmed so that respondents need only answer those questions that apply to them. Respondents have the option of leaving responses blank if they prefer not to answer, but a warning screen appears to ensure that no question is left blank accidentally.

Collection of vaginal specimens

A Dacron™ (DuPont, Wilmington, Delaware, USA) swab is used to collect vaginal specimens at months 0, 4, 8, 12, 18, and 24. Women are asked to abstain from intercourse a minimum of 24 hours before collection of the specimen. This minimizes risk of contamination with residual male epithelial cells, urethral secretions, and or/semen [90,158]. Self-collection methods have been shown to be valid for research and clinical purposes, and are acceptable to women [159-161]. Although participants in the feasibility study seemed to prefer to have their specimens collected by the nurse, they were also comfortable with the self-

collection approach, if taught by our nurse. Women also thought that self-collection was more practical for multiple specimens over time.

Because HITCH will describe transmission of HPV, and is not a study of cervical carcinogenesis, "wide area" sampling of the lower genital tract was preferred to measure HPV infection in the vaginal site. There is evidence that HPV DNA may be detected in vaginal sites before it is detected in the cervix [74]. Positivity for HPV is typically higher in vaginal than in cervical specimens [162].

At the clinic, the research nurse provides women with bilingual instructions for self-sampling, and remains available should women have questions before or during self-collection. Women are instructed to gently insert the Dacron™ swab into the vagina until physically it cannot go any further (at least five centimetres), then to rotate the swab inside the vagina for three full rotations. After self-sampling, the research nurse agitates the swab in a vial containing Preservcyt™ (Cytyc Corporation, owned by Hologic Inc., Bedford, Massachussetts, USA) then presses it against the side of the vial to express any remaining fluid. (Preservcyt™ is a proprietary liquid medium that contains methanol and special buffering agents. It is used for cervical cytology and molecular marker studies because it preserves the integrity of cellular material and of nucleic acids). The swab is then disposed of. All samples are stored in a refrigerator at 4ºC pending transfer to the laboratory.

Collection of epithelial cell specimens from the penis and scrotum

Previous research has found that men are willing to submit to penile skin swabbing and that this method of sampling yields adequate material for HPV-DNA detection compared to urine and urethral specimens [122,163]. At both clinic visits, men provide a specimen of epithelial cells from both the penis and scrotum for HPV testing. Because HPV is transmitted through skin-to-skin contact, it is possible that transmission may occur through contact with skin not covered by the condom [164]. Therefore, two specimens are obtained per male: one of the area that would be covered by a condom (the penis, i.e., the glans up to and including the

external opening of the meatus, coronal sulcus, penile shaft, and foreskin in uncircumcised men), and one of the area that would not be covered by a condom (scrotum).

Men are asked to abstain from sexual intercourse for the 24 hours preceding collection [90]. The research nurse wears latex gloves throughout the clinical examination and specimen collection. First, the research nurse conducts an external examination of the genital area to note circumcision status, and the presence of any relevant clinical findings (e.g., warts, lesions, erythema, abrasions, inflammation, discharges, tenderness, adenopathy). Specimens of epithelial cells from the penis and scrotum are then collected using gentle exfoliation with ultra-fine emery paper (3M 600A-grit Wetordry™ Tri-M-ite, Maplewood, Minnesota, USA) followed by swabbing with a cotton Dacron™ applicator moistened with normal saline using the technique described by Weaver and colleagues [122]. Gloves are changed between sampling the penile and scrotum sites. Prior to genital specimen collection, the research nurse shows men a sample emery strip and rubs it on the back of the participant's hand to relieve any anxiety about the procedure. Used emery papers are rolled up and placed into a vial with Preservcyt. After swabbing, the research nurse agitates the swab in a vial containing Preservcyt, then presses it against the side of the vial to express the solution. The swab is then disposed of. All samples are stored in a refrigerator at 4ºC pending transfer to the laboratory.

Collection of blood specimens

Blood is collected at all clinic visits. Subjects provide a 10 ml blood sample which is collected by venipuncture in a non-heparinized Vacutainer tube. Following the formation of a clot of red blood cells at room temperature, specimens are centrifuged at 2700 rpm for 5 minutes in a standard clinical centrifuge. Serum is aliquoted with Pasteur pipettes separately in three individual Nunc™ vials (Nalge Nunc International, part of Thermo Fisher Scientific, Rochester, New York, USA) and stored at -20ºC pending transfer to the laboratory.

Oral specimens

Participants provide an oral specimen for HPV-DNA testing at clinic visits for which both partners are attending (i.e., enrolment visit, 4-month follow-up visit, and subsequent follow-up visits when women enrol a new male partner and the accompanying follow-up visit for that new male partner). Specimens are collected using the technique of the International Agency of Research in Cancer (IARC) that was previously used in a study of HPV and oral cancer by Dr. Franco. [165] Due to safety and low-cost, Scope mouthwash (Procter & Gamble, Cincinnati, Ohio, USA) is used as a specimen transport medium. [166] Briefly, participants rinse their mouth with water then brush all areas of the mouth with a soft toothbrush. The toothbrush is then agitated in mouthwash to release exfoliated cells. Participants then gargle with fresh mouthwash, which is then expectorated into the specimen container. Mouthwash specimens are centrifuged at 3400 rpm for 10 minutes, after which the supernatant is discarded. Cell pellets are frozen at -20ºC pending transfer to the laboratory.

Collection of epithelial cells specimens from the hand

Participants provide a specimen of epithelial cells from the dominant hand for HPV-DNA testing at clinic visits for which both partner are attending. First the participant is asked to thoroughly wash their hands with soap and water, and dry them. Two specimens are then obtained by the research nurse: (1) brushings of the finger tips and under and around the nails; and (2) a swab of the palmar surface of the index and middle fingers. For specimen 1, the nurse brushes a cytobrush around the tips of the index and middle fingers. The cytobrush is agitated in Preservcyt to release cells. The method used for specimen 2 is exactly the same as that used for male genital specimens. Briefly, the skin is first gently abraded using sterilized ultra-fine emery paper (3M 600A-grit Wetordry™ Tri-M-ite), then swabbed with a Dacron™ applicator moistened with normal saline. Swabs are agitated in PreservCyt™, then discarded. The emery paper is placed in the vial with the PreservCyt™ solution. Specimens are stored at 4ºC pending laboratory processing.

This PhD dissertation does not include an analysis of findings from blood, oral or hand specimens but the description is added here for completeness, so that the reader may fully appreciate the design of the HITCH study.

HPV-DNA detection and typing using the Linear Array Genotyping Test (LA-HPV)

Specimens are tested by a polymerase chain reaction (PCR) protocol based on co-amplification of HPV and beta-globin DNA sequences. This protocol uses the enhanced PGMY09/11 primer system, which targets a conserved 450 base-pair segment of the L1 gene of most HPV genotypes and has been extensively utilized in epidemiologic studies [167] including those of our own McGill unit. The research prototype version of the assay, the line blot assay, performed very well against standard methods in initial validation studies [116,167-172] and in proficiency testing studies [163,173]. The line blot assay has been further optimized and is now commercially available from Roche Molecular Systems (Alameda, California, USA) under the designation Linear Array HPV genotyping test (LA-HPV) [174]. The reagents used for LA-HPV are standardized and produced under quality-controlled conditions. Amplification profiles and reagents were optimized to increase the sensitivity and reproducibility, mainly by avoiding competition during co-amplification of beta-globin and HPV DNA. The LA-HPV assay reliably detects the presence of as little as 10 genome copies of HR-HPV DNA [174], and has been shown to be more sensitive and to detect more types than the line blot assay. Thirty-six mucosal HPV genotypes can be detected with this technique: types 6, 11, 16, 18, 26, 31, 33, 34 (formerly known as type 64), 35, 39, 40, 42, 44 (formerly known as type 55), 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82 (including subtype IS39), 83, 84, and 89 (formerly known as CP6108). Negative, weak positive, and strong positive controls are included in each amplification run. Extensive safeguards to avoid contamination are used. Co-amplification of a beta-globin sequence ensures that the specimen contains human DNA and thus it serves to check for the integrity of the specimen. Samples that are both beta-globin and HPV-DNA negative are considered inadequate (see below for further information on how this is decided).

Provision of HPV-DNA results to participants

At enrolment, participants are told that the specimens are being gathered for research purposes only, and that they should contact their primary care provider for follow-up or care. Screening for other STIs is not part of the study protocol. Nevertheless, participants are encouraged to have follow-up care at the clinic (or with their usual provider) if signs or symptoms of STI are reported to or observed by the research nurse. Participants receive their HPV test results at the end of the study or when they leave the study, whichever is earlier. The research nurse provides results in a non-judgemental, sensitive and informative fashion. Participants are reassured that the vast majority of people clear their HPV infection. All women who test positive for HPV DNA are offered a Pap test at study exit. Regardless of their HPV results, all women are advised that regular Pap test screening should be part of their regular health care. Men are advised to seek care if they observe a growth on their genitals. Men and women are reassured that HPV is very common and that they should not feel any anxiety or shame. Learning of an STI diagnosis among those who believe they are in monogamous relationships may be upsetting. Participants are reassured that if they or their partners have an infection, it does not necessarily mean that they recently acquired it. Participants are told about ways to reduce risk of HPV acquisition and transmission, and how to discuss HPV-infection status with partners if appropriate. Pamphlets and films addressing common concerns are available on the study website (www.mcgill.ca/hitchcohort).

Data management

Data management is facilitated through the use of a secure web-based administrative database. This is used by study staff to record and manage study procedures. It was designed by Magma Communications (a division of Primus Telecommunications Canada Inc.) according to my strict specifications. The database is used to store participant information; record clinic visits; review due, overdue, and completed clinic visits and questionnaires; data entry of paper questionnaires (for rare occasions when this format is used); and for export of

data. Assigned rights to various areas of this tool are user-specific. The database can only be accessed from specific IP addresses as an additional security measure. The hosting company, Primus Telecommunications Canada Inc., carries out daily backups of the database. Primus has formal policies that cover personnel, physical and information security, including firewalls, restricted server and database access, and offsite storage of backup media. Paper records (including consent forms, participant's contact information, printed records of clinic visit details, and any paper questionnaires) are stored in locked filing cabinets at the clinics.

Status of the Cohort as of August 31, 2008

Recruitment began at the McGill study site in May 2005 and at the Concordia site in March 2006. As of August 31, 2008, 574 participants (278 females and 296 males) had enrolled. These represent 278 newly-recruited couples, 12 second male partners of already-enrolled women, and six men who re-enrolled with a new female partner, and for a total of 296 couples. Of the 278 newly-recruited couples, 49% (137/278) were recruited at the McGill site and 51% (144/278) at the Concordia site.

A total of 1,209 clinic visits had been attended as of August 31, 2008. The majority of these were attended by women (772/1209) because the study design requires only a single follow-up visit for men. Follow-up of participants is currently ongoing. Only 5% (15/278) of women had completed all six clinic visits as of August 31, 2008. Among the 208 women who attended at least one follow-up visit, a total of 244 person-years of observation have been accumulated.

The single male follow-up visit was added to the study design in fall 2006. A total of 147 men have attended a follow-up visit. Among these men, a total of 75 person-years of follow-up have been accumulated.

As of August 31, 2008, eight men and 21 women had been declared lost-to-follow-up. Most common reasons for drop-out were that participants had moved out of Montreal or that they were no longer interested in participating. Attrition

rates among women using the lifetable approach were 4% by month 4, 7% by month 12, and 15% by month 24. A visual examination of the hazard of loss-to-follow-up over time suggests that the greatest risk of withdrawing occurs between visits 1 and 2 (month 4). Few drop out between visits 2 and 3 (months 4 and 8). Then attrition slowly rises over time. These patterns suggest that there are two distinct groups of women who drop out. The first are women who decide they are unwilling to participate after the first visit. Those who return for the second visit are the dedicated women. Nevertheless, attrition gradually rises over time, typically because women's life plans change and they move out of Montreal.

Women who dropped out were more likely to have enrolled at the McGill site (13%) than at the Concordia site (2%). Enrolment data were compared between women who dropped out and those who did not. There were no statistically significant differences in terms of age, smoking status, lifetime number of vaginal sex partners, age at coitarche, monogamy with their HITCH partner, or HPV status.

Sample for Analysis

Analysis for this PhD thesis focussed on HPV infection outcomes observed at the enrolment visit. As of September 2008, HPV-DNA testing had been done for 542 participants (n=264 female, 278 male). For analysis of HPV prevalence, concordance between partners, and their relation with sexual behaviour, the data were restricted to:

- couples for whom HPV-DNA testing had been carried out for both partners (eliminated five couples);
- couples for whom a valid/non-missing HPV DNA result was available for both the male and female partner (eliminated one couple); and
- couples for whom the male was the first partner the woman recruited (eliminated nine second male partners).

This resulted in baseline HPV and sexual behaviour data from 526 participants, or 263 couples in total.

Definition of HPV outcomes

Male and female genital specimens were tested for presence of 36 HPV types: 6, 11, 16, 18, 26, 31, 33, 34 (formerly known as 64), 35, 39, 40, 42, 44 (formerly known as 55), 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82 (including subtype IS39), 83, 84, and 89 (formerly known as CP6108).

Specimens were also tested for presence of beta-globin which is a marker of human cells/DNA. These results indicate specimen adequacy. There are two bands for the beta-globin result in the LA-HPV assay. "B" is for the higher concentration of the probe and "b" for the lower concentration of the probe. The presence of a B band only indicates the presence of human DNA but in low quantities. The absence of a B band indicates the absence of cells. Interpretation of these markers was sex-specific. A female specimen was considered beta-globin positive and adequate if it had both bands (Bb); it was considered inadequate if one or both bands were absent unless the specimen was HPV-DNA positive. The vast majority of vaginal swabs (99.6%) were adequate. A male specimen was considered beta-globin positive and adequate if it had the higher concentration probe (Bb or only B); it was considered inadequate if a B band was absent unless the specimen was HPV-DNA positive. Ninety-eight percent of specimens from the penis and 91% of specimens from the scrotum were considered adequate.

In summary, the overall HPV-DNA result had three possible values: negative, positive, or inadequate. Inadequate results were eliminated from statistical analyses.

Research nurses collected samples of epithelial cells from two male genital sites: the penis and scrotum. I created an overall result for male genitalia by combining results from the two sites. If an HPV type was present in one specimen, but not the other, the overall result was considered positive for that type. If beta-globin was detected in one specimen, but not the other, the overall result was considered adequate. Future analyses will differentiate among these anatomic site-specific

results, but for the sake of simplicity and consistency with the present objectives I combined them for the thesis.

HPV types may be classified in terms of their oncogenic potential. Infection with high-risk oncogenic HPV types (HR-HPV) was defined as infection with any of the following 16 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, or 82. Infection with low risk HPV types (LR-HPV) was defined as infection with any of the following 20 types: 6, 11, 26, 34, 40, 42, 44, 53, 54, 61, 62, 67, 69, 70, 71, 72, 81, 83, 84, or 89.

Additionally, HPV types may be classified by species which are considered to be phylogenetically related as part of the Alphapapillomavirus genus (alpha, as short notation), which includes most mucosotropic HPV types. Presumably, types within the same species would behave similarly in terms of their transmissibility and natural history. The 36 mucosal HPV types detected by the LA-HPV assay are part of the alpha genus. Individual species were defined as follows.

- Alpha-3: any of HPV types 61, 62, 72, 81, 83, 84, 86, 87, or 89
- Alpha-7: any of HPV types 18, 39, 45, 59, 68, 70, 85
- Alpha-9: any of HPV types 16, 31, 33, 35, 52, 58, 67
- Alpha-10: any of HPV types 6, 11, 13, 74, 44, 55
- Alpha 15: HPV type 71

Definitions of demographic characteristics and risk factors

Measurement of these variables was based on the questions in the self-completed questionnaires (**Appendix C**).

The following variables were considered as possible risk factors for HPV infection. Unless otherwise indicated, these measures were available for both the male and female partner. Risk factors specific to a couple's behaviour are indicated with an asterisk (*).

- Age

- Male circumcision (as noted by the research nurse)
- Total number of vaginal sex partners in lifetime
- HITCH partner was the first vaginal sex partner
- Age at and years since coitarche
- Number of months couple was sexually active at enrolment ("sexual activity" was defined as mutual masturbation, oral sex, vaginal sex or anal sex)*
- Number of months since couple first had vaginal sex*
- Total number of couple's vaginal sex encounters*
- Frequency of vaginal intercourse per week*
- Number of days since couple's last vaginal sex encounter*
- Frequency of condom use*
- Number of protected vaginal encounters*
- Number of unprotected vaginal encounters*
- Frequency of mutual masturbation*
- Frequency of oral sex*
- Anal sex*
- Signs/symptoms of a genital infection

Individual-level variables were based on self-reported data. Couple-level variables, as indicated above with an asterisk (*), were based on both partners' reports. Responses were averaged when both partners provided them, with these additional coding rules.

- When one partner had a missing response (i.e., because they did not remember, did not respond, or whose response was clearly erroneous), the response provided by the other partner was used.
- When one partner denied an activity, but the other reported it, the couple was coded as having engaged in the activity.

Some questions included a "do not know" response category but most did not. Nevertheless, respondents had the option of leaving any question unanswered.

Unless otherwise stated, all "do not know" or unanswered responses were coded as missing and were excluded from analyses of that variable.

Statistical Analysis

Statistical analysis was conducted using SAS, version 9 (SAS Institute, Inc., Cary, North Carolina). Unless otherwise indicated, all p-values were two-sided. Statistical significance was based on the traditional $p < 0.05$ level. I first carried out exploratory analysis to determine the demographic characteristics of the sample, and for all measures of interest for the thesis objectives. This analysis was descriptive in nature and included means, medians, measures of spread, frequencies, and visual inspection of distributions. I compared means using t-tests and medians using the Kruskall Wallis nonparametric test. Sex differences in reports of continuous variables were tested using the paired t-test or the sign test if distributions were skewed.

The total number of vaginal sex encounters was a key measure, and is the basis of the estimation of the probability of HPV transmission per coital act. I gave special attention to the descriptive analysis of this variable. Participants reported how many times they had vaginal intercourse with their HITCH partner since the start of their relationship. They had the option of answering an actual number, or they could report the frequency per week or per month. For participants who opted to report a frequency per week or per month, I estimated the number of encounters by multiplying the frequency by the duration of time engaging in vaginal sex (which was further estimated by the interval between the reported date first engaged in vaginal sex and the questionnaire date). Agreement between partner's reports was assessed using the intra-class correlation coefficient (ICC). The ICC is a measure of how much one partner's report of the number of encounters corresponds with his/her partner's report. An ICC of zero implies no correlation between partner's reports. An ICC of one implies perfect agreement in partner's reports. For such data, one would like to observe a high value of the ICC which would indicate few discrepancies and high agreement.

Analysis for Thesis Objective 1 (to describe male and female HPV prevalence): This analysis consisted of straightforward estimation of the proportion of men and women with any HPV infection, any HR-HPV, any LR-HPV, and the prevalence rate for each of the 36 HPV genotypes identified by the LA-HPV assay.

Analysis for Thesis Objective 2 (to describe gender-specific risk factors for HPV infection): Gender-stratified analysis was used to identify correlates of HPV prevalence at enrolment. This analysis was guided by my assumptions regarding the underlying causal relationships between variables (**Appendix D**). I used cross-tabulations and simple logistic regression to examine crude, unadjusted associations with any HPV infection. I analysed risk factors for type-specific prevalence by treating each HPV-type outcome as an individual observation, such that each participant could have as many as 36 HPV-type outcomes. This approach allows for the assessment of the effect of the partner's HPV status *for the same HPV type under comparison*. To account for multiple observations within subjects, I used logistic regression with generalized estimating equations (GEE) with an exchangeable correlation structure. Results are reported as gender-stratified odds ratios (OR) with 95% confidence intervals (CI). I visually assessed the linearity assumption for continuous variables using empirical logit plots. I also hypothesized that the relations between risk factors and infection status could vary according to the infection status of the partner. For example, a protective effect of condoms, if one exists, can only be exerted upon exposure to an infected partner. Therefore I tested all pair-wise interactions between partner's HPV status and the other risk factors.

Analysis for Thesis Objective 3 (To describe type-specific HPV concordance/discordance in newly-formed relationships): The focus of analysis was the occurrence of type-specific HPV concordance among couples. This was defined as the presence of the same HPV type in both the female and male partner (e.g., both positive for HPV-16). The proportion of couples who were positive-concordant for at least one HPV-type was estimated. A Monte

Carlo test was used to test the null hypothesis that HPV status is independent in couples. For this test, 100,000 random samples of couples were simulated under the assumption that there is no association between HPV status in the male and female, using the marginal HPV type-specific prevalence for each gender. The observed number of HPV-positive type-concordant couples was then compared to the percentile distribution of the expected number of concordant couples under the assumption of independence. A one-sided p-value was calculated to test the null hypothesis of independence against the alternate hypothesis that the number of concordant couples is greater than that expected by chance.

I reviewed two-by-two contingency tables for each HPV-type comparison within couples, comparing HPV status of the female with HPV status of the male for each of the 36 HPV types. A chi-square or Fisher's exact test was used to test the null hypothesis of no association for each HPV type. To test the null hypothesis of no association for all types simultaneously, I used logistic regression with GEE, where the outcome was female HPV status for each of the 36 types, and the single independent variable was male HPV status for each type (as done in analysis for objective 2).

I also used McNemar tests to test the hypothesis that, when type-discordance is observed, it is as equally likely that a discordant couple is female HPV-positive/male HPV-negative as they would be male HPV-positive/female HPV-negative.

The proportion of positive concordance among "exposed" couples was estimated. (These were couples for whom at least one partner had detectable infection). I treated each HPV type-specific within-couple comparison as a single observation. Proportions were summarized over all types, HR-HPV, LR-HPV, and for alpha species 3/15, 7, 9 and 10. I included only the within-couple comparisons in which HPV was present in at least one partner. Observations for which both partners were negative for a particular type were excluded. For example, if neither partner was infected with HPV-16 then that couple contributed no information to the

estimation of the conditional proportion concordant among couples exposed to type 16. Robust standard errors were used in 95%CI estimation to account for repeated observations in couples.

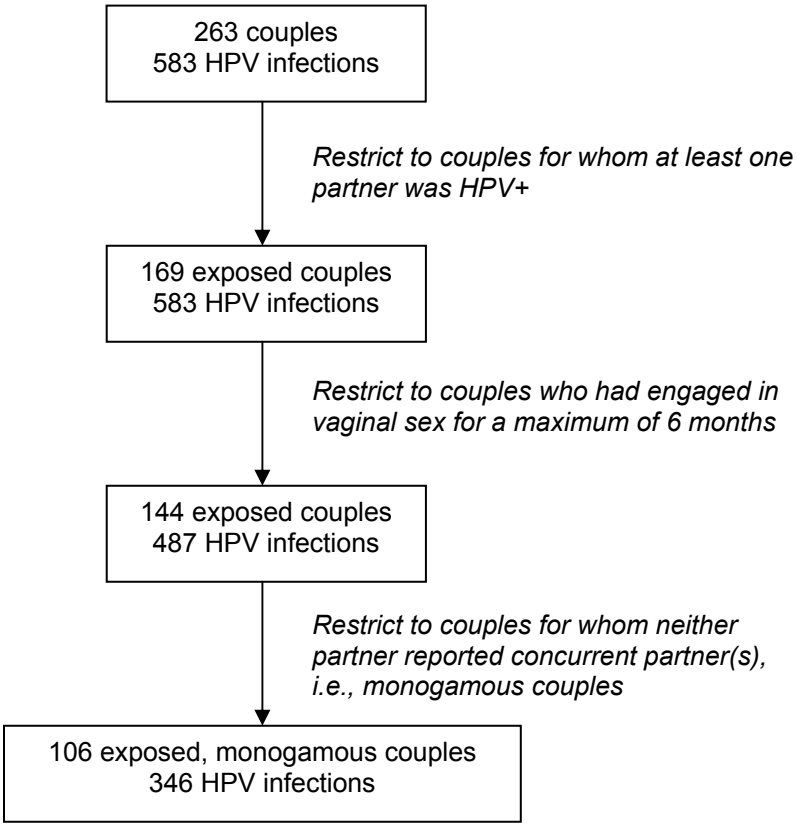
Finally, I examined patterns of type-specific HPV concordance over two time metrics: (1) number of months engaging in vaginal sex; and (2) the total number of vaginal sex encounters. This analysis was restricted to exposed couples who reported no other sexual partners since the start of their relationship. This was done to allow for the interpretation of these cross-sectional data as a snapshot of HPV distribution among exposed couples over time, without any new introduction of types from extra-dyadic partners.

Analysis for Thesis Objective 4 (to identify risk factors for type-specific HPV concordance versus discordance): Three restrictions were placed on the sample so that concordance could be interpreted as a proxy measure of recent transmission within the couple. These are depicted in **Figure 4.2**. First, I restricted to exposed couples, that is, those for which at least one partner had a detectable HPV type (n=169). This focused the analysis on couples for which there was an opportunity for HPV transmission. With this restriction, positive type concordance may be interpreted as a proxy measure of transmission under the following assumptions:

i) that all positive concordances represent transmissions;
ii) that all discordances represent non-transmissions; and,
iii) that there is no source of infection from outside the couple (i.e., no extra-dyadic partners).

The suitability of assumption i) was verified twice. Previous simulation analysis had suggested that most (but not all) positive-concordance at enrolment would represent recent HPV transmissions within the couple given the type-specific HPV prevalence rates observed in the previous McGill-Concordia Cohort Study. This assumption was verified again with HITCH data as part of the analysis for Objective 3.

Figure 4.2 Subsample for analysis of risk factors for positive HPV-type specific concordance



Second, I limited the analysis to exposed couples engaging in vaginal sex for six months or less (n=144). The suitability of assumption ii) requires no clearance of HPV infection present at the inception of the couple's relationship by the time of enrolment. As described in **Manuscript I**, the average duration of HPV infection is about one year among women, and is thought to be the same or less for men. The patterns of positive concordance and discordance over time revealed in the analysis of objective 3 (**Manuscript IV**) showed that the proportion concordant increased with each month engaging in vaginal sex, then declined beyond six months. This implies that by six months clearance may have occurred in partners who were originally infected when the relationship began. Therefore, the restriction to couples who had engaged in vaginal intercourse for less than six months will minimize violations of assumption ii).

Third, I limited the analysis to monogamous couples (n=106). This was done to ensure that the time period of exposure (i.e., since the initiation of the sexual relationship) was correct, and specifically that quantitative measures of sexual exposures were accurate. For example, imagine a couple who enrolled in HITCH five months after they began their sexual relationship. Say that the male partner had an extra-dyadic partner who transmitted HPV-16 to him three months after the initiation of his relationship with his HITCH partner. To interpret concordance with the HITCH partner as transmission, the analysis would have assumed that all of the couple's sexual exposures over their five-month relationship were exposed contacts when, in reality, only the last two months were exposed contacts. Therefore, eliminating all non-monogamous couples prevents overestimating the number of exposed acts of intercourse.

The focus of analysis was risk factors for type-specific HPV-positive concordance among couples. Each type-specific HPV infection detected in the couple was treated as an individual observation. Only those within-couple comparisons where HPV was present in at least one partner were included in the analysis. Observations for which both partners were HPV-negative for a particular type were excluded since there is no opportunity for transmission. For example, if neither partner is infected with HPV-35 then that couple contributes no information to an analysis of risk factors for concordance for HPV type 35. There were a total of 346 relevant observations among the 106 couples.

I identified risk factors for type-specific HPV concordance using logistic regression with GEE and exchangeable correlation to account for clustering by couple. Results were reported as ORs with 95%CIs based on empirical standard errors. First, I examined two-by-two contingency tables and calculated the crude ORs using logistic regression with a single independent variable at a time. The linearity assumption for continuous variables was assessed visually using empirical logit plots. Second, I calculated OR adjusted for the three variables considered *a priori* the most likely confounders: men's and women's lifetime number of vaginal sex partners and the couple's number of unprotected vaginal

sex encounters. Finally, risk factors that remained associated with concordance in step two were combined in a multiple logistic regression model to identify independent risk factors.

Analysis for Thesis Objective 5 (to estimate HPV transmission probabilities per partnership, βp, and per coital act, βa): Measures of the transmission probability are typically denoted with the Greek letter beta (β) in transmission dynamic theory [59]. Analysis carried out for objective 4 can be extended to estimate HPV transmission probabilities per partnership (β_p). The same assumptions apply. For an intercept-only model, the logistic regression equation

$$\log(\text{odds of concordance}) = \alpha$$

can be manipulated algebraically to estimate the probability of concordance, which is interpreted as an estimate of the transmission probability per partnership (β_p).

$$\beta_p = e^{\alpha} / (1 + e^{\alpha})$$

One can use simple probability theory to relate the probability of transmission per partnership (β_p) to the probability of transmission per coital act (β_a) using the following equation:

$$\beta_p = 1 - (1 - \beta_a)^{n_i}$$

where n_i is the number of coital acts accumulated during a couple's history of sexual intimacy [175]. This approach assumes a constant probability of transmission per coital act (β_a) over all acts of intercourse. For small values of β_a (e.g., <0.10), one can use log-log binomial regression for estimation [119]. This is a general linear model with a complementary log-log link, using the regression model $\{\log[-\log(1 - P)]\}=\log(n_i) + b_0 + b_1X_1$ where b_1 and X_1 are regression coefficients and covariates, respectively. In a model with no covariates, the exponentiated intercept is the β_a estimate. In a model with covariates, exponentiated coefficients can be interpreted as relative risks of the probability of transmission per act.

For the application of log-log binomial regression model, I considered each HPV type comparison within a couple as a single outcome, as done in the analysis for objective 4. There were a total of 345 HPV infections among the 105 couples who met the restriction criteria for this analysis and who had data on the number of acts of vaginal intercourse. GEEs with an exchangeable correlation structure were used to account for multiple measures within couples.

Preliminary analysis of inter-relations between all variables: Using an earlier dataset of 196 couples, I carried out extensive preliminary analysis to identify inter-relations between risk factors and female HPV infection, male HPV infection, and HPV-type concordance in couples for whom at least one partner was HPV+. Furthermore, I examined inter-relations between four critical risk factors (frequency of condom use, frequency of vaginal sex, and lifetime number of partners for women and men) and the remaining potential risk factors. These preliminary findings helped to guide later analyses with the complete dataset of 263 couples.

SECTION 5: CHARACTERISTICS OF HITCH PARTICIPANTS

Sociodemographic characteristics of participants and couples

Sociodemographic characteristics are shown in **Table 5.1**. On average women were a year and a half younger (mean 21.2 years) than their male partner (mean 22.7 years). Although there was no upper-age restriction for male eligibility, 95% of men were aged 28 or younger. About two-thirds of participants were born in Canada and reported either English Canadian (women: 38%; men 46%) or French Canadian (women: 21%; men: 21%) ethnicity. There was fair agreement between partners in the country of birth (Canada versus other, kappa = 0.37). In 50% of couples, both partners were born in Canada. For 21%, neither partner was born in Canada. There was approximately equal tendency for couples who were discordant by country of birth to be female Canada/male not (17%) or male Canada/female not (12%) (McNemar p=0.17). Both partners reported the same ethnicity in only 43% (112/259) of couples.

The majority of questionnaires (79%) were completed in English with the remainder in French (**Table 5.1**). There was good agreement in the language used to complete the questionnaire (kappa = 0.68). In the majority of couples (73%), both partners completed the questionnaire in English. For 16%, both partners completed the questionnaire in French. There was equal tendency for couples discordant by language to be female English/male French (5%) or female French/male English (6%) (McNemar p=0.68).

Most participants were of moderate to high socioeconomic status, as measured by the respondent's appraisal of the mother's education and financial situation in childhood/youth (**Table 5.1**).

Fifteen percent of women and 21% of men were current smokers (**Table 5.1**). Current smoking frequency between partners was correlated (Spearman rank ρ = 0.42, $p<0.0001$). There was fair agreement between the lifetime smoking status of partners (kappa = 0.32). If only one partner smoked, it tended to be the male

partner (ever smoked: McNemar OR = 2.25, $p<0.001$; current smoker: McNemar OR = 1.83, $p<0.05$). Nevertheless, in the majority of couples (72%) neither partner was a current, regular smoker.

Table 5.1. Sociodemographic characteristics of men and women enrolled in the HITCH Cohort Study, May 2005 – August 2008.

Characteristic	Women	Men	Difference (W-M)
Age			
N	263	263	263
Mean (SD)	21.2 (1.9)	22.7 (3.2)	-1.5 (2.9)
Median (IQR)	21 (20 – 22)	22 (20 – 24)	-1 (-3 – 0)
Range	18 – 26	18 – 38	-6 – 7
Born in Canada (n)	67% (263)	62% (263)	
Ethnicity (n)	263	263	
English Canadian	38%	46%	
French Canadian	21%	21%	
Black Canadian	1%	3%	
Latin American	6%	6%	
European	9%	5%	
American	5%	4%	
Arab/Middle East	3%	5%	
Jewish/Israeli	2%	1%	
South Asian	4%	2%	
East Asian	3%	1%	
Southeast Asian/Pacific	0.4%	1%	
Mixed ethnicity	4%	2%	
Other	2%	2%	
Not answered	1%	0.4%	
Questionnaire completed in English* (n)	79% (228)	79% (227)	
Mother's education > high school (n)	86% (263)	81% (263)	
Financial situation when growing up (n)	(263)	(263)	
Difficult	6%	7%	
Moderate	24%	35%	
Comfortable	47%	44%	
Very comfortable	24%	14%	
Current student (n)	98% (263)	71% (263)	
If not a student, employment status (n)	n/a	(71)	
Working full-time		64%	
Working part-time		20%	
Other answer		15%	
Not answered		1%	
Ever smoke 100+ cigarettes in lifetime (n)	40% (263)	53% (263)	
Current smoking frequency (n)	(263)	(263)	
*Never smoked regularly **	71%	66%	
Ex-smoker	13%	13%	
1-14 cigarettes/day	13%	15%	
15+ cigarettes/day	2%	5%	

*Data on questionnaire language was absent for the very first series of paper questionnaires (n=35 women, 36 men).

**Includes participants who never smoked 100+ cigarettes in their lifetime and those who did but never smoked "regularly" (defined as one or more cigarettes per day).

Sexual histories

Sexual histories of women and men are shown in **Table 5.2**. All enrolled couples were male-female couples, and most participants identified themselves as exclusively heterosexual. Nevertheless, 17% of women and 6% of men reported ever having a same-sex partner, and 14% of women and 3% of men reported a sexual orientation other than heterosexual (typically bisexual orientation).

All men and all but three women reported having engaged in vaginal sex (**Table 5.2**). For the majority, their HITCH partner was not their first vaginal sex partner. Both partners were in their first vaginal sex relationship in only 5% (12/260) of couples. There was no tendency for men versus women to be in their first relationship (McNemar OR for men versus women = 0.96, NS). On average, it had been 4.2 years since women's and 5.5 years since men's first vaginal sex encounter. Women reported having been sexually active a mean of one year less than men (paired t-test for difference, $p < 0.0001$).

The median number of lifetime vaginal sex partners was 5 for both men and women (**Table 5.2**). The mean was slightly higher among men (8.1) than women (6.2) and the mean difference was that the male reported 1.9 partners more than the woman (sign test for paired difference, $p = 0.05$).

Signs/symptoms of a genital infection were particularly common among women, but few reported an actual diagnosis of STI, even in their lifetime (**Table 5.2**).

Table 5.2. Sexual history characteristics of men and women enrolled in the HITCH Cohort Study, May 2005 – August 2008.

Characteristic	Women	Men	Difference (W-M)
Heterosexual orientation (n)	86% (263)	97% (263)	
Ever had same sex partner (n)	17% (263)	6% (263)	
Never had vaginal sex (n)	1% (263)	0% (263)	
HITCH first vaginal sex partner (n)	14% (263)	13% (263)	
Years since coitarche			
N	260	263	260
Mean (SD)	4.2 (2.3)	5.5 (5.5)	-1.23 (3.6)
Median (IQR)	4 (2 – 6)	5 (3 – 7)	-1 (-3 – 1)
Range	0 – 11	0 – 23	-19 – 9
P-value: paired t			<0.0001
Sign test			<0.0001
Total # of vaginal sex partners in lifetime (including HITCH partner, if applicable)			
N	263	261	261
Mean (SD)	6.2 (5.3)	8.1 (9.1)	-1.9 (8.7)
Median (IQR)	5 (2 – 9)	5 (2 – 11)	0 (-4 – 2)
Range	0 – 35	1 – 54	-39 – 24
P-value: paired t			0.0006
Sign test			0.05
Ever diagnosed with an STI* (n)	8% (251)	7% (258)	
Diagnosed with an STI since start of relationship with HITCH partner (n)	2% (249)	1% (255)	
Signs/symptoms of genital infection since start of relationship with HITCH partner* n)	64% (256)	17% (259)	
Painful urination, or difficulty urinating, or frequent urination	28%	8%	
Itching or burning sensation when urinating	24%	7%	
Blood in urine	9%	1%	
Abnormal genital discharge	18%	2%	
Sores in genital area	5%	5%	
Unusually painful or heavy menstrual period	13%	n/a	
Vaginal itching or burning	30%	n/a	
Lower back pain not caused by physical exertion	15%	n/a	

*Data on specific STIs were available but were not analysed due to small numbers reporting a diagnosis.

Characterization of couples' relationships and behaviours

Descriptive statistics are shown in **Tables 5.3 through 5.8**.

The vast majority of participants considered their HITCH partner to be their dating partner, and most partners agreed in their assessment of the relationship status (90% of couples) and whether their relationship was ongoing (86%) (**Table 5.3**).

According to the study protocol, couples were to have been sexually active for no more than six months at the time of enrolment. Sexual activity was defined as mutual masturbation, oral sex, vaginal or anal intercourse. Nearly 80% (209/263) of enrolled couples had been sexually active for six months or less (**Table 5.4**). Only 8% (20/263) had been sexually active together for more than nine months. The vast majority of couples agreed (within 0.5 months) in the duration they had been sexually active (86%, 218/253). Nevertheless, when disagreement occurred there was a tendency for women to report a slightly shorter length of time than men (mean 0.75 months less, p=0.02 signed rank test).

Eighty-three percent (216/260) of couples had engaged in vaginal sex for six months or less at enrolment, with only 6% (16/260) having done so for more than nine months (**Table 5.4**). The vast majority of couples agreed (within 0.5 months) in the duration they had been engaging in vaginal sex (88%, 212/242). There was no evidence of gender difference in the reported time since engaging in vaginal sex.

The data in **Table 5.4** show that the majority of HITCH couples met the important eligibility criterion of having been sexually active for six months or less. Nevertheless, a proportion did not. There are several potential reasons why this violation may have occurred, as outlined below.

- Eligibility screening was based on self-report, and some may have misreported this duration or misunderstood questions asked by the nurses.

- There were occasional delays between eligibility screening and the actual enrolment visit, such that a couple may have been eligible at the time of screening but were past the six-month point by the time of enrolment.
- Nurses were instructed to focus on time since the first vaginal sex encounter when there were uncertainties regarding eligibility; therefore some (13/263) were sexually active for longer than six months, but had been engaging in vaginal sex for less than six months.
- Couples who had been together, broke up for an extended period, then re-established their relationship were occasionally enrolled if the date they "got back together" was within six months. Unfortunately, the questionnaire was not designed to measure this. These couples cannot be identified, and would have reported the date of their first sexual encounter. Such couples are no longer enrolled in HITCH.

The delay in days between commencement of sexual activity and vaginal sex was calculated by estimating the difference between the averaged dates for these events as reported by the couple. A small proportion (7%, 19/260) reported a negative interval and were excluded from this calculation. Among the 241 couples who had a valid interval, the median delay was 0 days (mean 23, IQR 0-15, range 0-651 days), suggesting that for many couples the initiation of sexual activity and vaginal sex was simultaneous.

Data regarding the number of vaginal sex encounters is shown in **Table 5.5**. Most participants preferred to report this measure as a frequency per week (women: 83%; men: 76%). Couples had engaged in a median of 63 vaginal sex encounters by the time they were enrolled. The mean difference reported by men and women was zero, suggesting no gender difference in reporting. Nevertheless, there was considerable variation in the number of encounters reported by partners. The final column in **Table 5.5** shows that for the average couple, the difference in their reported number of encounters was 35% of the mean of their two individual reports. This amount of variation would be equally achieved by a report of 6.5 and

10 encounters by the male and female partner, or 65 and 100 encounters by the female and male partner. The intra-class correlation coefficient (ICC) was 0.428, indicating that 42.8% of the variation in the number of vaginal sex encounters was due to couple membership (i.e., differences between couples). This suggests that the remaining variability was due to discrepancies between partner's reports. When the data were log-transformed, the ICC was improved (0.743).

Some of the intra-couple variability likely resulted from the three options for reporting this information: an actual number or a frequency per week or per month. In 79% (191/241) of couples, both partners opted to use the same reporting method. Discrepancies were lesser among couples who used the same method (median difference/average=0.29) than in couples who used different methods (median 0.62, $p<0.0001$ Kruskall Wallis nonparametric test of medians).

Similarly, in 93% (225/241) of couples, both partners reported a frequency of vaginal sex rather than an actual number (although one may have reported per week and the other per month). Discrepancies in these couples were lesser (median difference/average=0.28) than in couples for whom at least one partner reported an actual number (median 0.57, $p<0.0001$ Kruskall Wallis nonparametric test of medians).

I estimated the total number of protected and unprotected vaginal sex encounters based on the total number of encounters and the couple's frequency of condom use (**Tables 5.5 & 5.6**). On average, couples engaged in a median of 26 unprotected and 25.6 protected encounters.

Additional data regarding vaginal sex behaviours are shown in **Table 5.6**. Couples reported a median of 4 vaginal sex encounters per week, with no evidence of a gender difference in reporting.

Couples reported a median of 2 days since their last vaginal sex encounter, with no evidence of a gender difference in reporting (**Table 5.6**). Participants were instructed to refrain from sexual activity for 24 hours prior to their enrolment

visit. According to these self-reports, only 3% (7/257) did not adhere to these instructions.

On average, couples reported using condoms "sometimes", defined as use 26-75% of the time (**Table 5.6**). Only nine percent of couples never used condoms. For the majority (64%, 164/258), there was agreement in the frequency that condoms were used. For those who were discrepant, the averaging of their reports resulted in more couples being assigned to the middle categories, and fewer in the extreme categories of never or always using condoms. There was no evidence of a gender difference in reporting.

Sexual behaviours other than vaginal sex are described in **Table 5.7**. Mutual masturbation and oral sex were commonplace. Twenty-three percent of couples also engaged in anal intercourse. Generally, there was excellent agreement in reporting of anal sex. When discrepancies occurred, men tended to report anal sex and women deny it (McNemar OR=3.3, p = 0.05).

Couples were characterized in terms of their past and concurrent partners. As shown in **Table 5.8**, the sample breaks down into: (1) a large group of monogamous couples who had previous vaginal sex experience (58%, 150/260); and roughly equal proportions of couples who (2) were monogamous and one or both partners were in their first vaginal sex relationship (23%, 59/260); and (3) couples for whom at least one partner had concurrent partners (23%, 60/263).

Table 5.3. Description of nature of sexual relationship by couples enrolled in the HITCH Cohort Study, May 2005 – August 2008.

	Women (n=263)	Men (n=263)
Partner status		
Husband/wife	0.4%	0.4%
Common-law partner	7.6%	9.9%
Dating partner	88.6%	87.4%
Friend	1.9%	1.1%
Casual acquaintance	0.8%	0.0%
Not sure – just met	0.4%	0.4%
Other	0.4%	0.8%
Relationship ongoing		
Ongoing & steady	93.9%	87.8%
Ongoing & sporadic	5.3%	9.9%
One/few times only	0.0%	1.1%
Other	0.8%	0.8%
Not answered	0.0%	0.4%

Table 5.4. Reports of the start and duration of the sexual relationship by couples enrolled in the HITCH Cohort Study, May 2005 – August 2008.

	Women	Men	Average of both reports*	Difference (W-M)**	Difference (W-M) in DATE, # days**
Number of months since couple first engaged in sexual activity					
N	257	259	263	253	253
Mean (SD)	4.8 (4.5)	5.5 (6.2)	5.1 (4.9)	-0.75 (4.59)	21.5 (133)
Median	4.2	4.2	4.2	0	0
(IQR)	(2.7–5.4)	(2.8–5.7)	(2.8–5.6)	(0–0)	(-3–9)
Range	0.3–47.5	0.3–48.1	0.3–42.1	-43–11	-328–1301
<2 mos			13%		
2-<4 mos			34%		
4-<6 mos			33%		
6-<9 mos			13%		
9+ mos			8%		
P-value: paired t				0.008	0.01
Signed rank				0.02	0.08
Number of months since couple first engaged in vaginal sex with each other					
N	250	252	260	242	242
Mean (SD)	4.3 (4.2)	4.7 (4.7)	4.6 (4.1)	-0.37 (3.51)	10.1 (10.1)
Median	3.9	3.9	3.9	0	0
IQR	(2.5–5.2)	(2.5–5.3)	(2.7–5.3)	(0–0)	(-2–5)
Range	0.2–47.5	0.2–36.7	0.2–42.1	-31–13	-381–945
<3 mos			30%		
3-<6 mos			53%		
6+ mos			17%		
P-value: paired t				0.10	0.14
Signed rank				0.15	0.39

*If one partner did not answer this question or provided an invalid answer (i.e., a date in the future or distant past), the answer of the other partner was used.

**Only applicable to couples for whom both partners provided a valid answer.

Participants who reported the month and year but not the day were coded as having begun sexual activity on the 15th of the reported month.

Table 5.5. Total number of vaginal sex encounters by couples enrolled in the HITCH Cohort Study, May 2005 – August 2008.

	Women	Men	Average of both reports	Difference (W-M)*	Abs(Diff) / AVG *
Total number of vaginal sex encounters since the start of their relationship **					
N	249	252	260	241	241
Reported a #	14%	20%			
*Reported a frequency per week***	82%	74%			
*Reported a frequency per month***	4%	6%			
Mean	79.1	90.2	86.0	-10.4	0.46
(SD)	(76.3)	(103)	(80.8)	(93.6)	(0.44)
Median	59	64	63	0	0.35
(IQR)	(34–99)	(30–113)	(34–115)	(-22–15)	(0.14–0.68)
Range	1–519	0–986	2–600	-772–395	0.00–2.00
24 or less			15%		
>24 to 60			33%		
>60 to 100			22%		
> 100 encounters			30%		
P-value: paired t				0.08	
Signed rank				0.21	
Total number of unprotected vaginal sex encounters since the start of their relationship †					
N			260		
Mean			39.0		
(SD)			(53.3)		
Median			26.0		
(IQR)			(9.1–51.0)		
Range			0–600		
None			11%		
One to 24			38%		
>24 to 60			24%		
>60 encounters			26%		
Total number of protected vaginal sex encounters since the start of their relationship ‡					
N			260		
Mean			46.9		
(SD)			(63.5)		
Median			25.6		
(IQR)			(6.0–62.2)		
Range			0–427		
None			9%		
One to 24			41%		
>24 to 60			31%		
>60 encounters			20%		

*Only applicable to couples for whom both partners provided a valid answer.

**For participants who opted to report a frequency per week or per month, the number of encounters was estimated by multiplying the frequency by the duration of time engaging

in vaginal sex (which was further estimated by the interval between the reported date first engaged in vaginal sex and the questionnaire date).

† The total number of *unprotected* vaginal sex encounters were calculated by multiplying the total number of vaginal sex encounters by the midpoint estimate of the frequency of encounters for which condoms were not used. (E.g. if condoms were used sometimes, then the number of unprotected encounters is calculated by multiplying the total number of encounters by 50%.)

‡ The total number of *protected* vaginal sex encounters were calculated by multiplying the total number of vaginal sex encounters by the midpoint estimate of the frequency of encounters for which condoms were used. (E.g. if condoms were used sometimes, then the number of protected encounters is calculated by multiplying the total number of encounters by 50%.)

Table 5.6. Characterization of vaginal sex behaviours by couples enrolled in the HITCH Cohort Study, May 2005 – August 2008.

Behaviour	Women	Men	Average of both reports	Difference (W-M)
Frequency of vaginal intercourse per week				
N	260	258	260	258
Mean (SD)	4.7 (3.4)	5.0 (7.1)	4.8 (4.4)	-0.33 (6.8)
Median	4.0	4.0	4.0	0.0
(IQR)	(3.0–5.0)	(3.0–6.0)	(2.7–6.0)	(-1.0–1.0)
Range	0.2–28.0	0.0–106.7	0.2–57.9	-97.7–21.0
<3 times/week			25%	
3-4 times/week			36%	
5-6 times/week			21%	
7+ times/week			16%	
P-value:				
paired t				0.43
Signed rank				0.74
Number of days since the couple last had vaginal sex together*				
N	247	243	257	233
Mean (SD)	5.4 (11.9)	5.8 (14.9)	5.6 (10.0)	-0.4 (18.3)
Median (IQR)	2 (1–4)	2 (2–4)	2.5 (1.5–5.0)	0 (-1–0)
Range	0–130	0–164	0–84.0	-160–126
<3			25%	
3-4			36%	
5-6			21%	
7+ times/week			16%	
P-value:				
paired t				0.76
Signed rank				0.70
Frequency couple used condoms for vaginal sex **				
N	260	258	260	258
Never (0%)	12%	17%	9%	
Rarely (1-25%)	28%	25%	26%	
Sometimes (26-75%)	16%	15%	28%	
Most times (76-99%)	22%	20%	18%	
Always (100%)	21%	22%	19%	
Mean (SD)				0.08 (0.81)
Median (IQR)				0 (0–0)
Range				-2–4
P-value: paired t				0.11
Signed rank				0.14

*Outliers who reported 365 days or more since the last vaginal sex encounter were coded as missing, and their partner's information was used for the average value.

**Condom use frequency was numerically coded as 0 = never, 1 = rarely, 2 = sometimes, 3 = most of the time, and 4 = always.

Table 5.7. Characterization of sexual behaviours other than vaginal intercourse among couples enrolled in the HITCH Cohort Study, May 2005 – August 2008.

	Women	Men	Average of both reports
Frequency of mutual masturbation of male partner			
N	263	263	263
Never (0%)	3%	2%	0%
Rarely (1-25%)	21%	16%	10%
Sometimes (26-75%)	36%	34%	65%
Most times (76-99%)	33%	40%	23%
Always (100%)	7%	8%	2%
Frequency of mutual masturbation of female partner			
N	263	263	263
Never (0%)	1%	0.4%	0%
Rarely (1-25%)	11%	11%	5%
Sometimes (26-75%)	36%	35%	63%
Most times (76-99%)	42%	41%	29%
Always (100%)	9%	12%	3%
Frequency of oral sex on male partner			
N	263	263	263
Never (0%)	2%	2%	1%
Rarely (1-25%)	21%	21%	13%
Sometimes (26-75%)	46%	44%	64%
Most times (76-99%)	28%	28%	20%
Always (100%)	4%	5%	1%
Frequency of oral sex on female partner			
N	263	263	263
Never (0%)	6%	6%	4%
Rarely (1-25%)	25%	24%	17%
Sometimes (26-75%)	41%	4-%	60%
Most times (76-99%)	25%	25%	17%
Always (100%)	2%	5%	0.4%
Anal sex			
N	263	263	261
No	81%	78%	77%
Yes	19%	21%	23%
Not answered	0%	1%	

Table 5.8. Report of concurrent* partners among couples enrolled in the HITCH Cohort Study, May 2005 – August 2008.

	Frequency	Percent
Woman reported concurrent sex partner(s)	39/263	15
Man reported concurrent sex partner(s)	36/263	14
Either partner reported concurrent sex partner(s)	60/263	23
Both partners reported concurrent sex partner(s)	15/263	6
Characterization of couple in relation to greater sexual network**		
Both first vaginal sex partners, monogamous	12/260	5
Female's first vaginal sex, monogamous	18/260	6
Male's first vaginal sex, monogamous	20/260	7
Male and female had previous partners, monogamous	150/260	58
Female had concurrent partners	24/260	9
Male had concurrent partners	21/260	8
Both had concurrent partners	15/260	6

* A concurrent sex partner was defined as reporting a sexual partner other than the HITCH partner since the start of that sexual relationship

** These are mutually exclusive categories assigned in ascending hierarchical order.

SECTION 6: HPV PREVALENCE AT ENROLMENT (MANUSCRIPT III)

Objectives 1 and 2 of the thesis were to describe male and female type-specific HPV prevalence among newly-formed couples, and to describe gender-specific risk factors for HPV infection, respectively. **Manuscript III** reports on analysis of 263 HITCH couples to address these objectives. There are several novel aspects for HPV research. It is the first report of the magnitude of the effect of one's partner's HPV infection status on the risk of being presently infected with this virus at the level of type-specific detection. It is also the first to show how the effect of condom use varies between men and women and by the HPV infection status of the partner.

Novel methods were also used. Although multiple HPV genotypes may be present in the genital tract, researchers typically group these types and analyse risk factors for any HPV infection, or occasionally HR-HPV or LR-HPV types are grouped together. In Manuscript III, I analysed the effect of risk factors for detection of specific HPV types using a logistic regression which considered each HPV type as its own observation. This approach is akin to conducting separate logistic regressions for each detectable type, then obtaining an average of the odds ratio estimates for each risk factor over all of the separate analyses. In practice, I analysed all HPV types simultaneously in a single logistic regression model within a generalized estimating equations (GEE) framework. The LA-HPV assay detects 36 HPV types, but HPV types 11, 26, 69, and 71 were excluded due to zero cells; therefore, each person contributed 32 outcomes. The GEE approach accounted for the fact that each person contributed multiple outcomes by adjusting standard errors and CIs for the actual number of participants. This ensured that statistical inferences were unbiased.

Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner

Running head: HPV infection in new couples

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SHORT SUMMARY:

In a study of newly-forming couples, the partner's status was the most important risk factor for detectable infection, and condoms exerted stronger protection among men than among women.

ABSTRACT:

Background: We evaluated the influence of the partner's human papillomavirus (HPV) status and sexual practices on prevalent HPV infection among new couples to study HPV transmission.

Methods: Women attending university or college in Montreal, Canada, and their male partners (N=263 couples) were enrolled in 2005-08. HPV typing was done in self-collected vaginal swabs and clinician-collected penis and scrotum swabs. The outcome measures were overall and type-specific HPV prevalence.

Results: HPV was detected in 56% of women and men. Prevalence was higher among persons with infected partners (85%) than in those whose partners were negative (19%). Type-specific detection was substantially higher among women (OR=55.2, 95%CI: 38.0-80.1) and men (OR=58.7, 95%CI: 39.8-86.3) if their partner harbored the type under consideration. Prevalence among women and men with ten or more lifetime partners was 15.4 (95%CI: 5.9-40.2) and 9.5 (95%CI: 4.4-19.8) times higher than among those with one partner. Frequent condom use was protective in men, particularly if his partner was HPV-infected (OR=0.64, 95%CI: 0.50-0.82). This effect was attenuated among women with an infected partner (OR=0.88, 95%CI: 0.69-1.11).

Conclusions: The current partner's status was the most important risk factor for prevalent HPV infection. Condoms exerted a stronger protective effect among men than among women.

Key words: human papillomavirus, young adults, prevalence, sex partners, condoms

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI). Population-based estimates of prevalence are 27% among US females aged 14-59 years, and 45% among women aged 20-24 [176]. HPV is also common in men [177-179]. Persistent infections with high-risk HPV types (HR-HPV) are the central causal factor for cervical cancer and are further responsible for a substantial proportion of many other anogenital neoplasms and head and neck cancers [34,51]. Infections with HPV types of low oncogenic risk (LR-HPV), such as HPV-6 and 11, are associated with benign anogenital lesions including condylomata acuminata (genital warts) [51].

Most HPV studies to date have focused on individuals although transmission involves contact between two people. In individual-based studies, the number of sexual partners is consistently the strongest risk factor for infection in women and men [77,97,98,176,180-182]. Women's estimates of their current male partner's number of partners are also predictive of infection [74,97,183]. Naturally,

such assessments are imprecise and can only be proxy measures of the underlying true risk factor — the partner's HPV status.

Couple-based studies have documented the importance of male sexual behavior on women's risk of HPV-related disease [66,184,185]. Couples in these studies are older with relationships of long duration. Because HPV is thought to be highly transmissible [30,186], the transmission event likely occurred years ago, and many infections would have resolved. Ideally, one would recruit newly-forming couples to study HPV infection closer in the time to the transmission event. To our knowledge, no such study has been published.

When two individuals initiate a sexual relationship, one or both may be HPV-infected due to previous sexual exposure. This establishes the opportunity for transmission, the likelihood of which will depend on the inherent transmissibility of the virus and susceptibility of the uninfected partner but also on the number and nature of sexual encounters. Frequent condom use may be protective. Evidence is available from some studies [72,93,187,188] but findings have been inconsistent [23,71,181,189]. If condom use truly protects, its effect can only be observed upon exposure to an infected partner. Moreover, use of condoms may be a marker for exposure to an infected partner, since they tend to be used with new or casual partners [124,135]. When information on partner's HPV status is lacking, the effect of condom use may be obscured.

Having information on the current partner's HPV status permits answering several questions. Does it correlate with prevalent HPV infection as strongly as the number of partners? How would the effect of condom use differ among

persons with and without an HPV-infected partner? To answer these questions, we analyzed data from a study of young heterosexual couples in new relationships.

MATERIALS AND METHODS

Study design

The HITCH Study (HPV Infection and Transmission among Couples through Heterosexual activity) is an ongoing longitudinal investigation initiated in May 2005. The study population consists of young (aged 18-24) women attending a university or junior college in Montreal, Canada and their male partners. Eligible women were currently sexually active with a male partner for no more than six months; had an intact uterus and no history of cervical lesions/cancer; and were not pregnant or planning to become pregnant in the next 24 months. Eligible male partners were aged 18 and older. Cross-sectional data obtained at enrolment are the object of the present analysis.

A self-selected volunteer sample was recruited through study promotion on campuses and at venues frequented by students. Promotional materials invited interested persons to visit the study website (www.mcgill.ca/hitchcohort) and to contact the research nurses. Of those making initial contact, 37% were eligible and of these, 58% enrolled. Participants attended the student health services clinics of either McGill or Concordia Universities and were compensated CDN $50 per clinic visit. All provided written informed consent. Study procedures and

documents were approved by the ethical review committees at McGill University, Concordia University, and Université de Montréal.

Men and women self-completed separate computerized questionnaires, and were assured that information would not be released to their partner. Sexual behavior within the couple (i.e., with their "HITCH partner") was measured since the start of their relationship, defined as the first encounter involving mutual masturbation, oral sex, and vaginal or anal intercourse. Participants reported condom use frequency using the following response categories: never (0% of the time), rarely (1-25%), some of the time (26-75%), most of the time (76-99%), and always (100% of the time), coded from 0 to 4. The responses provided by each partner were averaged for use in the primary analysis.

The outcome of interest was the presence of HPV DNA in genital specimens. Women self-collected vaginal swabs; they were instructed to gently insert a Dacron™ swab into the vagina until it could not go any further (at least 5 centimeters), then to rotate the swab inside the vagina for three full rotations. Clinician-obtained specimens of epithelial cells from the penis (i.e., the glans up to and including the external opening of the meatus, coronal sulcus, penile shaft, and foreskin in uncircumcised men) and scrotum were collected using previously described methods [122]. Briefly, the skin was first gently abraded using sterilized ultra-fine emery paper (3M 600A-grit Wetordry™ Tri-M-ite), then swabbed with a Dacron™ applicator moistened with normal saline. Vaginal and male genital swabs were agitated in PreservCyt™ and then discarded. Emery papers from male

specimens were placed in the vials with the PreservCyt™ solution. Specimens were stored at 4ºC before processing.

HPV testing and typing

Genital specimens were tested by a polymerase chain reaction protocol based on amplification of a 450 bp segment in the L1 HPV gene using the Linear Array HPV genotyping assay (LA-HPV) (Roche Molecular Systems) [174]. Thirty-six mucosal HPV genotypes can be detected with this technique: types 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89. Co-amplification of a ß-globin DNA sequence permitted determining whether the specimens were adequate for testing. To date, 99.6% of vaginal specimens and 100% of combined penile and scrotum epithelial specimens were considered adequate.

Statistical analysis

The analysis was restricted to enrolment visit data from couples recruited between May 2005 and August 2008. Six couples who did not have valid HPV-DNA results for both partners were excluded, resulting in a final sample of 526 participants, or 263 couples in total. Statistical analysis was conducted using SAS, version 9 (SAS Institute, Inc., Cary, North Carolina). All p-values were two-sided. Statistical significance was based on a $p < 0.05$. Individual-level sociodemographic and sexual history characteristics were based on self-report.

Couple-related characteristics (e.g., condom use frequency) were based on the average of both partners' reports.

We calculated overall and type-specific HPV prevalence rates and prevalence rate ratios with 95% confidence intervals (CI) for overall HPV prevalence within categories of risk factors. Three potential risk factors were evaluated: the HITCH partner's infection status, the participant's lifetime number of vaginal sex partners, and the couple's frequency of condom use. To allow for the type-specific comparison of HPV status between partners, we considered each HPV type as its own observation, such that participants could have as many as 36 HPV-type outcomes. We used logistic regression within a generalized estimating equations (GEE) framework with an exchangeable correlation structure. This approach ensures that statistical inferences are unbiased by adjusting standard errors and CIs for the actual number of participants. We report gender-stratified results as odds ratios (OR) with 95%CI. The linearity assumption for continuous variables was assessed visually using logit plots. We tested for interaction to assess whether the effects of number of partners and condom use differed according to the HITCH partner's infection status.

RESULTS

Participant characteristics are shown in table 1. Men were on average one year older than their female partner. Both partners were in their first vaginal sex relationship in only 5% (12/260) of couples. Women and men reported a median of five vaginal sex partners in their lifetime. Most (89% women, 87% men)

considered their HITCH partner to be their dating partner (i.e., boyfriend or girlfriend). A minority (8% women, 10% men) reported that their HITCH partner was their marital or common-law spouse.

At enrolment, couples had been sexually active together for a median of 4.2 months (interquartile range 2.8 to 5.6 months), and had engaged in vaginal sex for a median of 3.9 months (interquartile range 2.7 to 5.3 months). The mean frequency of vaginal sex was 4.8 times per week (standard deviation 4.4). Couples' frequency of condom use was 19% always (49), 18% (47) most of the time, 28% (74) some of the time, 26% (67) rarely, and 9% (23) never. In most couples (64%, 164/258), both partners reported the same frequency of condom use (kappa = 0.54, 95%CI 0.53-0.55). There was no evidence of a gender difference in reporting (median difference of female report minus male report = 0).

HPV prevalence

Overall HPV prevalence and prevalence of the ten most common and vaccine-preventable types (i.e., HPV-6, -11, -16, and -18) are shown in figure 1. One or more HPV types was detected in 56% of women (147/263) and 56% of men (147/263). Although prevalence rates exactly correspond, couples were not necessarily concordant for the same HPV type(s). Ignoring type, in 48% (125) both partners were HPV positive. In 36% (94) both partners were HPV negative. Among couples for whom at least one partner was infected (n=169), 64% were concordant for one or more types, 56% were male-positive/female-negative for

one or more types, and 51% were male-negative/female-positive for one or more types. Among HPV-infected participants, the mean number of types was 2.8 for women (range 1-10) and 2.8 for men (range 1-11).

Influences on overall HPV prevalence

As shown in table 2, prevalence was 4.5 times higher among those whose partner was positive for any HPV type than among those whose partner was negative for all types. Similarly, prevalence was higher among those with greater numbers of lifetime vaginal sex partners. No HPV infection was detected in the twelve couples for whom both reported no previous vaginal sex partners. Lower rates were observed among couples who used condoms more frequently (table 2); protection being significant for men (p=0.02, chi-square test for trend) but not for women (p=0.11).

Risk factors for type-specific HPV infection

Because there are many HPV genotypes, we examined whether the presence of a specific type in one partner corresponded with detection of the same type in the other. Results of a logistic regression analysis that treated HPV types as individual observations are shown in Table 3. HPV detection increased by 55.2 times (95%CI: 38.0-80.1) in women and 58.7 times (95%CI: 39.8-86.3) in men if their current partner was infected with the type under consideration, even after adjustment for lifetime number of partners and condom use.

The lifetime number of partners was positively associated with HPV type detection, but this relationship was modified by partner's HPV status among women ($p < 0.0001$, table 3). For women whose HITCH partner was negative for the HPV type being compared, prevalence was 1.09 (95%CI: 1.06-1.12) times higher for each additional male partner in her lifetime. In contrast, there was no effect of a woman's past sexual history if her HITCH partner was positive for the HPV type under consideration (OR=0.98, 95%CI: 0.94-1.02). Among men, HPV detection was 1.05 (95%CI: 1.03-1.06) times higher for each additional partner, regardless of his HITCH partner's HPV status.

More frequent condom use was associated with lower odds of HPV detection in men (OR=0.79, 95%CI: 0.70-0.90) but not women (OR=1.02, 95%CI: 0.87-1.18) (interaction $p = 0.04$) when the HPV status of the partner was ignored. The magnitude of protection increased among those whose HITCH partner was infected with the type under consideration but was more pronounced for men (OR=0.64, 95%CI: 0.50-0.81) than for women (OR=0.88, 95%CI: 0.69-1.11). The odds ratio estimates were not materially changed by use of the female or male partner's report of condom use frequency, or by additional adjustment for the frequency of mutual masturbation, oral sex, and engaging in anal sex (data not shown).

HITCH participants were asked to abstain from any sexual contact in the 24 hours preceding their clinic visit and 95% (250/263) complied. To identify evidence of possible contamination of genital specimens by recent sexual contact, the OR for the association between type-specific HPV detection and partner's

status was re-examined, stratified by time since the couple's last vaginal sex encounter. The OR was was 118 among the 7 couples who had sex the same day as the clinic visit (non-compliers), and was 38.6 among those who had sex one day prior (n=37), 91.0 two days prior (n=63), 50.0 three days prior (n=22), 26.2 four to six days prior (n=38), and 45.0 seven or more days prior to the clinic visit (n=44). There was no statistical evidence of effect modification by time since last intercourse.

DISCUSSION

Genital HPV infection was very common among persons with a new sex partner. The strongest risk factor was presence of infection in one's current sexual partner, which resulted in an over fifty-fold increase in the prevalence of type-specific infection. This is the first report that this association is of such high magnitude and is considerably greater than that for the lifetime number of partners. Male prevalence was negatively associated with more frequent condom use, but this relationship did not reach statistical significance in women.

By design, all couples who enrolled in the HITCH Cohort Study had recently initiated their sexual relationship. Prevalence is expected to be high in this population since acquisition of a new partner is an important risk factor for incident infection in women [74,98,190] and men [191]. In our previous study of Montreal female university students, HPV prevalence in cervical specimens was 29% [26], nearly half that observed in HITCH. Prevalence would be expected to be higher in vaginal than in cervical specimens [162]. HPV prevalence based on vaginal

swabs among US women aged 20-24 was 45% [176]. Studies of young male adults observed prevalence rates of 41% in Florida [177], 51% in a combined Florida-Arizona sample [178] and 65% in a predominantly university-based sample in Hawaii [179].

HPV infection among persons with a new partner may arise from three possible sources. One may have been infected from a previous sexual contact. The strong association between one's lifetime number of partners and prevalent HPV reflects the role of past exposure [66,71,77,97,98,176,180,182,192,193]. Alternatively, one may have been infected from the current partner. The very strong associations between HPV infection status in partners is evidence of recent transmission. This could not be explained by contamination from recent sexual contact. Due to the cross-sectional nature of these data, we cannot determine whether transmission was male-to-female or female-to-male. A third explanation is that HPV was transmitted from a concurrent partner after the relationship began. Nonetheless, we expect transmission from extra-dyadic partners to be minimal, as only 15% of women and 14% of men reported concurrent partnerships, and this was not associated with HPV detection (data not shown).

A protective effect of condoms on prevalent male infection has been reported by some [187,188] but not all studies (reviewed in [189]). Of three studies of male incidence, one observed a protective effect of condoms [72] but two others did not [71,181]. None of these previous studies had information on infection status of the female partner. Condom use was protective against prevalent HPV infection among male HITCH participants whose current partner was infected. The OR

indicated protection among women whose partner was infected, but it did not reach statistical significance. As for men, an effect of condom use has been inconsistently observed in women [23] but recent data suggest protection against incidence in women's first vaginal sex relationships [93]. Furthermore, in a longitudinal study of 25 heterosexual couples, there were fewer HPV transmissions in couples who always used condoms [186].

We propose two hypotheses for our observed gender difference. First, if male-to-female transmission is more efficient than female-to-male, then a protective effect may be overcome by a high transmission probability to women [30,136]. Alternatively, the stronger effect observed in men may be due to more rapid HPV clearance if at enrolment more condom-using men had cleared their infection. In a randomized controlled trial among male partners of women with cervical intraepithelial neoplasia, condom users experienced faster regression of HPV-associated penile lesions [194].

This analysis focused on the number of lifetime vaginal sex partners and frequency of condom use for vaginal sex within the couple under the assumption that penile-vaginal exposures were most likely to result in transmission to the genitalia. This was corroborated by the finding that HPV infection was not observed in any of the twelve couples for whom both partners were in their first vaginal sex relationship. Nevertheless, HPV transmission through other sexual activities is plausible [74,195]. The impact of sexual activities other than vaginal sex on HPV infection in HITCH couples will be the subject of a future paper.

Measurement error is a potential limitation for these findings. Estimates of one's lifetime number of partners may be imprecise [148]. Errors in the reporting of condom use frequency were minimized by collecting this information from both partners, and agreement was excellent between partners. We used accepted methods for cell sampling and a highly-sensitive HPV DNA detection method, but particularly for males, these methods are evolving [51].

Generalization of these results to other populations requires careful assessment of sample comparability. The challenge of recruiting research volunteers is well known [196], and this is compounded in studies of couples. Women enrolled in HITCH reported similar numbers of lifetime partners but more frequent sex compared to our previous study of women attending university in Montreal [26]. New couples who are willing to join an STI study may differ from those who are unwilling. In particular, new couples may be emotionally fragile. They may not yet have sufficient trust between partners to have the sensitive discussion needed to establish willingness of each partner to participate in an STI transmission study. We suspect participants had more liberal sexual attitudes, and would be more comfortable with communication of sexual matters, although this cannot be known with certainty. This should not bias our risk factor estimates, however, among populations with similar age profiles and sexual histories.

Our study is the first to document HPV prevalence in young adult heterosexual couples that are newly-forming. HPV prevalence was high (56%) and more common than in similar populations without a restriction to persons with a new sex partner. The partner's HPV status was the most important risk

factor for HPV infection, and condoms exerted a stronger protective effect among men than among women. These findings underscore the very high sexual transmissibility of HPV infection and should assist policymakers in devising strategies to supplement the preventive effect of prophylactic HPV vaccination.

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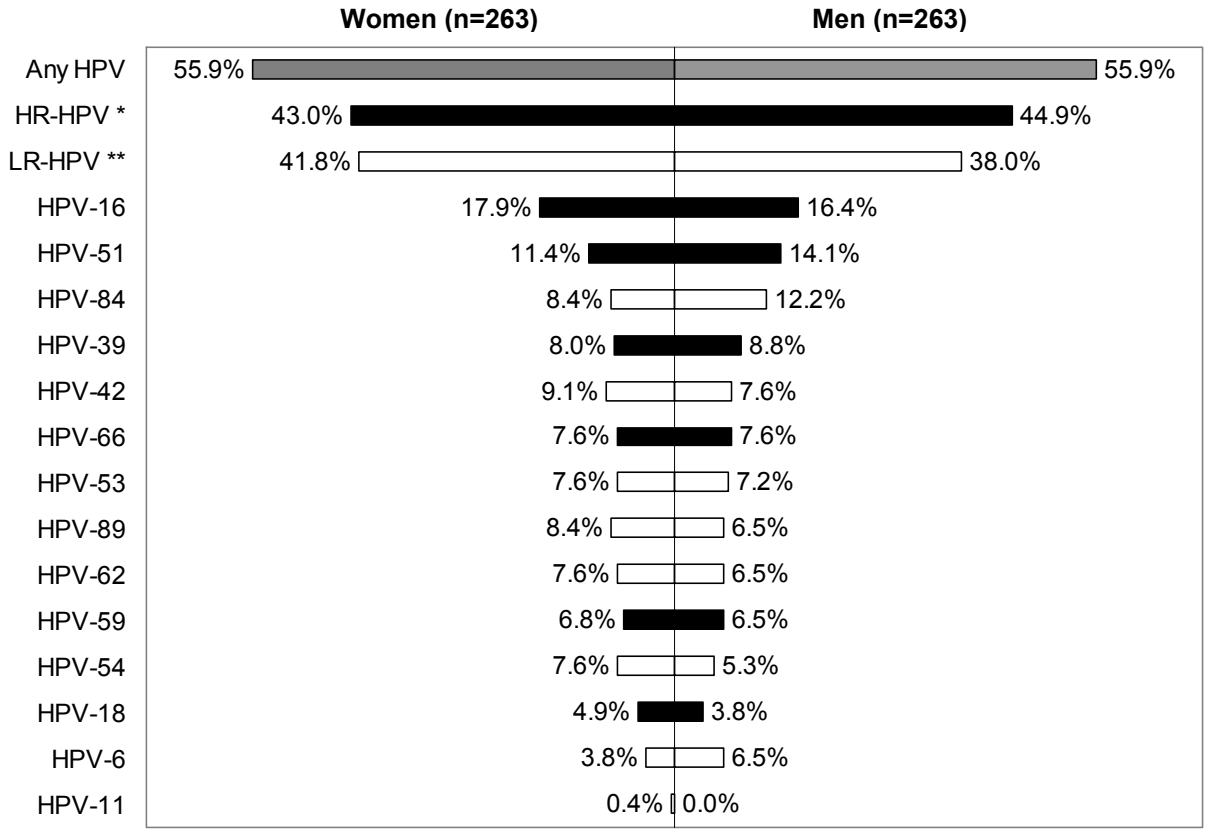

FIGURE 6.1. HPV prevalence at enrolment among women and men in a new sexual relationship, HITCH Cohort Study, 2005-2008.

* HR-HPV, high-risk oncogenic HPV type, defined as any of the following 16 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82. HR-HPV types indicated in black.

** LR-HPV, low risk HPV type, defined as any of the following 20 types: 6, 11, 26, 34, 40, 42, 44, 53, 54, 61, 62, 67, 69, 70, 71, 72, 81, 83, 84, 89. LR-HPV types indicated in white.

TABLE 6.1. Characteristics of women and men participating in the HITCH Cohort Study, Montreal, Canada, 2005-2008.

Characteristics	Women (n=263)	Men (n=263)
Median age in years (range)	21 (18 – 26)	22 (18 – 38)
Born in Canada (%)	67	62
Current student (%)	98	71
Mother's education greater than high school (%)	86	81
Self-identify as exclusively heterosexual (%)	86	97
Ever had vaginal sex (%)	99	100
HITCH partner is first vaginal sex partner (%)	14	13
Median number of vaginal sex partners in lifetime (range)	5 (0 – 35)	5 (1 – 54)
Median years since coitarche (range)	4 (0 – 11)	5 (0 – 23)
Monogamous since onset of sexual relationship with HITCH partner (%)	85	86

TABLE 6.2. Prevalence of any HPV infection by risk factor and gender among women and men in a new sexual relationship, HITCH Cohort Study, 2005-2008.

Gender	Risk factor	n	Prevalence, %	Prevalence Rate Ratio (95% CI)	P-value
Women and men	**At least one HPV type detected in partner**				<0.0001[a]
	Yes	147	85.0	4.48 (3.06 – 6.57)	
	1+ same type(s) detected	*109*	*100.0*		
	Only different type(s) detected	*38*	*42.1*		
	No	116	19.0	1.00	
Women	**Number of vaginal sex partners in lifetime**				<0.0001[b]
	None or one only	39	12.8	1.00	
	Two – four	88	44.3	3.46 (1.48 – 8.10)	
	Five – nine	81	69.1	5.39 (2.34 – 12.4)	
	Ten or more	55	85.4	6.67 (2.91 – 15.2)	
	Couple's frequency of condom use				0.11[b]
	Never	23	65.2	1.00	
	Rarely (1-25%)	67	59.7	0.91 (0.64 – 1.31)	
	Sometimes (26-75%)	74	58.1	0.89 (0.62 – 1.27)	
	Most of the time (76-99%)	47	51.1	0.78 (0.52 – 1.18)	
	Always	49	49.0	0.75 (0.50 – 1.13)	

Gender	Risk factor	n	Prevalence, %	Prevalence Rate Ratio (95% CI)	P-value
Men	**Number of vaginal sex partners in lifetime**				<0.0001[b]
	One only	35	22.9	1.00	
	Two – four	84	33.3	1.46 (0.74 – 2.88)	
	Five – nine	68	60.3	2.64 (1.39 – 5.00)	
	Ten or more	74	93.2	4.08 (2.21 – 7.52)	
	Couple's frequency of condom use				0.02[b]
	Never	23	69.6	1.00	
	Rarely (1-25%)	67	59.7	0.86 (0.61 – 1.20)	
	Sometimes (26-75%)	74	60.8	0.87 (0.63 – 1.21)	
	Most of the time (76-99%)	47	51.1	0.73 (0.50 – 1.08)	
	Always	49	42.9	0.62 (0.40 – 0.94)	

CI, confidence interval.

a) Chi-square test. b) Chi-square test for trend.

TABLE 6.3. Risk factors for detection of type-specific* HPV infection among women and men in a new sexual relationship.

	Women			Men		
	Percent positive	**Unadjusted Odds Ratio (95% CI)**	**Adjusted** Odds Ratio (95% CI)**	**Percent positive**	**Unadjusted Odds Ratio (95%CI)**	**Adjusted** Odds Ratio (95% CI)**
HPV type under analysis was detected in partner						
Yes	58.6	58.7 (40.4 – 85.3)	55.2 (38.0 – 80.1)	57.9	57.4 (39.4 – 83.6)	58.7 (39.8 – 86.3)
No	2.2	1.00	1.00	2.1	1.00	1.00
Number of vaginal sex partners in lifetime						
None or one only	0.6	1.00		1.2	1.00	
Two – four	3.2	5.12 (1.91 – 13.7)		1.8	1.51 (0.67 – 3.41)	
Five – nine	5.9	9.79 (3.72 – 25.8)		4.9	4.40 (2.01 – 9.66)	
Ten or more	9.0	15.4 (5.89 – 40.2)		10.0	9.47 (4.42 – 19.8)	
Average effect of each additional partner		1.07 (1.05 – 1.10)	1.04 (1.00 – 1.08)		1.05 (1.04 – 1.06)	1.05 (1.03 – 1.06)
If current partner:						
Positive for HPV type under analysis			*0.98 (0.94 – 1.02)a*			*1.04 (1.02 – 1.07)c*
Negative for HPV type under analysis			*1.09 (1.06 – 1.12)a*			*1.05 (1.03 – 1.07)c*

	Women			Men		
	Percent positive	**Unadjusted Odds Ratio (95% CI)**	**Adjusted** Odds Ratio (95% CI)**	**Percent positive**	**Unadjusted Odds Ratio (95%CI)**	**Adjusted** Odds Ratio (95% CI)**
Couple's frequency of condom use						
Never	5.7	1.00		6.9	1.00	
Rarely (1-25%)	5.0	0.88 (0.49 – 1.57)		5.7	0.81 (0.46 – 1.43)	
Sometimes (26-75%)	5.5	0.96 (0.53 – 1.75)		5.6	0.80 (0.45 – 1.42)	
Most of the time (76-99%)	4.3	0.73 (0.39 – 1.39)		3.9	0.54 (0.28 – 1.02)	
Always	4.2	0.73 (0.37 – 1.42)		2.6	0.36 (0.18 – 0.71)	
Average effect of each additional increase in frequency		0.92 (0.81 – 1.05)	1.02 (0.87 – 1.18)		0.79 (0.70 – 0.90)	0.75 (0.64 – 0.88)
If current partner:						
Positive for HPV type under analysis			*0.88 (0.69 – 1.11)b*			*0.64 (0.50 – 0.81)d*
Negative for HPV type under analysis			*1.19 (0.98 – 1.45)b*			*0.89 (0.73 – 1.07)d*

* Based on logistic regression analysis for which each HPV-type outcome represented an individual observation. HPV types 11, 26, 69, and 71 were excluded due to zero cells, resulting in 32 HPV-type outcomes for each individual participant. Among women, 4.88% (411/8,416) HPV-type outcomes were positive. Among men, 4.82% (406/8,416) HPV-type outcomes were positive. Generalized estimating equations used to account for multiple observations per-person; within-subject correlations were 0.054 among women and 0.056 among men.

** Variables included in model were partner's HPV status, lifetime number of partners, and frequency of condom use.

P-values for interactions with partner's HPV status: a) <0.0001; b) 0.06; c) 0.62; d) 0.04

SECTION 7: HPV CONCORDANCE AT ENROLMENT (MANUSCRIPT IV)

Objective 3 was to describe type-specific HPV concordance and discordance in newly-formed relationships. The key difference between this objective and objective 1 (the prevalence of HPV in men and women) is the unit of analysis. For objective 1 and in **Manuscript III**, the unit of analysis is the individual. For objective 3, the unit of analysis is the couple or partnership. **Manuscript IV** describes the results of such an analysis among the 263 HITCH couples at enrolment. Although other studies of HPV concordance among couples have been published, this is the first report of these data in a sample of newly-forming couples. It is also the first to report estimates of the proportion concordant by alpha species, by months engaging in vaginal intercourse, and by total number of vaginal sex encounters.

Distribution and genotype-concordance of human papillomavirus infections among couples in new sexual relationships

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Running head: HPV infections in young couples

ABSTRACT

Background: No studies have examined human papillomavirus (HPV) infections among couples at the onset of sexual relationships, a time at which transmission is likely to occur. Our objective was to describe the distribution of HPV infections among newly-formed couples using the partnership as the unit of analysis.

Methods: Women aged 18-24 attending a university or junior college in Montreal enrolled in a longitudinal study with their new male partners. Self-collected vaginal swabs and clinician-collected swabs from the penis and scrotum were tested for 36 HPV genotypes. Participants self-reported sexual behavior in computerized questionnaires. We analyzed patterns of genital HPV infection in 263 couples using data obtained at enrolment.

Results: Couples had engaged in vaginal sex for a median of 3.9 months. HPV was detected in 64% of couples. In 41%, both partners harbored the same HPV type, nearly four times more than expected if HPV status of partners were uncorrelated. In 583 type-specific HPV infections among 169 couples for whom at least one partner was infected, for 42% (95%CI 36%, 47%) the same type was observed in both partners. This rose from 25% at <2 months to 68% among those engaging in vaginal sex for 5-6 months.

Conclusions: Although HPV was common, detection of the same type in persons initiating a sex relationship would be rare given type-specific prevalence rates. The high degree of concordance suggests a high probability of transmission.

Genital human papillomavirus (HPV) is the most common sexually transmitted infection (STI).[2] The vast majority of these infections clear spontaneously.[61,189] The small proportion that persists may result in substantial morbidity, particularly if caused by high oncogenic risk HPV (HR-HPV) genotypes. The latter, especially HPV-16 and 18, are recognized unequivocally as the main causal factor for cervical cancer.[96] HR-HPV may also cause other anogenital neoplasms and head and neck cancers, so that as much as 5.2 percent of incident cancers worldwide are attributed to these infections.[33] Infections with types of low oncogenic risk (LR-HPV), such as HPV-6 and 11, are associated with benign lesions including genital warts or are completely subclinical.

Due to its sexually-transmitted nature, the study of HPV at the level of the sexual partnership is fundamental to our understanding of the epidemiology of these infections. Most research of HPV in couples has consisted of cross-sectional assessment of prevalent infection in both partners (reviewed in [197,198]). Study populations included STI clinic attendees [90], couples being evaluated for infertility [199], women referred for colposcopy and their partners [73,200], and also within the context of retrospective case-control studies of women with cervical intraepithelial neoplasia (CIN) or cervical cancer.[66,185,193] These studies documented the importance of male sexual behaviour for women's ultimate risk of HPV-related disease.[66,184,193] However, many of these studies found that observance of the same HPV type in both partners (i.e., HPV-type-specific positive concordance) was relatively low. In two studies, concordance was greater than expected by chance [73,90], and was associated with more recent sexual

intercourse [90] and higher viral load.[73] Methods of HPV-DNA detection and male specimen collection have been in a process of refinement, thus it is possible that some of these earlier studies had limited ability to detect HPV infections.

Couples in these previous studies tended to be older, with relationships of long duration. No studies specifically targeted newly-forming couples, and some excluded couples of less than six months duration.[66,193] Because most HPV infections are no longer detectable within 12-24 months [61,181], infections may have cleared in one or both partners by the time couples have been together for years.

The observation that HPV is more commonly observed in sexual partners than expected by chance provides evidence for the sexual transmission of HPV.[90] We hypothesize that the extent of concordance will be greatest among relatively young couples at the onset of their sexual relationship, because this is when the transmission event likely occurs. Acquisition of a new partner is an important risk factor for incident infection in women [74,98,190] and men [191], and HPV is thought to be highly transmissible.[30,31] Therefore, the objective of the current investigation was to describe the distribution of HPV infections among newly-forming heterosexual couples. We focused on the partnership as the unit of analysis.

METHODS

We analyzed cross-sectional enrolment data from the HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity). HITCH is an ongoing longitudinal investigation initiated in May 2005. The study population consists of young (aged 18-24) women attending a

university or junior college in Montreal, Canada and their male partners. Eligible women were willing to attend follow-up visits for two years; currently sexually active with a male partner for no more than six months; had an intact uterus and no history of cervical lesions/cancer; and were not currently pregnant or planning to become pregnant in the next 24 months. Eligible male partners were aged 18 and older and willing to participate for at least four months.

A self-selected volunteer sample was recruited through study promotion on campuses and at venues frequented by students. Promotional materials invited interested persons to visit the study website (www.mcgill.ca/hitchcohort) and to contact the research nurses. Of those making initial contact, 37% were documented as eligible, and of these 58% enrolled. Participants attended clinic visits at the student health services clinics of either McGill or Concordia Universities. They were compensated CDN $50 for a completed clinic visit. All provided written informed consent. Study procedures and documents were approved by the ethical review committees at McGill University, Concordia University, and Université de Montréal.

Men and women self-completed separate computerized questionnaires. Sexual behavior within the HITCH couple was measured since the start of their sexual relationship, defined as the first encounter involving mutual masturbation, oral sex, and vaginal or anal intercourse.

Participants were asked to abstain from oral, vaginal or anal sex for 24 hours prior to attending the clinic visit, at which time genital specimens were collected. Women self-collected vaginal swabs; they were instructed to gently

insert a Dacron™ swab into the vagina until physically it could not go any further (at least 5 centimeters), then to rotate the swab inside the vagina for three full rotations. Clinician-obtained specimens of epithelial cells from the penis (i.e., the glans up to and including the external opening of the meatus, coronal sulcus, penile shaft, and foreskin in uncircumcised men) and scrotum were collected using previously described methods.[122] Briefly, the skin was first gently abraded using sterilized ultra-fine emery paper (3M 600A-grit Wetordry™ Tri-M-ite), then swabbed with a Dacron™ applicator moistened with normal saline. Vaginal and male genital swabs were agitated in PreservCyt™, then discarded. Emery papers from male specimens were placed in the vials with the PreservCyt™ solution. Specimens were stored at 4ºC pending laboratory processing.

Genital specimens were tested by a polymerase chain reaction protocol based on amplification of a 450 bp segment in the L1 HPV gene using the Linear Array HPV genotyping assay (LA-HPV) (Roche Molecular Systems).[174] Thirty-six mucosal HPV genotypes can be detected with this technique: types 6, 11, 16, 18, 26, 31, 33, 34 (formerly known as type 64), 35, 39, 40, 42, 44 (formerly known as type 55), 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82 (including subtype IS39), 83, 84, and 89 (formerly known as CP6108). Co-amplification of a ß-globin DNA sequence permitted determining whether the specimens contained human cells and were thus adequate for HPV-DNA assessment. To date, 99.6% of vaginal specimens, 98% of penile epithelial specimens, and 91% of epithelial specimens from the scrotum were considered adequate.

Statistical analysis was conducted using SAS, version 9 (SAS Institute, Inc., Cary, North Carolina). The focus of the analysis was the occurrence of HPV type-specific positive concordance among couples (or "concordance", for short). This was defined as the presence of the same HPV type in both the female and male partner (e.g., both positive for HPV-16). "Discordance" was defined as the presence of a specific HPV type in one partner but not the other.

We calculated the proportion of couples that were concordant for at least one HPV type and compared this to the expected proportion that would have occurred due to chance alone. This was calculated using probability theory. For a single HPV type *"t"*, the probability of concordance is equal to the product of the male- and female-specific prevalence rates ($P_{mt} \times P_{ft}$). The probability that both partners do not share the same type *t* is $1 - (P_{mt} \times P_{ft})$. The probability that partners are not positive concordant for any type is the product of all the $(1 - P_{mt}P_{ft})$ quantitites over the 36 types, i.e., $\Pi(1 - P_{mt}P_{ft})$. This assumes that the probability of infection with one type is independent of infection with another type. Finally, the probability that a couple is concordant on one or more types due to chance is equal to $1 - \Pi(1 - P_{mt}P_{ft})$.

A Monte Carlo test was used to evaluate the null hypothesis that there is no association between HPV status of the male and female partner (i.e., HPV status is independent). For this test, 100,000 random samples of 263 couples were simulated. The marginal HPV type-specific prevalences for each gender were used to randomly assign infection status for all 36 types in each simulated couple. The result was a distribution of the expected proportion of concordant couples

under the null hypothesis of independence. The percentage of simulations with an expected proportion equal to or greater than the observed proportion is the 1-sided p-value for testing the null hypothesis against the alternate hypothesis that the proportion is greater than that expected by chance.

For each HPV type comparison within couples, we reviewed two-by-two tables and tested the null hypothesis of no association using Fisher's exact test. We calculated the ratio of the observed to the expected proportion concordant according to the marginal HPV type-specific prevalences. Among the discordant pairs, the odds ratio (OR) for male versus female positivity was calculated with 95% confidence intervals (CI). For single HPV types, McNemar test-based CIs were calculated. For grouped types (e.g., all HR-HPVs), robust standard errors were used in CI estimation to account for multiple observations per couple.

Furthermore, we estimated the proportion of HPV infections that were concordant among "exposed" couples (i.e., those for whom at least one partner had detectable infection). It is only in these exposed couples that there is an opportunity for HPV transmission to have occurred. We calculated the ratio of the observed to the expected proportion concordant according to the marginal HPV type-specific prevalences; this was summarized over all types, HR-HPV, LR-HPV, and for Alphapapillomavirus species 3/15, 7, 9, and 10.

Next, each type-specific HPV infection in a couple was treated as a single observation. The proportion of couple-level HPV infections for which both partners were infected was estimated, summarized over all types, HR-HPV, LR-HPV, and for Alphapapillomavirus species 3/15, 7, 9, and 10. There were a total

of 583 HPV infections among 169 exposed couples. Robust standard errors were used in 95%CI estimation to account for multiple observations per couple.

Finally, the cross-sectional proportions of infections for which both partners were infected were compared by time since the couple formed. The two time metrics were (1) number of months engaging in vaginal sex; and (2) the total number of vaginal sex encounters. This analysis was restricted to 126 exposed couples who reported no other sexual partners since the start of their relationship. This was done to allow for the interpretation of these cross-sectional data as a "snapshot" of HPV infections over time, without any new introduction of types from extra-dyadic partners. The effect of time was evaluated using logistic regression with generalized estimating equations (GEE) to account for multiple observations per couple.

RESULTS

On average women were 1.5 years younger (mean 21.2 years) than their male partners (mean 22.7 years). Most participants identified themselves as exclusively heterosexual (women: 86%; men: 97%). All men and all but three women reported having ever engaged in vaginal sex. Few reported that their HITCH partner was their first vaginal sex partner (women: 14%; men: 13%). The median number of lifetime vaginal sex partners was 5 for both men and women.

Most subjects (women: 89%, men: 87%) considered their HITCH partner to be their dating partner (i.e., boyfriend or girlfriend). At enrolment, couples had been sexually active together for a median of 4.2 months. All reported engaging

in mutual masturbation. Nearly all reported oral sex on the male (99%) and female partner (95%). All but three couples (99%) engaged in vaginal sex and had done so for a median of 3.9 months. (**Table 7.1**). The mean frequency of vaginal sex was 4.8 times per week; by the time couples enrolled they had engaged in a median of 63 vaginal sex encounters. Only 9% never used condoms. The majority of participants reported no other sex partner since the start of their relationship with their HITCH partner (women: 85%; men: 86%).

HPV distribution among couples

HPV was highly prevalent in the 263 couples with 64% (169) having one or more detectable types in at least one of the partners. For overall HPV prevalence (ignoring type), in half (47%, 125) of couples both partners were HPV positive. In 36% (94) both partners were HPV negative. An equal number of couples were male-positive/female-negative (8%, 22) or male-negative/female-positive (8%, 22). Among couples for whom both partners were positive for any HPV, 87% (109/125) were concordant for one or more types.

When type distribution was examined in all 263 couples, 41% (109) were concordant for at least one HPV type; 33% (86) were female positive, male negative for at least one type; and 36% (95) were male positive, female negative for at least one type. Based on the type-specific female and male prevalences and an assumption of independence for HPV status at couple formation, the expected proportion of concordance on one or more types is 11% due to chance alone. Moreover, none of the 100,000 simulations resulted in an expected proportion

concordant as high as the observed 41% under an assumption of independence (**Figure 7.1**). The mean expected proportion concordant was 11% and the maximum was 17%. The simulation results indicated that the Monte Carlo p-value is <0.00001 under the null hypothesis that partners' HPV infections are independent of each other.

Figure 7.2 shows the distribution of the most common and vaccine-preventable HPV types among couples. HPV-16 was the most common; in 22% of couples it was detected in one or both partners. **Table 7.2** shows the degree of concordance and discordance for each HPV type among the 263 couples. For most types, the proportion concordant was far greater than expected and there was strong evidence ($p < 0.001$) to reject the null hypothesis of independence of HPV infection between partners. When all HPV types were summed into a single two-by-two table (36 types * 263 couples = 8,496 observations), 2.5% were positive concordant, which was 13.4 more times than expected based on the individual type-specific prevalences.

Among type-discordant observations, odds ratios were calculated comparing the odds of a discordant pair being male-positive versus female-positive. There was little evidence for a pattern of discordance by gender for all HPV types combined (OR=1.1, 95%CI: 0.8-1.4), all HR types (OR=1.0, 95%CI: 0.8-1.4), all LR types (OR=1.1, 95%CI: 0.7-1.6), or individually for most of the common and vaccine-preventable types (**Figure 7.3**). The exception was HPV-6 which was eight times more likely to be present in the male when couples were discordant (95%CI 1.4, 46).

HPV infections in exposed couples

Patterns of HPV infections were examined among the 169 couples in which HPV was present in at least one partner (i.e., the "exposed" couples). These are the couples in which there was an opportunity for HPV transmission. Nearly two-thirds (64%, 109) of these couples were positive concordant for at least one HPV type; 51% (86) were female positive, male negative for at least one type; and 56% (95) were male positive, female negative for at least one type. The mean number of types present in these couples was 3.4 (SD 2.4, median 3, range 1-12). There were a total of 583 type-specific HPV infections of which 238 were concordant between partners.

Table 7.3 shows the proportion of exposed couples who were positive concordant on one or more types, overall, by oncogenicity, and by Alphapapillomavirus species. Given the conditioning on infection in at least one of the partners, the proportion concordant was higher than in **Figure 7.1** and **Table 7.2**, as one would predict. Even so, the extent of concordance was still higher than expected based on gender-specific prevalences.

Table 7.3 also shows results for the 583 HPV infections in these 169 couples. Recall that a couple-level HPV infection was defined as an instance of a specific HPV type *t* being detected in a couple (i.e., in at least one of the partners). The proportion of HPV infections for which both partners were infected was 42% (95%CI: 36%-47%). This proportion varied little by oncogenic category (HR: 43%; LR: 39%). The highest occurrence of infection in both partners was

observed for alpha 9 type infections (50%, 95%CI: 41%-59%). This was driven by HPV-16, for which 58% (95%CI: 45%-70%) of couple-level infections were present in both partners. For couples infected with alpha 9 type infections other than HPV-16, in 42% (95%CI: 30%-55%) both partner were infected, similar to other alpha species.

Couples were instructed to abstain from oral, vaginal or anal sex in the 24 hours preceding their clinic visit. The presence of type-specific HPV infections in both partners was highest among couples who had their most recent sexual contact one to two days prior to their visit (49%, n=93 couples). It was lower for those whose last contact was three to four days ago (29%, n=32), five to six days ago (33%, n=11), and one week ago or more (35%, n=26). It was also lower among the 3 couples who violated this instruction (33%).

Patterns of HPV infections in one or both partners among the exposed, monogamous couples by time since the couple formed are shown in **Figure 7.4**. The proportion of infections shared by both partners was higher among couples who had engaged in vaginal sex for longer, and peaked at 68% among couples who had engaged in vaginal sex for 5-6 months, or a total of 100-124 encounters. It was lower thereafter. These curvilinear associations were statistically significant ($p < 0.01$). Figure 7.4B suggests that there may be a second peak at 150 or more vaginal sex encounters, but a cubic term was not statistically significant ($p=0.76$).

DISCUSSION

Detection of one or more HPV types was observed in 64% of newly-formed couples. The presence of the same type in both partners was detected in 41% of couples which was far more frequently than expected by chance. Among couples in whom an HPV type was observed in one partner but not the other, there was little evidence for a pattern of discordance by gender with the exception of HPV-6 which was eight times more likely to be observed in males than females.

This is the first report of patterns of HPV infection among couples who recently initiated a sexual relationship. In previous couple-based studies that used PCR for HPV detection, the proportion concordant ranged from 2% to 47%, with most observations in the 20-40% range.[66,73,90,193,199-202] We observed 41% concordance among newly-formed couples which was in the upper limit of this range. Among exposed couples, this proportion was even higher at 64%.

There are two possible explanations for the concordance we observed. The first is coincidence. That is, both partners were infected from past partners. The second explanation is that one partner was infected when the couple initiated their sexual relationship and transmitted it to the other. By enrolment, HITCH couples had engaged in a median of 63 vaginal sex encounters, providing ample opportunity for transmission. Our best estimate is that 11% of couples would have been positive concordant due to chance alone, based on the type-specific prevalences observed in men and women. Yet we observed concordance in 41% of couples. Greater than expected concordance was also observed in two previous

studies.[73,90] Although some of the concordance we observed was likely coincidental, we conclude that the majority was not and instead was recent transmission within these newly-formed couples. The conclusion is consistent with the pattern of increasing concordance in exposed couples over months engaging in sex and with the total number of vaginal sex encounters. A longitudinal study of 25 couples documented high rates of genital HPV transmission.[186]

Concerning within-couple discordance, i.e., the observation of a given HPV type in one partner but not in the other, there are two possible explanations apart from possible sampling and detectability issues (see below). First, HPV may not have been transmitted (yet) from the infected to the uninfected partner. Alternatively, HPV may have been present in both partners (either due to coincidence or transmission), but by the time of enrolment it had cleared in one of the partners. The average time to clearance is thought to be no more than 12 months in women [61] and as short as six months among men.[181] Among exposed HITCH couples, the patterns of concordance and discordance over time suggest that these effects of clearance may become important once couples have engaged in vaginal sex for six months or longer.

Assuming that most concordance represented transmission and most discordance represented absence of transmission, the proportion of couple-level HPV infections for which both partners were infected can be interpreted as an estimate of the per-partner transmission probability. The overall estimate of this proportion was 42% (95%CI 36%, 47%) but this was a function of time. It rose to

a peak of 68% by 5-6 months engaging in vaginal sex, or 100 to 124 encounters. Estimates did not vary meaningfully when grouping HPV types by oncogenic potential or Alphapapillomavirus species, with one exception. HPV-16 infection in both partners occurred more commonly than other types (58%, 95%CI: 45%-70%) although confidence intervals overlapped. This finding cannot be entirely explained by higher prevalence of HPV-16, since after accounting for prevalence, concordance of HPV-16 was still 4.3 times higher than expected. The finding could be explained by higher transmissibility HPV-16, or by longer duration of these infections. Longer duration of HPV-16 infection has been observed in some studies of women (reviewed in [61]). Ultimately, longitudinal data are needed to verify type differences in transmissibility.

Measurement errors may have affected these results. We used accepted methods for cell sampling and the highly-sensitive HPV-LA for HPV DNA detection and genotyping, but particularly for males, these methods are evolving.[51] There are also concerns that observed concordance may not represent true infection in both partners, but cross-contamination due to recent sexual contact between partners. We instructed couples to refrain from oral, vaginal or anal sex in the 24 hours preceding their clinic visit and 95% complied. Concordance was highest among couples who reported their most recent contact one to two days prior to the clinic visit. Nonetheless, concordance was still high even among couples whose most recent contact was over one week ago. Finally, errors in the reporting of sexual behaviors were minimized by the collection of this information from both partners.

These data are consistent with HPV being highly transmissible. Researchers in this area should design studies to enable observation of rapid transmission soon after acquisition of a new partner. With the development of efficacious HPV vaccines, modelers have projected the public health and economic impact of various vaccination strategies.[13-19] Many of these projections use dynamic transmission models which require sound knowledge of the natural history of HPV acquisition, including the probability of transmission upon exposure. Our results may therefore be of utility to improve forecasting estimates.

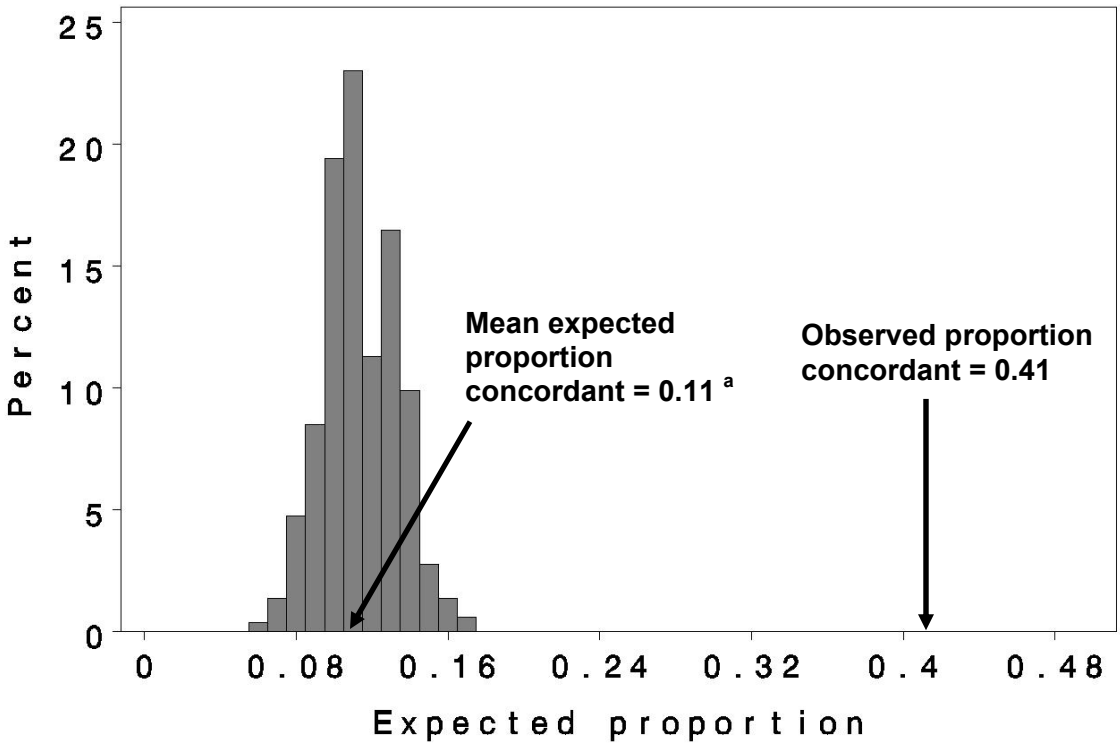

Figure 7.1. Observed proportion of couples concordant on one or more HPV types compared with the expected distribution of the proportion of concordant couples assuming independence in HPV type distribution between partners. a) The mean of 0.11 from 100,000 simulations of 263 couples exactly corresponded with the expected proportion calculated using probability theory, i.e., the probability that a couple is positive concordant on one or more types due to chance is equal to $1 - \Pi(1 - P_{mt}P_{ft})$, where P_{mt} is the observed prevalence of type *t* in the male and P_{ft} is the observed prevalence of type *t* in the female.

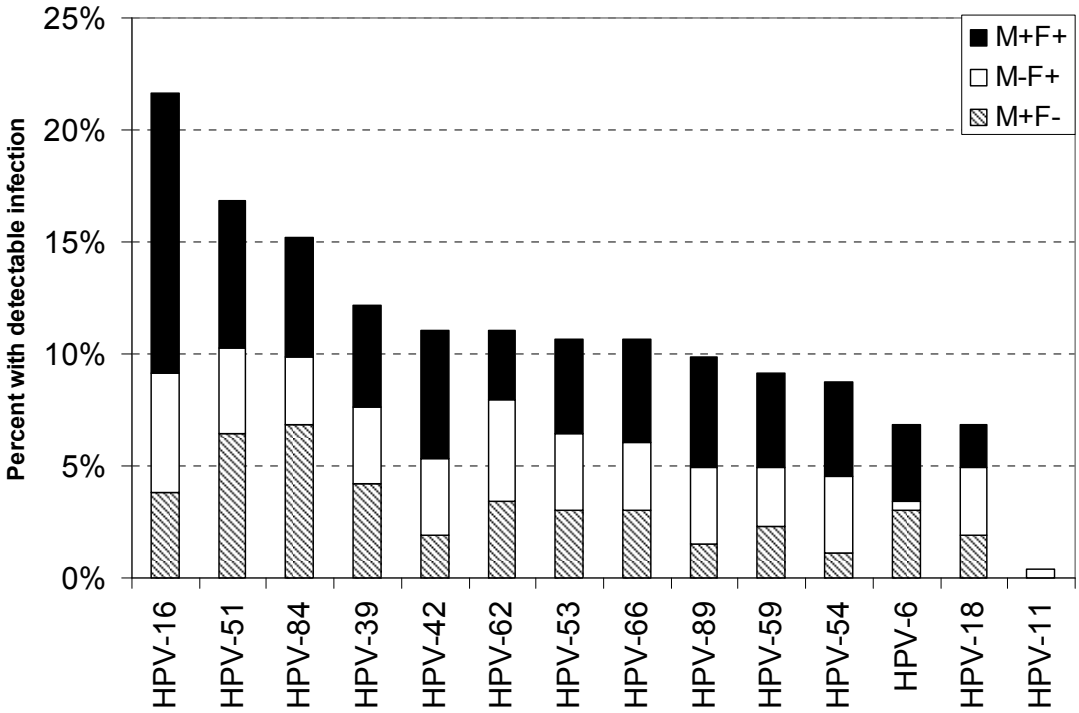

Figure 7.2. Distribution of ten most common and vaccine-preventable HPV types among newly-formed couples (n=263). Legend: M+F+: male positive, female positive; M-F+: male negative, female positive; M+F-: male positive, female negative.

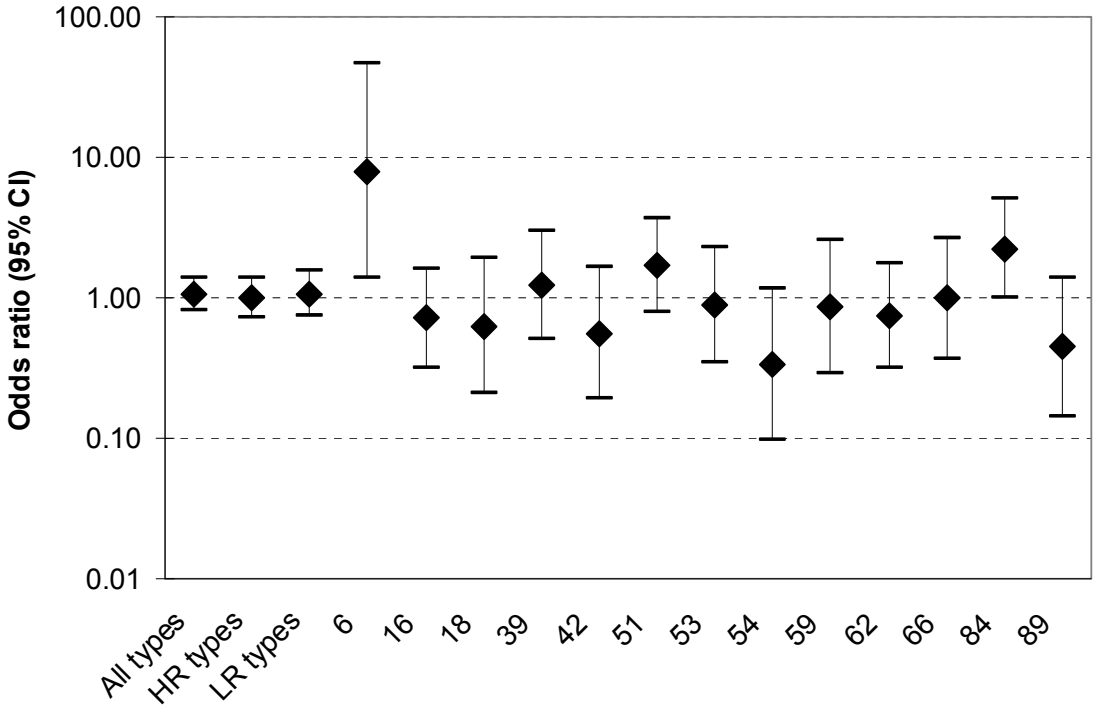

Figure 7.3. Odds ratio and 95% confidence interval for male positivity versus female positivity (i.e., M+F- relative to M-F+) among HPV-type discordant couples, shown for all types, high-risk (HR) types, low-risk (LR) types, and the most common and vaccine-preventable HPV types (although it is vaccine-preventable, HPV-11 is not shown as it was only observed in one male participant).

B

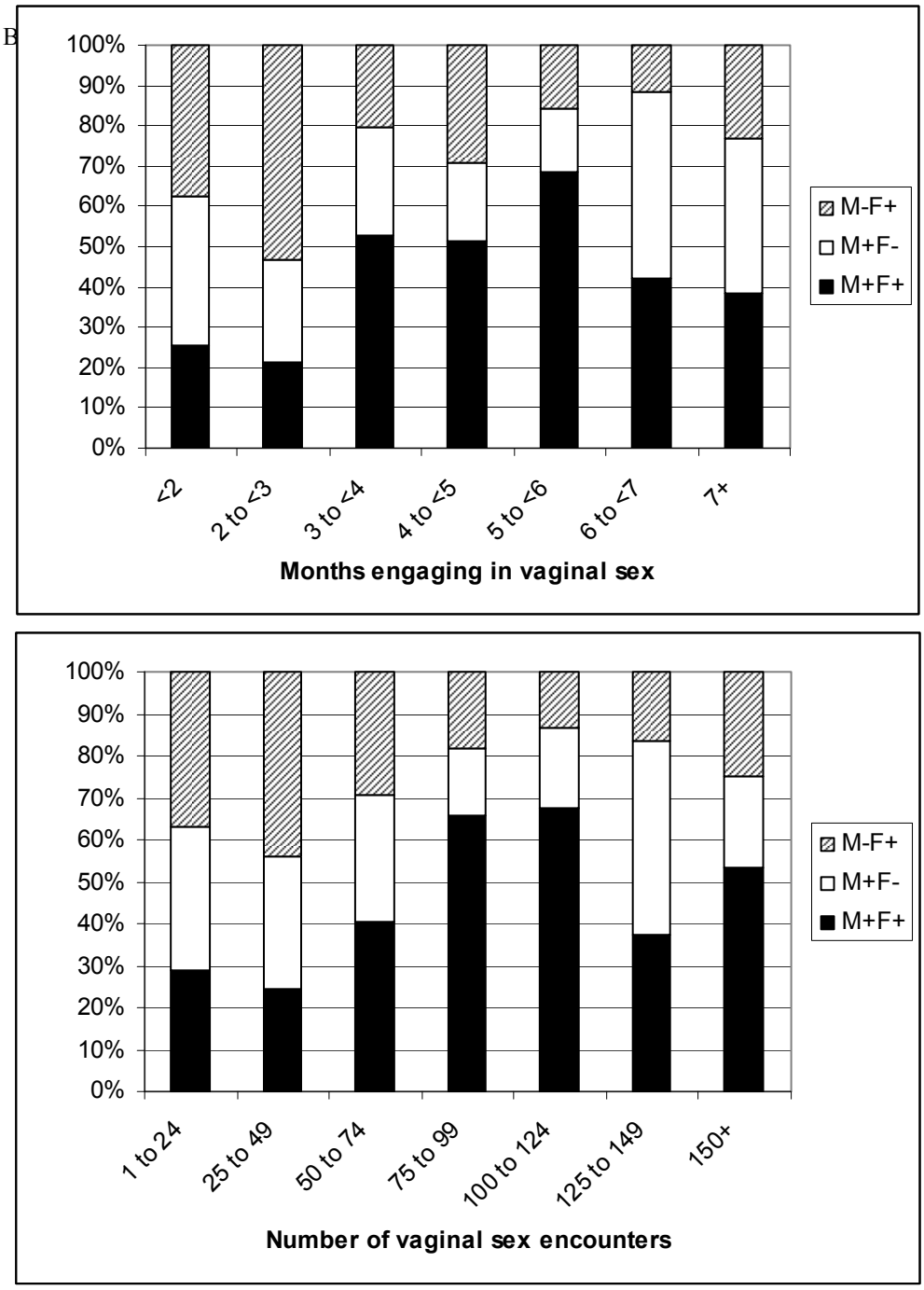

Figure 7.4. Patterns of HPV type concordance and discordance over time in 419 infections among 126 monogamous couples in whom at least one partner had detectable infection. A (top): by months engaging in vaginal sex; B (bottom): by total number of vaginal sex encounters. M+F+: male positive, female positive; M-F+: male negative, female positive; M+F-: male positive, female negative.

Table 7.1. Sexual behaviors reported by couples[a] at enrolment in the HITCH Cohort Study.

	Frequency	Percent [b]
Months engaging in vaginal sex		
<2 months	38	15
2 to <3 months	41	16
3 to <4 months	53	20
4 to <5 months	54	21
5 to <6 months	30	12
6 to <7 months	20	9
7 months or more	24	9
Frequency of vaginal sex		
<3 times/week	66	25
3-4 times/week	95	36
5-6 times/week	56	21
7 times/week or more	43	16
Total number of vaginal sex encounters		
1 to 24	39	15
25 to 49	63	24
50 to 74	46	18
75 to 99	34	13
100 to 124	24	9
125 to 149	18	7
150 or more	36	14
Frequency couple used condoms for vaginal sex		
Never (0%)	23	9
Rarely (1-25%)	67	26
Sometimes (26-75%)	74	28
Most of the time (76-99%)	47	18
Always (100%)	49	19
Engaged in anal sex		
Yes	59	23
No	202	77

Table 7.2. Proportion of concordant and discordant HPV infections, by HPV type (n=263 couples).

HPV type	M+F- % (n)	M-F+ % (n)	M+F+ % (n)	Ratio of observed % M+F+ concordant over expected (95%CI)
6	3.0% (8)	0.4% (1)	3.4% (9)	13.9 (6.4-26.5)
11	0.0% (0)	0.4% (1)	0.0% (0)	n/c
16	3.8% (10)	5.3% (14)	12.6% (33)	4.3 (3.0-6.0)
18	1.9% (5)	3.0% (8)	1.9% (5)	10.1 (3.3-23.6)
26	0.4% (1)	0.0% (0)	0.0% (0)	n/c
31	1.9% (5)	1.5% (4)	3.0% (8)	13.5 (5.8-26.6)
33	0.4% (1)	0.8% (2)	0.4% (1)	43.9 (1.1-244.5)
34	0.4% (1)	0.8% (2)	0.0% (0)	0.0 (0.0-482.9)
35	0.0% (0)	0.4% (1)	0.8% (2)	87.8 (10.6-316.9)
39	4.2% (11)	3.4% (9)	4.6% (12)	6.5 (3.4-11.4)
40	1.1% (3)	0.8% (2)	1.1% (3)	26.3 (5.4-76.9)
42	1.9% (5)	3.4% (9)	5.7% (15)	8.2 (4.6-13.6)
44	1.9% (5)	1.5% (4)	0.8% (2)	12.5 (1.5-45.3)
45	0.8% (2)	1.5% (4)	1.1% (3)	22.6 (4.6-65.9)
51	6.5% (17)	3.8% (10)	6.6% (20)	4.7 (2.9-7.3)
52	0.4% (1)	0.4% (1)	0.4% (1)	65.8 (1.6-366.8)
53	3.0% (8)	3.4% (9)	4.2% (11)	7.6 (3.8-13.6)
54	1.1% (3)	3.4% (9)	4.2% (11)	10.3 (5.2-18.5)
56	1.9% (5)	3.8% (10)	1.9% (5)	8.8 (2.9-20.5)
58	0.0% (0)	1.9% (5)	1.1% (3)	32.9 (6.8-96.2)
59	2.3% (6)	2.7% (7)	4.2% (11)	9.5 (4.7-16.9)
61	2.7% (7)	1.1% (3)	1.1% (3)	13.2 (2.7-38.5)
62	3.4% (9)	4.6% (12)	3.0% (8)	6.2 (2.7-12.2)
66	3.0% (8)	3.0% (8)	4.6% (12)	7.9 (4.1-13.8)
67	2.3% (6)	2.7% (7)	3.4% (9)	9.9 (4.5-18.7)
68	1.1% (3)	1.5% (4)	1.5% (4)	18.8 (5.1-48.2)
69	0.0% (0)	0.0% (0)	0.0% (0)	n/c
70	0.4% (1)	1.1% (3)	0.0% (0)	0.0 (0.0-322.0)
71	0.0% (0)	0.8% (2)	0.0% (0)	n/c

72	0.8% (2)	0.4% (1)	0.0% (0)	0.0 (0.0-482.9)
73	3.0% (8)	0.4% (1)	3.4% (9)	13.9 (6.4-26.5)
81	0.8% (2)	0.0% (0)	1.1% (3)	52.7 (10.8-153.9)
82	1.5% (4)	1.5% (4)	1.1% (3)	16.1 (3.3-47.1)
83	0.0% (0)	0.8% (2)	1.9% (5)	37.6 (12.2-87.8)
84	6.8% (18)	3.0% (8)	5.3% (14)	5.2 (2.9-8.8)
89	1.5% (4)	3.4% (9)	4.9% (13)	9.1 (4.9-15.6)
All types [a]	1.8% (169)	1.9% (176)	2.5% (238)	13.4 (11.2-16.8)

M-F+, male negative, female positive. M+F-, male positive, female negative. M+F+, male positive, female positive. n/c, not calculable

a) Based on a summary two-by-two table of all 36 HPV types combined, for a total of 9,468 observations from 263 couples.

Table 7.3. Positive concordance of HPV infections among 169 couples in which at least one partner was infected.

	Number of couples	Proportion of couples concordant for 1 or more HPV types	Ratio of observed % concordant over expected (95%CI)	Number of couple-level HPV infections [a]	Proportion of infections for which both partners are infected (95%CI)
All types	169	0.64	2.6 (2.1, 3.1)	583	0.42 (0.36, 0.47)
Grouping by oncogenicity					
HR-HPV: high-risk oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82	144	0.51	3.0 (2.4, 3.8)	339	0.43 (0.37, 0.49)
LR-HPV: low risk HPV types 6, 11, 26, 34, 40, 42, 44, 53, 54, 61, 62, 67, 69, 70, 71, 72, 81, 83, 84, 89	124	0.40	4.1 (3.1, 5.1)	244	0.39 (0.33, 0.46)

	Number of couples	Proportion of couples concordant for 1 or more HPV types	Ratio of observed % concordant over expected (95%CI)	Number of couple-level HPV infections [a]	Proportion of infections for which both partners are infected (95%CI)
Grouping by Alphapapillomavirus species					
3/15: HPV types 61, 62, 71, 72, 81, 83, 84, 86, 87, 89	85	0.22	4.3 (3.0, 5.9)	125	0.37 (0.29, 0.46)
7: HPV types 18, 39, 45, 59, 68, 70, 85	76	0.18	5.2 (3.6, 7.4)	98	0.36 (0.27, 0.46)
9: HPV types 16, 31, 33, 35, 52, 58, 67	86	0.28	3.3 (2.4, 4.3)	114	0.50 (0.41, 0.59)
10: HPV types 6, 11, 13, 74, 44	26	0.06	8.0 (3.8, 15)	30	0.36 (0.21, 0.55)
Other species	120	0.41	4.2 (3.3, 5.4)	216	0.43 (0.36, 0.51)

a) A couple-level HPV infection was defined as an instance of a specific HPV type *t* being detected in a couple (i.e., in at least one of the partners).

SECTION 8: RISK FACTORS FOR HPV CONCORDANCE

Objective 4 was to identify risk factors for type-specific HPV concordance versus discordance among newly-formed couples in whom at least one partner is positive for HPV. Three restrictions were placed on the study sample so that concordance could be interpreted as a proxy measure of recent transmission within the couple (**Methods** and **Figure 4.2**). I eliminated data from couples for whom neither partner was HPV-infected, who had engaged in vaginal sex for longer than six months, and for whom either partner reported concurrent partner(s) since the start of the sexual relationship with their HITCH partner.

Among the 106 couples in the subsample, 69% (73) were positive concordant for at least one HPV type; 49% (52) were female positive, male negative for at least one type; and 50% (53) were male positive, female negative for at least one type. The mean number of types present in these couples was 3.3 (SD 2.3, median 3, range 1-11). There were a total of 346 observations of a specific type being detected in one or both partners of which 43% (148) were concordant. The intra-class correlation coefficient (ICC) for positive HPV-type concordance was 0.29. This ICC indicates that 29% of the total variability in positive HPV-type concordance in the 346 HPV type comparisons is attributable to couple membership, suggesting moderate correlation within couples.

Table 8.1 shows the results of unadjusted analyses and crude estimates of ORs for a number of potential risk factors. Also shown in this table are associations adjusted for men's and women's lifetime number of partners and for the number of unprotected vaginal sex encounters, which are the most likely potential confounders. Age, smoking status, age at coitarche, years since coitarche, first vaginal sex relationships, the weekly frequency of vaginal sex, mutual masturbation, and oral sex did not have evidence ($p>0.1$) of an association with concordance independent of these covariates, and so they were excluded from further modeling.

Table 8.1. Risk factors for positive HPV-specific concordance among exposed couples.

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
HPV alpha species						
Alpha 3/15	n/a		72	43.1%	1.00	
Alpha 7 (18-related)			53	39.6%	1.00	
Alpha 9 (16-related			71	49.3%	1.00	
HPV-16			38	55.3%	1.7 (0.95,3.0)	1.8 (1.0,3.4)
Alpha 9 excluding HPV-16			33	42.4%	1.00	
Alpha 10 (6- and 11-related)			13	30.8%	1.00	
Other alpha species			137	41.6%	1.00	
Female age						
<20 years	14	66.7%	38	44.7%		
20-21 years	40	70.0%	140	41.4%		
22-23 years	32	65.6%	111	42.3%		
24 years and older	19	73.7%	57	45.6%		
Each additional year of age					0.98 (0.84,1.1)	1.00 (0.86,1.2)
Male age						
< 20 years	6	33.3%	10	20.0%	0.34 (0.071,1.7)	0.43 (0.097,1.9)
20-24 years	67	68.7%	222	43.2%	1.00	1.00
25 years and older	33	75.8%	114	43.9%	1.00	1.00
Couple's current smoking status						
Both current (1+/d)	11	81.8%	34	35.3%	1.00	1.00
Only male smokes	19	73.7%	64	46.9%	0.92 (0.47,1.8)	1.00
Only female smokes	9	44.4%	34	23.5%	0.37 (0.12,1.12)	0.45 (0.16,1.3)
Both non/ex-smokers	67	68.7%	214	45.8%	0.71 (0.31,1.6)	1.00

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
Female age at coitarche						
<16 years	28	75.0%	106	42.5%		
16-17 years	38	76.3%	116	47.4%		
18 years and older	39	56.4%	123	38.2%		
Each additional year of age					0.96 (0.83,1.1)	0.89 (0.75,1.07)
Male age at coitarche						
<16 years	24	75.0%	94	46.8%		
16-17 years	43	67.4%	140	42.1%		
18 years and older	39	66.7%	112	40.2%		
Each additional year of age					0.95 (0.85,1.07)	0.97 (0.86,1.10)
Female years since coitarche						
<2 years	7	71.4%	16	62.5%		
2 - <4 years	21	61.9%	69	39.1%		
4 - <6 years	46	58.7%	157	37.6%		
6+ years	31	87.1%	103	49.5%		
Each additional year					1.03 (0.90,1.17)	1.11 (0.95,1.3)
Male years since coitarche						
<2 years	7	42.9%	13	38.5%		
2 - <4 years	17	70.6%	45	42.2%		
4 - <6 years	26	69.2%	91	39.6%		
6+ years	56	71.4%	197	44.7%		
Each additional year					1.01 (0.94,1.09)	0.99 (0.92,1.07)
Number of vaginal sex partners in lifetime reported by female						
None or one only	6	66.7%	9	55.6%		
Two - four	31	61.3%	84	50.0%		
Five - nine	36	72.2%	100	46.0%		
Ten or more	33	72.7%	153	36.0%		
Each additional lifetime partner					0.94 (0.90,0.98)	0.94 (0.90,0.98)

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
Number of vaginal sex partners in lifetime reported by male						
None or one only	8	50.0%	18	33.3%		
Two - four	27	63.0%	63	39.7%		
Five - nine	30	70.0%	94	48.9%		
Ten or more	40	75.0%	168	41.7%		
Each additional lifetime partner					1.01 (0.98,1.04)	1.02 (0.99,1.05)
Female's first vaginal sex relationship	5	60.0%	8	50.0%	1.2 (0.26,5.2)	1.00 (0.25,4.0)**
No	100	69.0%	337	42.4%	1.00	1.00
Male's first vaginal sex relationship	8	50.0%	18	33.3%	0.54 (0.17,1.7)	0.73 (0.24,2.2)**
No	98	70.4%	328	43.3%	1.00	1.00
Months engaging in vaginal sex with HITCH partner						
Less than 2 months	21	57.1%	91	25.3%		
2 to <3 months	17	52.9%	47	21.3%		
3 to <4 months	27	77.8%	78	52.6%		
4 to <5 months	28	71.4%	86	51.2%		
5 to 6 months	12	83.3%	43	67.4%		
Each additional month					1.4 (1.1,1.7)	1.4 (1.1,1.7) †
Condom use with HITCH partner						
Never	8	87.5%	26	57.7%	1.00	1.00
Rarely (1-24%)	32	71.9%	105	48.6%	0.62 (0.17,2.2)	0.68 (0.23,2.0)‡
Sometimes (25-75%)	31	67.7%	118	37.3%	0.41 (0.11,1.5)	0.49 (0.16,1.4)‡
Most times (76-99%)	16	75.0%	43	53.5%	0.71 (0.18,2.8)	0.79 (0.23,2.7)‡
Always	18	50.0%	53	26.4%	0.25 (0.06,1.03)	0.26(0.074,0.94)‡

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
Number of vaginal sex encounters with HITCH partner						
24 or fewer	19	52.6%	76	29.0%		
25-49	27	55.6%	82	24.4%		
50-74	21	71.4%	58	43.1%		
75-99	11	90.9%	33	72.7%		
100-124	11	81.8%	34	70.6%		
125-149	6	83.3%	33	39.4%		
150 or more	10	80.0%	29	65.5%		
Each additional 10 encounters					1.09 (1.03,1.16)	1.09 (1.03,1.15) §
Number of unprotected vaginal sex encounters with HITCH partner						
None	11	45.5%	36	22.2%		
1-24 encounters	43	62.8%	138	37.0%		
25-60 encounters	24	75.0%	87	43.7%		
More than 60 encounters	27	81.5%	84	59.5%		
Each additional 10 encounters					1.12 (1.04,1.2)	1.12 (1.04,1.22)
Number of protected vaginal sex encounters with HITCH partner						
None	8	87.5%	26	57.7%		
1-24 encounters	53	64.2%	172	37.2%		
25-60 encounters	26	65.4%	79	39.2%		
More than 60 encounters	18	77.8%	68	54.4%		
Each additional 10 encounters					1.02 (0.96,1.09)	1.05 (0.98,1.11)
Couple's frequency of vaginal sex encounters per week						
Less than 3	23	51.2%	70	25.7%		
3 to 4	35	68.6%	110	40.9%		
5 to 6	30	76.7%	95	52.6%		
7 or more	17	76.5%	70	48.6%		
Each additional encounter per week					1.1 (0.98,1.2)	1.0 (0.90,1.1)

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
Days since last vaginal sex						
1 day or less	23	78.3%	84	48.8%	1.00	1.00
Two – four	58	70.7%	182	43.4%	0.66 (0.32,1.34)	0.85 (0.42,1.7))
Five – six	6	33.3%	20	35.0%	0.42 (0.067,2.7)	0.79 (0.14,4.6)
One week or longer	16	62.5%	55	30.9%	0.39 (0.15,1.03)	0.63 (0.23,1.7)
Frequency female was masturbated						
Never	0	n/a	0	n/a		
Rarely (1-24%)	2	100.0%	6	33.3%		
Sometimes (25-75%)	66	71.2%	233	43.8%		
Most times (76-99%)	33	60.6%	97	38.1%		
Always	5	80.0%	10	70.0%		
Each additional increase in frequency					1.1 (0.68,1.9)	1.2 (0.70,2.0)
Frequency male was masturbated						
Never	0	n/a	0	n/a		
Rarely (1-24%)	11	90.9%	36	55.6%		
Sometimes (25-75%)	62	67.7%	215	42.8%		
Most times (76-99%)	31	64.5%	91	38.5%		
Always	2	50.0%	4	25.0%		
Each additional increase in frequency					0.81 (0.53,1.2)	0.76 (0.50,1.15)
Frequency female received oral sex						
Never	7	85.7%	25	36.0%		
Rarely (1-24%)	15	73.3%	41	34.2%		
Sometimes (25-75%)	62	64.5%	204	44.6%		
Most times (76-99%)	22	72.7%	76	44.7%		
Always	0	n/a	0	n/a		
Each additional increase in frequency					1.0 (0.73,1.4)	0.96 (0.67,1.4)

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
Frequency male received oral sex						
Never	2	100.0%	4	50.0%		
Rarely (1-24%)	11	72.7%	38	26.3%		
Sometimes (25-75%)	67	74.6%	224	50.0%		
Most times (76-99%)	25	48.0%	79	29.1%		
Always	1	100.0%	1	100.0%		
Each additional increase in frequency					0.79 (0.52,1.2)	0.68 (0.44,1.04)
Any anal sex	22	86.4%	80	45.0%	1.1 (0.62,2.1)	0.94 (0.53,1.7)
No	82	64.6%	258	42.3%	1.00	1.00
Female had signs/symptoms of a genital infection since onset of relationship with HITCH partner						
Any of 8 symptoms	68	77.9%	212	51.4%	2.6 (1.3,5.1)	2.6 (1.3,5.1)
Painful, difficult, frequent urination	32	78.1%				
Itching, burning urination	23	78.3%				
Blood in urine	10	70.0%				
Abnormal discharge	21	81.0%				
Genital sores	5	80.0%				
Unusually heavy, painful period	16	93.8%				
Vaginal itching, burning	35	77.1%				
Lower back pain, not physical exertion	19	89.5%				
No symptoms	33	48.5%	123	26.0%	1.00	1.00

Risk factor	# couples	% any concordance	# HPV infections	% concordant	Crude OR (95%CI)	Adjusted* OR (95%CI)
Male had signs/symptoms of a genital infection since onset of relationship with HITCH partner						
Any of 5 symptoms	17	94.1%	51	60.8%	2.3 (1.2,4.4)	2.3 (1.1,4.7)
Painful, difficult, frequent urination	9	88.9%				
Itching, burning urination	4	75.0%				
Blood in urine	1	0.0%				
Abnormal discharge	2	100.0%				
Genital sores	7	100.0%				
No symptoms	88	63.6%	290	40.0%	1.00	1.00
Circumcision status						
Uncircumcised	51	70.6%	162	48.2%	1.6 (0.93,2.9)	1.8 (0.98,3.2)
Circumcised	55	67.3%	184	38.0%	1.00	1.00

* Unless otherwise indicated, these odds ratios are adjusted for men's and women's lifetime number of vaginal sex partners and total number of unprotected vaginal sex encounters.

** Adjusted only for total number of unprotected vaginal sex encounters because the variable "first vaginal sex partner" is colinear with the total number of vaginal lifetime partners.

† Adjusted only for the lifetime number of vaginal sex partners because there was strong correlation between the total number unprotected acts of vaginal sex and the duration of the vaginal sex relationship.

‡ Adjustment for number of lifetime vaginal sex partners (male & female) and total number of acts of vaginal sex (not unprotected acts).

§ Adjustment for number of lifetime vaginal sex partners (male & female only) since the number of unprotected acts is colinear with the total number of acts.

Table 8.2 shows the results of two multivariate logistic regression models. There were strong correlations between months engaging in vaginal sex, condom use frequency, and the number of unprotected and protected acts of vaginal sex. Therefore, model 1 included months engaging in vaginal sex and condom use frequency, whereas model 2 included the total number of unprotected and protected acts. Both models estimated the effects for HPV-16 exposure, men's and women's lifetime number of vaginal sex partners, signs/symptoms of genital infection, and male circumcision.

In both models in **Table 8.2,** I adjusted all covariates for the number of days since the last vaginal sex encounter to control for the possible effects of cross-contamination. An even better approach would be to stratify the analysis by days since the last vaginal sex contact, but with only 106 couples the data would have become sparse. Nevertheless, models 1 and 2 were repeated using data from the 80 couples who reported their last vaginal sex encounter at least two days before their clinic visit (**Table 8.3**).

The results of the models in **Tables 8.2 and 8.3** are summarized and discussed below.

Table 8.2. Risk factors for positive type-specific HPV concordance among exposed couples, according to multiple logistic regression (n=322 HPV infections among 96 exposed couples).

	Model 1	Model 2
RISK FACTOR	**Adjusted* OR (95%CI)**	**Adjusted** OR (95%CI)**
HPV-16	1.9 (0.9,3.9)	1.8 (0.9,3.6)
No	1.00	1.00
Number of vaginal sex partners in lifetime reported by female		
Each additional	0.94 (0.90,0.99)	0.94 (0.90,0.98)
Number of of vaginal sex partners in lifetime reported by male		
Each additional	1.05 (1.01,1.08)	1.04 (1.00,1.07)
Months engaging in vaginal sex with HITCH partner		n/a
Each additional	1.3 (1.1,1.6)	
Condom use with HITCH partner		n/a
Never	1.00	
Rarely (1-24%)	0.47 (0.16,1.4)	
Sometimes (25-75%)	0.33 (0.12,0.89)	
Most times (76-99%)	0.54 (0.20,1.5)	
Always	0.31 (0.084,1.1)	
Number of unprotected vaginal sex encounters with HITCH partner		
Each additional 10	n/a	1.10 (1.01,1.19)
Number of protected vaginal sex encounters with HITCH partner		
Each additional 10	n/a	1.04 (0.98,1.11)
Days since last vaginal sex		
1 day or less	1.00	1.00
Two - four	0.65 (0.31,1.4)	0.69 (0.31,1.5)
Five - six	0.23 (0.039,1.4)	0.34 (0.049,2.3)
One week or longer	0.58 (0.22,1.5)	0.66 (0.23,1.9)
Female had signs/symptoms of a genital infection since onset of relationship with HITCH partner		
Any of 8 symptoms	2.2 (0.94,5.0)	2.7 (1.2,5.9)
No symptoms	1.00	1.00
Male had signs/symptoms of a genital infection since onset of relationship with HITCH partner		
Any of 5 symptoms	2.1 (0.97,4.6)	2.0 (0.94,4.1)
No symptoms	1.00	1.00
Circumcision status		
Uncircumcised	2.2 (1.1,4.4)	2.3 (1.2,4.3)
Circumcised	1.00	1.00

*Adjusted for all variables shown in the model. Excludes the number of unprotected and protected vaginal sex encounters because these were strongly correlated with condom use frequency and months engaging in vaginal sex.

** Adjusted for all variables shown in the model. Excludes condom use frequency and months engaging in vaginal sex because these were strongly correlated with the number of unprotected and protected vaginal sex encounters.

Table 8.3. Risk factors for positive type-specific HPV concordance among exposed couples whose most recent sexual contact was at least two days ago (n=239 HPV infections among 80 exposed couples).

	Model 1	Model 2
RISK FACTOR	**Adjusted* OR (95%CI)**	**Adjusted** OR (95%CI)**
HPV-16	1.9 (0.8,4.6)	1.9 (0.8,4.5)
No	1.00	1.00
Number of vaginal sex partners in lifetime reported by female		
Each additional	0.94 (0.89,0.99)	0.93 (0.88,0.98)
Number of vaginal sex partners in lifetime reported by male		
Each additional	1.04 (1.00,1.09)	1.04 (1.00,1.08)
Months engaging in vaginal sex with HITCH partner		
Each additional	1.2 (0.95,1.6)	n/a
Condom use with HITCH partner		
Never	1.00	n/a
Rarely (1-24%)	1.30 (0.39,4.3)	
Sometimes (25-75%)	0.78 (0.29,2.1)	
Most times (76-99%)	0.98 (0.33,2.9)	
Always	0.76 (0.28,2.1)	
Number of unprotected vaginal sex encounters with HITCH partner		
Each additional 10	n/a	1.12 (1.01,1.23)
Number of protected vaginal sex encounters with HITCH partner		
Each additional 10	n/a	1.05 (0.99, 1.12)
Female had signs/symptoms of a genital infection since onset of relationship with HITCH partner		
Any of 8 symptoms	2.9 (1.1,7.6)	2.9 (1.1,7.7)
No symptoms	1.00	1.00
Male had signs/symptoms of a genital infection since onset of relationship with HITCH partner		
Any of 5 symptoms	1.7 (0.8,3.5)	1.7 (0.8,3.5)
No symptoms	1.00	1.00
Circumcision status		
Uncircumcised	2.4 (1.1,5.2)	2.5 (1.2,5.4)
Circumcised	1.00	1.00

*Adjusted for all variables shown in the model. Excludes the number of unprotected and protected vaginal sex encounters because these were strongly correlated with condom use frequency and months engaging in vaginal sex.

** Adjusted for all variables shown in the model. Excludes condom use frequency and months engaging in vaginal sex because these were strongly correlated with the number of unprotected and protected vaginal sex encounters.

HPV-16 infections were nearly twice as likely to be concordant than infections with other HPV types. This association did not quite reach statistical significance in the models in **Table 8.2.** The OR estimate of 1.9 remained the same in the models restricted to couples whose last contact was at least two days ago (**Table 8.3**). The finding may be an artifact and requires confirmation in a larger sample. If it is not artifactual, there are three reasons that could explain greater concordance of HPV-16 infections. First, this was the most common type observed in the study sample; therefore, this type is the most likely to be coincidentally present in both partners when they meet for the first time. Second, there is some evidence that the duration of HPV-16 infections is longer than that with other types [61,203]. Other HPV types may be more likely to clear in one partner by the time couples enrol, and so would be more likely to be discordant. Third, HPV-16 may be more transmissible than other types, which could explain in part why it is consistently the most prevalent type in many studied populations [1,189]. For example, if HPV-16 infections tend to have higher viral load than other types, then this could result in higher infectivity. Ultimately, longitudinal follow-up of HPV-discordant couples in HITCH and other studies would be needed to firmly establish differences in transmissibility by HPV type or alpha species.

Couples for whom the female partner had more lifetime number of partners were less likely to be concordant. The adjusted OR estimate was approximately 0.94 per additional lifetime vaginal sex partner in all four models in **Tables 8.2** and **8.3**. Assuming the association was not due to confounding by some unmeasured or mismeasured variable, the observed protective effect may be due to natural immunity acquired from women's previous HPV exposure. The more partners a woman has had, the more likely she has been exposed to HPV. In theory, such exposure would produce a primary immunological response involving B and T lymphocytes. This would be followed by the development of immune memory cells, which remember the antigenic composition of the pathogenic agent and can launch a rapid secondary immune response upon re-exposure. Many serological studies have shown that women are more likely than men to seroconvert

following natural infection [65,189,204]. However, the extent and duration of natural immunity is unknown and is an ongoing research question.

Alternatively, the finding that women's lifetime number of partners was negatively associated with concordance in the couple may be due to less efficient female-to-male transmission. In this scenario, a women with more past partners would tend to be the infected partner who has not yet transmitted HPV to her current male partner.

Couples for whom the male partner had more lifetime number of partners were more likely to be concordant. The adjusted odds ratio was approximately 1.04 per additional female partner the male reported. When the analysis limited to a comparison of concordance versus male-positive/female-negative discordance (i.e., the effect on female HPV status among women whose partners were infected), the OR was attenuated at 1.01. Conversely, when the analysis was limited to a comparison of concordance versus female-positive/male-negative discordance (i.e., the effect on male HPV status among men whose partners were infected), the OR was stronger at 1.11. My interpretation of these results is that for couples in whom the female is infected, if the male has had many previous partners, it is likely that he was infected from one of them rather than his current partner.

Ultimately, gender differences in transmission efficiencies must be evaluated using longitudinal follow-up of discordant couples. One study of 25 couples has published such results [186]. In that study, transmission was more efficient from the cervix to the penis (17.4 per 100 person-months of exposure, 95%CI 10.6-25.8) than from the penis to the cervix (4.9 per 100 person-months, 95%CI 1.6-10.0).

The more months a couple had engaged in vaginal sex, the greater the proportion concordant. This observation provides strong evidence for concordance being a marker of transmission within the couple, since as time passes there are more

opportunities for transmission to occur. Patterns of incident HPV infection among young women in Seattle, Washington were consistent with these HITCH results [74]. In that study, women were at elevated risk of incident vaginal infection in the 0-4 months, 5-8 months and 8-12 months following the report of a new partner (hazard ratios were 2.5, 3.4 and 2.4, respectively). Similarly, rates of HPV acquisition rise quickly with months since coitarche in women's first vaginal sex relationships [74,129,183]. Taken together, these findings support HPV transmisison occuring within months of the formation of a new partnership.

Condom use was associated with less concordance. Similarly, the number of unprotected vaginal sex encounters was positively associated with concordance, but the effect of the number of protected encounters was less and did not reach statistical significance. In the models restricted to couples whose last contact was at least two days ago (**Table 8.3**), the ORs for condom use frequency were attenuated but that for the number of unprotected encounters was not. Altogether, the pattern of these associations were consistent with the hypothesis that condoms confer some protection against HPV transmission. Notably, the association of condom use frequency with concordance remained after adjustment for months engaging in vaginal sex, which could confound the crude association because condom frequency was negatively associated with the duration of the couple's relationship. Yet protection against concordance was incomplete, even among couples who always used condoms. I also observed a protective effect of condoms against prevalent HPV among male participants with an HPV-infected partner, but the effect was weakened among women with an infected partner (**Manuscript III**).

The strongest corroborating evidence for condom efficacy against HPV comes from a study of 82 women living in Seattle who were in their first vaginal sex relationships [93]. In that study, incident HPV was observed three times less often among women who always used condoms compared with those who used them less than 5% of the time; nevertheless, rates of HPV acquisition among the

always-users were still non-negligible at 38 per 100 woman-years. HPV transmissions were observed less often among condom users in a longitudinal study of 25 couples who were followed for an average of 7.5 months [186]. Among the "non-transmitting" couples, 56% reported always using condoms at enrolment, whereas only 3% of the "transmitting" couples did so.

Concordance was over twice as common in couples for whom the female or male partner had signs/symptoms of a genital infection. This association was stronger for women's signs/symptoms than men's but confidence limits were wide. One cannot explain the association as signs/symptoms of genital infection being a marker for exposure to HPV since all were exposed in this analysis. Instead, these sign/symptoms must accelerate the transmission of HPV through enhanced susceptibility and/or infectivity, or they may lengthen the duration of HPV infections such that both partners were more likely remain infected by the time of enrolment. Genital infections may increase susceptibility to HPV infection via inflammation or microabrasions, or facilitate persistence of infection through immunologic mechanisms [92,203,205].

Concordance was twice as common in couples for whom the male partner was uncircumcised. This was a particularly interesting finding given that male circumcision was unrelated to HPV detection among all HITCH participants. Penile HPV prevalence did not significantly differ between circumcised (55%, 72/132) and uncircumcised men (54%, 67/123). Absence of an effect of male circumcision on prevalent infection has been observed elsewhere [121,122]. However other studies have observed a protective effect against prevalent male infection [163,188], incidence [71], and against cervical disease in female partners [184].

In contrast to the lack of effect against prevalent HPV among male HITCH participants, I observed two-fold protective effect of male circumcision against concordance in HPV-exposed couples. OR estimates for presence of a foreskin ranged from 2.2 to 2.5 in **Tables 8.2 and 8.3**. This association was further

pronounced with an OR of 2.7 when the outcome was concordance versus male negative/female positive discordance (i.e., the effect on male positivity versus negativity among men whose partners were infected).

Analysis of data from the South African Orange Farm randomized control trial of male circumcision observed a protective effect on HR-HPV detection in the urethra 21 months after randomization [206]; because that result was for HPV prevalence, not incidence, it cannot be known whether circumcision prevented HPV acquisition or aided clearance of infections present at study entry. A US study observed that circumcision was protective against HPV detection in the urethra and glans/corona, but as one sampled further from this site (e.g., the penile shaft and scrotum), or combined the result from all sampled genital sites, the effect was attenuated or absent [207]. Furthermore, a longitudinal study of men observed that circumcision was unrelated to HPV acquisition but was a strong predictor of clearance [181]. Taken together, these results suggest that male circumcision plays a role in the natural history of male HPV infection, and by extension women's risk of HPV-related disease, but the biological mechanism for this protection remains to be fully understood.

SECTION 9: PROBABILITY OF HPV TRANSMISSION

As for the analysis for objective 4 (risk factors for concordance), analysis for objective 5 (to estimate of HPV transmission probabilities) was restricted to the 106 exposed couples who had engaged in vaginal sex for no more than six months and who were monogamous (**Methods, Figure 4.2**.)

Probability of transmission per partnership (β_p)

The estimate of the *per partnership transmission probability*, β_p equals the probability of type-specific positive concordance, as calculated from the logistic regression model. This estimate was 46% with a 95%CI of 39% to 53%. **Table 9.1** shows additional estimates of β_p for selected characteristics. β_p was approximately two-fold greater in couples who had engaged in 100 or more acts for five to six months, compared with couples who had engaged in 25 acts or fewer for two months or less. The value of β_p was highest for couples who never used condoms compared with always-users, and for HPV-16 compared with other types, but confidence limits overlapped.

Table 9.1. Estimates of the per-partner HPV transmission probability, β_p.

Characteristic	# couples	# HPV infections	βp (95% CI)
All exposed couples	106	346	0.46 (0.39, 0.53)
25 or fewer acts	22	85	0.31 (0.20, 0.45)
100 or more acts	27	96	0.59 (0.48, 0.70)
Vaginal sex for 2 months or less	23	93	0.32 (0.20, 0.47)
Vaginal sex for 5-6 months	12	43	0.68 (0.55, 0.79)
Never used condoms	8	26	0.64 (0.35, 0.85)
Always used condoms	18	53	0.29 (0.15, 0.48)
Alpha 9 (HPV-16 & related)	53	71	0.49 (0.37, 0.61)
HPV-16	38	38	0.55 (0.39, 0.70)
Alpha 9 other than HPV-16	26	33	0.42 (0.26, 0.60)
HPV types other than alpha 9	101	275	0.45 (0.37, 0.52)

Probability of transmission per coital act (β_a)

The estimate of the probability of transmission per coital act (β_a) was estimated using log-log binomial regression. One of the 106 couples did not have information on the total number of vaginal sex encounters and was excluded. The overall estimate of β_a was 9 per 1000 (95%CI 7 – 12). **Table 9.2** shows additional estimates for selected characteristics. β_a was two- to four-fold greater in couples who had engaged in 25 or fewer acts in two months, compared with couples who had engaged in 100 or more acts for five to six months. The value of β_a was highest for couples who never used condoms compared with always-users, and for HPV-16 compared with other types, but confidence limits overlapped.

Table 9.2. Estimates of the per-act HPV transmission probability, β_a.

Characteristic	# couples	# HPV infections	βa (95% CI)
All couples	105	345	0.009 (0.007, 0.012)
Total of 25 or fewer acts	22	84	0.024 (0.014, 0.040)
Total of 100 or more acts	27	96	0.006 (0.004, 0.009)
Vaginal sex for 2 months or less	23	92	0.016 (0.008, 0.031)
Vaginal sex for 5-6 months	12	43	0.008 (0.004, 0.014)
Never used condoms	8	26	0.011 (0.005, 0.022)
Always used condoms	18	53	0.005 (0.002, 0.013)
Alpha 9 (HPV-16 & related)	53	71	0.012 (0.007, 0.018)
HPV-16	38	38	0.013 (0.007, 0.023)
Alpha 9 other than HPV-16	26	33	0.010 (0.005, 0.019)
HPV types other than alpha 9	101	274	0.009 (0.007, 0.012)

Interpretation

My *a priori* hypothesis was that the per-partner transmission probability, β_p, would average 60%. The β_p estimates based on cross-sectional HITCH data at enrolment ranged from 15% to 70% depending on the characteristics of the couple, and averaged 46%. The hypothesized estimate of 60% was based on a study of genital wart transmission, published by J.D. Oriel in 1971 long before HPV was known to cause warts [31]. Not knowing the duration of infectivity nor the incubation period, Oriel contacted the sexual partners of genital wart patients during the nine months before the warts appeared. No further details regarding sexual behaviour between partners was reported. A total of 62 of the 97 people who had had intercourse with a patient with genital warts subsequently developed warts, giving a point estimate of $\beta_p = 64\%$. The incubation period ranged from 3 weeks to 8 months and averaged 2.8 months.

I estimated β_p for couples who had engaged in vaginal sex for no more than six months; therefore this may explain the lower estimate of 46% from HITCH data compared to Oriel's study. Among couples who had engaged in vaginal sex for five to six months, β_p was 68% which is far closer to what he observed. Furthermore, I estimated β_p for HPV transmission, not transmission of HPV-related disease. It is possible that HPV infectiousness is greater among symptomatic, infected partners (i.e., those with genital warts or lesions) than among asymptomatic partners. One couple-based study observed greater concordance when one of the partners had high viral load [73]. There is also speculation that flat penile lesions play an important role in male-to-female transmission, given very low viral load among men without lesions [197]. Similarly, cervical lesions are associated with higher HPV viral load [208,209]. Future analysis of HITCH data will stratify transmission probability estimates by viral load values.

My *a priori* hypothesis was that the per-act transmission probability, β_a, would average between 5% and 40%. This was based on the simulation study, which

identified β_a values that were consistent with observed cumulative incidence among young women (**Manuscript II**). Conversely, the cross-sectional analysis of HITCH data produced lower β_a estimates in the range of 2 to 40 per 1000, and averaged 9 per 1000. The estimation of β_a is dependent upon accurate measures of the number of acts of intercourse, n_i. [119] If n_i tends to be over-estimated, then β_a will be under-estimated. Conversely, if n_i is under-estimated, then β_a will be over-estimated. In a study of Zambian couples, biological markers of unprotected sex suggested that at least half of unprotected acts were unreported [210]. Conversely, methodology studies have observed over-reporting of n_i in retrospective recall versus diary-based reporting [152 153]. HITCH used retrospective recall over a short interval. Measurement error in n_i was minimized in HITCH due to the collection of these data from both partners. Although no gender bias was evident in the reporting of n_i, 57% of its variation was due to discrepancies between partner's reports. This will have decreased the precision in the estimate of β_a. The considerable variation in reporting of n_i, even among new couples recalling behaviour over a short time period, has implications for all studies that aim to estimate β_a.

Therefore, possible explanations for the discrepancy between the simulation and HITCH estimates could be that HITCH couples over-reported their sexual intercourse frequency, or that it was under-estimated by women in the McGill-Concordia study, upon which the simulation was based. In the McGill-Concordia study, information was recalled over a six month interval, and was not substantiated by male partner reports. Moreover, limitations of both analytical methods may have resulted in their lack of comparability.

Estimation of β_a using a binomial model assumes that it is constant over all acts of intercourse and across persons. This may not be true as infectiousness and susceptibility may vary between persons and within persons over time. For example, it has been proposed that estimates of β_a are underestimated in discordant couples studies, due to the necessary exclusion of concordant couples

who experienced rapid transmission resulting from high infectiousness and/or high susceptibility [175]. The often-cited estimate of β_a for human immunodeficiency virus (HIV) is 1 per 1,000 acts of intercourse [211]. This averaged estimate is based on studies of discordant couples who typically had few cofactors (i.e., asymptomatic mid-stage HIV infection, circumcised men, and no concomitant genital infections). HIV infectivity is thought to be highest in the initial weeks following infection [Powers et al. 2008]; transmissions that occur during that time would never be observed in a discordant couples study because both partners would be infected by the time one could diagnose infection.

This underlines an important contribution of using enrolment data from HITCH to estimate β_a, despite its limitations. Restricting studies of transmission to longitudinal follow-up of HPV-discordant couples may present an incomplete picture of the true variability in β_a. Furthermore, β_a estimates among couples with few acts and with very short relationships suggest that β_a may indeed be as much as four times higher at 24 per 1000 (95%CI 14, 40) for the initial acts of intercourse. It remains to be seen whether β_a would be even greater when an infected partner has high viral load.

The range of 95%CI for β_a indicated that values above 50 per 1000 would be inconsistent with HITCH data. Despite the fact that these are lower estimates of β_a than those estimated by the simulation, they are still high when one considers that couples typically engage in not one but multiple acts of intercourse. The averaged estimate for β_a of 9 per 1000 for HPV is nearly 10-fold greater than the average estimate for HIV. Even the highest estimates for HIV are 6 per 1000 when the susceptible partner has genital ulcer disease [211], still lower than that estimated for HPV.

SECTION 10: DISCUSSION

Summary of results

The review of HPV epidemiology and transmission dynamics in **Manuscript I** revealed that HPV is a common STI, particularly among young women. Most infections are transient and clear within one to two years. There is strong evidence that transmission occurs primarily via sexual activity, most commonly vaginal and anal intercourse, although perinatal and non-sexual transmission does occur. The common tools for STI prevention, namely the promotion of abstinence or delay in sexual activity, monogamy, condoms, and treatment of existing infections, are not all equally applicable to HPV. Delay in coitarche and monogamy should reduce risk, but will not eliminate it, since HPV is highly prevalent and any sexual activity poses a risk. Condoms may provide some protection, but transmission may still occur via unprotected areas of genital skin. Currently, no treatment of existing infections is available to reduce the duration of infectiousness.

The simulation analysis reported in **Manuscript II** suggested that HPV per-act transmission probabilities that were consistent with observed cumulative incidence among young female university students ranged from a lower limit of 5% per act to an upper limit of 100% per act. Per-act transmissibility values of less than 5% were inconsistent with the observed data in the McGill-Concordia Cohort Study. The simulation also suggested that male HPV prevalence would have been at least as high as women's. These results were corroborated from a follow-up analysis using maximum likelihood methods. Nevertheless, the transmission probability estimates from these methods lacked precision. Much uncertainty remained regarding type differences. The prevalence of HPV among women's male partners was unknown, and the need to estimate that parameter obscures the interpretation of transmission to women.

Remaining analyses for the PhD thesis used enrolment data from 263 couples in the HITCH Cohort Study. In **Manuscript III**, genital HPV infection was shown to be high (56%) and more common than in similar populations without a restriction to persons with a new sex partner. The strongest risk factor for HPV detection was presence of infection in one's sexual partner, which resulted in an over fifty-fold increase in the odds of type-specific infection. Greater numbers of lifetime vaginal sex partners was correlated with higher prevalence, but did not matter for female prevalence if her current male partner was infected. Male prevalence was negatively associated with more frequent condom use, but this association did not reach statistical significance in women.

In **Manuscript IV**, the distribution of HPV infections was described using the couple as the unit of analysis. Detection of one or more HPV types was observed in 64% of couples. The presence of the same type in both partners was detected in 41% of couples which was far more frequently than expected by chance. Among couples in whom an HPV type was observed in one partner but not the other, there was little evidence for a pattern of discordance by gender with the exception of HPV-6 which was eight times more likely to be observed in males than females. Although some of the concordance I observed was likely coincidental, I concluded that the majority was not and instead was recent transmission within these newly-formed couples. The pattern of increasing concordance in exposed couples over months engaging in sex and with the total number of vaginal sex encounters suggest that transmission is most likely to have occurred within five to six months of vaginal sex activity, or by 100 to 124 encounters. Beyond this point, clearance in one or both partners could obscure the interpretation of concordance as transmission.

In **Section 8**, I analysed risk factors for positive type-specific HPV concordance among exposed couples. I interpreted these as risk factors for transmission due to the study design and restrictions placed on the subsample for analysis to enhance the validity of the inferences. Concordance was more likely than discordance for HPV-16 infections, when men had more lifetime vaginal sex partners, with more

months engaging in vaginal sex, and when either partner had signs or symptoms of a genital infection. Concordance was less likely when women had more lifetime vaginal sex partners, when couples always used condoms compared with never, and when the male partner was circumcised.

In **Section 9**, the estimation of transmission probabilities per partnership (average $\beta_p = 46\%$) and per coital act (average $\beta_a = 9$ per 1000) suggest that HPV is highly transmissible. The per-act transmission probability was less than that predicted by the simulation analysis in **Manuscript II**. HITCH data support some but not complete protection with condoms, and suggest HPV may be more transmissible in earlier acts of intercourse than later.

Internal validity

A unique strength of the HITCH Cohort Study is that it is the first large-scale study of HPV acquisition that involves the male partner. This is critically important for the study of an infectious pathogen that is transmitted person-to-person. Risk factors for infection in an individual-based study may be risk factors for contact with an infected partner, or risk factors for infection from that partner upon exposure. Conditioning on exposure to an infected partner allows for the identification of risk factors for transmission itself, and their interpretation as causal factors [212].

A second unique strength is that HITCH is the only study of HPV infection to limit enrolment to new couples, when most transmissions are believed to occur. This restriction allows for the analysis and interpretation of cross-sectional enrolment data as if they were from a retrospective cohort.

Additional strengths of the study include several design features for the measurement of sexual behaviour. These included self-completed computerized questionnaires and short recall periods. Furthermore, both partners answered specific questions about the sexual activities they engaged in as a couple. Partners frequently agreed in their responses, but when they disagreed the measures were

averaged for analysis. The questionnaire format and having two sources of information likely produced greater accuracy and precision of sexual behaviour measures than what would be achieved in an individual-based study. Nevertheless, estimates of the lifetime number of partners may be inaccurate and/or imprecise [148].

Detection of HPV outcomes used accepted methods of genital sampling, a state-of-the-art PCR protocol and was performed by an experienced laboratory that uses stringent quality control procedures for its HPV assays. Yet sampling errors in detection of HPV-DNA may occur; particularly among males these sampling methods are evolving. The transient nature of HPV infection means that a participant's HPV status at enrolment may not reflect his or her infection status over the course of the sexual relationship with the HITCH partner, even though this was short.

Measurement error of HPV status is an important limitation in the analyses for objectives 4 and 5, which involve the interpretation of positive type-concordance as a proxy measure of transmission. This interpretation depends on assumptions that may have been violated. First, it assumes that all positive type-specific HPV concordance represents transmission. The observed proportion of couples concordant on at least one HPV type was 41%. Based on observed type-specific prevalence in the sample and assuming random mixing with respect to HPV status at couple formation, only 11% of couples were expected to be positive concordant due to chance alone (**Manuscript IV**). As the assumption of mixing with respect to HPV status goes from random (independent) to assortative (like-with-like) mixing, even more couples would have been HPV-concordant by chance alone. (Analysis of all couples indicated fair correlation in lifetime number of partners, $\rho = 0.37$, suggesting some but not substantial assortative mixing by HPV status.) The implication of coincidental concordance is misclassification of "transmission events", and overestimation of the transmission probability.

Second, the interpretation of concordance as transmission assumes that all discordance represents non-transmissions. This may be violated if transmission had occurred but HPV infection was cleared in one of the partners by the time of enrolment (or whose viral load was below the detection threshold of the LA-HPV assay which is ten copies). I attempted to minimize such violations by restricting the analysis to couples who met the eligibility criteria (i.e., who had engaged in vaginal intercourse for six months or less). Nevertheless, the transient nature of HPV likely resulted in some violations of this assumption. The result is misclassification of "non-transmission", and underestimation of the transmission probability.

Violation of the above two assumptions would produce measurement error in estimates of transmission probabilities. They would also introduce error in the analysis of risk factors for positive discordance *when this outcome is interpreted as a proxy for transmission*. This error may be nondifferential and simply introduce noise and attenuated effects of the measured risk factors. Alternatively, they may introduce bias if the discordant outcomes (interpreted as non-transmissions) are in fact transmissions in couples in which the negative partner cleared their infection by the time of enrolment. In this situation, risk factors for concordance may be risk factors for transmission and/or risk factors for HPV persistence. Future analysis of these data could use quantitative bias adjustment to evaluate the likely direction of such bias, but it would depend heavily upon assumptions regarding clearance rates. A more useful comparison will be with risk factors identified in a future analysis of transmission observed during follow-up of HITCH participants.

In my analyses of HPV prevalence and concordance between partners, I focused on vaginal sex encounters as the exposures most likely to result in genital transmission. This was corroborated by the finding that HPV infection was not observed in any of the twelve couples for whom both partners were in their first vaginal sex relationship. Nevertheless, HPV transmission through non-penetrative sexual activity is plausible (**Manuscript I**). The identification of non-penetrative

transmission is complicated in this sample because virtually all couples reported engaging in vaginal sex. Genital transmission is more efficient through vaginal intercourse than other non-penetrative activities. In a study of female university students in Seattle, the cumulative incidence of HPV was 10% among virgins who engaged in finger-vulvar, penile-vulvar, or oral-penile non-penetrative contact and only 1% among virgins who reported no such contact; in contrast, the cumulative incidence of HPV infection among women who did engage in vaginal sex was 39% [74]. Although I believe it occurred less often than vaginal transmission, it is possible that non-penetrative activities led to infection in some HITCH participants. This would introduce error in the analysis of vaginal sex variables on HPV detection and concordance.

Concerns regarding potential cross-contamination between partners were repeatedly addressed in analyses of HITCH data. By "cross-contamination", I mean the detection in HPV in a participant's genital specimen which occurs only due to the presence of residual HPV-infected cells of the partner from recent sexual contact, as opposed to viral DNA present from a true, productive infection in the genital epithelium of the participant. The degree of association between partner's HPV status and the extent of concordance were greatest among partners who reported vaginal sex within one day of attending the clinic visit. Furthermore, the effects of condom use upon concordance were attenuated in a subanalysis limited to couples who had sex at least two days ago (although the estimate for effect of the total number of unprotected encounters was not). The sample size for risk factors for concordance was only 106 couples; therefore power to detect true differences between strata was limited. If the result is confirmed in a larger sample, it implies that condom-using couples are less likely to have cross-contaminated genital specimens. Nevertheless, in their entirety these data suggest that cross-contamination may play a possible role in the evaluation of HPV infection among sexually active persons. To the best of my knowledge, such concern has not arisen in individual-based studies but there is no reason why that should be the case. All research of HPV infection should record information on

the last sexual contact (at minimum the date, nature of the sexual encounter, and whether a condom was used), and examine the potential effects of cross-contamination.

Generalizability to other populations

The two unique strengths of the HITCH Cohort Study, that it is the first large-scale study of HPV acquisition that involves the male partner and that it limits enrolment to new couples, come at a cost that may affect generalizability and sample representativeness. (Naturally, the recruitment of partners doubles the sample size, also affecting the study budget and administrative burden.) The challenge of recruiting research volunteers for studies of individuals is well known [196] and this is compounded in studies of couples. Among eligible couples who refused to enrol in HITCH, a common reason was that one partner wished to participate but the other did not. Despite this challenge, the HITCH Cohort Study enrolled 328 couples as of December 31, 2008. No other study that has attempted to enrol asymptomatic couples has approached this sample size. Sample sizes for other studies are typically in the order of no more than 100 couples, and some had as few as 25 couples [90,186,213]. One other couple-based STI-related study that restricted enrolment to new couples had a sample size of 96 couples [88,214].

Among people documented as being eligible for HITCH, 58% enrolled. New couples who are willing to join an STI study may differ from those who are unwilling. Women recruited for the HITCH Cohort Study reported a similar number of lifetime vaginal sex partners but more frequent sex compared to women recruited for the McGill-Concordia Cohort Study [26]. Moreover, new couples may be emotionally fragile. They may not yet have sufficient trust between partners to have the sensitive discussion needed to establish willingness of each partner to participate in an STI transmission study. I suspect participants had more liberal sexual attitudes, and would be more comfortable with communication of sexual matters, although this cannot be known with certainty.

The HITCH sample under-represents partnerships of extremely short duration. The per-act probability of transmission in a single encounter may be different than among couples who have engaged in repeated acts of intercourse, but the former would be difficult to assess in a research study. The analysis for this PhD thesis was further limited to couples who had engaged in sex for no more than six months. Rates of transmission among couples in long-standing partnerships would require longitudinal rather than cross-sectional data. The only published study to date did document back-and-forth transmission of HPV in one of 25 couples, suggesting that reinfection may be important for persistent infections [186]. Continued follow-up of HITCH couples will help to characterize such transmission patterns over time.

Participants in HITCH were highly educated given the eligibility criterion for women to be attending a university or junior college/CEGEP. Participants' reports of their mother's education status also suggest that the sample was one of mid- to high socioeconomic status. Generalizability to couples of lower socio-economic status could be questioned, but I believe the results would be similar. In a study of demographic and geospatial patterns of STI infections in the United Kingdom, there was a strong effect of ethnicity on genital wart incidence, but very little effect of socioeconomic status [215].

In summary, the estimates of risk factor effects and transmission probabilities are probably generalizable to populations of comparable age and with similar sexual histories and prevalence rates.

Public health impact

The results from these analyses suggest high HPV prevalence in young adults and particularly those with new partners, which is corroborated by many previous studies in women and men (Manuscript I)[1,61,176,189]. Fortunately, for most individuals these infections are benign. They will eventually be cleared by the body's immune system, and will not lead to adverse health consequences.

Furthermore, HPV infections among young women rarely lead to cervical disease [203].

Infections with HPV-6 and 11 may produce external genital warts. Although these are not fatal, they may be bothersome, provoke anxiety, and can result in substantial health care costs, particularly for those that recur. Little is known about rates of genital wart diagnoses in Canada but in both the United States and United Kingdom rates have dramatically increased in recent years [54]. Among HITCH participants, only one male was infected with HPV-11. Four percent of women and six percent of men were infected with HPV-6, and among discordant couples HPV-6 was eight times more likely to be detected in the male. These data suggest that the introduction of the quadrivalent Gardasil™ vaccine could have an important impact in reducing genital warts in women, and in men through indirect effects by reduced female-to-male transmission.

The challenge with HPV infections is that although most people clear their infections, a small proportion does not. Those with persistent HR-HPV infection experience an increased risk of cervical and other HPV-related anogenital cancers in the future. Epidemiological research to date has not revealed distinct characteristics of women with a high probability of progression to persistent infection and eventually disease. Older age, smoking, oral contraceptive use, concomitant genital infections, nutrition, parity, and host immune factors have been associated with persistence and progression, but findings are inconsistent and observed effect sizes are not large [69]. Therefore, the prevention of HPV infection in the first place is most desirable to reduce the burden of HPV-related disease. The results in this PhD thesis suggest that condoms may prevent some infection, particularly among males; therefore their continued promotion is warranted for HPV prevention and sexual health in general. Reducing one's number of partners is a STI-prevention tool that also applies to HPV prevention, but its potential impact is limited given high prevalence among youth. Sexual contact with one infected partner is sufficient, and the chances of that one partner being infected are high unless they have had no previous partners (**Manuscript**

III). At a minimum, having one partner at a time, and extending the gap between sequential partners, may reduce transmission since HPV infections, if present, would have time to clear [74].

In Canada, the target age for HPV vaccination among girls is prior to the onset of sexual activity, specifically girls aged 9 to 13 [11]. HITCH data support this policy. The results from the simulation exercise (**Manuscript II**) and enrolment data from HITCH were consistent with high transmissibility, and a high likelihood of encountering an infected partner in one's first sexual relationship. The speed with which an infection moves through a population depends on the probability of being exposed to an infected partner (i.e., the contact rate), the duration of the infection, and the probability of HPV infection upon exposure to an infected partner (**Manuscript I**). Based on the theory of transmission dynamics, a high probability of infection implies the need for high vaccine coverage to have an impact on population-level HPV infection.

Conclusion

The findings from the research carried out for this PhD thesis are consistent with a high likelihood of HPV transmission when there is repeated sexual contact between an infected and uninfected partner. Results from the simulation analysis suggested that HPV is highly transmissible. This was confirmed by the cross-sectional analysis of enrolment data from the HITCH Cohort Study, albeit to a lesser degree. Among the 263 couples enrolled between 05/2005 and 08/2008, HPV prevalence was 56% among women and men. In nearly two thirds (169) of couples, at least one partner was infected with one or more types. The current partner's status was the most important risk factor for prevalent infection. Analysis of the patterns of type-specific concordance and discordance revealed that the extent of concordance was far greater than expected, and was consistent with rapid transmission between partners. There was evidence for a protective effect of condoms, but protection was incomplete and was stronger among men than among women. These results are likely to influence prevention efforts for cervical cancer and other HPV-related disease, including behavioural and other strategies to reduce risk. Results will also provide improved estimates of HPV transmission parameters to be used in models of the population health impact and cost effectiveness of vaccination and screening strategies.

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Appendix A

Ethics certificate and McGill consent forms

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

SEP 15 2008

September 9, 2008

Dr. Eduardo L. Franco
Division of Cancer Epidemiology
Gerald Bronfman Building
546 Pine Avenue West
Montreal Quebec H2W 1S6

RE: IRB Study Number A09-M77-04A

Dear Dr. Franco,

Thank you for submitting an application for Continuing Review for the above-referenced study entitled, *HPV Infection and Transmission Among Couples Through Heterosexual Activity. The HITCH Cohort.*

The study progress report underwent review and full Board re-approval for the study was provided on September 8, 2008. The ethics certification renewal form (enclosed) is valid until September 7, 2009.

If any study modifications or unanticipated study developments occur prior to the next annual review, including study terminations, please notify the IRB promptly. Regulation does not permit the implementation of study modifications prior to IRB review and approval.

Sincerely,

Roberta Palmour

Roberta Palmour, PhD
Co-Chair
Institutional Review Board

cc: A09-M77-04A

McGill Faculty of Medicine

Institutional Review Board

- Continuing Review Form -

DATE OF I.R.B. APPROVAL

SEP 0 8 2008

Faculty of Medicine
McGill University

Principal Investigator: **Eduardo Franco**

Department/Institution: **Oncology**

IRB Review Number **A09-M77-04A** Study Number (*if any*): Review Interval: **Annual**

Title of Research Proposal: HPV Infection and Transmission among Couples through Heterosexual activity: The HITCH Cohort

INTERIM REPORT (PLEASE CHECK OR SPECIFY)

Current Status of Study : Active Study √ On Hold ☐ Closed to Enrolment ☐

Interim Analysis ☐ Final Analysis ☐ Study Not Activated ☐**

**If the study has not become active at McGill, please enclose correspondence to explain or provide explanation:

McGill hospital(s) where study is being conducted and has received acceptance of local Research Ethics Board(s) (*if applicable*):

Douglas: ☐ JGH: ☐ MUHC/MCH (Mtl Children's): ☐ MUHC/MCI (Mtl Chest Ins).: ☐ MUHC/MGH: ☐

MUHC/MNH-MNI: ☐ MUHC/RVH: ☐ Shriners Hospital ☐ SMH: ☐ Other: ☐

McGill hospital(s) where study is being conducted and has NOT received acceptance of local Research Ethics Board(s) (*if applicable*):

In the case of a clinical trial, has the lead sponsor registered the study in the WHO Clinical Trials Registry http://isrctn.com/ Yes ☐ No ☐ or the NIH Clinical Trials Registry http://www.clinical.trials.gov ? Yes ☐ No ☐

If study sponsorship or financial support has changed, please provide correspondence to explain; enclosed: **NA**

Total number of subjects to be enrolled in the study: **1500** Number of subjects to be enrolled at McGill sites: **750**

Number of subjects enrolled by McGill PI to date: **278 (137♀ 141♂)** **TOT FROM ALL SITES = 573 (281♀ 292♂)**
Number of subjects enrolled by McGill PI since the last review: **57**

Have any of these subjects withdrawn from the study, and if yes, how many? Yes ☒ **5 SUBJECTS** No ☐

Has the study been revised since the last review? Yes ☐ No ☒

Has the consent form been revised since the last review? Yes ☐ No ☒ Date of current consent form **January 2008**

Are there any new data since the last review that could influence a subject's willingness to provide continuing consent?: **NO**

Have there been any Serious Adverse Experiences (SAEs)?: Yes ☐ No ☒

Have all SAEs and Safety Reports relevant to the study been reported to the IRB?: Yes ☐ No ☐ N/A ☒

SIGNATURES:

Principal Investigator: E. Franco Date: September 2nd, 2008

IRB Chair: Roberta Salomon Date: September 8, 2008

PATIENT INFORMED CONSENT FORM FOR WOMEN

<u>Research Project</u>: HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity)

Principal investigator: Dr. Eduardo Franco

Purpose of the study

You have been asked to take part in a study of human papillomavirus (HPV, for short) infection and transmission among young women and men. We will enrol a total of 600 couples in this study.

Should you decide to participate in this study, you will be given a copy of this consent form. It provides you with a detailed description of the study, describing all the procedures that will be followed. If you have any questions concerning what is explained here, don't hesitate to ask us. Please take all the time you need to read this form.

HPV infection and its consequences

It is now understood that over 99% of cervical cancers are caused by HPV. The cervix is the opening of the uterus. Cancer of the cervix was the most common cancer in Canadian women before Pap smear screening, and is still the most common cancer among women in some other countries. Furthermore, HPV may also cause penile cancer, although this is very rare. Some types of HPV cause skin or genital warts. These HPV types are unrelated to cancer. Genital warts can be treated but in many cases they will disappear by themselves because of the body's immune defences.

Most HPVs that cause cancer of the cervix are sexually transmitted. These sexually transmitted viruses are very frequent. So much so that more than 50% of women and men will have this type of infection at one point of their life or another. Fortunately, over 99% of women who have this virus will never get cervical cancer. Penile cancer occurs in less than one of 100,000 men. Most HPV infections go away by themselves and do not cause precancer or cancer. However, some will. For these reasons, a better understanding of HPV transmission between men and women will help prevent these infections, and reduce peoples' risk of cancer.

Regular Pap tests can prevent a substantial portion of cervical cancers. The Pap test, or cervical cytology, is the test that your doctor collects during a routine gynecologic examination. The sample collected is sent to a laboratory and examined under a microscope in order to detect precancerous cells. These cells can then be treated and cancer can be prevented. There is no equivalent test for detecting precancerous lesions in men.

Much is yet to be learned about HPV

Testing for HPV is a new screening strategy for women that may detect more cases of precancerous cervical lesions than the traditional Pap test. As more and more physicians order these tests, there is increased awareness of how common such infections are among Canadian women. Depending on age, 15%-40% have them. It is believed that a similar proportion of Canadian men also have HPV. Women, their partners, and their physicians are left with many unanswered questions on how the infection is transmitted, how much risk there is after a sexual encounter, and what they can do to protect themselves. This project will be the first in Canada to try to find answers to these questions.

What is required for participation in our study

Women eligible for the HITCH Cohort are:

- aged 18 to 24 years old;
- enrolled at any university or college/CEGEP in Montreal with plans to remain in Montreal for at least the next two years;
- currently heterosexually active with a male partner with whom they began having sexual relations in the past 6 months, and whose partner is willing to enroll in HITCH;
- willing to comply with follow-up for at least 24 months;
- have an intact uterus;
- have no history of cervical lesions/cancer; and
- not currently pregnant or planning to become pregnant in the next 24 months.

If you are eligible and consent to enrol in the HITCH Cohort, you will be asked to visit the Student Heath Service Clinic six times for the collection of hand, mouth, blood and vaginal specimens for HPV testing over the 24 months of your participation. You will also be asked complete 10 computerized surveys over these 24 months.

Your partner will be asked to visit the clinic twice to provide a hand, mouth, and blood sample, a sample of penile epithelial (skin) cells and to complete a computerized survey. His second visit will take place 4 months after his first visit.

The first clinic visit

You must abstain from any form of intercourse (or oral sex) for at least 24 hours prior to your visit to the McGill University Student Heath Service Clinic. At your first visit, a research nurse will collect a blood sample. She will also collect a sample of epithelial (skin) cells from your hands and mouth. She will also provide you with instructions for the self-collection of a vaginal HPV specimen. The research nurse will also show you how to use the computerized survey. You will then collect your first vaginal HPV specimen and complete your first survey in a private room at the clinic. The research nurse will be available at all times should you need help. You will then be given an access code so that you can fill out subsequent surveys through a secure Internet website. This first visit will last about one hour.

Subsequent clinic visits

You will visit the clinic at months 4, 8, 12, 18, and 24 of your participation. You must abstain from vaginal intercourse for at least 24 hours prior to each visit. At the clinic you will meet with the research nurse briefly who will collect a blood sample, and then you will collect a vaginal specimen for HPV testing in a private room. Each visit will last about 15 minutes.

Internet surveys

You will be asked to log on to a secure Internet site using a confidential access code to complete surveys. This is done every 2 months for the first year and every 3 months in the second year of your participation. The survey will ask questions about your medical and sexual history, sexual behaviour with your current partner, contraceptive use, and smoking habits. Each survey will take about 30 minutes to complete. Help will be available through email and telephone should you need assistance.

Laboratory testing of vaginal and blood specimens

The vaginal, mouth and hand specimens collected for HPV testing will be sent to the laboratory and will be tested for the most common types of HPV that can cause cervical cancer. We also ask your permission to test the sample for the presence of immune response polymorphisms that could explain whether or not you are more or less resistant to HPV infection.

The blood sample will be tested for antibodies against HPV infection.

We also ask your permission to keep your specimens for future studies about HPV infection using more refined technologies not yet available for this study.

Benefits

By participating in this study you will be contributing to our understanding of HPV transmission between women and men. Our results could help in the design of cancer prevention programs. Your participation will help to determine the best ways to prevent HPV infection and cervical cancer, which may benefit you at a later time in your life, and that, will certainly benefit others. The results of this study will help provide the women and men who are diagnosed with HPV with accurate information about ways they can prevent infection and transmission to others.

Risks

The risks in this study are minimal as the collection of a vaginal specimen for HPV testing is a safe procedure. There is a possibility that a slight discomfort might be felt during the insertion of the sampler to collect the specimen.

Blood samples will be collected from a vein, usually in the inner arm. One tube of blood (about 2 teaspoons, or 10 mL) will be taken. This will usually involve one needle prick. There may be some discomfort when the blood is drawn. Other possible side effects from

blood drawing include faintness, inflammation of the vein, pain, bruising, or bleeding at the site of puncture. There is also a slight possibility of infection.

The nurse will collect a sample of cells from your mouth using a soft toothbrush and you will be asked to rinse with a mouthwash. This procedure should not cause any discomfort.

The collection of a sample of epithelial (skin) cells from the hand is a safe procedure. There is a possibility that a slight discomfort might be felt during the procedure. Some may experience redness and mild swelling but this will disappear in at most a few hours. A burning sensation may also happen but it will be transient.

In the surveys, you will be asked a number of questions about medical history and sexual activity, some of which are of a sensitive nature.

Confidentiality

The results from the laboratory testing of your specimens and the responses you give in the surveys will be treated in strict confidentiality. Neither will be disclosed to your partner. No names or other information that could identify you as a patient will be released. All the data from this study will be analyzed in aggregate statistical form only, again with no names linked to any data.

The actual specimens will not be made available to investigators that are not involved with this study, nor will they be sold for commercial use. They will only be used for the purposes outlined in this consent form. They will be securely stored for as long as they are needed for the verification of laboratory results, testing with additional methods, and for research audit purposes. Your name will not be linked to any specimen.

Your rights

Your participation in this study is completely voluntary. You are free to withdraw from the study at any time. Your decision to withdraw will have no effect on your current or future health care at this university.

There are no costs to you, direct or indirect.

You will be given $40 in appreciation of your time for each visit to the clinic to provide blood and vaginal specimens. You will also be given an additional $10 for each survey that you complete.

End of study participation

At the end of your 24 months of participation, or if you withdraw, you will receive the results of your HPV tests. HPV testing of vaginal specimens is for research purposes only. The HITCH Cohort strongly advises you to have annual Pap test with your primary care provider. Women who receive an HPV positive result will be offered a Pap test at the McGill University Student Heath Service Clinic. Precancerous cells and lesions detected in a Pap test can be treated and cancer can be prevented. Women with an HPV-positive test but a normal Pap test should be reassured that their infection is not causing precancer or cancer

changes. Annual Pap tests are the best way to prevent cervical cancer. In the absence of a clinically apparent genital wart no treatment is required if a man is found to have a positive HPV test.

Some women and men who receive an HPV-positive result may worry that they are at risk for future cancer, and some may be upset that they have an infection that was sexually transmitted. Counselling will be available to all who request it or who appear to be under stress. It is important to know that an HPV infection can last for a very long time. Thus, a positive test for an HPV infection does not mean that it was recently acquired.

Additional information

If at any time during your study participation you have questions about HPV or this study, you may speak with Gail Kelsall, the research nurse, at the McGill Student Heath Service Clinic (514-398-6017) or telephone or email Ann Burchell, Project Coordinator, at 514-398-5249, hitch.cohort@mcgill.ca.

Ethical approval

The Institutional Review Board of the Faculty of Medicine, McGill University, has accepted this research project.

<u>Research Project</u>: HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity)

Principal investigator: Dr. Eduardo Franco

CONSENT

My signature on this form indicates that the information regarding my participation in this research has been explained to my satisfaction and I agree to participate as a study subject. In no way does this waive my legal rights nor release the investigators, nor involved institutions from their legal and professional responsibilities. I am free to withdraw from this study at any time. My continued participation should be as informed as my initial consent, so I am free to ask for clarification or new information throughout my participation. I understand that if I have any questions concerning matters related to this research, I may call Gail Kelsall, Research Nurse, at 514-398-6017, or Ann Burchell, Project Coordinator, at 514-398-5249.

Name of participant	Signature of participant	Date

Name of witness	Signature of witness	Date

PATIENT INFORMED CONSENT FORM FOR MEN

<u>Research Project</u>: HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity)

Principal investigator: Dr. Eduardo Franco

Purpose of the study

You and your partner have been asked to take part in a study of human papillomavirus (HPV, for short) infection and transmission among young women and men. We will enrol a total of 600 couples in this study.

Should you decide to participate in this study, you will be given a copy of this consent form. It provides you with a detailed description of the study, describing all the procedures that will be followed. If you have any questions concerning what is explained here, don't hesitate to ask us. Please take all the time you need to read this form.

HPV infection and its consequences

It is now understood that over 99% of cervical cancers are caused by HPV. The cervix is the opening of a woman's uterus. Cancer of the cervix was the most common cancer in Canadian women before Pap smear screening, and is still the most common cancer among women in some other countries. Furthermore, HPV may also cause penile cancer, although this is very rare. Some types of HPV cause skin or genital warts. These HPV types are unrelated to cancer. Genital warts can be treated but in many cases they will disappear by themselves because of the body's immune defences.

Most HPVs that cause cancer of the cervix and penis are sexually transmitted. These sexually transmitted viruses are very frequent. So much so that more than 50% of women and men will have this type of infection at one point of their life or another. Fortunately, over 99% of women who have this virus will never get cervical cancer. Penile cancer occurs in less than one of 100,000 men. Most HPV infections go away by themselves and do not cause precancer or cancer. However, some will. For these reasons, a better understanding of HPV transmission between men and women will help prevent these infections, and reduce peoples' risk of cancer.

Regular Pap tests can prevent a substantial portion of cervical cancers. The Pap test, or cervical cytology, is the test that a doctor collects during a woman's routine gynecologic examination. The sample collected is sent to a laboratory and examined under a microscope in order to detect precancerous cells. These cells can then be treated and cancer can be prevented. There is no equivalent test for detecting precancerous lesions in men.

Much is yet to be learned about HPV

Testing for HPV is a new screening strategy for women that may detect more cases of precancerous cervical lesions than the traditional Pap test. As more and more physicians order these tests, there is increased awareness of how common such infections are among Canadian women. Depending on age, 15%-40% have them. It is believed that a similar proportion of Canadian men also have HPV. Women, their partners, and their physicians are left with many unanswered questions on how the infection is transmitted, how much risk there is after a sexual encounter, and what they can do to protect themselves. This project will be the first in Canada to try to find answers to these questions.

What is required for participation in our study

Men are eligible for the HITCH Cohort if they are currently sexually active with a female partner with whom they began having sexual relations in the past 6 months, and whose partner is eligible for and willing to enrol in HITCH.

If you are eligible and consent to enrol in the HITCH Cohort, you will be asked to visit the Student Heath Service Clinic **twice** for the collection of a hand, mouth, and blood sample, a sample of penile epithelial (skin) cells for HPV testing and to complete a computerized survey. Your second visit will take place 4 months after your first visit.

Your partner will be asked to visit the clinic six times for the collection of hand, mouth, and vaginal specimens for HPV testing over the 24 months of her participation. She will also be asked complete 10 computerized surveys over these 24 months.

Clinic visit

You must abstain from any form of intercourse (or oral sex) for at least 24 hours prior to your visit to the McGill University Student Heath Service Clinic. During your visit, a research nurse will collect a sample of epithelial (skin) cells from your hands and mouth. Next, she will conduct an external examination of your genitals. The research nurse will then collect a sample of penile epithelial (skin) cells for HPV testing. She will also collect a blood sample and show you how to use the computerized survey. You will then complete the survey in a private room at the clinic. The survey will ask questions about your medical and sexual history, sexual behaviour with your current partner, contraceptive use, and smoking habits. The research nurse will be available at all times should you need help. This visit will last about one hour.

Laboratory testing of mouth, hand, penile epithelial cells and blood specimens

The samples collected for HPV testing will be sent to the laboratory and will be tested for the most common types of HPV that can cause cancer. We also ask your permission to test the sample for the presence of immune response polymorphisms that could explain whether or not you are more or less resistant to HPV infection.

The blood sample will be tested for antibodies against HPV infection.

We also ask your permission to keep your sample for future studies about HPV infection using more refined technologies not yet available for this study.

Benefits

By participating in this study you will be contributing to our understanding of HPV transmission between women and men. Our results could help in the design of cancer prevention programs. Your participation will help to determine the best ways to prevent HPV infection and cervical cancer, which may benefit you, your partner, or spouse at a later time in your lives, and that will certainly benefit others. The results of this study will help provide the men and women who are diagnosed with HPV with accurate information about ways they can prevent infection and transmission to others.

Risks

The risks in this study are minimal as the collection of a sample of epithelial (skin) cells from the hand, penis and scrotum for HPV testing is a safe procedure. There is a possibility that a slight discomfort might be felt during the procedure. Some men may experience redness and mild swelling but this will disappear in at most a few hours. A burning sensation may also happen but it will be transient.

Blood samples will be collected from a vein, usually in the inner arm. One tube of blood (about 2 teaspoons, or 10 mL) will be taken. This will usually involve one needle prick. There may be some discomfort when the blood is drawn. Other possible side effects from blood drawing include faintness, inflammation of the vein, pain, bruising, or bleeding at the site of puncture. There is also a slight possibility of infection.

The nurse will collect a sample of cells from your mouth using a soft toothbrush and you will be asked to rinse with a mouthwash. This procedure should not cause any discomfort.

In the surveys, you will be asked a number of questions about medical history and sexual activity, some of which are of a sensitive nature.

Confidentiality

The results from the laboratory testing of your specimens and the responses you give in the survey will be treated in strict confidentiality. Neither will be disclosed to your partner. No names or other information that could identify you as a patient will be released. All the data from this study will be analyzed in aggregate statistical form only, again with no names linked to any data.

The actual specimens will not be made available to investigators that are not involved with this study, nor will they be sold for commercial use. They will only be used for the purposes outlined in this consent form. They will be securely stored for as long as they are needed for the verification of laboratory results, testing with additional methods, and for research audit purposes. Your name will not be linked to any specimen.

Your rights

Your participation in this study is completely voluntary. You are free to withdraw from the study at any time. Your decision to withdraw will have no effect on your current or future health care at this university.

There are no costs to you, direct or indirect.

You will be given $50 in appreciation of your time for each visit to the clinic and completed survey.

Provision of HPV result

You will receive the results of your HPV tests at the end of your partner's participation in the study. HPV testing of penile epithelial (skin) cells is for research purposes only. The HITCH Cohort strongly advises that your partner has an annual Pap test with her primary care provider. Precancerous cervical cells and lesions detected in a Pap test can be treated and cancer can be prevented. Women with an HPV positive test but a normal Pap test should be reassured that their infection is not causing precancer or cancer changes. Annual Pap tests are the best way to prevent cervical cancer among women. In the absence of a clinically apparent genital wart no treatment is required if a man is found to have a positive HPV test.

Some men and women who receive an HPV-positive result may worry that they are at risk for future cancer, and some may be upset that they have an infection that was sexually transmitted. Counselling will be available to all who request it or who appear to be under stress. It is important to know that an HPV infection can last for a very long time. Thus, a positive test for an HPV infection does not mean that it was recently acquired.

Additional information

If at any time during your study participation you have questions about HPV or this study, you may speak with Gail Kelsall, the research nurse at the McGill Student Heath Service Clinic (514-398-6017) or telephone or email Ann Burchell, Project Coordinator, at 514-398-5249, hitch.cohort@mcgill.ca.

Ethical approval

The Institutional Review Board of the Faculty of Medicine, McGill University, has accepted this research project.

Research Project: HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity)

Principal investigator: Dr. Eduardo Franco

CONSENT

My signature on this form indicates that the information regarding my participation in this research has been explained to my satisfaction and I agree to participate as a study subject. In no way does this waive my legal rights nor release the investigators, nor involved institutions from their legal and professional responsibilities. I am free to withdraw from this study at any time. My continued participation should be as informed as my initial consent, so I am free to ask for clarification or new information throughout my participation. I understand that if I have any questions concerning matters related to this research, I may call Gail Kelsall, Research Nurse, at 514-398-6017, or Ann Burchell, Project Coordinator, at 514-398-5249, hitch.cohort@mcgill.ca.

Name of participant	Signature of participant	Date

Name of witness	Signature of witness	Date

Appendix B

Offprints of Manuscripts I and II with waivers from publishers

Waivers from co-authors of Manuscripts III and IV

Available online at www.sciencedirect.com
ScienceDirect
Vaccine 24S3 (2006) S3/52–S3/61
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Chapter 6: Epidemiology and transmission dynamics of genital HPV infection

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Abstract

This chapter provides an overview of the epidemiology of human papillomavirus (HPV) infection, with a focus on the dynamics of sexual transmission. We explore concepts related to the spread of sexually transmitted infections, including population prevalence, duration of infectivity, patterns of sexual contacts, and transmissibility, including modifiers of susceptibility and infectivity. HPV prevalence and incidence are high in most studies, particularly amongst young women. There is strong evidence that transmission occurs primarily via sexual activity, most commonly vaginal and anal intercourse. Although the duration of infectivity may be short, current evidence suggests that HPV is highly transmissible. The implications of transmission dynamics for the success of future HPV vaccines are discussed.

Keywords: Human papillomavirus; Prevalence; Transmission

1. Introduction

This chapter provides an overview of the epidemiology of HPV infection, with a focus on the dynamics of sexual transmission. We explore concepts related to the spread of sexually transmitted infections (STI), including population prevalence (an indicator of the burden of disease and of the probability of encountering an infected partner), duration of infectivity, patterns of sexual contacts, and transmissibility, including determinants of susceptibility and infectivity [1,2]. The implications for a future HPV vaccine are also discussed.

2. Prevalence

Genital HPV infection is the most common STI among women [1]. HPV infects the mucosal areas of the cervix, vagina, vulva, and anus. Detection of HPV types by polymerase chain reaction (PCR) assays varies greatly by age and by geography, as shown in a pooled analysis conducted by the International Agency for Research on Cancer (IARC) [3] and in a meta-analysis of published studies [46].

2.1. Age-specific prevalence and geographic variation of HPV infection in women

Among asymptomatic women in the general population, the prevalence of HPV infection ranges from 2 to 44% [4]. A recent meta-analysis estimated HPV prevalence among women with normal cytology using data from 78 published studies [46]. As shown in Table 1, the adjusted global prevalence was 10.41% (95% confidence interval, CI: 10.2–10.7%), with considerable variation by region. No data were available for Oceania. The number of women harboring HPV-DNA worldwide is estimated to be 291 million, and around 105 million women worldwide will have an HPV-16 or -18 infection, the most common oncogenic types in cervical carcinomas, at least once in their lifetime. The IARC

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Table 1
HPV prevalence[a] estimated from a meta-analysis of 78 studies of women with normal cytology, by world regions

	No. of studies	No. of women tested	No. of women HPV+	Adjusted HPV prevalence, % [95% CI]
Global estimate	78	157,879	15,764	10.41 [10.16–10.67]
Africa	8	6226	1429	22.12 [20.87–23.43]
America	24	40,399	6291	12.95 [12.41–13.51]
Europe	27	70,129	4649	8.08 [7.77–8.41]
Asia	19	41,125	3395	7.95 [7.53–8.40]

[a] Adjusted for region, study type, study design, publication year, sampling collection device, cell storage medium, HPV assay, primer used and youngest and oldest age of each included study (adapted from [46]).

pooled analysis used the same PCR method to evaluate specimens collected systematically throughout the world, and largely corroborates these observations [3].

The meta-analysis also indicated that prevalence is highest for young women and decreases in the middle age groups (see Fig. 1). At age 65 and older, an increase of the HPV prevalence is observed in the crude analysis. However, the adjustment for potential confounding factors (such as study design, sampling collection device, and HPV assay) results in a flattening of the age-specific prevalence in these age groups. The crude and adjusted estimates are not statistically significantly different in the ≥60 age group. This pattern is observed in many studies all over the world, with the exception of Asia, where the age-specific curves decrease smoothly with increasing age and no second peak is observed [4,47]. The reasons for the second peak and its geographic variation are unclear, but may be influenced by one or more non-mutually exclusive mechanisms [4]; for example, reactivation of previously undetectable infections acquired earlier in life could occur due to a gradual loss of type-specific immunity or to a sudden loss due to hormonal influences during the postmenopausal years. The second peak could also originate from acquisition of new infections due to sexual contacts with new partners later in life. Also plausible is a cohort effect, for example, the varying prevalence at different ages may reflect the changing experience of successive birth cohorts in being exposed to HPV in different eras. Because the changes in sexual morals over the last several decades have affected some cultural groups more than others, this explanation cannot be ruled out. Further, birth cohort differences in cofactors that may affect HPV progression or clearance (e.g., smoking, parity, oral contraceptives) and competing risks (e.g., mortality due to other causes) could also be involved. Finally, in populations without routine screening, a dip in prevalence in middle-aged women may not occur because underlying lesions remain undiagnosed and untreated.

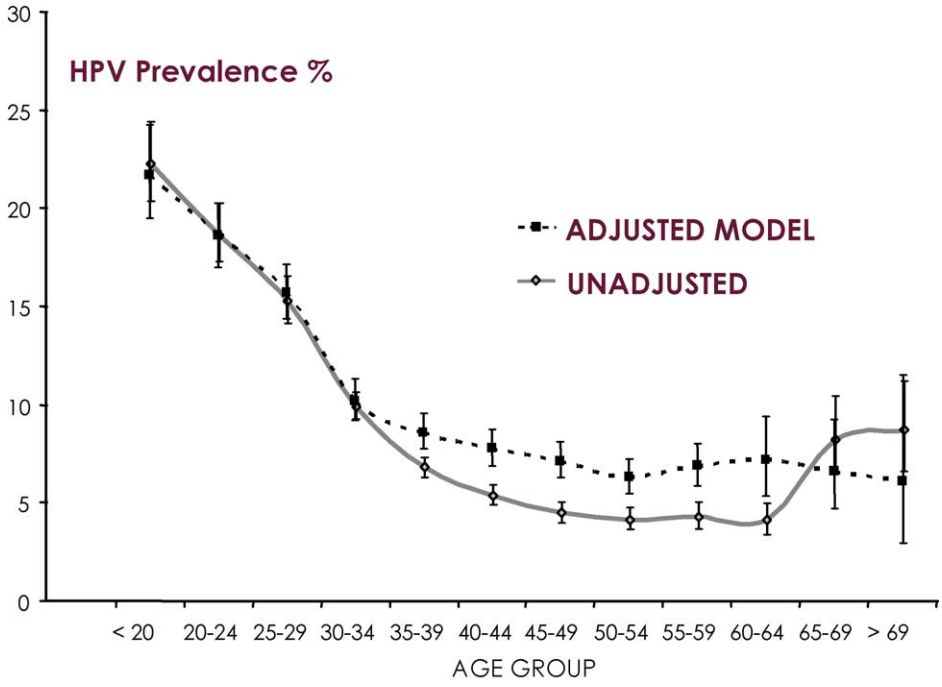

Fig. 1. Age-specific HPV prevalence among women with normal cytology. Crude and adjusted estimates are presented based on the meta-analysis of 78 studies. Age-specific prevalence estimates were calculated by means of logistic models based on a discriminatory analysis that included geographical area, study type, study design, youngest and oldest age values of each study, publication year, sampling collection device, cell storage medium, HPV assay, primer used and HPV type-specific assay. Adapted from [46].

Geographic and cultural variations in sexual behaviour of women and their male partners may result in differential rates of new HPV acquisition, and older men's behaviour may be more critical than women's. Data from 29 countries indicate, with considerable regional homogeneity, that 80% of men and 65% of women aged 40–80 years were sexually active in the past year, with the exception of Asia, where both men and women reported lower sexual activity [5]. In this same study, 5–11% of men compared to 1–6% of women reported more than one current partner (E.O. Laumann, personal communication, 2006). It will be difficult to elucidate the causes of age-related changes without frequent and long-term follow up of cohorts in multiple settings [4].

2.2. HPV prevalence in men

HPV-DNA has been clearly identified in the male genitalia, anal mucosa and oral cavity. Sampling methods for HPV-DNA in men are more variable and have not been thoroughly validated, and there are also difficulties associated with collecting cell specimens by exfoliation of cornified epithelium, which further contributes to the heterogeneity in methods. Partridge and Koustky [6] have reviewed 13 studies, and observed an HPV prevalence ranging from 3.5 to 45% for all types, and 2.3 to 34.8% for high-risk (HR) HPV. In all but one study, the most common type was HPV-16. The prevalence of low-risk (LR) HPV ranged from 2.3 to 23.9%. Penile HPV prevalence increased with the increasing number of sexual partners and with the number of sex worker partners [6,7]. Homosexual and bisexual men have been observed to have a particularly high prevalence of HPV (see Chapter 16). Few HPV serological studies have been conducted among males. The largest one reported lower seropositivity than among women and a peak prevalence among men aged between 30 and 39 [8]. Overall, the HPV data in men suggest that HPV prevalence in men (7.9%) is lower than in women (17.9%) and penile tissues may be less receptive to HR-HPV types [6].

3. Duration

The duration of infectivity is an important component of the rate of spread of an STI in a population, with infections of longer duration having a potentially greater impact [1]. Longitudinal research has consistently shown that most HPV infections detected by molecular hybridisation techniques are transient and are no longer detectable within 1–2 years [4] (see Chapter 5). HR infections seem to persist longer than LR ones [4]. Among HR types, there is some evidence that HPV-16 may persist longer than other types. This suggests that the rate of spread of HR-HPV in populations, including HPV-16, would be greater than for LR-HPV, assuming equivalent sexual contact patterns and transmissibility.

HPV infection among men seems also to be of short duration, with most infections no longer detectable after 1 year [9,10], although there is some evidence that more HR than LR male infections persist [10,11].

It is not known whether HPV is sufficiently infectious to result in transmission for the entire duration of detectable infection. Infectiousness may vary with viral load, since HPV positivity has been shown to correlate with viral load in the partner [12], but few data are available.

4. Incidence

The key measure to determine the spread of an STI is incidence, that is, the number of new HPV infections in a susceptible population over time. Other demographic influences notwithstanding, young women have high rates of HPV acquisition, although the influence of age is not so clear for men. Several studies have reported cumulative incidences of 40% or greater after 3 years of follow-up [4]. Rates of HPV infection in young women are high following first sexual intercourse ("sexual debut"), and remain high with acquisition of each new sexual partner [13,14]. As with prevalence, incidence in women tends to decline with age, although second peaks are sometimes observed in older women [15,16]. Incidence rates are generally higher for HR-HPV types than for LR types, with varying estimates according to the population studied and the number of HPV types tested [4]. Incidence rates for HPV-16 tend to be higher than those observed for other HPV types [4]. Co-infection with multiple HPV types and sequential infection with new types are common, and the risk of acquiring new HPV types appears to be independent of prior infection with other types [4].

Few studies have evaluated HPV acquisition in men. Nevertheless, the evidence suggests that incidence is similarly high among men than among women, with cumulative incidences ranging from 14 to 21% within 3–8 months of follow up [6].

5. Routes of infection

Data supporting sexual intercourse as the primary route of genital HPV infection include documented transmission of genital warts between sexual partners [17], concordance in sexual partners for type-specific and HPV-16 variant-specific HPV-DNA (see Table 2), the rarity of genital HPV infection in women who have not had vaginal intercourse [18], the strong and consistent associations between lifetime numbers of sexual partners and HPV prevalence in women [18] and men (albeit less consistently) [6], and increased risk of HPV acquisition from new and recent sexual partners [19]. Sexual intercourse includes both vaginal and anal intercourse. Receptive anal sex is strongly associated with HPV detection in the anal canal in homosexual and bisexual men [6], and to a lesser degree for women [20]. One explanation for the latter is that some anal HPV infections in women may occur due to viral shedding of cervical or vaginal HPV infections in vaginal discharge [20].

Table 2
Review of studies of HPV-type-concordance among couples

Reference	Population	Sample	Age	Relationship duration	Finding
Hippeläinen et al. [36]	Women with abnormal Pap smear and their male partners (Finland)	270 couples	♀: mean 27 (range 15–62); ♂: mean 32 (range 17–74)	Median: 18 months; mean: 41 months; range: 1–300	6% (15/270) of couples were HPV-positive concordant for the same type
Kyo et al. [37]	Women evaluated for infertility or who had cervical intraepithelial neoplasia (CIN) or cervical cancer, and their male partners (Japan)	53 couples	Not reported	All married for 2+ years	17% (9/53) of couples were HPV-16 positive concordant. In couples where at least one partner had HPV ($n = 26$), 35% were concordant. Discordancy was more likely to be female positive and male negative than female negative and male positive
Baken et al. [29]	Heterosexual partners attending STD clinic (Seattle, USA)	50 couples, 45 with HPV result	♀: mean 26; ♂: mean 29	Unspecified	29% (13/45) of couples were concordant for the same HPV type. In couples where at least one partner had HPV ($n = 41$), 32% were concordant. Concordance decreased with time since last intercourse
Castellsagué et al. [7]	Women enrolled in case-control studies for cervical neoplasia, and their husbands (Spain and Columbia)	816 couples, 431 with HPV result	♂: mean 45	Excluded relationships <6 months duration	(66%) 286/431 of couples were HPV-positive. Of these, 2% (7/286) were HPV-positive-type-concordant
Franceschi et al. [38]	Women enrolled in case-control studies for invasive cervical carcinoma (ICC) and *in situ* cervical cancer (CIS), and their husbands (Spain, Columbia, Brazil, Thailand, and the Philippines)	964 couples	♂: median 45, 50, and 38 for husbands of control women, women with ICC, and women with CIS, respectively	Excluded relationships <6 months duration	HPV-16 positive concordance observed in 0.02% (1/465), 4% (17/383) and 3% (4/116) of couples where the wife was a control, an ICC case, or a CIS case, respectively.
Bleeker et al. [12]	Women with CIN lesion and their male partners (The Netherlands)	238 couples, 181 with HPV result	♀: mean 34.7 (range 19–55); ♂: mean 37.6 (range 22–58)	Mean: 10.6 years; range: 0.6–35 years	37% (67/181) of coupes have type-specific HPV-positive concordance. In couples where HPV was present in at least one partner, 38% (67/176) were type-positive concordant. Increasing association between viral load in one partner and HPV positivity in the other

Although plausible, mechanisms other than sexual intercourse are less common routes of genital HPV infection (see Table 3). While oral and digital infection with genital HPV types clearly occurs, the risk of transmission by digital–genital or oral–genital contact appears to be minimal. Similarly, HPV infection by perinatal transmission or in children also occurs, as both HPV-DNA and serum antibodies have been detected in infants and children. The data suggest that this is rare and unlikely to result in persistent infection.

6. Sexual behaviour leading to exposure to HPV

A knowledge of patterns of sexual behaviour and sexual networking in populations is fundamental for the understanding of HPV transmission dynamics [21]. Generally, the trend in many Western countries is that sexual behaviours and attitudes have become more permissive over time [1]. Many aspects of sexual behaviour affect the likelihood of encountering an HPV-infected partner (Table 4).

6.1. Sexual debut

Several cross-sectional studies have reported that earlier sexual debut or shorter intervals between menarche and sexual debut are risk factors for prevalent HPV infection [22]. However, the reasons for this relationship are unclear. Earlier intercourse may be a marker for other risky sexual behaviour, such as greater lifetime numbers of partners and concurrent partnerships [1]. Indeed, one study has reported that the association of HPV-DNA acquisition with age at first intercourse is mediated by other sexual behaviour variables [23]. In a

Table 3
Review of selected studies evaluating HPV transmission via non-sexual intercourse contact

Reference	Population	Findings
Genital HPV infection associated with sexual contact other than intercourse		
Marrazzo et al. [39]	Cross-sectional study of women who have sex with women, including 21 women reporting only female sexual partners (USA)	HPV-DNA detected in genital tract specimens from 19% of women reporting only female sexual partners
Sonnex et al. [40]	Cross-sectional study of 14 men and 8 women with genital warts (UK)	27% of subjects tested positive for the same HPV-DNA type in both finger brush and genital samples
Winer et al. [19]	Longitudinal study of female university students, including 148 women reporting no history of vaginal intercourse at enrolment (USA)	The 24-month cumulative incidence of HPV-DNA infection in virgin women was 7.9% (95% CI: 3.5–17.1); any type of non-intercourse sexual contact (finger–vulvar, penile–vulvar or oral–penile) reported by virgin women was associated with an increased risk of HPV infection.
Oral HPV infection associated with oral sex		
Coutlée et al. [41]	Cross-sectional study of 178 (158 ♂, 20 ♀) HIV+ and 109 HIV− (73 ♂, 36 ♀) individuals (Canada)	32 of 287 (11.2%) oral samples tested positive for HPV-DNA; a univariate association between unprotected oral sex and oral HPV (odds ratio, OR = 5.5; 95% CI: 1.6–18.4) was no longer apparent after adjustment for other sexual behaviour variables and genital infections
Winer et al. [19]	Longitudinal study of 603 female university students (USA)	Only 5 of 2619 (0.02%) oral samples tested positive for HPV-DNA; there was no association between oral HPV and report of oral–penile contact in the past 12 months (hazard ratio, HR = 0.5; 95% CI: 0.07–3.5).
Kreimer et al. [42]	Cross-sectional study of 190 (108 ♂, 82 ♀) HIV+ and 396 HIV− (231 ♂, 165 ♀) individuals (USA)	18 of 583 (3.1%) oral samples tested positive for HPV-DNA; associations between oral sex and oral HPV were inconsistent and varied according to HIV serostatus and reports of oral sex with same-sex vs. opposite-sex partners; ORs for ≥2 vs. 0–1 recent oral sexual partners: HIV-negative 0.2 (95% CI: 0.0–1.2); HIV-positive 12.8 (95% CI: 3.1–52.7)
Rintala et al. [43]	Longitudinal study of 131 heterosexual married couples (Finland)	The 24-month cumulative incidence of oral HPV-DNA in both men and women was around 10%; oral HPV was not associated with oral sex habits
HPV infection in children and infants		
Smith et al. [44]	Longitudinal study with type-specific HPV-DNA testing in 574 mother–infant pairs (USA)	1.6% of oral and genital samples taken from infants a median of 65 h post delivery were positive for HPV-DNA. Type-specific concordance between mother and infant pairs was less than 1%. At 3-month follow-up, no HPV-DNA was detected in any of the infants tested
Dunne et al. [45]	Cross-sectional HPV-16 seroprevalence survey of 1316 children aged 6–11 (United States)	2.4% of children were seropositive, with higher prevalence in boys than girls (3.5% vs. 1.2%) and in children >7 years than in children ≤7 years (3.3% vs. 0.4%)

recent longitudinal study of 15–19-year-old women sampled within 1 year since sexual debut, the risk of HPV infection increased with the interval between menarche and first intercourse, probably due to the tendency of older women to form partnerships with older, more sexually experienced partners [14]. Biological mechanisms, including cervical immaturity, inadequate production of protective cervical mucus and increased cervical ectopy, may make younger women and adolescents more susceptible to HPV infection [22].

In developed countries, the age at sexual debut appears to be decreasing over time [1], although some recent data suggest a reversal of this trend in the United States [24]. In developing countries, there is considerable variability in the prevalence of virginity, age of sexual debut and premarital sex among women aged 15–24 [25]. In 10 countries from sub-Saharan Africa, Latin America and the Caribbean, the prevalence of premarital sex was greater in countries in sub-Saharan Africa (see Fig. 2a) [25]. However, in Latin America, there is evidence that the prevalence of virginity among young women is declining over time and premarital sex is increasing (see Fig. 2b) [25]. The trend of increased exposure to HPV at younger ages has important implications for vaccination programs.

6.2. Number of partners and acquisition of new partners

The associations between numbers of new and recent sexual partners and likelihood of detecting HPV-DNA in female genital tract specimens are strong and consistent [18,19]. The rate of acquisition of partners (contact rate) plays a key role in STI transmission dynamics [2]. Population surveys show heterogeneity in the number of lifetime and recent sexual partners, with a majority having none or one partner, and a

Table 4
Proposed risk factors* for HPV acquisition and transmission, according to hypothesized mechanism of action: Summary of results from published epidemiologic studies

	Hypothesized to affect likelihood of exposure to HPV-infected partner	Hypothesized to affect likelihood of transmission upon exposure through effects on. . .	
		Infectivity/duration	Susceptibility
Early age at sexual debut	↑		↑
Greater number of partners	↑		
Similarity or dissimilarity between individuals and their sexual partner(s)	↑/↓		
Acquisition of new partner	↑		
Concurrent/extra-dyadic partners	↑		
Short intervals between partners	↑		
Concomitant infection with other STI	↑	↑	↑
Male circumcision	↓	↓	↓
Condoms	↑/↓	↓	
Immune suppression (e.g., HIV infection, transplantation)			↑
Certain human leukocyte antigen (HLA) complex alleles and haplotypes		↑	↑
Hormonal contraceptives		↑	↑
Diet deficient in certain micronutrients		↓	
Smoking		↑	↑

Refer to text for details and strength of evidence.
* Arrows indicate the direction of the association, i.e., whether they increase or decrease risk via the proposed mechanism.

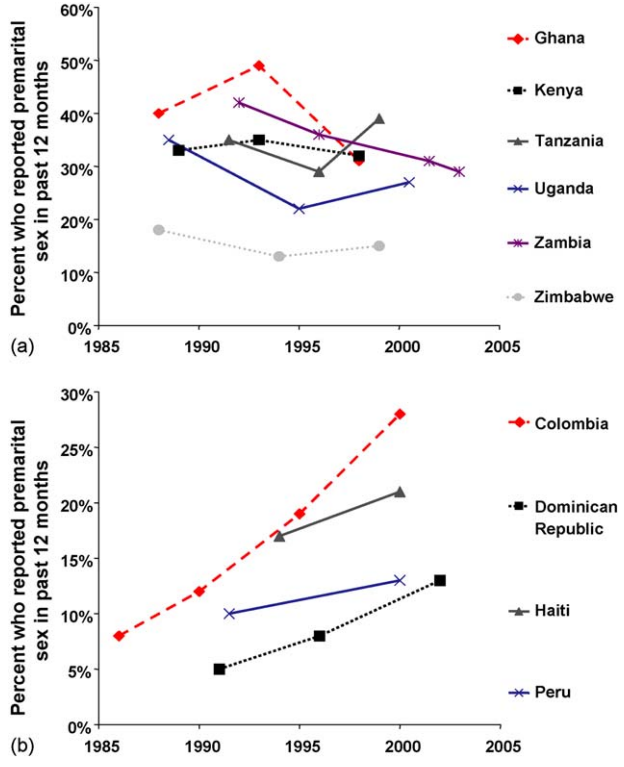

Fig. 2. (a) Percentage of never-married women aged 15–24 years who reported sex in the past 12 months in selected countries in Africa. Adapted from [25]. (b) Percentage of never-married women aged 15–24 years who reported sex in the past 12 months in selected countries in Latin America and the Caribbean. Adapted from [25].

minority having multiple partners [1]. More sexual partners and non-spousal/non-cohabitating partners are more often reported among men than women, and among the young than the old [1,25]. Gender differences could be explained by a small proportion of women having sex with many partners (e.g., sex workers), or by under-reporting of sexual activity by women or men's over-reporting [25].

6.3. *Characteristics of partners and sexual networks*

The characteristics of male partners are critical for female HPV acquisition. In case-control studies of cervical cancer, male partners of cases report higher numbers of partners than those of controls [19]. Female HPV prevalence and acquisition have been positively associated with women's estimates of their male partners' lifetime number of partners [13] or not knowing a male partner's prior sexual history [13,14].

Patterns of sexual networking are also critical for transmission dynamics [21]. Sexual networks are made up of individuals who are sexually connected, either directly or indirectly. Important network features that increase the chances of transmission are larger network size, higher contact rates and the patterns of sexual mixing or partner choice [21]. Random mixing occurs when an individual is equally as likely to have sex with any other individual [2]. Assortative mixing occurs when similar individuals tend to form contacts, whereas dissortative mixing occurs when individuals tend to form contacts with individuals who are different from them, and it is the latter that tends to increase the risk for STI transmission [21]. Most surveys show that mixing tends to be moderately assortative with respect to age, race/ethnicity or number of sexual partners [2], but not always [1,21]. For

example, in many cultures women tend to form partnerships with older men [2,21]; this could explain, in part, the high HPV prevalence among younger women, and its geographical variation.

"Core groups", or groups of highly sexually active individuals with many partners, are believed to contribute disproportionately to the spread of most STIs [2,21]. HPV infection is not restricted to core groups, however, as it is also relatively common among moderately sexually active individuals [1,18]. This may be due to inherent biological properties of HPV as a virus that is well adapted to be transmitted by skin-to-skin contact and to infect only the epithelial lining of susceptible body areas without the need to invade connective tissue or to be disseminated regionally or systemically, in addition to the generally silent nature of the infection. Bridging occurs when sexual linkages are formed between members of high and low prevalence subpopulations, which provide a conduit for infection between them [1]. For example, STI transmission between the homosexual and heterosexual populations is possible through bisexual activity [26], and could have implications for female-only vaccination strategies.

Should HPV vaccines reduce HPV transmission in the general population, HPV could then become concentrated in core groups, and the behaviours of these highly sexually active individuals will be of greater importance for research and prevention [26]. Direct targeting of vaccines to core groups would not be expected to reduce HPV population prevalence, given the lessons learned from the hepatitis B vaccine (see Chapter 14).

6.4. Concurrency and serial monogamy

The timing of sexual partnerships plays a role in determining STI spread. An example is sexual partner concurrency, in which sexual partnerships overlap each other in time [21]. Concurrent partnerships are not uncommon—they are reported by 32–54% of adolescents and 12–40% of adults in the US [27]. Since awareness of whether one's partner has other partners has been shown to be poor [27], this implies that long-term monogamy on the part of one partner may not necessarily reduce the risk of infection.

The timing of non-overlapping partnerships, or serial monogamy, may also be important. A survey of sexual behaviour in the US found that, among serially monogamous women, the mean gap between partners was 8 months for women aged 15–19, 11 months for women in their twenties, and 18 months for women aged 30–44 [28]. Given the average duration of HPV infection among women, serial monogamy must contribute to HPV transmission. Knowing a partner for more than 8 months has been associated with a lower risk of HPV acquisition among women [13], which could be explained by clearance or waning infectivity in the male. Likewise, intercourse with a partner who has had no other recent partners would be expected to reduce infection risk [13].

7. Transmissibility and factors affecting transmission

7.1. Probability of transmission upon exposure

To our knowledge, there have been no published reports of the transmissibility of HPV based on empirical data [18]. A study of the transmissibility of genital warts, conducted before HPV was identified as the causal agent, observed that 60% of sexual partners of patients with warts subsequently acquired them [17]. This suggests high transmissibility, at least for HPV types that cause genital warts.

To date, research on HPV in couples has consisted of cross-sectional assessment of prevalent HPV infection in both partners, rather than transmission per se (Table 2). Most, but not all, of these studies found relatively poor concordance for type-specific HPV positivity. In two studies, however, the HPV-type-specific positive concordance was greater than expected by chance [12,29]. Concordance was associated with more recent sexual intercourse [29] and higher viral load [12]. Methods for HPV testing among men are in the process of being refined, and it is possible that some of these previous studies have limited ability to detect HPV infections. Nevertheless, HPV status in couples where the woman has cervical lesions is likely not reflective of those in couples where the female is lesion-free. Furthermore, couples in these studies tended to be older, with relationships of long duration. The transmission event likely occurred years prior to enrolment, and many infections would have resolved. To study HPV transmission, one would ideally recruit relatively young couples that have newly formed relationships.

A stochastic computer simulation study has investigated values of HPV transmissibility that were consistent with observed incidence among female university students [30]. The probability of HPV transmission per coital act ranged from 5 to 100%, with a median of 40%. Similarly, Barnabas et al. [31] have recently estimated the per-partner male-to-female transmission probability as 60% for HPV-16 using Finnish data on seroprevalence. This is identical to the observed per-partner transmission probability for genital warts [17].

These results suggest that HPV is more transmissible than other viral STIs, but is comparable to bacterial STIs. Studies of HIV or herpes simplex virus-2 (HSV-2)-discordant couples indicate that the probability of transmission is 1 per 1000 acts of intercourse [2,32]. Per-partnership transmission probabilities for bacterial STIs range from 20% for chlamydia, 50% for gonorrhoea and 60% for syphilis to 80% for *Haemophilus ducreyi*, the causal agent of genital ulcers [2]. With high transmissibility, vaccines would need to reduce infectivity several-fold in breakthrough infections to stop the chain of transmission. This could happen by a reduction in viral load.

7.2. Factors affecting the probability of transmission

A number of factors may influence the probability of transmission of an STI, such as viral load, other STIs, cir-

cumcision, use of condoms, immune mediators of susceptibility or infectivity and nutrition (Table 4). Cervical infection with other STIs, such as *C. trachomatis*, may increase susceptibility to HPV infection by cervical inflammation or microabrasions, or facilitate persistence of HPV infection through immunological mechanisms [33]. The similar sexual behaviour risk-factor profiles for HPV and other STIs, however, make it difficult to discern whether other STIs are simply markers for exposure to HPV or act as true cofactors by increasing susceptibility or infectivity [4].

Evidence for male circumcision as a risk factor for genital HPV infection in both men and women is conflicting [6]. One study has reported a protective association against prevalent HPV infections and repeat detection of prevalent infections at a 1-year follow-up visit, but not against detection of new infections [10]. Male circumcision has not been linked to female HPV acquisition, although some, but not all, case-control studies have reported that male partners of women with cervical cancer are less likely to be circumcised than male partners of control women [19]. If male circumcision does contribute to the spread of HPV infection, it is unclear whether it affects men's susceptibility to infection and/or infectivity and persistence upon infection.

Use of condoms is an effective barrier against genital HIV transmission; however, data for other STIs, including HPV, are equivocal [34]. Condom use appears to offer some protection against developing high-grade cervical neoplasia and invasive cervical cancer [34], and have been shown to promote regression of cervical neoplasia and penile lesions and clearance of infection in men and women (see Chapter 5). Nonetheless, most studies evaluating the relationship between condom use and HPV infection have failed to demonstrate a protective effect of condoms [34]. This may, in part, be due to a tendency for condoms to be used more often in casual relationships, where the probability of encountering an infected partner is higher [4]. Data from a recent prospective cohort study of female university students enrolled prior to or within 2 weeks of their first intercourse, however, did show a more than three-fold protective effect of condoms on HPV acquisition [48]. Even with consistent condom use, however, HPV infections can still be transmitted through contact with areas of unprotected genital skin. Furthermore, a protective effect of condom use, even if one exists, may diminish over multiple sex acts in ongoing relationships due to high infectivity [30].

Increased genital HPV prevalence has been observed in men and women with immunodeficiencies, regardless of the cause. High HPV prevalence has been consistently observed among HIV-seropositive populations of women and men (see Chapter 16). Some HLA class II polymorphisms have also been shown to influence risk of acquisition and clearance of HPV infections [4].

While there is evidence to suggest that hormonal factors may influence susceptibility to HPV infection [18], associations between hormonal contraceptive use and HPV infection have been inconsistent [35]. Hormonal contraception may increase susceptibility to infection (e.g., by increased ectopy [35]) or it may also be confounded by unmeasured sexual behaviours. Most studies have not reported associations between hormonal contraceptive use and HPV infection, independent of sexual behaviour [35]. Risk of persistent HPV infection seems to be negatively associated with consumption of fruits and vegetables, dietary intake or circulating levels of vitamins C and E, and several carotenoids [4].

Finally, the effect of smoking on HPV acquisition is unclear. Most studies in both men and women have failed to associate smoking with HPV detection, or positive associations were attenuated after controlling for sexual behaviour [18,19]. One study has reported a significant positive association between current smoking and incident HPV infection, even after controlling for measured sexual behaviour variables [13]. While one explanation for this finding is that smoking increases susceptibility to infection, smoking may also be a proxy measure of unmeasured sexual behaviours.

8. Implications for vaccines and future research

There is strong evidence that transmission occurs primarily via sexual activity, most commonly vaginal and anal intercourse, although perinatal and non-sexual transmission does sometimes occur. The common tools for STI prevention, namely the promotion of abstinence or delay in sexual activity, monogamy, condoms and treatment of existing infections, are not all equally applicable to HPV. Delay in coitarche and monogamy should reduce risk, but will not eliminate it, since HPV is highly prevalent and any sexual activity poses a risk. Condoms may provide some protection, but transmission may still occur via unprotected areas of genital skin. Currently, no treatment of existing infections is available to reduce the duration of infectiousness.

The features of transmission dynamics have important implications for future HPV vaccines. With longer duration of infectivity, more frequent formation of sexual partnerships that facilitate exposure between infected and susceptible individuals, and/or higher transmissibility, the extent of vaccine coverage necessary to reduce population HPV prevalence increases. Many of these issues vary across populations, thereby suggesting that the potential vaccine impact will be population-specific, even with equivalent coverage. Furthermore, the nature of transmission dynamics will reduce the impact of vaccines in the face of vaccine failure. This would include scenarios where the vaccine has no effect in some individuals, if the vaccine does not fully eliminate susceptibility, or if there is loss of protective immunity over time.

To further our understanding of HPV transmission dynamics, data on acquisition and persistence among heterosexual men as well as homosexual and bisexual men are urgently needed. The natural history of HPV infection and patterns of viral load, and how this impacts on infectiousness, remains to be understood in both men and women. Frequent and long-term follow-up of women is necessary to determine the causes

of age-related changes in HPV positivity. In particular, longitudinal studies of older women are needed to evaluate whether new partner acquisition is associated with HPV detection at all ages, and patterns of viral load by age. Ideally, studies of HPV acquisition would also determine the HPV status of sexual partners.

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Original Contribution

Modeling the Sexual Transmissibility of Human Papillomavirus Infection using Stochastic Computer Simulation and Empirical Data from a Cohort Study of Young Women in Montreal, Canada

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The authors estimated plausible ranges of the probability of human papillomavirus (HPV) transmission per coital act among newly forming couples by using stochastic computer simulation. Comparative empirical data were obtained in 1996–2001 from a cohort study of female university students in Montreal, Canada. Female prevalence and frequency of sexual intercourse and condom use were set equal to those in the cohort. Simulations included 240 combinations of male prevalence, the relative risk for protected versus unprotected sex, and per-act transmission probabilities. Those that produced expected HPV incidence within the 95% confidence interval observed in the cohort were selected. The observed 6-month cumulative incidence following acquisition of a new partner was 17.0% (95% confidence interval: 11.4, 23.0). Expected incidences consistent with those from cohort findings occurred in 54/240 simulations. The range of per-act transmission probabilities was 5–100% (median, 40%). Male HPV prevalence was the same as or greater than that for women in all consistent simulations. Varying condom effectiveness did not produce better-fitting data. This simulation suggests that HPV transmissibility is several-fold higher than that for other viral sexually transmitted infections such as human immunodeficiency virus or herpes simplex virus 2. With high transmissibility, any potential protective effect of condoms would disappear over multiple intercourse acts, underlining the need for an effective HPV vaccine.

disease transmission; papillomavirus, human; sexually transmitted diseases; uterine cervical neoplasms

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; STI, sexually transmitted infection.

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI). Cervical HPV infection is found in 5–40 percent of asymptomatic women of reproductive age (1), and as many as 75 percent of adults may eventually be infected in their lifetime (2). Risk rises with increasing number of sexual partners, younger age at sexual debut, and recent acquisition of new partners (3–7). The vast majority of these infections will be transient (3, 8–12). However, a substantial increase in risk of cervical neoplasia exists for women who develop persistent, long-term infections with oncogenic HPV types (3, 9, 13–15). It is now well established that HPV infection is the central, probably necessary cause of cervical cancer (16).

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The acknowledgment that cervical cancer is caused by an STI has produced a change from a noninfectious to an infectious disease paradigm, with corresponding changes in prevention strategies. There is currently great enthusiasm concerning the possible application of HPV testing as an adjunct to Papanicolaou cytology screening for cervical cancer (17) and widespread interest in the development of HPV vaccines (18). However, assessments of the potential impacts of these proposed strategies are hampered by limited information on the sexual transmissibility of HPV. To date, most natural history models that predict the impact of HPV testing and vaccination strategies have been based on empirical data that have come exclusively from epidemiologic studies of women (19–23). A better understanding of the sexual transmission dynamics of HPV would lead to more informed decision making when different prevention strategies are compared through more valid mathematical prediction models.

In the absence of empirical data on HPV transmissibility, computer simulation may be a useful tool for estimation. The objective of this study was to simulate probabilities of HPV transmission per coital act in a hypothetical population to estimate plausible ranges for this parameter that would be coherent with observed rates of HPV incidence among young, sexually active women enrolled in a cohort study we previously conducted in Montreal, Canada (24).

MATERIALS AND METHODS

Hypothetical populations of newly forming heterosexual couples were simulated. Acquiring a new partner has been shown to be a key determinant of HPV acquisition (7). Therefore, newly forming rather than long-standing couples were the object of analysis.

McGill-Concordia Cohort Study

The source of empirical data was the prospective McGill-Concordia Cohort Study of young female university students in Montreal, Canada. Women attending either the McGill or Concordia university health services clinics were recruited for a study of the natural history of HPV infection and cervical neoplasia. The study methodology is described in detail elsewhere (24). In brief, 621 female participants were followed for 24 months at 6-month intervals in 1996–2001. At each visit, a cervical specimen was collected and tested for 27 HPV types using L1 consensus primers MY09/MY11 and HMB01 and the line blot assay (Roche Molecular Systems, Basel, Switzerland) (25). Women also self-completed questionnaires, which collected information on sexual history and behavior since the last visit. In the overall cohort, baseline cervical HPV prevalence was 29 percent for any type, 22 percent for high-risk oncogenic types, 15 percent for low-risk types, and 7 percent for HPV-16 (24).

Of 2,058 follow-up study visits, there were 238 visits by 182 women in which a new sexual partner was reported since her last visit, and no other partners (''new partner visits''). Empirical estimates of simulation parameters and cumulative HPV incidence were based on data from these new partner visits. Each new partner visit was assigned two time points: time t, the visit at which a new sexual partner was reported; and time $t - 1$, the visit immediately preceding time t. The median duration of the interval between $t - 1$ and t was 6 months (range, 3–28 months). Cumulative incidence of any new type of HPV was calculated using the Kaplan-Meier method. To account for repeated event times, the 95 percent confidence interval was estimated using bootstrap sampling of the 182 women who reported at least one new partner visit (26).

Simulation approach

A stochastic Monte Carlo computer simulation produced hypothetical cohort data for a population of 10,000 newly forming heterosexual couples. The assumed values for fixed and variable parameters used in the simulations are summarized in table 1.

The first step was to assign the initial type-specific HPV positivity for each hypothetical female. Doing so involved drawing a random variable from the standard uniform distribution, which was compared with the observed type-specific prevalence at time $t - 1$ (table 1). For example, HPV-16 prevalence was 4.37 percent. Then, if the drawn random variable was less than or equal to 0.0437, the hypothetical female was assigned to be HPV-16 positive at time $t - 1$ (i.e., female HPV-16 positivity ~ Bernoulli (0.0437)). Because HPV prevalence among male partners of McGill-Concordia cohort women was unknown, the male-to-female prevalence rate ratio at time $t - 1$ was varied from 0.5 to 2.0. With respect to HPV status, random mixing of males and females was assumed. That is, HPV positivity in one partner was considered independent of that in the other when the couple was initially formed.

Simulated data on the frequency of intercourse over a 6-month interval were then generated for each couple. Intercourse frequencies per month were set to be the same as in the cohort (table 1) using randomly drawn numbers from the gamma distribution, rounded to the nearest integer, that most closely matched the empirical distribution (shape $= 1$, scale $= 10$). Each couple was also randomly assigned condom use frequency. It was assumed that women interpreted ''sometimes'' as condom use 50 percent of the time and ''regularly'' as condom use 75 percent of the time.

Given the uncertainty regarding condom efficacy, the relative risk of HPV transmission for a single act of protected versus unprotected intercourse was varied from 0.1 to 1.0 (table 1). A lower bound of 0.1 was selected because it most closely approximates that for another viral STI, human immunodeficiency virus (HIV), for which considerable data on condom effectiveness have been accumulated (27).

To simulate the incidence of HPV in the female partner, per-act transmission probabilities were varied from 0.001 to 1.0 (table 1). A lower bound of 0.001 was selected because it is the estimate for HIV given conditions of low viral load and long-standing partnerships (28, 29). For each act of intercourse between HPV-discordant couples, a variable was randomly drawn from the standard uniform distribution and transmission events were assigned, taking into account condom use.

TABLE 1. Fixed and variable parameters used in simulations of male-to-female transmission of HPV*,†

Parameter	Value
Fixed	
Type-specific HPV prevalence (%) among women at time $t - 1$	
6	1.75
11	0.44
16	4.37
18	2.62
26	0.00
31	3.06
33	2.18
35	0.00
39	2.62
40	0.00
42	0.44
45	3.06
51	1.75
52	2.18
53	2.62
54	2.18
55	0.44
56	1.75
57	0.00
58	2.18
59	0.87
66	2.18
68	0.87
73	0.00
82	0.87
83	0.87
84	4.37
Monthly intercourse frequency	
Mean	9.48
Median	7.00
Standard deviation	9.95
Gamma shape parameter	1
Gamma scale parameter	10
Condom use frequency (%)	
Never	13
Sometimes	26
Regularly	61
Variable	
Male-to-female HPV prevalence rate ratio (5 levels)	
	0.50
	0.67
	1.00
	1.50
	2.00
Relative risk for condom use per coital act (4 levels)	
	0.10
	0.25
	0.50
	1.00
Probability of HPV transmission per coital act (12 levels)	
	0.001
	0.010
	0.025
	0.050
	0.100
	0.150
	0.200
	0.300
	0.400
	0.500
	0.750
	1.000

* HPV, human papillomavirus.

† Fixed parameters were based on observed data from the McGill-Concordia Cohort Study (refer to the text for details).

The simulation outputs a data set with the type-specific HPV status of simulated women for times $t - 1$ and t. Kaplan-Meier analysis was used to calculate the expected cumulative incidence of any new HPV type at 6 months, as it would have been observed in a hypothetical cohort.

For each of the 240 possible combinations of the male-to-female prevalence rate ratio, relative risk for condom use, and per-act transmission probability value, 100 simulations of 10,000 couples were run. Resulting cumulative incidences were averaged over the 100 simulations to provide the best estimate of what would be expected under those conditions. Expected incidences were then compared with the 95 percent confidence interval for the observed cumulative incidence. Simulated conditions that produced expected cumulative incidences within this range were considered compatible.

RESULTS

Cumulative incidence of HPV infection at 6 months for women reporting the 238 new partner visits in the McGill-Concordia Cohort Study was 17.0 percent (95 percent confidence interval: 11.4, 23.0). Of 240 simulations, 54 (22.5 percent) produced expected cumulative incidences that fell within the range of 11.4–23.0 percent.

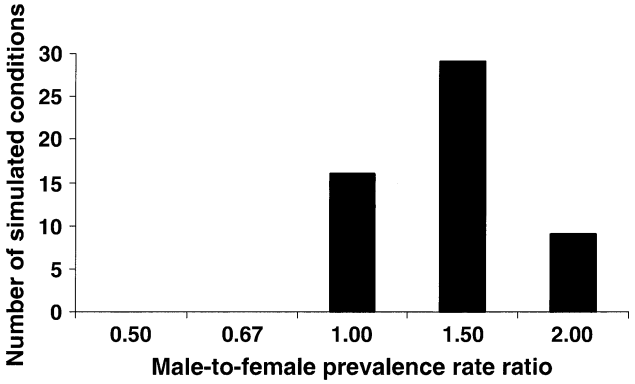

FIGURE 1. Frequency distribution of values of the male-to-female human papillomavirus (HPV) prevalence rate ratio in 54 simulated conditions that were consistent with the observed cumulative incidence of any new HPV type among women in the McGill-Concordia Cohort Study, Montreal, Canada, 1996–2001.

All of the simulations that produced expected incidences consistent with the observed data assumed that men's prevalence was the same as women's, or greater (figure 1). The highest proportion (54 percent) of consistent simulations assumed that men's prevalence was 1.5 times that for women.

Figure 2 shows that values of consistent per-act transmission probabilities ranged from 0.05 to 1.00, with a median of 0.40. Per-act transmission probabilities of 0.001–0.025 were not consistent with the observed cohort data. The median per-act probability values were 0.625 when the male-to-female prevalence rate ratio was assumed to equal 1.0, 0.30 when the prevalence rate ratio was assumed to equal 1.5, and 0.10 when the prevalence rate ratio was assumed to equal 2.0.

The probability of transmission over a specific number of acts, n, can be estimated with the following equation: Probability (infection) $= 1 - (1 - \lambda)^n$, where λ is the per-act transmission probability (28). At the median value of the per-act transmission probability for all consistent simulations (0.40), a woman would have a 99.6 percent probability of becoming infected within 11 acts of intercourse.

Figure 3 shows that no single estimate of per-act effectiveness of condoms produced better-fitting data. Simulations with relative risks ranging from 0.1 to 1 gave expected cumulative incidences that fit the observed data.

Figures 4 and 5 show how the relation between the expected 6-month cumulative incidence of any new HPV type and the per-act transmission probability varies with the male-to-female prevalence rate ratio, under the assumption that condoms offer no protection (figure 4) and that they offer fourfold protection (figure 5). The observed 95 percent confidence interval for cumulative incidence in the McGill-Concordia Cohort Study is shown for comparison (dotted area). If one assumes that HPV prevalence is equivalent in men and women, then the per-act transmission probability most consistent with the observed data is greater than 0.20. However, if one assumes that HPV is more prevalent among women than among men, then the per-act transmission probability may be as low as 0.03.

Similarly, figure 6 shows how the relation between the expected 6-month cumulative incidence of any new HPV type and the per-act transmission probability varies with the assumed protective effects of condoms if male HPV prevalence is 1.5 times that of females. All simulated relative risk values for condom effectiveness were compatible with the observed data, but higher condom effectiveness implies higher transmissibility. That is, if the relative risk is 1, then the plausible range for transmissibility is about 0.09–0.40; if the relative risk is 0.1, then this range shifts to 0.16–1.00.

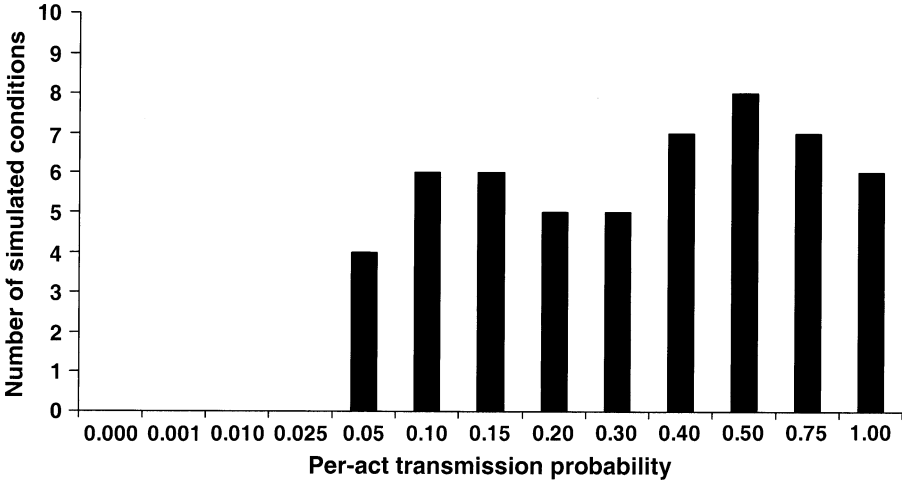

FIGURE 2. Frequency distribution of human papillomavirus (HPV) transmission probabilities per coital act in 54 simulated conditions that were consistent with the observed cumulative incidence of any new HPV type among women in the McGill-Concordia Cohort Study, Montreal, Canada, 1996–2001.

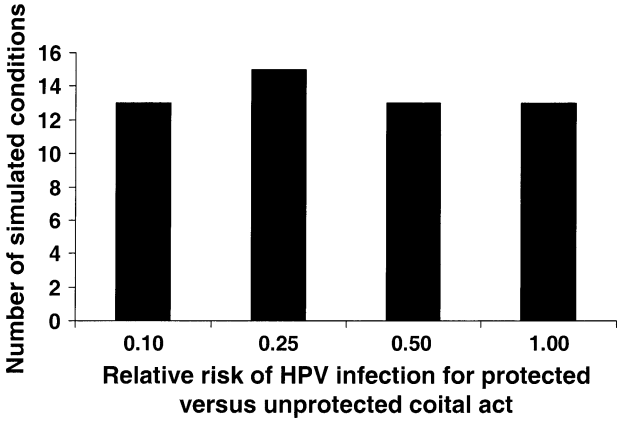

FIGURE 3. Frequency distribution of values of the relative risk of the effectiveness of condoms for a protected versus an unprotected coital act in 54 simulated conditions that were consistent with the observed cumulative incidence of any new human papillomavirus (HPV) type among women in the McGill-Concordia Cohort Study, Montreal, Canada, 1996–2001.

The Monte Carlo standard error of the expected incidence was estimated for each of the 240 configurations of parameter values. Consider, for example, the instance in which the male-to-female prevalence rate ratio was 1.5, the relative risk for condom use was 0.25, and the per-act transmission probability value was 0.40. Over 100 replications of a simulation of 10,000 couples, the mean incidence rate was 0.19900. The standard error of the mean, or Monte Carlo standard error, was calculated by dividing the observed standard deviation of the 100 estimates by sqrt(100), and it equaled 0.00047. Over the 240 sets of parameter values, this Monte Carlo standard error ranged from 0.00002 to 0.0007, indicating considerable precision in the simulated incidence rates.

The 95 percent confidence interval for observed incidence was chosen for comparison since it is the conventional level of confidence for the dispersion of parameter values in most decision-making situations in public health. For comparison, other interval boundaries were also used. For instance, the 99 percent confidence interval for observed incidence was 10.6, 25.3; although more (66/240) simulated conditions produced expected incidence values that were consistent with this interval than with the 95 percent confidence interval, the parameter values in those 66 simulated conditions were identical. Comparison with the 50 percent confidence interval (15.0, 18.8) led to fewer (12/240) simulated conditions being consistent with the observed rate. Per-act transmissibility values were in the lower range (0.05–0.30), whereas the male-to-female prevalence ratio was in the higher end of the range (1.5–2.0). The values of the risk ratio for condom use were no different from those obtained using the 95 percent confidence interval.

Further analysis was carried out to determine the influence of specific assumptions. Results were similar when incidence density, rather than cumulative incidence, was used as the comparative outcome (data not shown). The

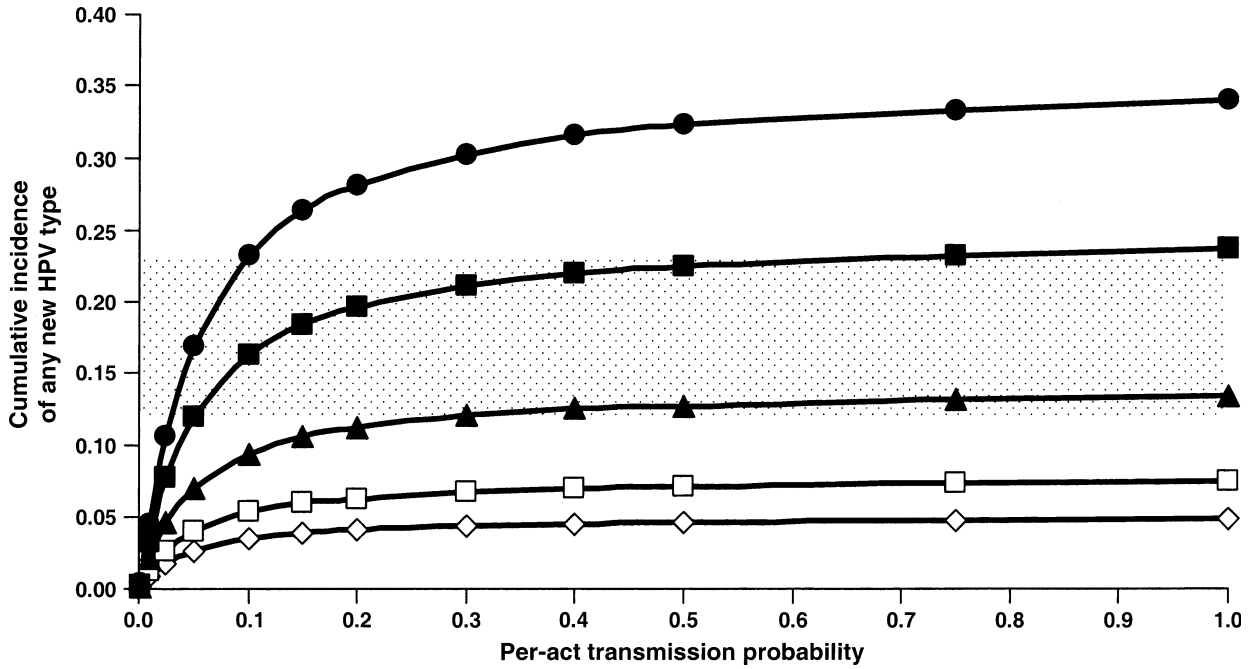

FIGURE 4. Expected cumulative incidence of human papillomavirus (HPV) in a simulated cohort of 10,000 women, by transmission probability per coital act and male-to-female prevalence rate ratio (◇ = 0.50, □ = 0.67, ■ = 1.00, ▲ = 1.50, ● = 2.00). The empirically observed 95 percent confidence interval (0.114, 0.230) for the incidence among women in the McGill-Concordia Cohort Study, Montreal, Canada, 1996–2001, is dotted. Results assume no protective effect of condoms.

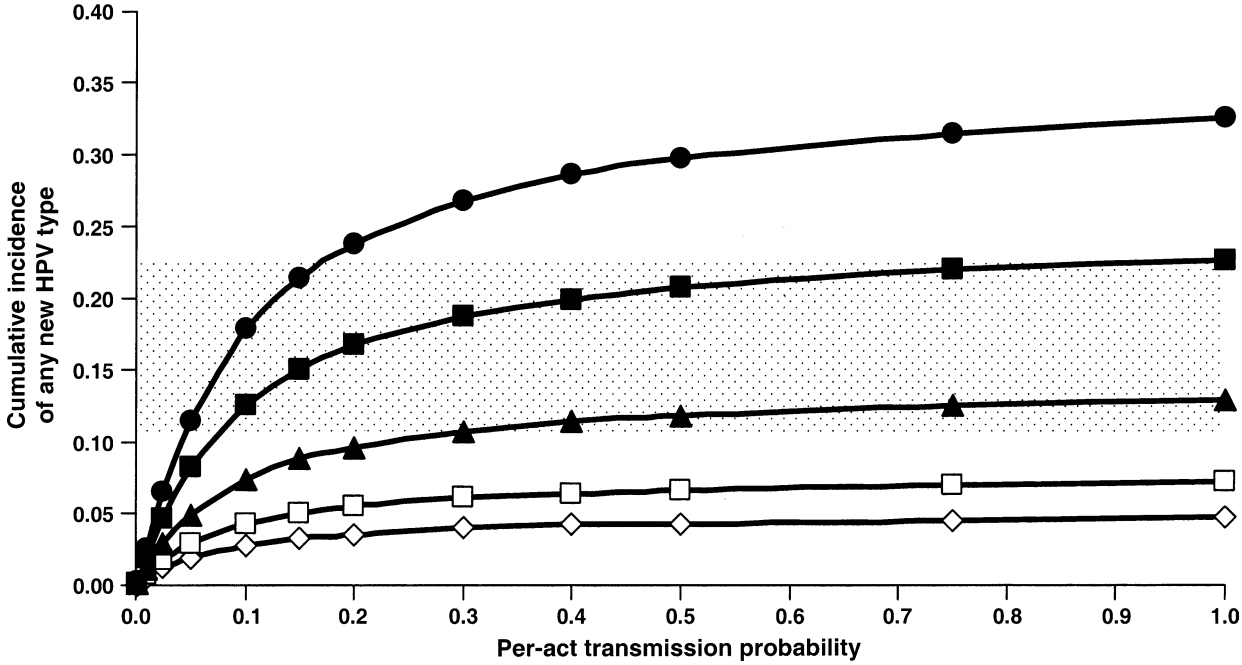

FIGURE 5. Expected cumulative incidence of human papillomavirus (HPV) in a simulated cohort of 10,000 women, by transmission probability per coital act and male-to-female prevalence rate ratio (◇ = 0.50, □ = 0.67, ■ = 1.00, ▲ = 1.50, ● = 2.00). The empirically observed 95 percent confidence interval (0.114, 0.230) for the incidence among women in the McGill-Concordia Cohort Study, Montreal, Canada, 1996–2001, is dotted. Results assume that condoms offer fourfold protection.

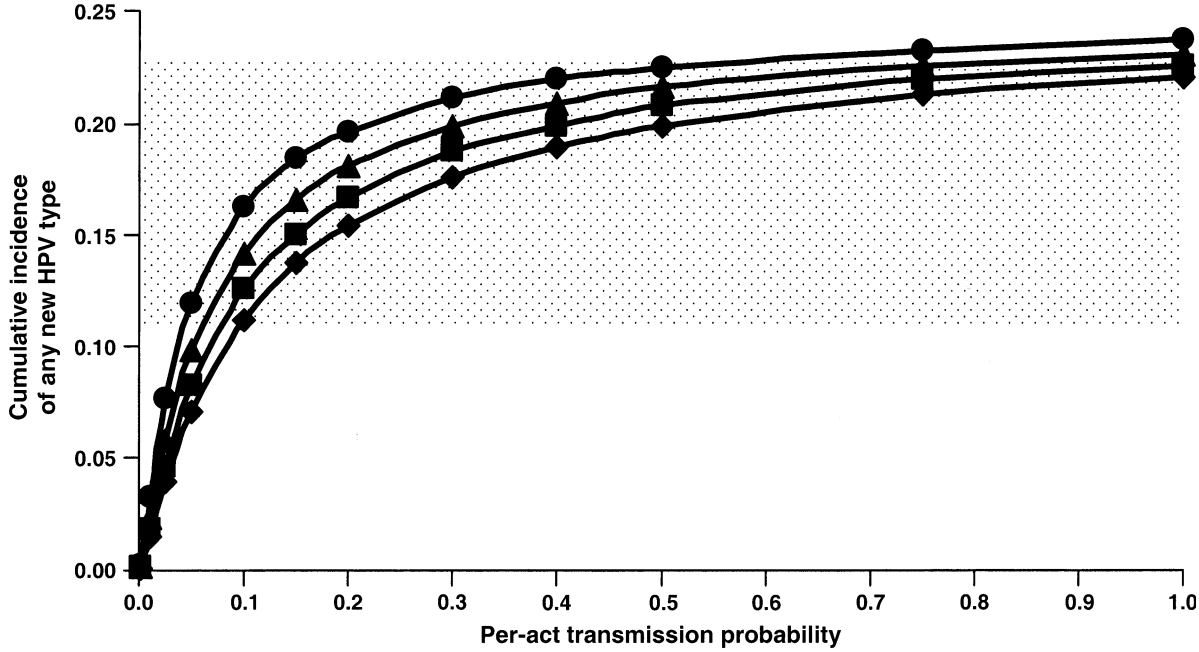

FIGURE 6. Expected cumulative incidence of human papillomavirus (HPV) in a simulated cohort of 10,000 women, by transmission probability per coital act and relative risk of transmission of HPV infection (◆ = 0.10, ■ = 0.25, ▲ = 0.50, ● = 1.00) for a protected versus an unprotected act. The empirically observed 95 percent confidence interval (0.114, 0.230) for the incidence among women in the McGill-Concordia Cohort Study, Montreal, Canada, 1996–2001, is dotted. Results assume that male prevalence is 1.5 times that among women.

simulations presented above assumed that regular condom use reported by women in the McGill-Concordia Cohort Study indicated use 75 percent of the time; results were similar when regular use was assumed to be 95 percent of the time (data not shown). Finally, random assignment of female HPV positivity at time $t - 1$, sexual frequency, and condom use frequency assumes that these factors are uncorrelated. To test this assumption, HPV incidence was simulated among the observed 238 new partner visits using reported data on female HPV positivity at time $t - 1$ and sexual and condom use frequency, and results were similar (data not shown).

DISCUSSION

The modeled HPV per-act transmission probabilities that were consistent with observed cumulative incidence among young female university students ranged from a lower limit of 5 percent per act to an upper limit of 100 percent per act. At the median, 40 percent per act, the probability of male-to-female transmission would reach virtually 100 percent with only 11 acts of intercourse. Per-act transmissibility values of less than 5 percent were inconsistent with the observed data.

The results suggest that HPV prevalence among male partners of this university student population in Montreal was equal to or greater than that among women. Other research of HPV prevalence in both sexes of the same university student population has reported slightly less to equivalent prevalence among males compared with females (7, 30, 31). In sexually transmitted disease clinic populations, higher prevalence was observed among males compared with females in Denmark and Greenland (32). However, comparison of sex-specific prevalence within the same population assumes that sexual networks are confined to that population. This assumption may not be true if female students have partners outside the student population. Partnership studies would be needed to verify the true infection status of women's partners.

STI transmission dynamics involve three distinct components: 1) transmissibility from an infected to an uninfected partner upon exposure, 2) the likelihood of sexual exposures between infected and uninfected persons, and 3) the duration of the infection (2). The first, transmissibility, can be measured empirically only in studies of couples (33, 34). One such study, conducted by Oriel (35), examined the transmission of genital warts before HPV was identified as the causal agent. Participants were patients at a hospital's venereology department in London, England. Sexual partners of the index patient in the 9-month period before and after the appearance of warts were recorded for 97 patients. Sixty percent (53/88) of the sexual partners of the index patients subsequently developed warts, suggesting high transmissibility.

To our knowledge, there have been no published reports of the transmissibility of HPV itself based on data from couples, but it is thought to be high (36, 37). Unlike most STIs, HPV is not concentrated in ''core groups''—small groups of highly sexually active individuals (2, 37). An epidemiologic pattern of high prevalence among moderately sexually active individuals may result from either a long duration of infectivity and/or high infectivity (37). There is evidence that the duration of HPV infection is short for women (3, 10, 24, 38), and the same may also be true for men (39). This evidence suggests that high transmissibility may explain the observed prevalence in most populations.

The estimated per-act transmission probabilities for HPV in this simulation study were high in comparison with other viral STI but were comparable to those presumed for bacterial STI. Studies of HIV-discordant couples indicate that the probability of HIV transmission is 1 per 1,000 acts of intercourse (28). This probability is believed to increase as much as 10-fold with high seminal viral load, which may occur during acute primary infection or when either partner is coinfected with other STIs (29, 40). Even in such circumstances, the range of plausible HPV per-act transmission probabilities indicates that HPV would still be considerably more infectious than HIV. Similarly, the probability of transmission of herpes simplex virus type 2 is estimated to be 1 per 1,000 acts among stable, long-standing couples (41). Transmission probabilities for other STIs are available; however, they are typically reported as the probability of transmission *per partnership*, not per coital act, and are considered an average across partnerships of varying duration. They range from 20 percent for *Chlamydia* and 50 percent for gonorrhea (42) to 60 percent for syphilis (43) and 80 percent for *Haemophilus ducreyi*, the infectious agent for genital ulcers (42). The higher rate of transmission of the latter two agents is related at least in part to the presence of genital ulcers that increase transmission of STI.

The present study used stochastic computer simulation to model HPV transmissibility. Deterministic models have also been developed for HPV, specifically to estimate the population impact of vaccination (36, 44). Hughes et al. (36) assumed a per-partner male-to-female transmission probability of 0.8 based on the epidemiology of HPV. Using Finnish HPV-16 seroprevalence data for calibration, Barnabas and Garnett (44) estimated a per-partner male-to-female transmission probability of 0.6 for that type. Both of these values are consistent with the range of per-act transmission probabilities deemed plausible in this simulation study.

The high per-act transmission probability estimated in this simulation study suggests that women exposed to an infected partner would acquire HPV within the first acts of intercourse. Consistent with high transmissibility, neither the frequency of sex nor the number of sex acts was associated with incident HPV infections among women in the McGill-Concordia cohort (data not shown).

This simulation study relied on the accuracy of the measured cumulative incidence of HPV in the Montreal cohort. In any given 6-month period in which women reported a single new partner, and no other partners, the cumulative incidence was 17.0 percent (95 percent confidence interval: 11.4, 23.0). This rate is consistent with that for women starting their first sexual relationship (45), where cumulative incidence of any type of HPV was 20 percent at 6 months following the first act of intercourse. A concern in any study of HPV among sexually experienced women is the possibility that ''incident'' infections may be reactivation of previously latent infections. In the McGill-Concordia Cohort

Study, such misclassification would have been uncommon since women were young (aged 18–24 years). Furthermore, the 6-month cumulative incidence among women who reported no sexual activity was nearly five times less, at 3.8 percent, than among women who reported a new partner.

Epidemiologic investigations of HPV also have to contend with sampling variability due to anatomic site chosen for the specimen, collection method, sample processing, and assay error. The McGill-Concordia Cohort Study used accepted methods for cell sampling and HPV testing. Nevertheless, sampling and assay variability is an issue that our simulation work did not address. Such variability is likely to become compounded in studies involving both partners, especially given that the sampling methods for males are evolving. Results from modeling may therefore be complementary to empirical studies of transmission.

Assumptions must be made in any simulation exercise, and this study was no exception. The simulation of couples assumed random mixing of men and women, at least with respect to HPV status. Surveys of sexual behavior show that mixing may not be random; rather, it may tend to be moderately assortative, such that ''like'' mix with ''like'' (2, 46). High rates of HPV even among moderately sexually active populations (37) suggest that an assumption of random mixing with respect to HPV status may not be untenable. Nevertheless, if substantial assortative mixing was present, our simulation would have resulted in an underestimation of per-act transmissibility.

This simulation assumed that couples remained together and that no partnerships dissolved. This assumption, if violated, would have led to an underestimate of transmissibility, but this bias was minimized by the short time interval for simulation (6 months). Furthermore, per-act transmission probabilities were presumed constant. It is possible that the risk of STI transmission varies with the number of acts, and future efforts to study transmissibility should examine this issue (47). The random assignment of female HPV positivity at time $t - 1$, sexual frequency, and condom use frequency in the hypothetical couples presumes that these variables are uncorrelated. Such correlations were not influential when they were simulated, nor did analysis of the cohort itself reveal correlation among these variables. It was also assumed that women who reported ''regular'' condom use had in fact used condoms 75 percent of the time. Regular use was not assumed to indicate 100 percent use of condoms; even among those who always use them, partial condom use can occur (i.e., not applying the condom before insertion, removing the condom sometime during intercourse, and condom breakage or slippage). As many as 38 percent of young heterosexual condom users report delaying application of the condom at least occasionally (48–50). Nevertheless, when the simulations were repeated assuming that regular use indicated use 95 percent of the time, the results were nearly equivalent.

Whether or not condoms provide any level of protection against HPV transmission remains a subject of debate (51). In vitro studies demonstrate that latex condoms are impermeable to all known sexually transmitted pathogens (52), although they cannot protect the entire surface of the genital epithelium from infection. HPV research has found equivocal results (27, 51). A paradoxical effect is occasionally reported, such that condom use appears to increase risk of HPV infection (5, 51, 53). Methodological issues that have limited the evaluation of condom effectiveness include imprecise measurement and the inability to distinguish with whom participants use condoms or the infection status of that partner (51, 54).

A critical implication of high transmissibility found in this simulation is that condoms may not offer effective protection over multiple acts of intercourse, which could explain an absence of observed effects in many empirical studies. A protective effect of condoms, even if one exists, is virtually lost with high infectivity (55). Simulated conditions in this study showed that high per-act transmission probabilities result in substantial transmission, even with a 10-fold protective effect of condoms. Although condoms may offer protection in relatively brief encounters involving few acts of intercourse, they would be ineffective in partnerships where multiple sex acts occur in an ongoing relationship. For example, if the true per-act transmission probability is 40 percent, transmission occurs within 11 acts of intercourse. If condoms reduce risk of transmission by half to 20 percent, then transmission would occur within 24 acts, which is within about 10 weeks according to the intercourse frequency reported by women in the McGill-Concordia Cohort Study.

That the simulation was unable to provide an estimate of the effect of condoms leads to study design considerations for observational studies of transmission among couples. It would not be possible to obtain more specific estimates of condom effectiveness using the McGill-Concordia study design, which was similar to other longitudinal studies of HPV in young women (3, 4, 7, 38). To obtain an estimate of the relative risk of infection for a protected versus an unprotected act, and to finely distinguish it from the per-act transmission probability, one would need to compare couples who always used condoms correctly with couples who never used them. Alternatively, one could study couples who engaged in few acts of sexual intercourse.

Fortunately, HPV vaccines are a promising alternative to condoms. Preliminary evidence from proof-of-principle trials shows great promise for vaccines against HPV-16 alone (56), HPV-16 and -18 (57), and HPV-6, -11, -16, and -18 (58). The findings of this simulation study provide a strong rationale for maximizing coverage of an HPV vaccine upon licensure and for considering the benefits of extending vaccination to young men before they engage in sexual activity. A second implication is that high transmissibility will magnify the impacts of poor vaccine coverage, poor ''take,'' or waning of immunity over time. Close monitoring of population coverage and vaccine effectiveness over time will be necessary. A first generation of validated natural history models has been used to assess the potential impact of changes in these parameters on long-term vaccine efficacy (21–23). However, these Markov models have been built exclusively on the basis of probabilistic assumptions consistent with findings from epidemiologic studies of the natural history of HPV and cervical neoplasia in women. The approach described here may provide the HPV transmissibility framework that could be incorporated into these

models to enhance their ability to make projections of vaccine efficacy under a wider range of scenarios than has been possible with the first-generation models.

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From: Eduardo Franco [eduardo.franco@mcgill.ca]
Sent: March 5, 2009 3:52 AM
To: Ann Burchell, Mrs
Cc: Candida Pizzolongo, Mrs.
Subject: Permission

Dear Ann,

As a coauthor of the following papers, I hereby grant permission to you to include the following papers in your PhD thesis:

"Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner"

and

"Distribution and genotype concordance of human papillomavirus infections among couples in new sexual relationships".

This email will be placed in your personnel file.

Best wishes,

EF

Eduardo L. Franco, Professor of Epidemiology and Oncology Director, Division of Cancer Epidemiology, McGill University
546 Pine Avenue West, Montreal, QC, Canada H2W1S6
Phone: 1-514-398-6032, Fax: 1-514-398-5002

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Département d'épidémiologie, biostatistique et santé au travail

1020 Pine Avenue West
Montreal (Quebec) Canada H3A 1A2

Fax: (514) 398-4503

March 5, 2009

To whom it may concern:

I grant permission to Ann Burchell to include the following papers in her PhD thesis, for which I am a co-author:

> Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner;
>
> Distribution and genotype concordance of human papillomavirus infections among couples in new sexual relationships.

Please do not hesitate to contact me if you require further information.

Yours sincerely,

James A. Hanley, Ph.D.
Professor

Ann Burchell, Mrs

From: Pierre-Paul Tellier, Dr.
Sent: March 5, 2009 11:15 AM
To: Ann Burchell, Mrs; Eduardo Franco, Dr.
Cc: Candida Pizzolongo, Mrs.
Subject: Waiver allowing Ann Burchell to include HITCH papers in my thesis

Good day

"I grant permission to Ann Burchell to include the following papers in her PhD thesis, for which I am a co-author: Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner; and Distribution and genotype concordance of human papillomavirus infections among couples in new sexual relationships."

Best of luck!

Pierre-Paul Tellier MD
Associate Professor Family Medicine

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Montréal, le 8 mars 2009

M^me^ Ann Burchell

Dear Ann,

I grant permission to Ann Burchell to include the following papers in her PhD thesis, for which I am a co-author: Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner; and Distribution and genotype concordance of human papillomavirus infections among couples in new sexual relationships.

François Coutlée, M.D., FRCP, CSPQ
Microbiologiste-Infectiologue Tél.: 514-890-8000, poste 25162 Fax.: 514-412-7512

Appendix C

Enrolment questionnaires (paper format)

FEMALE RESPONDENT
ENROLMENT

Thank you very much for agreeing to complete this survey for the HITCH Cohort Study. Your help will ensure that the study will be able to answer questions about how HPV is transmitted, how much risk there is after a sexual encounter, and what women and men can do to protect themselves.

The survey will ask questions about you, your health and sexual history, recent sexual behaviour, and your knowledge of and attitudes toward human papillomavirus (HPV). It should take about 30 minutes to complete. Please use a pencil to write your answers. Most questions require that you simply circle the response that applies to you. Other questions ask for a specific answer, such as your age, a date, or another number. Depending on your answer for some questions, you may be told to skip past some questions or go to a different part of the questionnaire. Please read these skip instructions carefully. They are to save you time so that you won't have to answer questions that do not apply to you.

The HITCH Cohort Study enrolls couples who recently initiated a sexual relationship. A number of questions will ask about the partner who enrolled with you. Please refer to him for all questions that mention your "HITCH partner".

There are no right or wrong answers to any question. Since we will be using this survey with many people with different experiences, you may find that some of the questions do not seem to apply to you. Other questions will definitely be relevant. Many questions ask you to think back over your adult years, or over the past several months, to recall specific information. Please take your time to consider each question carefully.

Remember that all your answers are completely confidential. You can leave blank any question that you do not want to answer. If you cannot possibly remember the information, you can also leave the question blank, but we encourage you to try to answer all questions. A good guess is always better than no information at all. If you would like to tell us more about any specific items, please use the available space at the end of the questionnaire.

Let's begin!

Please record your HITCH ID number, today's date, and the time you started filling out the survey here.

ID number: _____

Today's date: _____

Time at start of survey: _____

General Information

The first part of the questionnaire concerns general information about you and where you live.

1. What is your date of birth?

dd	*mm*	*yyyy*

2.a) In what country where you born?

country

→ b) If born in Canada, indicate province.

province

3. What is your current marital status? Please circle your answer.

1	*Single/never married*
2	*Unmarried but living with a partner*
3	*Married*
4	*Divorced/separated*
5	*Widowed*

4. The Montreal area is made up of many ethnic groups. We would like to know in which group you would place yourself. Circle the most appropriate category.

1	*French Canadian*
2	*English Canadian*
3	*Black Canadian*
4	*Aboriginal*
5	*Latin American*
6	*Greek*
7	*Italian*
8	*South Asian*
9	*East Asian*
0	*Other (specify)*

5.a) What is/was your father's (or primary male caregiver's) highest level of education that he completed?

1	*No formal education*
2	*Grade 8 or less*
3	*Some high school*
4	*High school graduate*
5	*Some community college or CEGEP*
6	*Community college or CEGEP graduate*
7	*Some university*
8	*University graduate (including undergraduate, graduate and postgraduate studies)*
0	*Other (specify)*

b) What is/was your mother's (or primary female caregiver's) highest level of education that she completed?

1	*No formal education*
2	*Grade 8 or less*
3	*Some high school*
4	*High school graduate*
5	*Some community college or CEGEP*
6	*Community college or CEGEP graduate*
7	*Some university*
8	*University graduate (including undergraduate, graduate and postgraduate studies)*
0	*Other (specify)*

c) On average, would you say that your family's financial situation while growing up was…

1	*Difficult*
2	*Moderate*
3	*Comfortable*
4	*Very comfortable*

d)Are you presently enrolled at McGill/Concordia or at another educational institution?

0	*No*
1	*Yes (specify)*

→ ***If no, go to question 5f.***

e) How are you presently enrolled as a student?

1	*Undergraduate student*
2	*Graduate studies – Diploma, Master's, or Doctoral Program*
3	*Community college or CEGEP student*
0	*Other (e.g. Trainee, Postdoctoral studies, Sabbatical) (specify)*

f) What is the highest level of education that you have completed?

1	*No formal education*
2	*Grade 8 or less*
3	*Some high school*
4	*High school graduate*
5	*Some community college or CEGEP*
6	*Community college or CEGEP graduate*
7	*Some university*
8	*University graduate (including undergraduate, graduate and postgraduate studies)*
0	*Other (specify)*

g) What is your current employment status? Circle one only.

1	*Working full time (30 hours/week or more)*
2	*Working part time (<30 hours/week)*
3	*Not working due to full-time studies*
4	*On parental leave*
5	*Looking for work*
6	*Temporarily off sick*
7	*No longer able to work*
8	*No longer wish to work*
9	*Homemaker*
0	*Other (specify)*

6. How long have you lived in Montreal?

	OR	
# months		*# years*

Smoking History

The following questions are about your tobacco smoking habits.

7. Have you smoked a total of at least 100 cigarettes (4 or more packs) in your lifetime?

0	*No*
1	*Yes*

→ ***If no, go to question 12.***

8. Have you ever smoked cigarettes regularly, that is, one cigarette or more each day for a year or more?

0	*No*
1	*Yes*

→ ***If no, go to question 12.***

9. At what age did you start to smoke regularly?

Age in years

10.a) Do you still smoke regularly?

0	*No*
1	*Yes*

If no →

b) At what age did you stop smoking regularly?

Age in years

11. On average, how many cigarettes have you smoked a day since you began smoking regularly? (If you have stopped smoking regularly, please consider only those periods during which you were smoking regularly).

 # cigarettes per day

Reproductive History

12. At what age did you have your first menstrual period?

Age in years

13. To the best of your knowledge, are you currently pregnant?

0	*No*
1	*Yes*
7	*Do not know*

14.a) Have you ever been pregnant? (If you are currently pregnant, please answer yes.)

0	*No*
1	*Yes*

→ ***If no, go to Lifetime Sexual History on page 7.***

b) How many times? (If you are currently pregnant, include this pregnancy.)

 # pregnancies

Lifetime Sexual History

The next questions are about your sexual history. We realize this is a personal subject, but it is very important to the study of HPV. Please take the time to recall this information as accurately as possible. Remember that all the information you give will be kept entirely confidential.

Throughout this survey, we will refer to various specific sexual acts. These terms are explained below so that everyone attaches the same meanings to them. Please be sure to read these definitions. If you need any further help or explanation, please ask the Research Nurse.

partners or sexual partners:	People who have had sex together—whether once, or just a few times, or as regular partners, or as married partners
genital area:	A man's penis or a woman's vulva and vagina—that is, the sex organs
oral sex:	A man's or a woman's mouth on a partner's genital area
vaginal sex or vaginal sexual intercourse:	A man's penis in a woman's vagina. This is what most people usually think of as "having sex" or "sexual intercourse"
anal sex or anal sexual intercourse:	A man's penis in a sexual partner's anus or rectum
mutual masturbation:	Hand stimulation of a (*woman/man's*) genital area by (*his/her*) partner, NOT involving intercourse (vaginal, oral, or anal)
sexual activity:	Mutual masturbation, oral sex, vaginal sex, or anal sex
sexual intercourse:	This includes oral, vaginal, and anal sex

15.a) Please think about all the people with whom you have engaged in sexual intercourse (oral, vaginal or anal). In total, with how many people—male or female—have you engaged in sexual intercourse in your lifetime?

☐
Approximate #

b) How many were male?

☐
Approximate #

c) How many were female?

☐
Approximate #

16. How old were you when you first had vaginal sexual intercourse?

☐ *OR* ☐
Age in years *Check here if never had vaginal sexual intercourse*

→ ***If never, go to question 18.***

17. Throughout your life, what is the number of male partners with whom you have had vaginal sexual intercourse?

☐
Approximate #

18. Do you consider yourself to be:

1	*Heterosexual/straight*
2	*Bisexual*
3	*Lesbian/homosexual*
0	*Other (specify)*

Sexual Activity with Enrolled HITCH Partner

The next questions are about the male partner who enrolled in HITCH with you. We will refer to him as your "HITCH partner".

19. What are his initials? (If you prefer, you can use an alias or nickname for this partner. Please choose one that you will remember later.)

Initials/Alias

20. What is his date of birth?

dd	*mm*	*yyyy*

21.a) Is he your…

1	*Husband*
2	*Common-law or live-in partner (living together)*
3	*Dating partner/boyfriend*
4	*Friend*
5	*Casual acquaintance*
6	*Not sure –we just met*
0	*Other (specify)*

b) Do you consider your sexual relationship with him to be…

1	*Ongoing and steady/regular*
2	*Ongoing but sporadic/on and off*
3	*One or a few times only*
0	*Other (specify)*

22. When did you first engage in sexual activity with him? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex. (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

23. Have you and your HITCH partner ever discussed the following since the start of your sexual relationship?

	No	*Yes*	*Do not remember*
i) Pregnancy prevention	*0*	*1*	*7*
ii) Sexually transmitted disease prevention	*0*	*1*	*7*
iii) **His** sexual history	*0*	*1*	*7*
iv) **Your** sexual history	*0*	*1*	*7*
v) Whether **he** ever had a sexually transmitted disease	*0*	*1*	*7*
vi) Whether **you** ever had a sexually transmitted disease	*0*	*1*	*7*
vii) Whether **he** had ever been tested for sexually transmitted diseases (including HIV/AIDS)	*0*	*1*	*7*
viii) Whether **you** had ever been tested for sexually transmitted diseases (including HIV/AIDS)	*0*	*1*	*7*

24. To the best of your knowledge…

a) …what is the number of female partners with whom he has had vaginal intercourse in his lifetime, including you (if applicable)?

☐ *Approximate #* *OR* ☐ *Check here if do not know*

b) …has he ever had a sexually transmitted infection (e.g., chlamydia, gonorrhea, syphilis, genital herpes, pubic lice, HIV, hepatitis B)?

0	*No*
1	*Yes*
7	*Do not know*

c) …is he circumcised?

0	*No*
1	*Yes*
7	*Do not know*

The next series of questions are about sexual activities you may have engaged in with your HITCH partner since you first started your sexual relationship.

25. Since the start of your sexual relationship with your HITCH partner, how many times did you engage in sexual activities with him? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex.

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*	

During those sexual encounters…

26. …how often did you masturbate him?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

27. …how often did he masturbate you?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

28. …how often did you give him oral sex?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

29. …how often did he give you oral sex?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

30.a) Have you ever had vaginal intercourse with your HITCH partner?

0	*No*
1	*Yes*

→ ***If no, go to question 33.***

b) When did you first have vaginal intercourse with him? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

c) When was the last time you had vaginal intercourse with him?

dd	*mm*	*yyyy*

d) Since the start of your sexual relationship with your HITCH partner, how many times did you have vaginal intercourse with him?

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*

31. How often did you use condoms for vaginal intercourse with him? (This includes male and female condoms.)

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

→ ***If never, go to question 33.***

32. **When you used condoms** for vaginal intercourse with your HITCH partner…

a) …did the condom ever break or slip off?

0	*No*
1	*Yes*
7	*Do not remember*

b) …did you always put the condom on before starting to have vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

c) …did you ever take the condom off then continue to have unprotected vaginal intercourse with him?

0	*No*
1	*Yes*
7	*Do not remember*

33. Have you ever had anal intercourse with your HITCH partner?

0	*No*
1	*Yes*

Sexual Activity with Other Partners

The next questions are about sexual activities you may have engaged in with someone other than your HITCH partner.

34. Since the start of your relationship with your HITCH partner, did you engage in sexual activity with someone else? **Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex.**

0	*No*
1	*Yes*

→ ***If yes, go to question 36.***

35. Is your HITCH partner the only person with whom you have ever engaged in sexual activity **in your lifetime**?

0	*No*
1	*Yes*

→ ***If yes, go to question 39 on page 16.***

→ ***If no, complete one pink OP Form for the last person with whom you engaged in sexual activity before your HITCH partner, then go to question 39 on page 16.***

<u>Do not answer questions 36-38.</u>

36. Since the start of your relationship with your HITCH partner, how many **other** sexual partners did you have?

Approximate #

→ ***If 5 or fewer other partners, complete a pink OP Form for each of these partners, then go to question 39 on page 16.***
<u>Do not answer questions 37-38.</u>

→ ***If more than 5 other partners, advance to question 37.***

37. Since the start of your relationship with your HITCH partner, how many **other** sexual partners were ongoing sexual partners? That is, partners with whom you had an **ongoing sexual relationship** (e.g. dating partner, husband, common-law partner)?

Approximate #

→ ***Complete a pink OP Form for each of these partners, then advance to question 38.***

38. Since the start of your relationship with your HITCH partner, how many **other** sexual partners were sexual partners with whom you did **<u>not</u> have an ongoing sexual relationship**? (e.g. one-night stands or flings)?

Approximate #

→ ***Complete one purple AP Form for all of these partners combined, then advance to question 39.***

Contraceptive History

Here we would like to know about methods of birth control or family planning that you and your partner(s) may have used **in your lifetime**.

39. The following is a list of common birth control methods. Please read the list and indicate if you have **ever** used any of them **in your lifetime**.

	Never used it	*Used it but not regularly*	*Used it regularly (at least 75% of the time for 3 or more consecutive months)**
i) Mirena, an intrauterine device (IUD) with progestin	*0*	*1*	*2*
ii) Loop, coil, or other intrauterine device (IUD), not including Mirena	*0*	*1*	*2*
iii) Hormonal contraceptive (e.g. birth control pill, Depo-Provera injections, vaginal ring, Norplant, the patch), not including Mirena	*0*	*1*	*2*
iv) Condom	*0*	*1*	*2*
v) Spermicide foam, jelly, cream, or suppository	*0*	*1*	*2*
vi) Diaphragm	*0*	*1*	*2*
vii) Cervical cap	*0*	*1*	*2*
viii) Sponge	*0*	*1*	*2*
ix) Vaginal douche	*0*	*1*	*2*
x) Rhythm, calendar, or natural method	*0*	*1*	*2*
xi) Withdrawal/pulling out	*0*	*1*	*2*
xii) Emergency contraception (the "morning-after pill")	*0*	*1*	*2*

*If you first engaged in vaginal intercourse less than 3 months ago, then consider regular use as use at least 75% of the time since you began having vaginal intercourse.

→ ***If no use of Mirena (i) or hormonal contraceptives (iii), go to question 42.***

40. How old were you when you first used a hormonal contraceptive (e.g. birth control pill, Depo-Provera injections, vaginal ring, Mirena)?

Age in years

41. For how long did you use hormonal contraceptives? Add together all periods during which you took any hormonal contraceptive.

	OR	
# months		*# years*

Next we would like to know about methods of birth control or family planning that you and your partner(s) may have used **since the start of your sexual relationship with your HITCH partner**.

42. During that time, did you use any protection to keep from getting pregnant? (For example, hormonal contraceptives, spermicides, condoms, the rhythm method.)

0	*No*
1	*Yes*

→ ***If no, go to question 44.***

43. Which of the following birth control methods have you used since the start of your sexual relationship with your HITCH partner?

	Did not use it	*Used it but not regularly*	*Used it regularly (at least 75% of the time)*
i) Mirena, an intrauterine device (IUD) with progestin	*0*	*1*	*2*
ii) Loop, coil, or other intrauterine device (IUD) (not including Mirena)	*0*	*1*	*2*
iii) Hormonal contraceptive (e.g. birth control pill, Depo-Provera injections, vaginal ring, Norplant, the patch), not including Mirena	*0*	*1*	*2*
iv) Condom	*0*	*1*	*2*
v) Spermicide foam, jelly, cream, or suppository	*0*	*1*	*2*
vi) Diaphragm	*0*	*1*	*2*
vii) Cervical cap	*0*	*1*	*2*
viii) Sponge	*0*	*1*	*2*
ix) Vaginal douche	*0*	*1*	*2*
x) Rhythm, calendar, or natural method	*0*	*1*	*2*
xi) Withdrawal/pulling out	*0*	*1*	*2*
xii) Emergency contraception (the “morning-after pill”)	*0*	*1*	*2*

Medical History

The next questions refer to your medical history.

44. The Pap test (sometimes called a Pap smear) is a way to examine cells collected from the cervix (the lower, narrow end of the uterus). The main purpose of the Pap test is to find abnormal cell changes that may arise from cervical cancer or before cancer develops. Doctors and other specially trained health care professionals may perform Pap tests during a pelvic exam. While a woman lies on an exam table, the clinician inserts a speculum into her vagina to widen it. A sample of cells is taken from the cervix with a wooden scraper and/or a small cervical brush. The specimen (or smear) is placed on a glass slide and preserved with a fixative, or is rinsed in a vial of fixative, and is sent to a laboratory for examination.

    Since you became sexually active, how many times have you had a Pap test?

0	*Never*
1	*Once*
2	*2-3 times*
3	*4-5 times*
4	*6-10 times*
5	*More than 10 times*

→ ***If never, go to question 46.***

45. When was your last Pap test?

mm	*yyyy*

46. Did a doctor **ever** tell you that you had one of the following conditions?

				→ **If yes:** Were you told this since the start of your sexual relationship with your HITCH partner?		
	No	*Yes*	*Don't remember*	*No*	*Yes*	*Don't remember*
i) Trichomonas vaginal infection	*0*	*1*	*7*	*0*	*1*	*7*
ii) Venereal warts, condylomas, or papilloma virus infection	*0*	*1*	*7*	*0*	*1*	*7*
iii) Chlamydia	*0*	*1*	*7*	*0*	*1*	*7*
iv) Genital herpes	*0*	*1*	*7*	*0*	*1*	*7*
v) Syphilis	*0*	*1*	*7*	*0*	*1*	*7*
vi) Gonorrhea	*0*	*1*	*7*	*0*	*1*	*7*
vii) Ulcers or genital sores	*0*	*1*	*7*	*0*	*1*	*7*
viii) HIV	*0*	*1*	*7*	*0*	*1*	*7*
ix) Hepatitis B	*0*	*1*	*7*	*0*	*1*	*7*
x) Ureaplasma hominis	*0*	*1*	*7*	*0*	*1*	*7*
xi) Vaginal yeast infection, thrush, or candidiasis	*0*	*1*	*7*	*0*	*1*	*7*
xii) Bacterial vaginosis	*0*	*1*	*7*	*0*	*1*	*7*

47. Since the start of your sexual relationship with your HITCH partner, did you have any of the following signs/symptoms?

	No	*Yes*	*Don't remember*
i) Painful urination, or difficulty urinating, or frequent urination	*0*	*1*	*7*
ii) Itching or burning sensation when urinating	*0*	*1*	*7*
iii) Blood in urine	*0*	*1*	*7*
iv) Abnormal vaginal discharge (i.e. different colour, consistency, or odor)	*0*	*1*	*7*
v) Sores in the genital area	*0*	*1*	*7*
vi) Unusually painful or heavy period	*0*	*1*	*7*
vii) Vaginal itching or burning	*0*	*1*	*7*
viii) Lower back pain not caused by physical exertion	*0*	*1*	*7*

Knowledge of HPV

This section is about your knowledge of and attitudes towards HPV. Please remember that you can speak with the Research Nurse after completing the survey if you have questions about HPV, cervical cancer, or penile cancer.

48. Before enrolling in the HITCH Cohort Study, had you ever heard of human papillomavirus, or HPV?

0	*No*
1	*Yes*

49. Please indicate whether the following statements are TRUE or FALSE.

		True	*False*	*Don't know*
i)	HPV can cause cervical cancer in women	*1*	*2*	*7*
ii)	Men can carry HPV	*1*	*2*	*7*
iii)	Genital warts cause cervical cancer in women	*1*	*2*	*7*
iv)	HPV can be cured with antibiotics	*1*	*2*	*7*
v)	A person may be infected with HPV and not know it	*1*	*2*	*7*
vi)	HPV can cause penile cancer in men	*1*	*2*	*7*
vii)	HPV causes genital herpes	*1*	*2*	*7*
viii)	Condoms protect against HPV	*1*	*2*	*7*
ix)	Having multiple sex partners increases one's risk for HPV	*1*	*2*	*7*
x)	Regular Pap tests can help to prevent complications from HPV	*1*	*2*	*7*
xi)	HPV is the most common sexually transmitted infection	*1*	*2*	*7*

50. Please indicate whether the following statements are TRUE or FALSE.
A person can get HPV from…

		True	*False*	*Don't know*
i)	Sharing a plate, fork, or glass with someone who has HPV	*1*	*2*	*7*
ii)	Unprotected sexual intercourse with a someone who has HPV	*1*	*2*	*7*
iii)	Oral sex with someone who has HPV	*1*	*2*	*7*
iv)	Kissing (with exchange of saliva) someone who has HPV	*1*	*2*	*7*
v)	Sharing a washroom or shower with someone who has HPV	*1*	*2*	*7*

51. What do you think are your chances of becoming infected with HPV?

1	*Almost certain I will not*
2	*Very small chance*
3	*Some chance*
4	*Large or very large chance*
5	*Almost certain that I will get infected*
6	*I am already infected*

52. What do you think are your chances of developing cervical cancer?

1	*Almost certain I will not*
2	*Very small chance*
3	*Some chance*
4	*Large or very large chance*
5	*Almost certain that I will get develop cervical cancer*
6	*I have already been diagnosed with cervical cancer*

HPV Vaccine

The last set of questions are about HPV vaccines. In the summer of 2006, an HPV vaccine became available for young women in Canada. Prior to this time, the vaccine was only available to women who were participating in clinical trials.

53.a) Have you received the HPV vaccine?

0	*No*
1	*Yes*
7	*Don't know*

→ ***If no or don't know, go to question 54.***

b) Did you receive the vaccine as part of participation in a clinical trial?

0	*No*
1	*Yes*
7	*Don't know*

c) How many injections of the HPV vaccine have you received, including booster shots?

#

d) When was your last injection of the HPV vaccine? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

→ ***Go to question 55.***

54. If the HPV vaccine is offered to you in the future, how likely is it that you will choose to be vaccinated?

1	*Very likely*
2	*Somewhat likely*
3	*Neutral*
4	*Somewhat unlikely*
5	*Very unlikely*

55. Please use the space below if you have any additional information you feel would be important for us to know.

Please record the time you stopped filling out the survey here.

Time finished survey: _____

This brings us to the end of this survey. Please take a moment to review you answers in all sections of the questionnaire. Again, try to answer all questions. A good guess will be more useful to the study than leaving the question blank.

Thank you very much for your participation!

OTHER PARTNER (OP) FORM:
FEMALE RESPONDENT AT ENROLLMENT

Please record your HITCH ID number, today's date, and the number for this OP form (e.g., form 2 of 3 if this is the second OP form out of 3 completed).

ID number: _____

Today's date: _____

OP Form number: _____ **of** _____

If instructed to fill out a pink OP form at question 35:

➔ Complete one pink OP form for the last person with whom you engaged in sexual activity before your HITCH partner.

If instructed to fill out pink OP form(s) at question 36:

➔ Complete one pink OP form for each sexual partner you reported in question 36, to a maximum of 5 pink forms.

If instructed to fill out pink OP form(s) at question 37:

➔ Complete one pink OP form for each sexual partner you reported in question 37, to a maximum of 5 pink forms.

OP1. Was this sexual partner…

1	*Male*
2	*Female*
3	*Transgendered male (female to male)*
4	*Transgendered female (male to female)*

If your sexual partner was transgendered, please respond to the remaining questions based on the anatomy of your partner. For example, if your partner identified as male, but had female genitals, respond to the following questions as if this sexual partner were female.

OP2. What are his/her initials? (If you prefer, you can use an alias or nickname for this partner. Please choose one that you will remember later.)

Initials/Alias

OP3. What is his/her date of birth?

dd	*mm*	*yyyy*

OR

Approximate age in years

OP4.a) Is/was he/she your…

1	*Husband*
2	*Common-law or live-in partner (living together)*
3	*Dating partner/boyfriend/girlfriend*
4	*Friend*
5	*Casual acquaintance*
6	*Not sure–we just met*
7	*Ex-husband*
8	*Ex-common-law partner*
9	*Ex-dating partner*
10	*Client partner for commercial sex (i.e., he/she paid you money to have sex with you)*
11	*Commercial sex worker (i.e., you paid him/her money to have sex with you)*
0	*Other (specify)*

b) Do you consider your sexual relationship with him/her to be…

1	*Ongoing and steady/regular*
2	*Ongoing but sporadic/on and off*
3	*One or a few times only*
4	*Our sexual relationship was ongoing but has now ended*
0	*Other (specify)*

→ ***If sexual partner was female, go to OP6.***

OP5. To the best of your knowledge…

a) …what is the number of female partners with whom he has had vaginal intercourse in his lifetime, including you (if applicable)?

☐ *Approximate #* *OR* ☐ *Check here if do not know*

b) …has he ever had a sexually transmitted infection (e.g. chlamydia, gonorrhea, syphilis, genital herpes, pubic lice, HIV, hepatitis B)?

0	*No*
1	*Yes*
7	*Do not know*

c) …was he circumcised?

0	*No*
1	*Yes*
7	*Do not know*

OP6.a) When did you first engage in sexual activity with him/her? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex. (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

b) When was the last time you engaged in sexual activity with him/her?

dd	*mm*	*yyyy*

c) How many times in total did you engage in sexual activity with him/her?

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*

d) How many times did you engage in sexual activity with him/her since the start of your relationship with your HITCH partner? (If you did not engage in sexual activity with him/her during that time, please answer zero.)

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*

→ ***If partner is female, end OP Form and return to the main, white questionnaire.***

OP7.a) Have you ever had vaginal intercourse with him?

0	*No*
1	*Yes*

→ ***If no, go to OP10.***

b) When did you first have vaginal intercourse with him? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

c) When was the last time you had vaginal intercourse with him?

dd	*mm*	*yyyy*

d) How many times in total did you have vaginal intercourse with him?

	OR		*OR*	
Approximate #		*Approximate # times per week*		*Approximate # times per month*

e) How many times did you have vaginal intercourse with him since the start of your relationship with your HITCH partner? (If you did not have vaginal intercourse with him during that time, please answer zero.)

	OR		*OR*	
Approximate #		*Approximate # times per week*		*Approximate # times per month*

OP8. How often did you use condoms for vaginal intercourse with him? (This includes male and female condoms.)

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

→ ***If never, go to OP10.***

OP9. **When you used condoms** for vaginal intercourse with him …

a) …did the condom ever break or slip off?

0	*No*
1	*Yes*
7	*Do not remember*

b) …did you always put the condom on before starting to have vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

c) …did you ever take the condom off then continue to have unprotected vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

OP10. Have you ever had anal intercourse with him?

0	*No*
1	*Yes*

END OF PINK OP FORM

Please return to the main, white questionnaire

AGGREGATED PARTNERS (AP) FORM:
FEMALE RESPONDENT AT ENROLMENT

Please record your HITCH ID number and today's date.

ID number: _____

Today's date: _____

Please complete the following questions for the sexual partner(s) you reported in **question 38**.

AP1. How many were…

	Approximate #
i) male	
ii) female	
iii) Transgendered male (female to male)	
iv) Transgendered female (male to female)	

If a sexual partner was transgendered, please respond to the remaining questions based on their anatomy. For example, if your partner identified as male, but had female genitals, respond to the following questions as if this sexual partner were female.

AP2. How many were your…

	Approximate #
i) husband	
ii) common-law or live-in partner (living together)	
iii) dating partner/boyfriend/girlfriend	
iv) casual acquaintance	
v) ex-husband	
vi) ex-common-law partner	
vii) ex-dating partner	
viii) client partner for commercial sex (i.e., they paid you money to have sex with you)	
ix) commercial sex worker (i.e., you paid them money to have sex with you)	

AP3. Since the start of your relationship with your HITCH partner, how many times did you engage in sexual activities with these **other sexual partners**? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex.

	Approximate #	*OR*		*OR*	
			Approximate # times per week		*Approximate # times per month*

→ ***If all partners were female, end purple AP form and return to main, white questionnaire.***

AP4.a) Since the start of your relationship with your HITCH partner, did you engage in **vaginal intercourse** with any of these **other** male partners?

0	*No*
1	*Yes*

→ ***If no, go to AP8.***

b) With how many did you engage in vaginal intercourse?

Approximate #

c) Since the start of your relationship with your HITCH partner, how many times did you have vaginal intercourse with these **other** partners?

	Approximate #	*OR*		*OR*	
			Approximate # times per week		*Approximate # times per month*

d) When was the last time you had vaginal intercourse with any of these **other** partners?

dd	*mm*	*yyyy*

AP5. Since the start of your relationship with your HITCH partner, how often did you use condoms for vaginal intercourse with these **other** partners? (This includes male and female condoms.)

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

→ ***If never, go to AP7.***

AP6. **When you used condoms** for vaginal intercourse with these **other** partners…

a) …did the condom ever break or slip off?

0	*No*
1	*Yes*
7	*Do not remember*

b) …did you always put the condom on before starting to have vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

c) …did you ever take the condom off then continue to have unprotected vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

AP7. Since the start of your relationship with your HITCH partner, did you have **anal intercourse** with any of these **other** male partners?

0	*No*
1	*Yes*

END OF PURPLE AP FORM

Please go to question 39 in the main, white questionnaire.

MALE RESPONDENT

Thank you very much for agreeing to complete this survey for the HITCH Cohort Study. Your help will ensure that the study will be able to answer questions about how HPV is transmitted, how much risk there is after a sexual encounter, and what men and women can do to protect themselves.

The survey will ask questions about you, your health and sexual history, recent sexual behaviour, and your knowledge of and attitudes toward human papillomavirus (HPV). It should take about 30 minutes to complete. Please use a pencil to write your answers. Most questions require that you simply circle the response that applies to you. Other questions ask for a specific answer, such as your age, a date, or another number. Depending on your answer for some questions, you may be told to skip past some questions or go to a different part of the questionnaire. Please read these skip instructions carefully. They are to save you time so that you won't have to answer questions that do not apply to you.

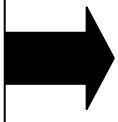
The HITCH Cohort Study enrolls couples who recently initiated a sexual relationship. A number of questions will ask about the partner who enrolled with you. Please refer to her for all questions that mention your "HITCH partner".

There are no right or wrong answers to any question. Since we will be using this survey with many people with different experiences, you may find that some of the questions do not seem to apply to you. Other questions will definitely be relevant. Many questions ask you to think back over your adult years, or over the past several months, to recall specific information. Please take your time to consider each question carefully.

Remember that all your answers are completely confidential. You can leave blank any question that you do not want to answer. If you cannot possibly remember the information, you can also leave the question blank, but we encourage you to try to answer all questions. A good guess is always better than no information at all. If you would like to tell us more about any specific items, please use the available space at the end of the questionnaire.

Let's begin!

Please record your HITCH ID number, today's date, and the time you started filling out the survey here.



ID number: _____

Today's date: _____

Time at start of survey: _____

General Information

The first part of the questionnaire concerns general information about you and where you live.

1. What is your date of birth?

dd	*mm*	*yyyy*

2.a) In what country where you born?

country

→ b) If born in Canada, indicate province.

province

3. What is your current marital status? Please circle your answer.

1	*Single/never married*
2	*Unmarried but living with a partner*
3	*Married*
4	*Divorced/separated*
5	*Widowed*

4. The Montreal area is made up of many ethnic groups. We would like to know in which group you would place yourself. Circle the most appropriate category.

1	*French Canadian*
2	*English Canadian*
3	*Black Canadian*
4	*Aboriginal*
5	*Latin American*
6	*Greek*
7	*Italian*
8	*South Asian*
9	*East Asian*
0	*Other (specify)*

5. a) What is/was your father's (or primary male caregiver's) highest level of education that he completed?

1	*No formal education*
2	*Grade 8 or less*
3	*Some high school*
4	*High school graduate*
5	*Some community college or CEGEP*
6	*Community college or CEGEP graduate*
7	*Some university*
8	*University graduate (including undergraduate, graduate and postgraduate studies)*
0	*Other (specify)*

b) What is/was your mother's (or primary female caregiver's) highest level of education that she completed?

1	*No formal education*
2	*Grade 8 or less*
3	*Some high school*
4	*High school graduate*
5	*Some community college or CEGEP*
6	*Community college or CEGEP graduate*
7	*Some university*
8	*University graduate (including undergraduate, graduate and postgraduate studies)*
0	*Other (specify)*

c) On average, would you say that your family's financial situation while growing up was…

1	*Difficult*
2	*Moderate*
3	*Comfortable*
4	*Very comfortable*

d)Are you presently enrolled at McGill/Concordia or at another educational institution?

0	*No*
1	*Yes (specify)*

→ ***If no, go to question 5f.***

e) How are you presently enrolled as a student?

1	*Undergraduate student*
2	*Graduate studies – Diploma, Master's, or Doctoral Program*
3	*Community college or CEGEP student*
0	*Other (e.g. Trainee, Postdoctoral studies, Sabbatical) (specify)*

f) What is the highest level of education that you have completed?

1	*No formal education*
2	*Grade 8 or less*
3	*Some high school*
4	*High school graduate*
5	*Some community college or CEGEP*
6	*Community college or CEGEP graduate*
7	*Some university*
8	*University graduate (including undergraduate, graduate and postgraduate studies)*
0	*Other (specify)*

g) What is your current employment status? Circle one only.

1	*Working full time (30 hours/week or more)*
2	*Working part time (<30 hours/week)*
3	*Not working due to full-time studies*
4	*On parental leave*
5	*Looking for work*
6	*Temporarily off sick*
7	*No longer able to work*
8	*No longer wish to work*
9	*Homemaker*
0	*Other (specify)*

6. How long have you lived in Montreal?

	OR		*OR*	
# months		*#years*		*Check here if do not live in Montreal*

Smoking History

The following questions are about your tobacco smoking habits.

7. Have you smoked a total of at least 100 cigarettes (4 or more packs) in your lifetime?

0	*No*
1	*Yes*

→ ***If no, go to Lifetime Sexual History on page 6.***

8. Have you ever smoked cigarettes regularly, that is, one cigarette or more each day for a year or more?

0	*No*
1	*Yes*

→ ***If no, go to Lifetime Sexual History on page 6.***

9. At what age did you start to smoke regularly?

Age in years

10.a) Do you still smoke regularly?

0	*No*
1	*Yes*

If no →

b) At what age did you stop smoking regularly?

Age in years

11. On average, how many cigarettes have you smoked a day since you began smoking regularly? (If you have stopped smoking regularly, please consider only those periods during which you were smoking regularly).

 # cigarettes per day

Lifetime Sexual History

The next questions are about your sexual history. We realize this is a personal subject, but it is very important to the study of HPV. Please take the time to recall this information as accurately as possible. Remember that all the information you give will be kept entirely confidential.

Throughout this survey, we will refer to various specific sexual acts. These terms are explained below so that everyone attaches the same meanings to them. Please be sure to read these definitions. If you need any further help or explanation, please ask the Research Nurse.

partners or sexual partners:	People who have had sex together—whether once, or just a few times, or as regular partners, or as married partners
genital area:	A man's penis or a woman's vulva and vagina—that is, the sex organs
oral sex:	A man's or a woman's mouth on a partner's genital area
vaginal sex or vaginal sexual intercourse:	A man's penis in a woman's vagina. This is what most people usually think of as "having sex" or "sexual intercourse"
anal sex or anal sexual intercourse:	A man's penis in a sexual partner's anus or rectum
mutual masturbation:	Hand stimulation of a (*woman/man's*) genital area by (*his/her*) partner, NOT involving intercourse (vaginal, oral, or anal)
sexual activity:	Mutual masturbation, oral sex, vaginal sex, or anal sex
sexual intercourse:	This includes oral, vaginal, and anal sex

12.a) Please think about all the people with whom you have engaged in sexual intercourse (oral, vaginal or anal). In total, with how many people—female or male— have you engaged in sexual intercourse in your lifetime?

☐ *Approximate #*

b) How many were female? ☐ *Approximate #*

c) How many were male? ☐ *Approximate #*

13. How old were you when you first had vaginal sexual intercourse?

☐ *Age in years* *OR* ☐ *Check here if never had vaginal sexual intercourse*

→ ***If never, go to question 15.***

14. Throughout your life, what is the number of female partners with whom you have had vaginal sexual intercourse?

☐ *Approximate #*

15. Do you consider yourself to be:

1	*Heterosexual/straight*
2	*Bisexual*
3	*Gay/homosexual*
0	*Other (specify)*

Sexual Activity with Enrolled HITCH Partner

The next questions are about the female partner who enrolled in HITCH with you. We will refer to her as your "HITCH partner".

16. What are her initials? (If you prefer, you can use an alias or nickname for this partner. Please choose one that you will remember later.)

Initials/Alias

17. What is her date of birth?

dd	*mm*	*yyyy*

18.a) Is she your…

1	*Wife*
2	*Common-law or live-in partner (living together)*
3	*Dating partner/girlfriend*
4	*Friend*
5	*Casual acquaintance*
6	*Not sure –we just met*
0	*Other (specify)*

b) Do you consider your sexual relationship with her to be…

1	*Ongoing and steady/regular*
2	*Ongoing but sporadic/on and off*
3	*One or a few times only*
0	*Other (specify)*

19. When did you first engage in sexual activity with her? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex. (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

20. Have you and your HITCH partner ever discussed the following since the start of your sexual relationship?

	No	*Yes*	*Do not remember*
i) Pregnancy prevention	*0*	*1*	*7*
ii) Sexually transmitted disease prevention	*0*	*1*	*7*
iii) **Your** sexual history	*0*	*1*	*7*
iv) **Her** sexual history	*0*	*1*	*7*
v) Whether **you** ever had a sexually transmitted disease	*0*	*1*	*7*
vi) Whether **she** ever had a sexually transmitted disease	*0*	*1*	*7*
vii) Whether **you** had ever been tested for sexually transmitted diseases (including HIV/AIDS)	*0*	*1*	*7*
viii) Whether **she** had ever been tested for sexually transmitted diseases (including HIV/AIDS)	*0*	*1*	*7*

21. To the best of your knowledge…

a) …what is the number of male partners with whom she has had vaginal intercourse in her lifetime, including you (if applicable)?

☐ *OR* ☐

Approximate # *Check here if do not know*

b) …has she ever had a sexually transmitted infection (e.g., chlamydia, gonorrhea, syphilis, genital herpes, pubic lice, HIV, hepatitis B)?

0	*No*
1	*Yes*
7	*Do not know*

The next series of questions are about sexual activities you may have engaged in with your HITCH partner since you first started your sexual relationship.

22. Since the start of your sexual relationship with your HITCH partner, how many times did you engage in sexual activities with her? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex.

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*	

During those sexual encounters…

23. …how often did you masturbate her?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

24. …how often did she masturbate you?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

25. …how often did you give her oral sex?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

26. …how often did she give you oral sex?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

27.a) Have you ever had vaginal intercourse with your HITCH partner?

0	*No*
1	*Yes*

→ ***If no, go to question 30.***

b) When did you first have vaginal intercourse with her? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

c) When was the last time you had vaginal intercourse with her?

dd	*mm*	*yyyy*

d) Since the start of your sexual relationship with your HITCH partner, how many times did you have vaginal intercourse with her?

	OR		*OR*	
Approximate #		*Approximate # times per week*		*Approximate # times per month*

28. How often did you use condoms for vaginal intercourse with her? (This includes male and female condoms.)

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

→ ***If never, go to question 30.***

29. **When you used condoms** for vaginal intercourse with your HITCH partner…

a) …did the condom ever break or slip off?

0	*No*
1	*Yes*
7	*Do not remember*

b) …did you always put the condom on before starting to have vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

c) …did you ever take the condom off then continue to have unprotected vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

30.a) Have you ever had anal intercourse with your HITCH partner?

0	*No*
1	*Yes*

→ ***If no, go to question 31.***

b) Since the start of your sexual relationship with your HITCH partner, how many times did you have anal intercourse with her?

	OR		*OR*	
Approximate #		*Approximate # times per week*		*Approximate # times per month*

c) How often did you use condoms for anal intercourse with her?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

Sexual Activity with Other Partners

The next questions are about sexual activities you may have engaged in with someone other than your HITCH partner.

31. Since the start of your relationship with your HITCH partner, did you engage in sexual activity with someone else? **Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex.**

0	*No*
1	*Yes*

→ ***If yes, go to question 33.***

32. Is your HITCH partner the only partner with whom you have ever engaged in sexual activity **in your lifetime**?

0	*No*
1	*Yes*

→ ***If yes, go to question 36 on page 16.***

→ ***If no, complete one blue OP Form for the last person with whom you engaged in sexual activity before your HITCH partner, then go to question 36 on page 16.***

<u>Do not answer questions 33-35.</u>

33. Since the start of your relationship with your HITCH partner, how many **other** sexual partners did you have?

Approximate #

→ ***If 5 or fewer other partners, complete a blue OP Form for each of these partners, then go to question 36 on page 16.***
<u>Do not answer questions 34-35.</u>

→ ***If more than 5 other partners, advance to question 34.***

34. Since the start of your relationship with your HITCH partner, how many **other** sexual partners were ongoing sexual partners? That is, partners with whom you had an **ongoing sexual relationship** (e.g. dating partner, wife, common-law partner)?

Approximate #

→ ***Complete a blue OP Form for each of these partners, then advance to question 35.***

35. Since the start of your relationship with your HITCH partner, how many **other** sexual partners were sexual partners with whom you did **<u>not</u> have an ongoing sexual relationship**? (e.g. one-night stands or flings)?

Approximate #

→ ***Complete one green AP Form for all of these partners combined, then advance to question 36.***

Medical History

The next questions refer to your medical history.

36. Did a doctor **ever** tell you that you had one of the following conditions?

				→ **If yes:** Were you told this since the start of your sexual relationship with your HITCH partner?		
	No	*Yes*	*Don't remember*	*No*	*Yes*	*Don't remember*
i) Trichomonas genital infection	*0*	*1*	*7*	*0*	*1*	*7*
ii) Venereal warts, condylomas, or papilloma virus infection	*0*	*1*	*7*	*0*	*1*	*7*
iii) Chlamydia	*0*	*1*	*7*	*0*	*1*	*7*
iv) Genital herpes	*0*	*1*	*7*	*0*	*1*	*7*
v) Syphilis	*0*	*1*	*7*	*0*	*1*	*7*
vi) Gonorrhea	*0*	*1*	*7*	*0*	*1*	*7*
vii) Ulcers or genital sores	*0*	*1*	*7*	*0*	*1*	*7*
viii) HIV	*0*	*1*	*7*	*0*	*1*	*7*
ix) Hepatitis B	*0*	*1*	*7*	*0*	*1*	*7*
x) Ureaplasma hominis	*0*	*1*	*7*	*0*	*1*	*7*

37. Since the start of your sexual relationship with your HITCH partner, did you have any of the following signs/symptoms?

	No	*Yes*	*Don't remember*
i) Painful urination, or difficulty urinating, or frequent urination	*0*	*1*	*7*
ii) Itching or burning sensation when urinating	*0*	*1*	*7*
iii) Blood in urine	*0*	*1*	*7*
iv) Abnormal discharge from penis	*0*	*1*	*7*
v) Sores in the genital area	*0*	*1*	*7*

Knowledge of HPV

This section is about your knowledge of and attitudes towards HPV. Please remember that you can speak with the Research Nurse after completing the survey if you have questions about HPV, penile cancer, or cervical cancer.

38. Before enrolling in the HITCH Cohort Study, had you ever heard of human papillomavirus, or HPV?

0	*No*
1	*Yes*

39. Please indicate whether the following statements are TRUE or FALSE.

		True	*False*	*Don't know*
i)	HPV can cause cervical cancer in women	*1*	*2*	*7*
ii)	Men can carry HPV	*1*	*2*	*7*
iii)	Genital warts cause cervical cancer in women	*1*	*2*	*7*
iv)	HPV can be cured with antibiotics	*1*	*2*	*7*
v)	A person may be infected with HPV and not know it	*1*	*2*	*7*
vi)	HPV can cause penile cancer in men	*1*	*2*	*7*
vii)	HPV causes genital herpes	*1*	*2*	*7*
viii)	Condoms protect against HPV	*1*	*2*	*7*
ix)	Having multiple sex partners increases one's risk for HPV	*1*	*2*	*7*
x)	Regular Pap tests can help to prevent complications from HPV	*1*	*2*	*7*
xi)	HPV is the most common sexually transmitted infection	*1*	*2*	*7*

40. Please indicate whether the following statements are TRUE or FALSE. A person can get HPV from…

		True	*False*	*Don't know*
i)	Sharing a plate, fork, or glass with someone who has HPV	*1*	*2*	*7*
ii)	Unprotected sexual intercourse with a someone who has HPV	*1*	*2*	*7*
iii)	Oral sex with someone who has HPV	*1*	*2*	*7*
iv)	Kissing (with exchange of saliva) someone who has HPV	*1*	*2*	*7*
v)	Sharing a washroom or shower with someone who has HPV	*1*	*2*	*7*

41. What do you think are your chances of becoming infected with HPV?

1	*Almost certain I will not*
2	*Very small chance*
3	*Some chance*
4	*Large or very large chance*
5	*Almost certain that I will get infected*
6	*I am already infected*

42. What do you think are your chances of developing penile cancer?

1	*Almost certain I will not*
2	*Very small chance*
3	*Some chance*
4	*Large or very large chance*
5	*Almost certain that I will get develop penile cancer*
6	*I have already been diagnosed with penile cancer*

HPV Vaccine

The last set of questions are about HPV vaccines. Although an HPV vaccine is not currently licensed for men, some men may have received it if they participated in a clinical trial of the vaccine. It may become available for all men in the future.

43.a) Have you received the HPV vaccine?

0	*No*
1	*Yes*
7	*Don't know*

→ ***If no or don't know, go to question 44.***

b) Did you receive the vaccine as part of participation in a clinical trial?

0	*No*
1	*Yes*
7	*Don't know*

c) How many injections of the HPV vaccine have you received, including booster shots?

#

d) When was your last injection of the HPV vaccine? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

→ ***Go to question 45.***

44. If the HPV vaccine is offered to you in the future, how likely is it that you will choose to be vaccinated?

1	*Very likely*
2	*Somewhat likely*
3	*Neutral*
4	*Somewhat unlikely*
5	*Very unlikely*

45. Please use the space below if you have any additional information you feel would be important for us to know.

Please record the time you stopped filling out the survey here.

Time finished survey: _____

This brings us to the end of this survey. Please take a moment to review you answers in all sections of the questionnaire. Again, try to answer all questions. A good guess will be more useful to the study than leaving the question blank.

Thank you very much for your participation!

OTHER PARTNER (OP) FORM:
MALE RESPONDENT

Please record your HITCH ID number, today's date, and the number for this OP form (e.g., form 2 of 3 if this is the second OP form out of 3 completed).

ID number: _____

Today's date: _____

OP Form number: _____ **of** _____

If instructed to fill out a blue OP form at question 32:

➔ Complete one blue OP form for the last person with whom you engaged in sexual activity before your HITCH partner.

If instructed to fill out blue OP form(s) at question 33:

➔ Complete one blue OP form for each sexual partner you reported in question 33, to a maximum of 5 blue forms.

If instructed to fill out blue OP form(s) at question 34:

➔ Complete one blue OP form for each sexual partner you reported in question 34, to a maximum of 5 blue forms.

OP1. Was this sexual partner…

1	*Male*
2	*Female*
3	*Transgendered male (female to male)*
4	*Transgendered female (male to female)*

If your sexual partner was transgendered, please respond to the remaining questions based on the anatomy of your partner. For example, if your partner identified as male, but had female genitals, respond to the following questions as if this sexual partner were female.

OP2. What are her/his initials? (If you prefer, you can use an alias or nickname for this partner. Please choose one that you will remember later.)

Initials/Alias

OP3. What is her/his date of birth?

dd	*mm*	*yyyy*

OR

Approximate age in years

OP4. Is/was she/he your…

1	*Wife*
2	*Common-law or live-in partner (living together)*
3	*Dating partner/girlfriend/boyfriend*
4	*Friend*
5	*Casual acquaintance*
6	*Not sure–we just met*
7	*Ex-wife*
8	*Ex-common-law partner*
9	*Ex-dating partner*
10	*Client partner for commercial sex (i.e., they paid you money to have sex with you)*
11	*Commercial sex worker (i.e., you paid them money to have sex with you)*
0	*Other (specify)*

b) Do you consider your sexual relationship with her/him to be…

1	*Ongoing and steady/regular*
2	*Ongoing but sporadic/on and off*
3	*One or a few times only*
4	*Our sexual relationship was ongoing but has now ended*
0	*Other (specify)*

OP5. To the best of your knowledge…

a) …what is the number of male partners with whom she/he has had vaginal and/or anal intercourse in her/his lifetime, including you (if applicable)?

☐ *Approximate #* *OR* ☐ *Check here if do not know*

b) …has she/he ever had a sexually transmitted infection (e.g. chlamydia, gonorrhea, syphilis, genital herpes, pubic lice, HIV, hepatitis B)?

0	*No*
1	*Yes*
7	*Do not know*

OP6. a) When did you first engage in sexual activity with her/him? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex. (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

b) When was the last time you engaged in sexual activity with her/him?

dd	*mm*	*yyyy*

c) How many times in total did you engage in sexual activity with her/him?

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*

d) How many times did you engage in sexual activity with him/her since the start of your relationship with your HITCH partner? (If you did not engage in sexual activity with her/him during that time, please answer zero.)

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*

→ ***If male partner, go to OP10.***

OP7. Have you ever had vaginal intercourse with her?

0	*No*
1	*Yes*

→ ***If no, go to OP10.***

b) When did you first have vaginal intercourse with her? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

c) When was the last time you had vaginal intercourse with her?

dd	*mm*	*yyyy*

d) How many times in total did you have vaginal intercourse with her?

	OR		*OR*	
Approximate #		*Approximate # times per week*		*Approximate # times per month*

e) How many times did you have vaginal intercourse with her since the start of your relationship with your HITCH partner? (If you did not have vaginal intercourse with her during that time, please answer zero.)

	OR		*OR*	
Approximate #		*Approximate # times per week*		*Approximate # times per month*

OP8. How often did you use condoms for vaginal intercourse with her? (This includes male and female condoms.)

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

→ ***If never, go to OP10.***

OP9. **When you used condoms** for vaginal intercourse with her…

a) …did the condom ever break or slip off?

0	*No*
1	*Yes*
7	*Do not remember*

b) …did you always put the condom on before starting to have vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

c) …did you ever take the condom off then continue to have unprotected vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

OP10.a) Have you ever had anal intercourse with her/him?

0	*No*
1	*Yes*

→ ***If no, end OP Form and return to the main, white questionnaire.***

b) When did you first have anal intercourse with her/him? (If you only know the approximate date, specify the month and year.)

dd	*mm*	*yyyy*

c) When was the last time you had anal intercourse with her/him?

dd	*mm*	*yyyy*

d) How many times in total did you have anal intercourse with her/him?

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*

→ ***If zero, end OP Module.***

e) How often did you use condoms for anal intercourse with her/him?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

END OF BLUE OP FORM

Please return to the main, white questionnaire.

AGGREGATED PARTNERS (AP) FORM: MALE RESPONDENT

Please record your HITCH ID number and today's date.

ID number: _____

Today's date: _____

Please complete the following questions for the sexual partner(s) you reported in **question 35**.

AP1. How many were…

	Approximate #
i) male	
ii) female	
iii) Transgendered male (female to male)	
iv) Transgendered female (male to female)	

If a sexual partner was transgendered, please respond to the remaining questions based on their anatomy. For example, if your partner identified as male, but had female genitals, respond to the following questions as if this sexual partner were female.

AP2. How many were your…

	Approximate #
i) wife	
ii) common-law or live-in partner (living together)	
iii) dating partner/girlfriend/boyfriend	
iv) casual acquaintance	
v) ex-wife	
vi) ex-common-law partner	
vii) ex-dating partner	
viii) client partner for commercial sex (i.e., they paid you money to have sex with you)	
ix) commercial sex worker (i.e., you paid them money to have sex with you)	

AP3. Since the start of your relationship with your HITCH partner, how many times did you engage in sexual activities with these **other sexual partners**? Remember that by sexual activity, we mean mutual masturbation, oral, vaginal, and/or anal sex.

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*	

→ ***If no female partners, go to AP7.***

AP4.a) Since the start of your relationship with your HITCH partner, did you engage in **vaginal intercourse** with any of these **other** female partners?

0	*No*
1	*Yes*

→ ***If no, go to AP7.***

b) With how many did you engage in vaginal intercourse?

Approximate #

c) Since the start of your relationship with your HITCH partner, how many times did you have vaginal intercourse with these **other** partners?

Approximate #	*OR*	*Approximate # times per week*	*OR*	*Approximate # times per month*	

d) When was the last time you had vaginal intercourse with any of these **other** partners?

dd	*mm*	*yyyy*

AP5. Since the start of your relationship with your HITCH partner, how often did you use condoms for vaginal intercourse with these **other** partners? (This includes male and female condoms.)

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

→ ***If never, go to AP7.***

AP6. **When you used condoms** for vaginal intercourse with these **other** partners …

a) …did the condom ever break or slip off?

0	*No*
1	*Yes*
7	*Do not remember*

b) …did you always put the condom on before starting to have vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

c) …did you ever take the condom off then continue to have unprotected vaginal intercourse?

0	*No*
1	*Yes*
7	*Do not remember*

AP7.a) Since the start of your relationship with your HITCH partner, did you have **anal intercourse** with any of these **other** partners?

0	*No*
1	*Yes*

→ ***If no, end green AP form and return to main, white questionnaire.***

b) With how many did you engage in anal intercourse?

Approximate #

c) Since the start of your relationship with your HITCH partner, how many times did you have anal intercourse with these **other** partners?

	Approximate #	*OR*		*OR*	
			Approximate # times per week		*Approximate # times per month*

d) When was the last time you had anal intercourse with any of these **other** partners ?

dd	*mm*	*yyyy*

e) Since the start of your relationship with your HITCH partner, how often did you use condoms for anal intercourse with these **other** partners?

0	*Never (0%)*
1	*Rarely (1-25%)*
2	*Some of the time (26-75%)*
3	*Most of the time (76-99%)*
4	*Always (100%)*

END OF GREEN AP FORM

Please go to question 35 in the main, white questionnaire.

APPENDIX D
Hypothesized causal relationships for HPV prevalence at enrolment

Causal diagrams were constructed to illustrate the hypothesized causal relations for HPV detection at enrolment. Directed acyclic graphs (DAGs) are one type of causal diagram that could be used. Briefly, these diagrams depict causal associations between variables. Single-headed arrows represent direct cause and effect relations. Strictly speaking, the diagrams I show below are do not exactly follow the guidelines for drawing DAGs. I chose to use the representation suggested by Clarice Weinberg to depict effect-modifying cofactors.[1] She suggests drawing an arrow from an effect modifier to the arrow between the purported cause and effect, rather than only drawing arrows between causes as done for DAGs.

Let

L_m = Lifetime number of sex partners reported by the male (i.e. his past exposure)

L_f, = Lifetime number of sex partners reported by the female (i.e. her past exposure)

$HPV_{m\,0}$ = Male's HPV status at couple formation, time 0, unmeasured

$HPV_{f\,0}$ = Female's HPV status at couple formation, time 0, unmeasured

$HPV_{m\,1}$ = Male's HPV status at enrolment, time 1

$HPV_{f\,1}$ = Female's HPV status at enrolment, time 1

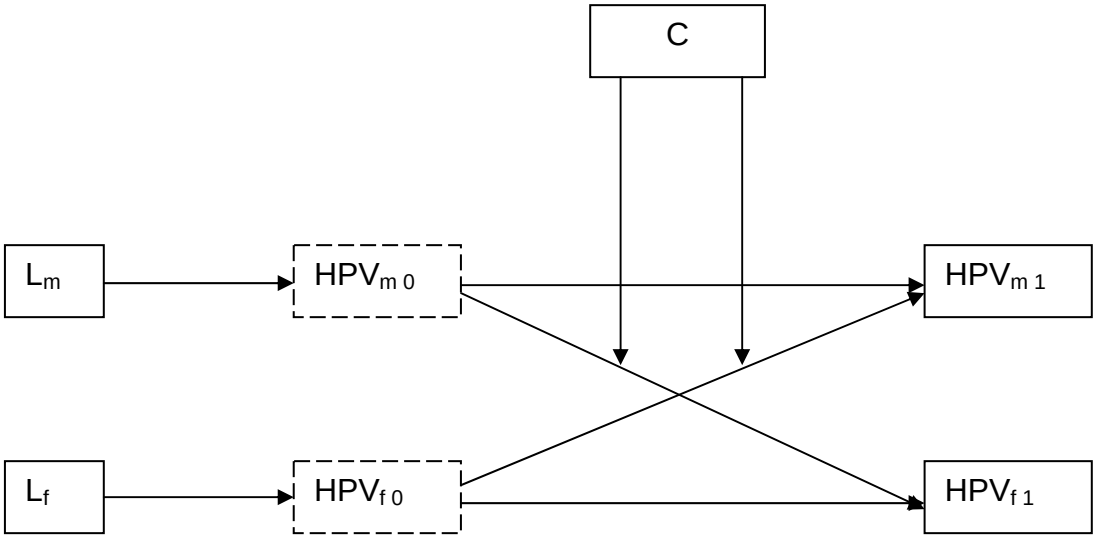
C = frequency of condom use by the couple between time 0 and time 1

Figures D.1a and D.1b represent the proposed relations between lifetime number of partners, HPV status at couple formation (time 0, unmeasured), condom use, and HPV status at enrolment (time 1). HPV status at time 0 does not vary with condom use, and condom use (C) does not vary with HPV at time 0, and C is not an independent cause of HPV at time 1. Neither HPV at time 0 nor C is a cause of the other. However, there may be effect modification for combinations of values of HPV at time 0 and C on HPV at time 1. That is, condom use may affect the probability of transmission from one partner to another, denoted as the arrow between HPV status of one partner at time 0 and HPV status of the other partner at time 1. Note that the condom use variable C could be replaced with other measures that quantify or qualify the sexual contacts by the couple between times 0 and 1. Moreover, for simplicity I excluded ancillary factors in the causal diagrams that may affect persistence of HPV, such as hormonal contraceptive use, smoking, age, genetic susceptibility factors, or viral characteristics.[2]

Furthermore, other effect modifications are possible between lifetime number of partners and current partner's HPV status. For simplicity, consider the analysis of women's prevalence where the outcome is $HPV_{f\,1}$. Neither $HPV_{m\,0}$ nor L_f is a cause of the other. (However, L_f may cause $HPV_{m\,1}$ via $HPV_{f\,0}$.) Both $HPV_{m\,0}$ and L_f may modify the association of the other with $HPV_{f\,1}$. For example, if past exposure to HPV through multiple partners generates some degree of natural immunity, then L_f may modify the association between $HPV_{m\,0}$ (measured by proxy using $HPV_{m\,1}$) and $HPV_{f\,1}$. Alternatively, if HPV is highly transmissible such that infection in the woman's current partner virtually ensures transmission to her, then the association between L_f and $HPV_{f\,1}$ may be attenuated or absent when the current partner is HPV+. These potential effect modifications are depicted in Figures D2.a and D.2b (similar to Weinberg's Figure 1).

Figure 1. Underlying diagram depicting hypothesized causal relations between lifetime number of partners, condom use, and HPV status.

a. Showing measured and unmeasured (dashed outline) variables.



b. Showing only measured variables.

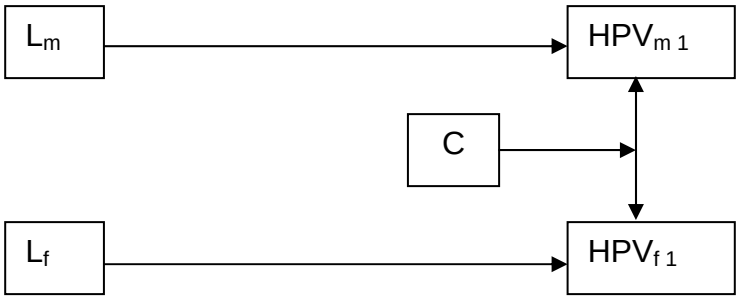
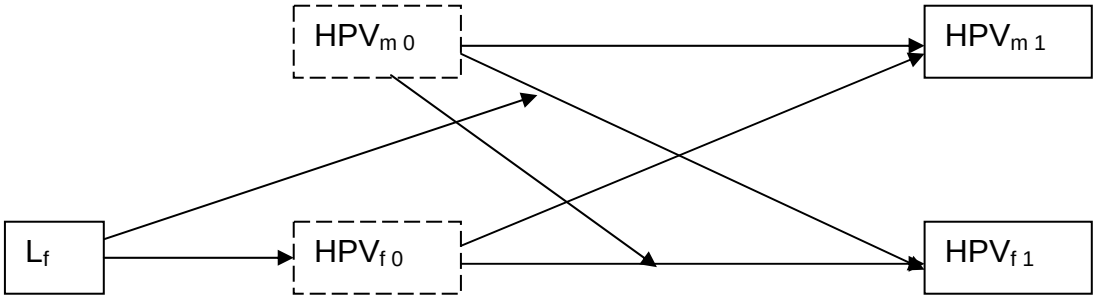
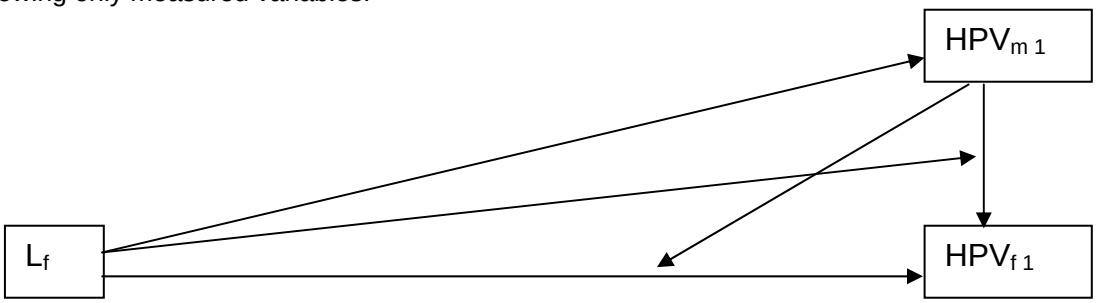


Figure 2. Proposed relations between women's lifetime number of sex partners, the current male partner's HPV status, and women's current HPV status.

a. Showing measured and unmeasured (dashed outline) variables.



b. Showing only measured variables.



References

1. Weinberg C. Can DAGs clarify effect modification? *Epidemiology.* 2007;18:569-572.

2. Moscicki AB, Schiffman M, Kjaer S, Villa L. Updating the natural history of HPV and anogenital cancer . *Vaccine.* 2006;24:S42-S51.