Prevalence and determinants of human papillomavirus (HPV) infection in Inuit women of Nunavik, Quebec

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ABSTRACT

Objectives:

To study the prevalence and determinants of human papillomavirus (HPV) infection among Inuit women in Nunavik, Quebec.

Methods:

We recruited a cohort of Inuit women seeking routine care and living in communities in Nunavik. Baseline demographic and lifestyle data was collected and cervical specimens were tested for HPV-DNA using the PGMY-Line blot assay.

Results:

Overall and high-risk (HR) HPV prevalence were 28.9% and 20.4%, respectively. Co-infections were observed in 40% of HPV-positive subjects. The most common HPV type was HPV-16; other prevalent HR types included HPV-31, HPV-52, and HPV-58. The most prevalent papillomavirus species were alpha-9 and alpha-3. In multivariate logistic regression, age (OR: 0.95; 95% CI: 0.93-0.98) and ten or more lifetime sexual partners (OR: 2.25; 95% CI: 1.41-3.60) were associated with HR-HPV infection.

Conclusions:

HPV prevalence is elevated when compared to most Canadian populations. Age and markers of sexual activity appear to be risk factors for HR-HPV infection.

RÉSUMÉ

Objectif:

Déterminer la prévalence et les déterminants du VPH chez les femmes inuites du Nunavik, Québec.

Méthode:

Nous avons recruté une cohorte de femmes vivant au Nunavik. Des données démographiques et reliées au mode de vie ont été récoltées. Des échantillons de cellules du col de l'utérus ont été analysés à l'aide du *PGMY-Line blot assay* afin de détecter de l'ADN-VPH.

Résultats:

La prévalence du VPH et du VPH-HR était respectivement de 28.9% et 20.4%. Les types de VPH-HR les plus répandus étaient VPH-16, VPH-31, VPH-52 et VPH-58. Une analyse de régression logistique multivariée a révélé que l'âge (RC: 0.95; 95% CI: 0.93-0.98) et avoir dix partenaires sexuels ou plus au cours d'une vie (RC: 2.25; CI: 1.41-3.60) sont associés à l'infection au VPH-HR.

Conclusion:

La prévalence du VPH est élevée comparée à la majorité des populations canadiennes. L'âge et des marqueurs d'activité sexuelle sont des facteurs de risque pour l'infection au VPH-HR.

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DEDICATION

I would like to dedicate this work to my father, Gregory Douglas, who always took pride in his work and knew the value of leaving a positive footprint, whether in a professional or an interpersonal context.

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AG-US	atypical glandular cells of undetermined significance
ASC-H	squamous cells of undetermined significance, high-grade squamous intraepithelial lesion cannot be excluded as a possibility
ASCUS	atypical squamous cells of undetermined significance
CI	confidence interval
CIN	cervical intraepithelial neoplasia
HC1	hybrid-capture I
HC2	hybrid-capture II
HPV	human papillomavirus
HR	high-risk
HR-HPV	high-risk human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
LCR	long control region
LR	low-risk
LR-HPV	low-risk human papillomavirus
LSIL	low-grade squamous intraepithelial lesion
OC	oral contraceptive
OR	odds ratio
Pap	Papanicolaou
PCR	polymerase chain reaction
SIL	squamous intraepithelial lesion
STI	sexually transmitted infection

1 LITERATURE REVIEW

1.1 HUMAN PAPILLOMAVIRUS (HPV)

Human papillomavirus (HPV) is the most common viral sexually transmitted infection (STI) globally, with a prevalence of about 440 million infections worldwide[1]. It is estimated that 75% of Canadians will acquire at least one HPV infection in their lifetime[2]. To date, over 100 HPV genotypes (simply known as 'types') have been identified, 40 of which infect the anogenital and upper digestive tracts.

HPV infection has been recognized as the main biological precursor to cellular changes leading to cervical cancer in women[3]. It has also been linked to more rare cancers of the anus, penis, vulva and vagina and has been associated with a proportion of mouth and oropharyngeal cancers. HPV types are classified as high-risk (HR), of which there are approximately 15 types, and low-risk (LR), based on their oncogenic potential in cervical cancer[3]. The high-risk HPV (HR-HPV) types 16 and 18 have been observed in approximately 70% of cervical intraepithelial neoplasia (CIN) lesions, the precursors to invasive cervical cancer. LR types such as HPV-6 and -11 are not associated with the development of these lesions, but may manifest in the form of genital warts (condyloma). Indeed, these two LR types are estimated to cause 90% of genital warts[4].

1.1.1 Classification Systems of HPV

HR and LR classifications have been derived based on epidemiological evidence including pooled analysis of case-control studies with a common protocol[5]. Although not all HPV types have been classified with certainty epidemiologically, there has been general agreement on the importance of a subset of types (Table 1.1) [5].

Table 1.1: Epidemiologic classification of HPV types

Epidemiologic Classification	HPV Types
High-risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Probable high-risk	26, 53, 66
Low-risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108
Undetermined risk	34, 57, 83

Phylogenetic classification of papillomaviruses groups HPV types into genera and species according to their genetic linkage[6]. The largest genus is that of the alpha-papillomaviruses, which cause mucosal and cutaneous lesions in humans and primates and includes 59 HPV types. These alpha HPV types, in turn, have been categorized into genetically-related species (Figure 1.1). HPV types within a phylogenetic group often share similar biological and pathological properties, and phylogenetic classifications have been used to further substantiate epidemiologic classifications. For example, the HPV types in the alpha-7 papillomavirus species (HPV 18, 39, 45, 59, 68, and 70) are associated with HR mucosal lesions, whereas the alpha-2 papillomavirus species (HPV 3, 10, 28, 29, 77, 78, and 94) more frequently cause cutaneous lesions and are generally LR.

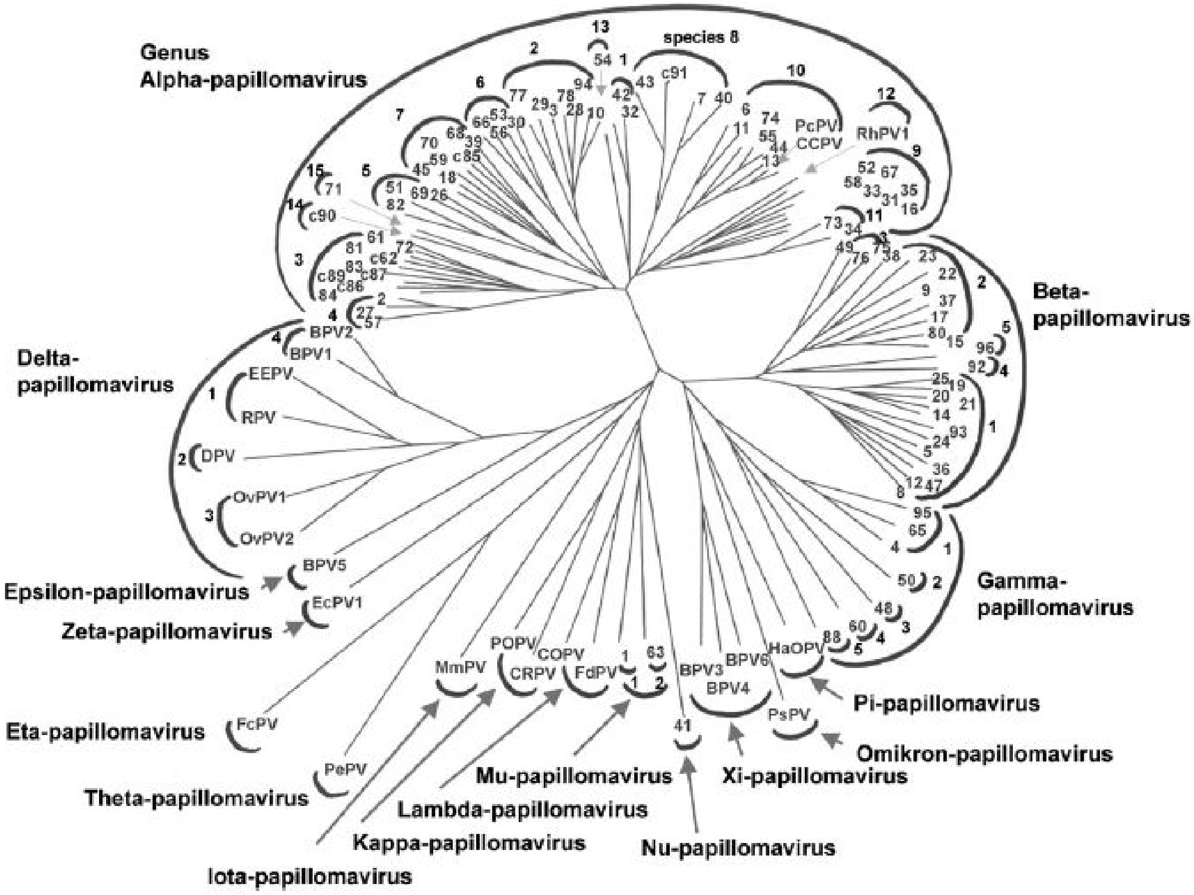

Figure 1.1: Phylogenetic tree with sequences of 118 papillomavirus types[6]

Numbers at the end of branches refer to individual HPV types, c-numbers refer to candidate HPV types and all other abbreviations refer to animal-specific papillomavirus types. The outermost semi-circles refer to HPV genera (e.g. alpha-papillomavirus genus, beta-papillomavirus genus), the inner semi-circles indicate HPV species and are identified by the number beside the grouping. For example, in the top right, HPV types 16, 31, 33, 35, 52, 58, and 67 form HPV species 9 in the alpha-papillomavirus genus (alpha-9 species).

Reprinted from *Virology*, 324, de Villers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H, Classification of papillomaviruses, 17-27, Copyright 2004, with permission from Elsevier.

HPV types are not entirely uniform in their genetic profiles; rather, some intra-type diversity exists. For this reason, HPV types may be further sub-classified into molecular variants, which are defined as having less than 2% variation in nucleotide sequence coding regions and less than 5% variation in non-coding regions[7, 8]. Sequence analysis of the non-coding long control region (LCR) of HPV-16 has driven the recognition of five classes of HPV-16 variants, which are based on geographical relatedness: European, Asian, Asian-American and two African classes of variants[9]. Studies of intra-type diversity of HPV-18 have revealed similar patterns to that of HPV-16[10].

It has been hypothesized that molecular variants of a given HPV type could also have varying oncogenic potential. The main rationale for this hypothesis is that, in the case of HPV genotypes, the same regions of the genome which contain nucleotide differences that create type classifications also contain variations that classify the virus by oncogenic potential. Limited research has investigated the association between geographic relatedness of molecular variants and the risk for development and progression of cervical lesions. Two studies, one conducted in female university students in Seattle[11] and another conducted in a Brazilian cohort[12], have shown that infection with non-European variants of HPV-16 is associated with a higher risk of progression to CIN[11] and that infection with non-European variants of HPV-16 and HPV-18 is associated with higher risk of high-grade lesions than European variants[12].

1.1.2 Natural History of HPV Infection

HPV infections are transmitted sexually by direct contact between an infected individual's epithelium (skin or mucosa) and the epithelium of another individual. In vertical transmission, infection is mediated by contact between a child and the maternal genital tract during delivery. Finally, it is hypothesized that HPV infections of the head and neck are a result of transmission by oral mucosal contact.

In the sexual transmission of HPV in women, the virus principally infects the cervical transformation zone, which is a rapidly proliferating junction between the columnar epithelium and squamous epithelium that lines the cervix. It is in this region that the HPV virus enters basal epithelial cells of the basement membrane, which may be facilitated by the presence of micro-abrasions. The virus then begins its replication outside the host genome (episomal replication) and may either induce productive infections (resulting in condyloma) or cellular transformations, depending on the viral type. The main difference between HR- and LR-HPV types is that HR types can integrate into the host cell genome, which appears to confer their oncogenic potential.

Most HPV infections, regardless of whether they involve HR or LR types, are asymptomatic and transient: they are thought to clear spontaneously by the shedding of virus-infected endocervical cells. Across many studies, the average time to clearance ranges from between 4 and 20 months, but most data indicate that less than half of women will remain positive at 12 months[13]. Further, research suggests that only persistent infections with HR-HPV types (particularly HPV-16 and -18) will lead to the development and maintenance of severe cellular dysplasia (disordered growth) and *in situ* cancer. In fact, the risk of cervical intraepithelial neoplasia (CIN) is proportional to the number of cervical specimens testing positive for HPV[14]. In addition, women with persistent HR-HPV infections are approximately 300 times more likely to develop high-grade squamous intraepithelial lesions (HSIL)[15], which represent the precursor to invasive cervical cancer in its pathogenesis[16]. A persistent HPV infection is generally defined by the detection of the same HPV type (and variant) two or more times, with a given time interval between tests[17]. There is not widespread agreement, however, on the period of time for which an HPV infection must be present for it to be defined as persistent.

1.2 CERVICAL CANCER

Cervical cancer is the only cancer for which a necessary cause has been identified: HPV infection has been detected in 99.7% of a large international collection of cervical cancer specimens[3, 18]. The relative risk for the association between HPV infection and cervical neoplasia has been estimated to be between 20 and 70, which is greater than that for smoking and lung cancer[19]. HPV infection is not, however, a sufficient cause for cervical cancer. Although HPV is a ubiquitous viral STI, cervical cancer develops in only a small number of women. Viral factors, as well as behavioural and lifestyle factors of the human host, are thought to modulate the effect of HPV infection on the risk for development of cervical cancer.

1.2.1 Natural History of Cervical Cancer

The natural history of cervical cancer has not yet been well described, but it is believed that cervical cancer tends to progress very slowly from precancerous lesions of the cervix to *in situ* carcinoma and invasive cancer. The average latency period between HPV infection and the development of invasive cancer is estimated to be between 20 and 30 years, with a minimum latency of approximately 7 years. Rarely, a rapid progression from the development of precancerous lesions to invasive cancer in the span of less than a year has been observed[20].

Intraepithelial lesions are considered the earliest morphological changes associated with cancer. Cervical lesions are classified according to the cytopathology results of a Papanicolaou (Pap) smear, which is used to detect cellular dysplasia (disordered growth) in cervical cancer screening. Cytological classification was formerly divided into three grades of cervical intraepithelial neoplasia (CIN): CIN1, CIN2 and CIN3, which represent mild dysplasia, moderate dysplasia and severe dysplasia or carcinoma *in situ*, respectively.

The CIN Papanicolaou classes represent categories ranging from abnormal growth to invasive cancer, but are not considered a strictly step-wise progression from precancerous lesions through to development of cancer. In order to emphasize the different tendencies of cervical lesions to progress to cancer, a new classification system was developed in 1988, updated in 2001, and is now in widespread use. The so-called Bethesda system[21] describes both squamous and glandular epithelial cellular abnormalities as outlined in Table 1.2.

Table 1.2: Bethesda classification of epithelial cellular abnormalities

Squamous cells
Atypical squamous cells • Atypical squamous cells of undetermined significance (ASC-US) • Atypical squamous cells of undetermined significance, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) **Squamous intraepithelial lesions (SIL)** **Low-grade squamous intraepithelial lesion (LSIL)** • Koilocytosis • CIN1 (mild dysplasia) **High-grade squamous intraepithelial lesion (HSIL)** • CIN2 (moderate dysplasia) • CIN3 (severe dysplasia and carcinoma *in situ*) **Squamous cell carcinoma (invasive cancer)**
Glandular cells
Atypical **Atypical glandular/endocervical cells, favour neoplastic** **Endocervical adenocarcinoma *in situ*** **Adenocarcinoma**

When a Pap smear shows abnormal cells, the follow-up is usually a colposcopic examination of the cervix with a directed collection of biopsies. The histopathologic result of a biopsy is the degree of dysplasia or carcinoma, which ranges from mild to severe dysplasia, and carcinoma *in situ* to invasive carcinoma. Many cervical lesions spontaneously regress, but others may persist and progress to cancer, a process which is likely modulated by a host of factors including HPV type, persistence of HPV infection, viral load, and the age of the patient[22]. Most cases of mild dysplasia and about half the cases of moderate dysplasia will regress back to normal cytology within two years of diagnosis[23].

1.3 EPIDEMIOLOGY OF HPV

HPV is an extremely common STI, having prevalence estimates of between 5% and 40% in asymptomatic women of reproductive age[24]. A peak in prevalence in women less than 25 years of age has been observed between studies, with decreasing prevalence in older women. Some studies have reported a second peak in prevalence amongst women in their 40s, 50s and older, an observation which may be explained by increasing persistence of infections[25]. In addition, HR-HPV types seem to predominate in women under 25 years of age, while LR and indeterminate-risk types are most prevalent in women over 55 years of age[26].

1.3.1 Canadian Prevalence Estimates

All estimates of the prevalence of HPV infection in Canada are based on studies in selected female populations including patients attending primary care, university health, and STI/HIV clinics, since HPV is not nationally notifiable and no population-based studies have been published to date[27]. The reported overall prevalence of HPV has ranged from 11% to 33%. Across individual age groups, the prevalence has ranged from approximately 3% to 42%, which highlights the importance of age as a risk factor for acquiring HPV infection[27]. A summary of the HPV point prevalence estimates found in Canadian studies using polymerase chain reaction (PCR) or hybrid-capture (HC) methods can be found in Table 1.3.

Table 1.3: Point prevalence of HR- and LR-HPV infection in Canadian studies using PCR, hybrid capture I (HC1) and/or hybrid capture II (HC2) methods*

Study	Population	Age range (years)	N	% HPV	Test
Healey et al.[28]	Women undergoing routine screening, from 19 communities in Baffin and Keewatin regions of Nunavut (86% Inuit)	13 – 79 13-20 21-30 31-40 > 40	1,290 240 480 331 239	25.8 (HR) 42.1 31.3 13.9 15.1	HC2
Ratnam et al.[29]	Routine screening in 10 regions of Newfoundland	18-69 < 25 25-34 35-44 45+	2,098 401 1,098 536 59	10.8 (HR) 16.7 11.7 5.0 3.6	HC1 (69%) HC2 (31%)
Richardson et al.[30]	Women attending university health centre in Montreal	Most 18-24 (3% over 30)	375	22.7 (HR, LR) 11.8 (HR) 6.2 (LR)	MY09/11†
Richardson et al.[31]	Women attending university health centre in Montreal	17-42 (Mean: 23, Median: 21)	621	29.0 (HR, LR) 21.8 (HR) 14.8 (LR)	MY09/11†
Sellors et al.[32, 33]	Family practice clinics for cytologic cervical screening. Proportional random sampling from 6 health planning regions in Ontario	15-49[32] 15-19 20-24 25-29 30-34 35-39 40-44 45-49 >50[33]	955 89 125 159 163 157 144 118 156	12.7 (HR) 15.7 24.0 16.4 12.3 9.6 8.3 3.4 8.3	HC2
Young et al.[34]	Winnipeg inner-city clinic (42% Aboriginal)	Age range not reported (73% < 30)	1,263	33 (HR, LR)	MY09/11‡

* Adapted from "Reported point prevalence of cervical high-risk and low-risk HPV in Canadian studies using PCR or hybrid capture I (HC1) and/or II (HC2) methods"[35]
HC2: hybrid-capture II for HR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68
GP5+/6+: General primer 5+/6+ polymerase chain reaction (PCR) with bi-directional sequencing; comparison with known HPV types
HC1: hybrid-capture I for HR types 16, 18, 31, 33, 35, 45, 51, 52, and 56
†MY09/11: PCR with dot-blot hybridization for HR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and LR types 6, 11, 26, 40, 42, 53, 54, 55, 57, 66, 73, 82 (MM4), 83 (MM7), 84 (MM8)
‡MY09/11: PCR with dot-blot hybridization for HR types 16, 18, 31, 33, 35 and LR types 6, 11

1.3.2 Co-infection with Multiple HPV Types

Although cervical cancer is typically associated with a single HPV type (a monoclonal event), the surrounding cervical epithelium may simultaneously be infected with multiple HPV types[17]. Prevalence of multiple infections seems to vary considerably between populations – in IARC prevalence surveys of cytologically normal women across the world, the prevalence of multiple infections ranged from 0.4% to 8.3% overall[36]. In the Ludwig-McGill cohort[37], one-fifth of all women who tested positive for HPV during follow-up had a multiple infection detected. Multiple type infections have been more commonly reported in younger women[38, 39], in women with abnormal cytology[26, 40] and in those with impaired immune function[41, 42]. Being infected with a given HPV type does not appear to influence concurrent infection with a phylogenetically related type[13, 39, 43].

The results of research examining the association between multiple infections and cervical neoplasia have been inconsistent, some suggesting an association between multiple infections and the development or progression of cervical neoplasia[40, 44-46] and others not showing that infection with multiple HPV types elevates the risk for precancerous lesions or invasive cancer[26, 41, 47, 48]. There is a growing body of evidence, however, that suggests that certain HPV types may act synergistically in cervical carcinogenesis[46, 49].

1.4 EPIDEMIOLOGY OF CERVICAL CANCER

Cervical cancer is the world's second most common cancer in women[3]. Approximately 1 million women were estimated to have cervical cancer in 2005 and upwards of 250,000 deaths were attributed to the disease[50]. The average age at diagnosis is estimated to be approximately 50 years of age, although women as young as 17 years of age have been reported to have cervical cancer. Approximately 80% of cervical cancer cases are experienced by women in developing countries[51].

In Canada, there are approximately 1,500 new cases of invasive cervical cancer each year and 420 deaths due to the disease[52]. The age-specific incidence of cervical cancer peaks first in 40-year-old women, then drops, and peaks again in women over 70 years of age[27]. Overall, incidence and mortality rates have been dropping since the 1970s, which has been attributed to successful Pap cytology screening programs whose implementation began in the 1960s.

Since then, the Pap smear has been considered the most effective tool for cervical cancer prevention that is available. It was reported in the 2003 Canadian Community Health Survey[53] that 79% of eligible Canadian women aged 18 to 69 years had a Pap test in the previous 3 years. However, a series of studies have shown that approximately 60% of Canadian women who develop cervical cancer have not been screened in the previous 3 years[54].

In Canada, approximately 70% of all cervical cancers are squamous cell carcinomas and about 25% are adenocarcinomas and adenosquamous carcinomas[54]. In the past 30 years there has been a steady decline in the incidence of squamous cell carcinoma both in Canada and internationally. However, the incidence of adenocarcinoma has been steadily increasing, particularly in younger age groups[55, 56]. This trend should raise concern, since adenocarcinoma patients show poorer prognosis than those with squamous cell carcinomas. Additionally, traditional Pap testing seems to be less effective at detecting this type of cervical cancer[57], since adenocarcinomas tend to develop further into the endocervical canal. Modifications to the Pap smear sampling method appear to improve collection of these endocervical cells by using cervical brushes in combination with a spatula with an extended tip, rather than using a spatula alone[58].

1.5 RISK FACTORS FOR CERVICAL CANCER

Cervical cancer has been shown to act like an STI with regards to its epidemiologic risk factors. The most consistently reported risk factors for the development of CIN precursor lesions and cervical cancer are markers of sexual activity, including lifetime number of sexual partners, an early onset of sexual activity and the sexual behaviour of a woman's male partners[59]. Biologically, an early age at first intercourse may increase susceptibility to cervical cancer because cervical tissue undergoes many changes during puberty which make it more vulnerable to damage.

Not having undergone regular Pap screening increases the risk of developing cervical cancer, and is one of the most significant risk factors for poor outcomes in women with the disease[60]. Smoking has been identified as a risk factor for cervical cancer in several studies, especially in long-term smokers,[61] and may act in synergy with high viral loads to cause the disease[62]. The detection of nicotine metabolites in the cervical mucus of female smokers may evidence a direct carcinogenic effect of smoking[63]. It should be noted that, given the association between smoking and sexual activity, and the impossibility of fully adjusting for the confounding effects of sexual behaviour, the association between smoking and cervical cancer is unlikely to be definitively confirmed[19].

Long-term use of oral contraceptives (OCs), for more than 12 years, has been associated with an increased risk for cervical cancer, while the use of barrier contraceptive methods has been associated with a decreased risk[59]. The risk of OC use seems to be higher for adenocarcinomas than for squamous cell carcinomas, even when adjusted for important confounding variables[64]. Assessing the effect of OC use is complex, however, considering its high correlation with sexual activity and Pap screening history[65].

Number of live births has been reported as a consistent risk factor for cervical cancer. In fact, a linear trend in the association between parity and cervical cancer risk has been observed in populations in North, Central and South America[66]. Diets rich in beta-carotene, vitamin C and, to a lesser extent, vitamin A, have been found to be protective against cervical cancer[67, 68]. Together, between-country differences in diet and parity, in combination with differences in screening coverage and quality, may account for differences in cervical cancer incidence rates[69].

Persistent HPV infection has been identified as an important risk factor for cervical cancer and is understood as the true biological precursor to the development of cervical abnormalities. Persistence is most likely related to a combination of factors, including host susceptibility and viral factors. Specifically, it has been associated with older age and infection with HR and multiple HPV types[70-72].

1.6 RISK FACTORS FOR HPV INFECTION

Numerous risk factors for HPV infection have been identified through cross-sectional and prospective cohort studies in many different populations[13]. These determinants include age at first sexual intercourse, lifetime and recent number of sexual partners, smoking, OC use, presence of other STIs (chlamydia, herpes simplex virus), chronic inflammation, immunosuppression (e.g. due to HIV infection) and parity[30, 73-77]. Across all studies, the most consistent determinants of HPV infection are markers of sexual activity and age. Most studies show a dramatic decline in risk for HPV infection in women aged 30 years or older, which seems to be independent of sexual activity. A second peak in HPV prevalence in peri- and post-menopausal women has been reported in numerous epidemiologic studies[26, 38, 78-80].

Determinants of HPV infection not only vary across populations, but also across HR- and LR-HPV type classifications. In a study of Montreal university students, Richardson et al.[31] found that markers of sexual activity, including lifetime frequency of sexual intercourse and lifetime number of oral sex partners, were associated with HR-HPV infection but not infection with LR-HPV types. Rousseau et al.[81] found a strong association between markers of sexual activity and both HR and LR infections, but observed a strong negative association with age only with regards to HR-HPV types.

1.7 HPV AND CERVICAL CANCER IN ABORIGINAL POPULATIONS

In Canada, efforts to enhance cervical cancer screening programs as well as to initiate HPV vaccination campaigns aim to further decrease the incidence of the disease in Canadian women. In this context, the large discrepancies in cervical cancer rates across disparate Canadian populations are of serious concern. In particular, Aboriginal women in Canada suffer disproportionately from cervical cancer, as they do in North America in general[82]. Among First Nations populations in Saskatchewan[83], Manitoba[84] and Ontario[85], incidence rates have been reported to range from two to six times higher than the general population. Age-standardized rates are three times higher in the Canadian Inuit than the general population[83].

Not only do Aboriginal women suffer disproportionately from cervical cancer, but their outcomes are also poorer when compared to other populations. Standardized mortality rates for cervical cancer were four times higher for Quebec Aboriginal women than for the province overall between 1988 and 2004[86]. Elevated mortality rates have also been reported among First Nations groups in British Columbia[87].

Despite the fact that Canadian Aboriginals have higher cervical cancer incidence and mortality than the general Canadian population, only sparse data exist that describe the prevalence of HPV and other risk factors for cervical cancer in these populations. In fact, data of this nature are limited for Aboriginal populations in general. To date, data have been collected on Greenlandic Inuits[88], New Mexico American Indians[89, 90], and Alaska Aboriginals[91], yet the methods these studies employed are now considered to be lacking in both sensitivity and specificity. The results, however flawed, showed that Aboriginal populations had lower prevalence of HPV infection than general populations, despite higher risks for cervical cancer. Recent studies using more advanced PCR techniques showed Guarani Indians of Argentina[92] and American Indian women of the Northern Plains[93] to have elevated rates of HPV infection.

In Canada, a few studies have examined HPV infection in Aboriginal populations and some differences in prevalence have been observed when comparing to non-Aboriginal groups (Table 1.3). In particular, a study of primarily Inuit women in the territory of Nunavut showed higher rates of HR-HPV infection than in other Canadian studies, which is consistent with the elevated rate of cervical cancer in this population[28]. In addition, a higher prevalence of HR-HPV in Inuit women aged 13 to 20 years (32%) compared to non-Inuit women of the same age (12%) may suggest that HPV infection is acquired at an earlier age in the Aboriginal women. In contrast, a study of women attending a primary care clinic in Winnipeg[34] did not find a significant difference between the prevalence of HPV (any type) in Aboriginal versus non-Aboriginal women. However, HR types 18, 31, 33 and 35 and LR types 6 and 11 were more commonly detected in Aboriginal women than non-Aboriginal women. HPV-18 represented the most prevalent HR-HPV type found in Aboriginal study subjects.

2 RATIONALE AND STUDY SETTING

2.1 STUDY SETTING

This study was conducted in Nunavik, an arctic and sub-arctic region of Northern Quebec that covers approximately 500,000 square kilometres (Figure 2.1). The population of Nunavik is comprised of 12,000 people scattered across 14 coastal communities that lie on Hudson Bay and Ungava Bay. Approximately ninety percent of residents of Nunavik self-identify as Inuit.

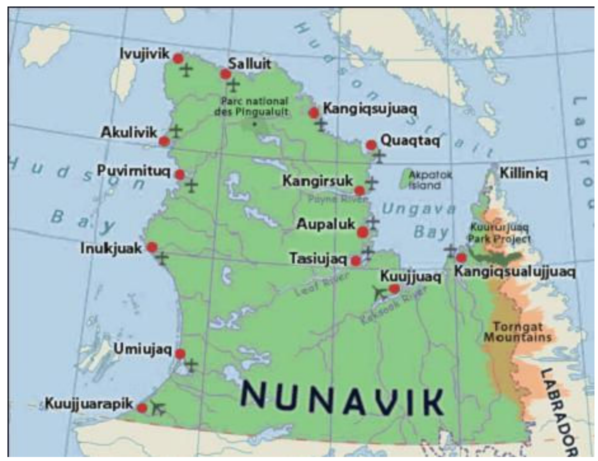

Figure 2.1: Nunavik, Quebec[94] (left), Communities in Nunavik[95] (right)

The population of Nunavik is predominantly young and growing. Between 1995 and 2000, the growth rate for this region was 10.5%[96], as compared to only 1.4% in Quebec overall[97]. The age pyramid of Nunavik (Figure 2.2) resembles that of some of the world's lowest-income nations, with more than fifty percent of the population aged less than 20 years. The Quebec Inuit experience a unique set of socioeconomic and cultural challenges in their daily lives which manifest at the community level in the form of unusually high rates of suicide and teenage pregnancy, as well as elevated incidence of STIs, including chlamydia and gonorrhea[98]. The impact of chronic diseases such as diabetes is also of growing public health concern.

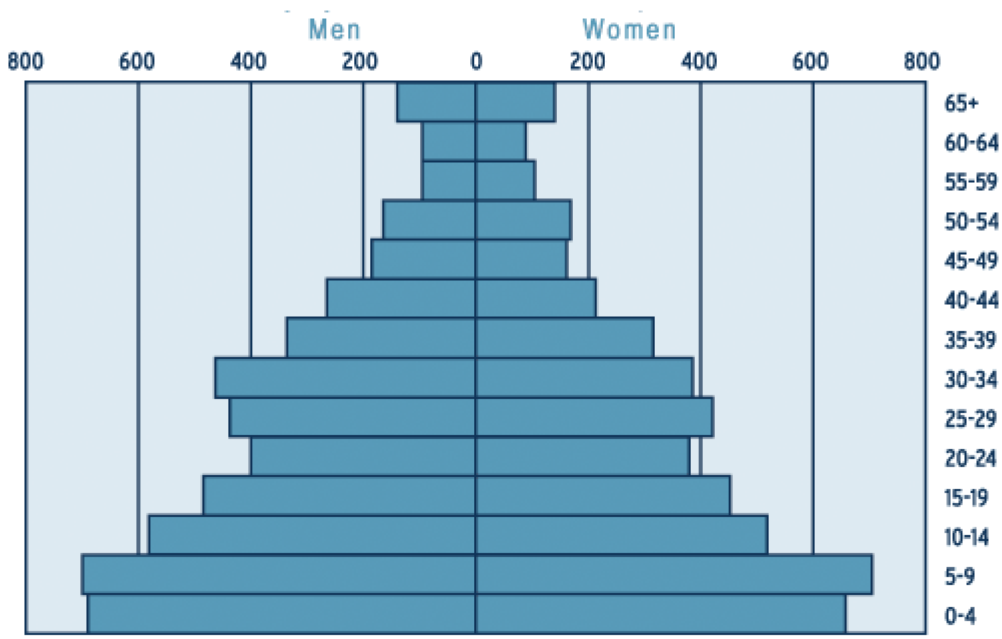

Figure 2.2: Age pyramid of the Nunavik population, 2000[99]

2.2 RATIONALE

HPV infection is a highly prevalent STI, whereas cervical cancer only strikes women who have cofactors that put them at risk for development and progression of the disease. Differing access to preventive screening likely contributes to large regional differences in cervical cancer rates, but viral and host cofactors may also play an important role. Widespread efforts have been made to study the epidemiology of HPV and cervical cancer in geographically and sociodemographically disparate populations in order to better understand these relationships. This type of epidemiologic information will become increasingly valuable as HPV vaccination and HPV-DNA testing become integrated into cervical cancer screening and prevention programs.

In Canada, Inuit women represent a particularly high-risk group for cervical cancer. Whereas cervical cancer accounts for 10% of all cancers worldwide[100] and 4.4% of cancers in the developed world[101], cervical cancer accounts for approximately 15% of female cancers in the Canadian Inuit[102]. One-fifth of Canada's Inuit population live in the province of Quebec[103], the majority of whom inhabit the self-governing region of Nunavik. Recent data suggest that women living in Nunavik are at a three times higher risk for developing cervical cancer than Quebec women overall[104]. Between 1971-1984, cervical cancer accounted for 28% of female cancers in Nunavik (15 cases)[105], and between 1987-1997 it represented 11% of all deaths due to cancer and 2% of all-cause mortality[98]. While the incidence of adenocarcinoma is increasing in Canada and worldwide, cases of cervical cancer reported in Nunavik are almost exclusively of the squamous cell carcinoma type.

To date, there are no published studies using PCR detection techniques that describe the prevalence of HPV infection in Quebec Inuit populations. In light of the high burden of cervical cancer in Quebec Inuit women, the reporting of such epidemiologic data, as well as the investigation of risk factors for HR-HPV infection, may be quite important in informing future vaccination and screening efforts. To this end, the purpose of this study is to provide an understanding of the prevalence and determinants of HR-HPV infection in a population of Inuit women residing in Nunavik, Quebec.

2.3 OBJECTIVES

The main objectives of this study were to:

1) Determine the type- and age- specific prevalence of HPV in a population of Inuit women residing in Nunavik, Quebec.
2) Determine the sociodemographic and behavioural predictors of HR-HPV infection in this population.

The primary hypotheses were that this population experiences a high prevalence of HPV infection, and of HR-HPV in particular, when compared to other Canadian populations, and that age and markers of sexual activity are the most important predictors of prevalent HR-HPV infection.

3 METHODOLOGY

3.1 STUDY DESIGN

3.1.1 Overview

A cross-sectional survey study design was used to answer the primary study questions. This analysis was conducted using data from a prospective cohort study of Inuit women who live in communities on Ungava Bay and Hudson Bay in Nunavik, Quebec. The current analysis utilizes information from a baseline questionnaire and corresponding cytology and HPV-DNA test results.

3.1.2 Target Population

The target population for this study was all Inuit women aged 15 to 69 years residing in Nunavik, Quebec between January 2002 and December 2007. In 2001, the total population for the Nunavik region was 9,600, ninety percent of whom self-identified as Inuit. Approximately half of the self-identified Inuit residents of Nunavik were women and roughly 2,480 were females between the ages of 15 and 69[106].

3.1.3 Sample Size Calculation

There are no published data on the prevalence of HPV infection or cervical lesions amongst the Inuit of Nunavik. A 26% prevalence of HR-HPV and 7.2% prevalence of cervical lesions have been reported previously in Nunavut[28]. The pilot study for this project detected a 7.5% prevalence of HPV infection and 11.9% prevalence of cervical lesions amongst Inuit women of Kuujjuaq. It was also estimated that with 400 eligible women and an 80% participation rate, that nearly 300 subjects could be enrolled in the study, with at least one Pap smear per year. Table 3.1 outlines the sample size needed for varying prevalence rates and levels of precision.

Table 3.1: Sample size calculations for estimating varying prevalence of HPV*

Expected HPV Prevalence	Precision (%)	Sample size (n)
7	3.0	278
10	3.5	282
15	4.0	306
20	5.0	246

* At a 95% confidence level

3.1.4 Eligibility Criteria

Women were eligible for the study if they:

1) Self-identified as Inuit
2) Were between 15 and 69 years of age
3) Were born in Nunavik, Quebec
4) Had an intact uterus and had no current referral for hysterectomy
5) Did not report use of vaginal medication in the previous 2 days
6) Did not report treatment for cervical disease in the previous 6 months, since these procedures may artificially increase clearance of HPV infection
7) Were no more than 12 weeks pregnant, since Pap tests are not recommended after 12 weeks in order to avoid spontaneous miscarriage, and changes to the cervix occur during pregnancy that may interfere with cytological tests[107]

3.1.5 Subject Recruitment

For the vast majority of study subjects, the sampling frame consisted of all women presenting for a regularly scheduled Pap test at clinics in one of the four participating communities of Kuujjuaq, Kangiqsualujjuaq, Kangiqsujuaq and Kangirsuk between January 2002 and December 2007. These communities are all located on Ungava Bay and were chosen based on the number of inhabitants. A small number of women were recruited when they presented for a mobile mammography screening program in communities along the coast of Hudson Bay and Ungava Bay between August and October 2004. Nurse practitioners systematically asked all non-enrolled women about their wish to participate in the study and, if they were interested, determined their eligibility. Written informed consent was obtained from all eligible subjects by means of a standardized consent form (Appendix 1). All enrolled participants were asked questions from a standardized questionnaire

(Appendix 2) by a nurse practitioner, who recorded the responses on the questionnaire form. The questionnaire collected information about sociodemographic and behavioural characteristics, as well as potential confounding factors. Cervical specimens were collected for both a Pap smear and HPV-DNA testing. When subjects enrolled through a clinic visit (not mobile mammography screening) presented for a subsequent visit requiring a Pap test, a cervical specimen was collected for follow-up. Any women who wished to withdraw from the study were able to do so at any time.

3.1.6 Ethical Considerations

Ethics approval was obtained from the McGill Institutional Review Board and the Tulattavik Health Centre, which provides services to study participants (Appendix 4).

3.2 DATA COLLECTION

3.2.1 Questionnaire

A questionnaire (Appendix 2) was administered at baseline by a nurse practitioner which collected information on sociodemographic characteristics, reproductive and sexual history, medical history, and some lifestyle factors, including smoking history and alcohol use. The study instrument was adapted from a previously validated questionnaire developed by one of our co-investigators (EL Franco) for use in community-based HPV surveys and was provided in English, French and Inuktitut. This research tool was validated by a steering committee composed of members drawn from the Nunavik community, the Tulattavik Health Centre, and the Nunavik Regional Board of Health and Social Services. It was further piloted by a group of ten Inuit women in order to ensure its comprehensibility and ease of use as a study instrument. The Inuktitut version of the questionnaire was back-translated into English in order to ensure accuracy of translation.

3.2.2 Medical Chart Review

Additional information on the medical history of study subjects, including their reproductive history, diagnosis of STIs, major surgeries, organ transplants, immunosuppression and use of steroid medications, was extracted from the medical charts by members of the research team. A standardized data retrieval form was used for review of medical charts (Appendix 3).

Reviewing patients' charts was also the primary means for retrieving the results of Pap tests performed at baseline and throughout the study period.

3.2.3 Cervical Specimen Testing and DNA Extraction

Cervical specimens were systematically collected at baseline and at each follow-up visit requiring a Pap test. Ectocervical and endocervical cells were collected with a Dacron swab and used to perform a Pap smear. After specimen collection, the swab was immersed in a tube containing 1.5 ml of a methanol-based liquid, PreservCyt (Cytyc Corporation, Boxborough, MA) which preserves the integrity of epithelial cells. Cell suspensions were kept at 4°C until they were transported on wet ice to the laboratory of Dr. François Coutlée in Montreal for HPV typing. The cervical smear slides were transported to Quebec City and read blindly by an experienced cytopathologist. Cytology results were sent back to the treating physician in the patient's respective community and placed in the medical chart. Cytopathology reports were based on the Bethesda classification system for cytological diagnoses[21].

3.2.4 HPV-DNA Testing and Typing

After cervical cell suspensions were centrifuged at 13000 x g for 15 minutes at 22°C, the supernatant was discarded, the cell pellet was left to dry and it was resuspended in 300 µl of 20 mM Tris buffer, pH 8.3. DNA was purified with Master pure[108] (Epicentre, Madison, WI). The quality of DNA samples was assessed by amplification of a 268-bp region of the human β-globin gene using GH20 and PC04 primers. HPV-DNA was detected by PCR amplification using PGMY09-PGMY11 primers and quality-controlled Line blot assay (Roche Diagnostics), as described previously[109]. Specimens were coded and provided to laboratory personnel who were blind to any information about the subjects from which the samples originated. Standard precautions were taken to prevent contamination.

HPV genotyping was accomplished using oligonucleotide probes to identify 26 genital HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68, 73, 82 (IS39 and MM4 subtypes), 83 and 84. After April 2004, an extended line blot strip was used that probed for an additional 10 genotypes: 61, 62, 64, 67, 69, 70, 71, 72, 81 and 89 (CP6108).

Samples were considered HPV-positive if they were positive for any of the 36 HPV types and also positive for β-globin. Samples were considered HPV negative if they were not positive for any of the HPV types, but were positive for β-globin. Subjects with a negative β-globin result, whether positive or negative for HPV, were not considered to have a baseline HPV result of acceptable quality.

3.2.5 Data Management

At recruitment, study participants were each assigned a unique identifier that was used to link information collected from the questionnaire and medical chart review, as well as HPV-DNA test results. In order to ensure the confidentiality of participants, all identifying information except for this unique ID was excluded from the databank used in statistical analyses. Access to data collection sheets and consent forms was restricted to members of the research team.

3.3 STATISTICAL ANALYSIS

3.3.1 Inclusion in Dataset

A dataset was built for the purpose of examining predictors of prevalent HR-HPV infection. Subjects were included in this dataset if they met eligibility criteria for the study, had completed a baseline questionnaire and consent form and had a baseline HPV-DNA test result within 70 days of the questionnaire. A baseline cytology result was considered as such if the specimen was collected within a 30-day period of the baseline HPV-DNA test result. If a matching cytology result was not available, women were still included in the analysis but with a missing value for their baseline cytology. The variable for Pap history (having had a Pap test in the previous 3 years) was measured by using the baseline HPV test as time zero and looking back three years for a record of Pap testing.

3.3.2 Study Variables

HPV Status

HPV types were classified as either HR or LR based on their oncogenic potential[5]. Probable HR types were grouped with those with more established evidence for HR oncogenic potential: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. Unclassified types were grouped with LR types: 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84 and 89 (CP6108)[49].

Independent Variables

Information on sociodemographic, medical history and lifestyle variables was collected through administration of the baseline questionnaire and review of enrolled subjects' medical charts. The main independent variables on which information was collected were:

Sociodemographic Variables:

- Age (years)
- Community (Kuujjuaq, Kangiqsualujjuaq, Kangiqsujuaq, Kangirsuk, Other)
- Marital status (Single, married, divorced, widowed, living with partner)
    - Re-categorized as 0 = Married or living with partner
      1 = Single (single, divorced, widowed)
- Employment status (0 = Unemployed; 1 = Employed)
- Level of education (Less than Grade 9, Grade 9 to 13, More than Grade 13)
    - Re-categorized as 0 = Less than Grade 9
      1 = More than Grade 9

Lifestyle Variables:

- Current smoker (0 = No; 1 = Yes)
- Ever smoked (0 = No; 1 = Yes)
- Current alcohol use (0 = No; 1 = Yes)
- Current birth control use (0 = No; 1 = Yes)
- Type of birth control used (OCs, medroxyprogesterone injection, condom, etc)

  Variable created for the use of hormonal contraceptives:
    - 0 = Does not use hormonal contraceptives
    - 1 = Uses hormonal contraceptives (OCs, medroxyprogesterone injection)

Sexual Behaviour

- Age at first sexual intercourse (years)

- Number of lifetime sexual partners (0 = Fewer than 10; 1 = More than 10)
- Number of sexual partners in the past year
- Number of sexual partners in the past month

<u>Gynaecological and Obstetric Events</u>

- Currently pregnant (0 = No; 1 =Yes)
- Lifetime number of deliveries
- Self-reported STI history (0 = No; 1 = Yes)

3.3.3 Coverage of Target Population and Selection Bias

Coverage of the target population was evaluated by utilizing 2001 Census data that includes information on the Aboriginal status of Canadian populations. The overall and age-specific coverage was calculated for the female Aboriginal (predominantly Inuit) populations for each of Kuujjuaq, Kangiqsualujjuaq, Kangiqsujuaq and Kangirsuk, the communities from which the largest proportion of study subjects were recruited. Overall and age-specific coverage for the combined population of these four communities was also calculated.

It was considered unfeasible to record the exact numbers of women invited into the study or to collect even basic sociodemographic information on women who chose not to participate. Nurse practitioners from each study community carried out recruitment in addition to their usual clinical and administrative duties. It was important to avoid overburdening these staff as well as to seek both their and the patients' acceptance of the study by not being overly intrusive or demanding of personal information. In addition, the high turnover rate of healthcare staff in these communities meant that a simplified research protocol had a greater chance of sustainability.

Selection bias was evaluated in a limited way by comparing characteristics of the study population with those of the general population of Nunavik. Published statistics on all female residents of Nunavik (Inuit and non-Inuit) as well as those concerning only Inuit women living in Nunavik were used in this assessment.

3.3.4 HPV-DNA Prevalence

The prevalence of HPV infection was calculated by type, oncogenic risk grouping and alpha-papillomavirus species. Age-specific prevalence was calculated for women aged 15-19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years and 60-69 years. Wilson's method, with a continuity correction, was used to calculate 95% confidence intervals (CIs) for type-specific HPV prevalence[110]. CIs were reported only for HPV types whose prevalence had a relative standard error of less than 50%. For prevalence by type and phylogenetic species, co-infections contributed to multiple categories

3.3.5 Clustering Analysis

We investigated whether any joint infections occurred with a greater frequency than would be expected under the assumption of no association between types[111]. Expected frequencies were compared with observed frequencies to detect types that co-occur. We used Fisher's exact test to identify possible patterns in type associations rather than to formally test the significance of these associations.

3.3.6 Determinants of HR-HPV Infection

Missing Data

The proportion of missing data was evaluated for each variable that was considered in the analysis of determinants of prevalent HR-HPV infection. All variables which were plausibly of interest in the modeling process were explored in univariate and multivariate analysis. Because a small proportion of missing data was observed across many variables, the complete case dataset containing all variables of interest (n=408) was substantially smaller than the overall study population (n=554). Univariate and multivariate analyses were carried out on both a complete case dataset and multiply imputed datasets. All univariate and multivariate analyses on the complete case dataset were completed using SAS Statistical Software version 9.1. Multiple imputation and related analyses were performed in the statistical computing program R version 2.4.1.

Complete Case Analysis

Univariate Analysis

Univariate unconditional logistic regression was performed on all independent variables to explore their association with the outcome of HR-HPV infection. Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were calculated. Relationships between variables were explored using correlation matrices, scatter plots and cross-tabulations of categorical variables.

Variable Selection and Multivariate Analysis

We examined the effect of each variable, independent of age, by generating age-adjusted ORs and 95% CIs. Model selection was then accomplished by placing in a multivariable model variables that have been found to be associated with HPV infection (HR or overall HPV) in the literature, as well as variables that could be considered potential confounders, proxies for confounding factors, or that were important for face validity. The effect of indicators of sexual activity or proxies for these indicators (age at first sexual intercourse, number of lifetime sexual partners, number of sexual partners in the previous month or year, and marital status) when included in or excluded from the model was investigated and only two of the variables were placed in the final model in the interest of parsimony. A multivariate unconditional logistic regression was performed using a complete dataset for all the variables selected for inclusion in the final multivariable model. ORs and their associated 95% CIs were calculated for the association between each independent variable and the outcome of prevalent HR-HPV infection, adjusted for all other variables in the model. The presence of statistical interaction was investigated by including interaction terms in the multivariable model and examining the resulting effect estimates and associated CIs.

3.3.7 Multiple Imputation Analysis

Multiple Imputation

Multiple imputation is a widely accepted and useful strategy for dealing with missing data, which generates a *set* of plausible values for each missing data point rather than replacing them with single values. The imputed datasets generated through this process are analyzed by standard statistical procedures and the results are subsequently combined for inference (Figure 3.1).

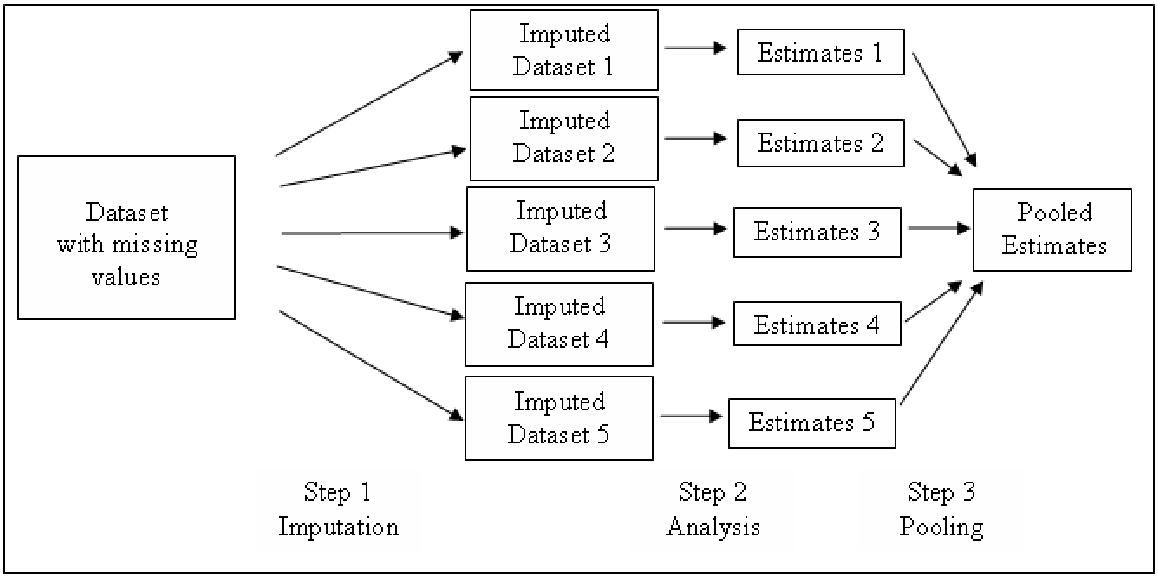

Figure 3.1: Schematic diagram of multiple imputation method
Three steps are involved in multiple imputation: First, imputed datasets are created, which fill in a range of plausible values for missing data points in the original dataset (in this example, five imputations are performed). Next, a standard analysis (e.g. linear or logistic regression) is performed on each imputed dataset, generating separate estimates for each. Finally, estimates from all the imputed datasets are pooled.

All variables which were considered for the complete case analysis and those which could possibly predict missingness for variables with missing data were included in the imputation dataset. The MICE package for the statistical computing program R version 2.4.1 was used for multiple imputation and subsequent analyses. The program includes three main commands: *mice*, which imputes missing data according to models specified in a prediction matrix, supplied by the user; an analysis command, such as *glm.mids,* which performs logistic regression on each of the imputed datasets; and *pool* which averages across the parameter estimates for the regression in the previous step. In our imputation analysis, the prediction matrix allowed information from all other variables in the dataset to predict missingness in each variable with missing data. Exploratory work was performed on a single imputed dataset for ease of analysis and all other analyses were performed using ten imputed datasets. Univariate analysis, variable selection and multivariate analysis were performed in the same manner as for the complete case analysis.

4 RESULTS

4.1 RECRUITMENT AND ELIGIBILITY

Between January 2002 and December 2007, 629 women were recruited into the cohort. A total of 554 women met the eligibility criteria, completed a baseline questionnaire and had an adequate HPV-DNA test result. These women were included in the analysis of prevalence at baseline. Women were excluded from the analysis because they were ineligible for the study or because they did not have a matching baseline questionnaire and adequate HPV-DNA test result. The baseline characteristics of the study population were not significantly different if these women were included in or excluded from the dataset. Twenty-two baseline HPV-DNA test results were excluded because they were negative for β-globin. Further type-specific testing detected HPV-DNA in five of these samples, but they were still considered of inadequate quality to be included in the analysis.

4.2 COVERAGE OF TARGET POPULATION

The coverage of the target populations for the individual communities of Kuujjuaq, Kangiqsualujjuaq, Kangiqsujuaq and Kangirsuk was between 42% and 71%. The combined coverage for these communities was 57%. The study captured 58% of 15-19 year-olds, 68% of 20-24 year-olds, 59% of 25-44 year-olds, 38% of 45-54 year-olds and 66% of 55-64 year-olds in these villages. The age distribution of the study subjects roughly mirrored that observed in the target population, both when the populations of these villages were pooled together (Figure 4.1) and when they were examined separately.

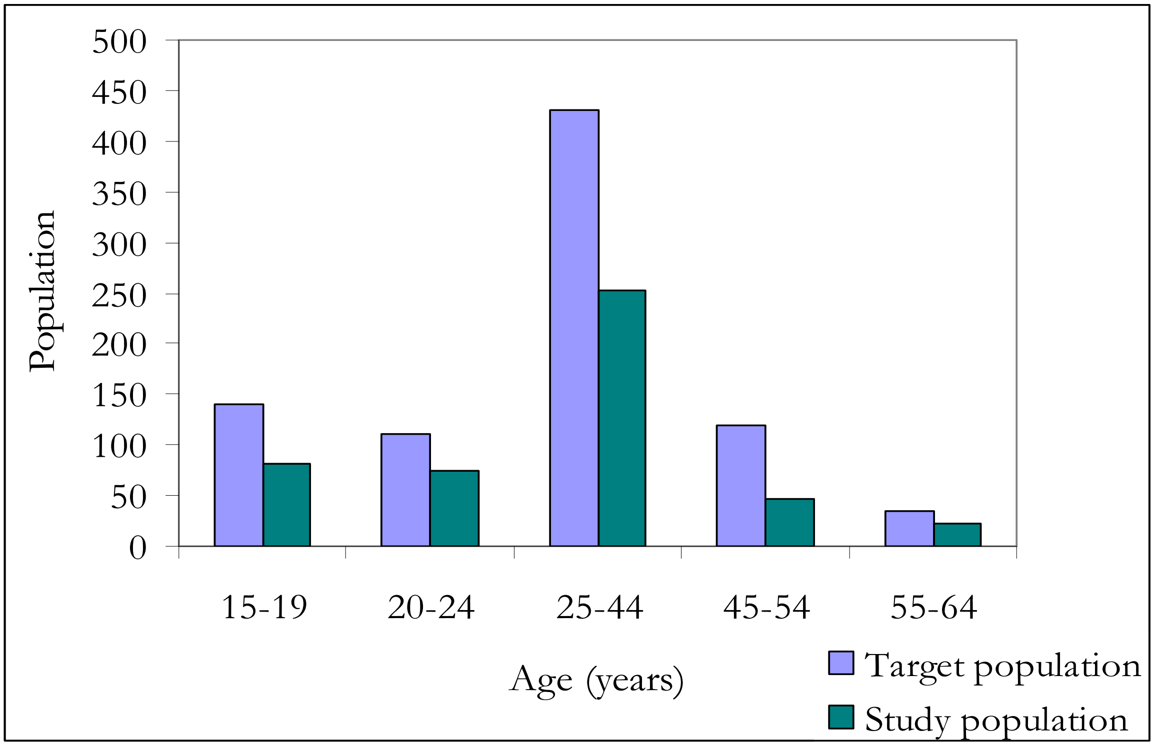

Figure 4.1: Age distribution of target and study populations for the four primary participating communities

Data for the target population (Kuujjuaq, Kangiqsualujjuaq, Kangiqsujuaq, and Kangirsuk) was retrieved from the 2001 Census Aboriginal Profiles: http://www12.statcan.ca/english/Profil01/AP01/Index.cfm?Lang=E

4.3 COMPARING SELF-REPORT AND MEDICAL CHART REVIEW

Information on several variables, namely history of STI and lifetime number of deliveries, was collected through both self-report and medical chart review. Because there was substantially more missing information for the variables when collected through medical chart review, the self-reported data was preferentially included in the analysis. In order to determine if substantial bias could be introduced by using the self-reported data, concordance between the two sources was examined by means of a kappa statistic. The simple kappa coefficient for self-reported versus physician-reported history of STI was 0.30 (95% CI: 0.21, 0.40) which is considered fair agreement. Although agreement was not ideal, the substantial amount of missing data for physician-reported history of STI (19% missing) justified retaining self-reported history of STI in the dataset. For the lifetime number of deliveries (0, 1-3, ≥4), the simple kappa coefficient was 0.80 (95% CI 0.75 – 0.86) which suggests very good agreement between the two measures. Self-reported lifetime number of deliveries was retained in the dataset since information was more complete for this variable than for the physician-reported measure.

4.4 CHARACTERISTICS OF THE STUDY POPULATION BY HPV STATUS

4.4.1 Sociodemographic Characteristics

Table 4.1 presents the sociodemographic characteristics of the study population. Almost half of the participants were from Kuujjuaq, a fifth were from Kangiqsualujjuaq and smaller proportions were from Kangiqsujuaq (10.3%), Kangirsuk (10.8%) and a collection of other communities, mostly on Hudson Bay (11.4%). There were no striking differences in the prevalence of either HPV overall or HR-HPV across the villages. There was higher prevalence of HPV-16/18 in subjects from Kangirsuk (13.3%) and Kuujjuaq (8%) to a lesser extent, as compared to subjects from the other communities (3.5-5.4%).

Table 4.1: Distribution of HPV status by sociodemographic characteristics (N=554)

	N (%)	% Any HPV-DNA	% Any HR	% Only LR	% HPV-16/18
Community					
Kuujjuaq	263 (47.5)	30.0	22.8	7.2	8.0
Kangiqsujuaq	57 (10.3)	26.3	22.8	3.5	3.5
Kangiqsualujjuaq	111 (20.0)	28.8	18.0	10.8	5.4
Kangirsuk	60 (10.8)	30.0	23.3	6.7	13.3
Other	63 (11.4)	25.4	9.5	15.9	4.8
Age (years)					
15-19	81 (14.6)	58.0	46.9	11.1	25.9
20-29	163 (29.4)	30.1	24.5	5.5	6.7
30-39	122 (22.0)	25.4	17.2	8.2	4.1
40-49	67 (12.1)	13.4	4.5	9.0	0.0
50-59	89 (16.1)	16.9	7.9	9.0	2.2
60-69	32 (5.8)	28.1	12.5	15.6	3.1
Marital status					
Married or living with partner	307 (55.4)	19.5	13.7	5.9	5.2
Single	242 (43.7)	40.1	28.1	12.0	9.9
Missing	5 (0.9)	60.0	60.0	0.0	0.0
Education					
< Grade 9	212 (38.3)	23.6	14.2	9.4	4.7
> Grade 9	324 (58.5)	31.8	23.5	8.3	9.0
Missing	18 (3.2)	38.9	38.9	0.0	5.6
Employed					
No	165 (29.8)	37.6	24.8	12.7	9.7
Yes	379 (68.4)	24.8	17.9	6.9	5.8
Missing	10 (1.8)	40.0	40.0	0.0	20.0

The mean age of participating women was 35.5 years (SD=14.4) and women ranged from 15 to 69 years of age (median=32.2). The prevalence of all categorizations of HPV infection was highest in the youngest age group; prevalence of infection decreased with age, but showed a second peak in women aged 60-69 years.

The study population was approximately evenly divided between women of single marital status and those who were married or living with a partner. There was a higher prevalence of HPV in single women across all categorizations of infection. Nearly 40% of subjects had less than a grade nine education. Women with lower education had a lower prevalence of HPV overall, HR-HPV and HPV-16/18. They had slightly elevated detection of only LR types than their more educated counterparts. Approximately 70% of study subjects were employed at baseline. There was higher prevalence of HPV in the unemployed subjects across all categorizations of infection.

4.4.2 Cigarette Smoking and Alcohol

Most women (93%) had a previous smoking history and 71% were current smokers at baseline (Table 4.2). Women who were not current smokers had a lower prevalence of HPV overall, HR-HPV and HPV-16/18. Nearly 70% of study subjects drank alcohol at baseline. Women who were not current users of alcohol had a lower prevalence of HPV overall, HR-HPV and HPV-16/18. A slightly higher proportion of women who did not drink had only LR types detected when compared to those who used alcohol.

Table 4.2: Distribution of HPV status by cigarette smoking and alcohol consumption (N=554)

	N (%)	% Any HPV-DNA	% Any HR	% Only LR	% HPV-16/18
Current smoker					
No	155 (28.0)	20.6	12.3	8.4	5.2
Yes	393 (70.9)	32.1	23.4	8.7	8.1
Missing	6 (1.1)	33.3	33.3	0.0	0.0
Current alcohol use					
No	178 (32.1)	24.2	12.9	11.2	5.1
Yes	372 (67.1)	31.2	23.9	7.3	8.3
Missing	4 (0.7)	25.0	25.0	0.0	0.0

4.4.3 Reproductive Health Characteristics

Table 4.3 displays reproductive health characteristics of the study subjects. Most subjects (87.4%) did not report being pregnant at baseline. The mean number of lifetime deliveries was 3 (median=3; range 0 to 14). Most women (84%) had given birth to at least one child. A higher proportion of women with no deliveries were positive for HPV overall, HR-HPV and HPV-16/18 than women who had given birth to a child. Women who did not provide information on deliveries (n=42, 7.6% of study population) had higher prevalence of HPV infection across all categorizations when compared with women who reported having given birth to one or more child.

Birth control was being used by 35% of women, and 22% of subjects used a hormonal form of contraception (birth control pill or medroxyprogesterone injection). There was slightly higher prevalence of HPV overall, HR-HPV and HPV-16/18 observed both in women who used birth control generally and those who used hormonal contraceptives specifically versus those who did not, but the differences were not substantial. Of the women for whom information on Pap screening history was available (n=460), 71% had a Pap test in the previous three years. There were no substantial differences in the prevalence of HPV infection in women who had a history of Pap screening versus those who did not.

Sixty-six percent of women had a positive self-reported history of STI. Women with a positive history of STI had a slightly higher prevalence of HR-HPV and HPV-16/18 and slightly lower prevalence of only LR-HPV detected than those with a negative history.

Table 4.3: Distribution of HPV status by reproductive health characteristics (N=554)

	N (%)	% Any HPV-DNA	% Any HR	% Only LR	% HPV-16/18
Currently pregnant					
No	484 (87.4)	27.5	18.8	8.7	6.8
Yes	50 (9.0)	36.0	28.0	8.0	10.0
Missing	20 (3.6)	45.0	40.0	5.0	10.0
Lifetime deliveries					
0	81 (14.6)	46.9	39.5	7.4	19.8
1 – 3	231 (41.7)	28.1	19.9	8.2	5.2
≥ 4	200 (36.1)	20.5	12.0	8.5	3.5
Missing	42 (7.6)	38.1	26.2	11.9	11.9
Current use of birth control					
No	342 (61.7)	28.1	18.4	9.6	6.7
Yes	191 (34.5)	30.9	23.6	7.3	8.4
Missing	21 (3.8)	23.8	23.8	0.0	4.8
Current use of hormonal contraceptive					
No	416 (75.1)	27.9	18.0	9.9	6.0
Yes	119 (21.5)	33.6	28.6	5.0	11.8
Missing	19 (3.4)	21.1	21.1	0.0	5.3
History of Pap test in previous 3 years					
No	135 (24.4)	29.6	20.0	9.6	7.4
Yes	325 (58.7)	30.5	23.3	7.1	8.3
Missing	94 (17.0)	22.3	10.6	11.7	3.2
Self-reported history of STI					
No	176 (31.8)	30.1	18.2	11.9	5.7
Yes	363 (65.5)	28.7	21.5	7.2	8.0
Missing	15 (2.7)	20.0	20.0	0.0	6.7

4.4.4 Sexual Behaviour Characteristics

All women in the study population reported previously having had sexual intercourse. The mean age at first sexual intercourse (Table 4.4) was 15.5 years (SD =2.5) and the median age was 15 years. Age at first sexual intercourse ranged from 7 to 30 years of age in the study population. Overall HPV prevalence, HR-HPV prevalence and HPV-16/18 prevalence were all higher in women whose age at first sexual intercourse was less than 15 years of age. Thirty-four percent of the study population reported having ten or more lifetime sexual partners. The prevalence of HPV overall, HR-HPV and HPV-16/18 was higher in these women than those with fewer than ten lifetime sexual partners. The detection of only LR-HPV did not vary considerably between the two groups. The median number of sexual partners in the previous year was one. Almost thirty percent of women had two or more sexual partners in the previous year. These women had a higher prevalence of HPV overall, HR-HPV and HPV-16/18 than those with one or fewer partners. Most women (68%) had one sexual partner over the previous month. Women with two or more sexual partners over the previous month had higher prevalence of HPV overall, HR-HPV and HPV-16/18 than women with fewer partners.

Table 4.4: Distribution of HPV status by sexual behaviour characteristics (N=554)

	N (%)	% Any HPV-DNA	% Any HR	% Only LR	% HPV-16/18
Age at first sexual intercourse (years)					
> 20	31 (5.6)	12.9	6.5	6.5	3.2
15-20	205 (37.0)	26.3	18.0	8.3	4.9
< 15	278 (50.2)	32.7	24.5	8.3	10.4
Missing	40 (7.2)	27.5	15.0	12.5	0.0
Lifetime number of sexual partners					
<10 partners	328 (59.2)	25.0	16.2	8.8	7.0
≥ 10 partners	188 (33.9)	37.2	28.7	8.5	9.0
Missing	38 (6.9)	21.1	15.8	5.3	0.0
Number of sexual partners in previous year					
0	52 (9.4)	19.2	7.7	11.5	0.0
1	315 (56.9)	23.2	15.9	7.3	6.0
≥2	154 (27.8)	42.2	32.5	9.7	11.7
Missing	33 (6.0)	36.4	27.3	9.1	9.1
Number of sexual partners in previous month					
0	109 (19.7)	27.5	16.5	11.0	3.7
1	376 (67.9)	26.9	19.4	7.4	8.0
≥ 2	43 (7.8)	46.5	34.9	11.6	9.3
Missing	26 (4.7)	34.6	26.9	7.7	7.7

4.5 HPV-DNA PREVALENCE

HPV-DNA was detected in 28.9% of subjects (n=160) and 32 different HPV types were identified. The most common HPV types detected (Figure 4.2) were HPV-16 (5.6%), HPV-31 (3.6%), HPV-61 (3.6%) and HPV-84 (3.1%). Of the HPV-positive women, 70.6% (n=113) were infected with at least one HR type and 46.3% (n=74) had exclusively HR types, for an overall HR prevalence of 20.4%. Infections with HPV-16 or HPV-18 (n=40) comprised 25% of all HPV infections and 35.4% of all HR infections.

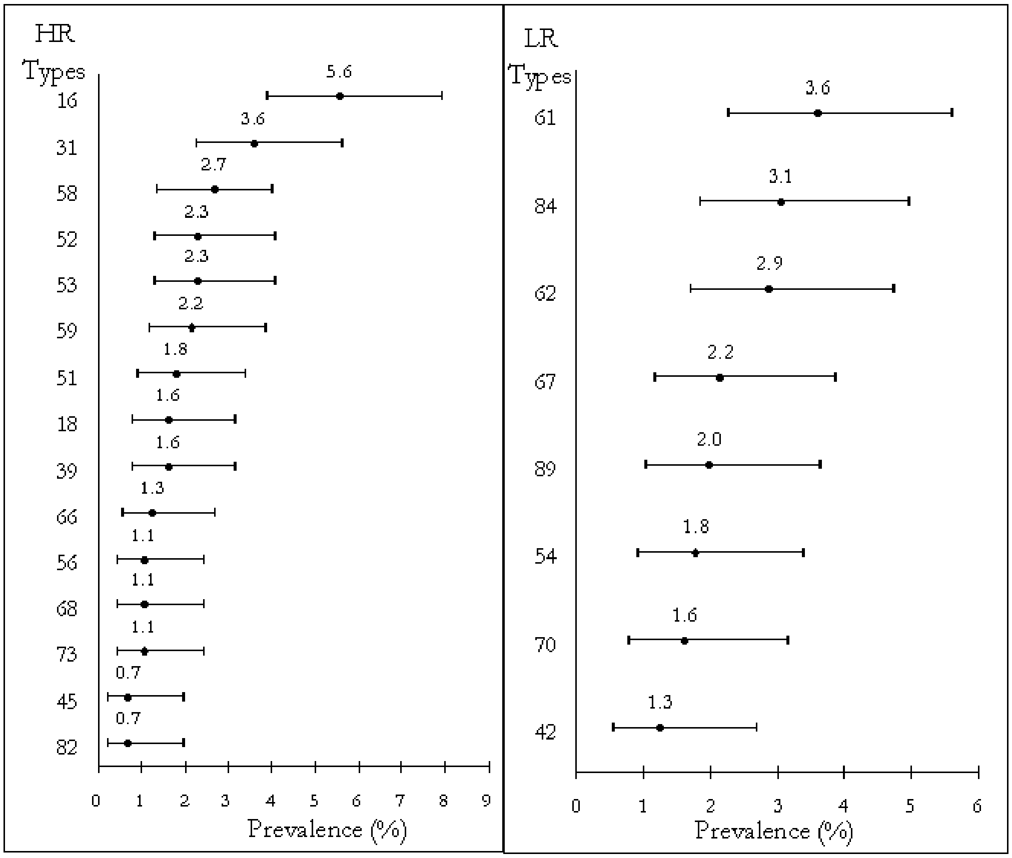

Figure 4.2: Detection of HR- and LR-HPV types among all subjects (N=554)
*Point estimates with 95% confidence intervals are displayed for the most common HR- and LR-HPV types. HPV types with a relative standard error of more than 50% are not presented. Listed here are the other HPV types that were tested for, with the number of infections in parentheses: HPV-26 (n=1), HPV-33 (n=3), HPV-35 (n=2), HPV-6 (n=2), HPV-11 (n=0), HPV-40 (n=1), HPV-55(n=4), HPV-64 (n=0), HPV-69 (n=0), HPV-71 (n=0), HPV-72 (n=2), HPV-81 (n=3), HPV-83 (n=2).

The most common HR types (Figure 4.2) after HPV-16 and HPV-31 were HPV-58 (n=15, 2.7%), HPV-52 (n=13, 2.3%) and HPV-53 (n=13, 2.3%). HPV-18 was detected in 1.6% of subjects (n=9). Among LR types, HPV-6 was detected in only 0.4% of subjects (n=2) and HPV-11 was not detected in this population. The most common LR types (Figure 4.2) after HPV-61 and HPV-84 were HPV-62 (n=16, 2.9%), HPV-67 (n=12, 2.2%), and HPV-89 (n=11, 2%). The most prevalent papillomavirus species overall (Table 4.5) were alpha-9 (n=96, 60% of infections), alpha-3 (n=71, 44%) and alpha-7 (n=49, 31%).

Table 4.5: Frequency of HPV species and types in single and multiple infections (N=160)

HPV species/type	All infections† N (%)	Single infections n	Multiple infections‡ n
Species A1	7 (4.4)	0 (0.0)	7 (10.9)
42	7 (4.4)	0	7
Species A3	71 (44.4)	26 (27.1)	45 (70.3)
61	20 (12.5)	6	14
62	16 (10.0)	4	12
72	2 (1.3)	2	0
81	3 (1.9)	1	2
83	2 (1.3)	1	1
84	17 (10.6)	6	11
89	11 (6.9)	6	5
Species A5	15 (9.4)	6 (6.3)	9 (14.1)
26	1 (0.6)	1	0
51	10 (6.3)	4	6
69	0 (0.0)	0	0
82	4 (2.5)	1	3
Species A6	26 (16.3)	7 (7.3)	19 (29.7)
53	13 (8.1)	4	9
56	6 (3.8)	0	6
66	7 (4.4)	3	4
Species A7	49 (30.6)	19 (19.8)	30 (46.9)
18	9 (5.6)	2	7
39	9 (5.6)	4	5
45	4 (2.5)	2	2
59	12 (7.5)	3	9
68	6 (3.8)	2	4
70	9 (5.6)	6	3
Species A8	1 (0.6)	0 (0.0)	1 (1.6)
40	1 (0.6)	0	1
Species A9	96 (60)	35 (36.5)	61 (95.3)
16	31 (19.4)	13	18
31	20 (12.5)	6	14
33	3 (1.9)	0	3
35	2 (1.3)	1	1
52	13 (8.1)	7	6
58	15 (9.4)	4	11
67	12 (7.5)	4	8
Species A10	6 (3.8)	0 (0.0)	6 (9.4)
6	2 (1.3)	0	2
11	0 (0.0)	0	0
55	4 (2.5)	0	4
Species A11	6 (3.8)	0 (0.0)	6 (9.4)
64*	0 (0.0)	0	0
73	6 (3.8)	0	6
Species A13	10 (6.3)	3 (3.1)	7 (10.9)
54	10 (6.3)	3	7
Species A15	0 (0.0)	0 (0.0)	0 (0.0)
71	0 (0.0)	0	0

Total	160 (100)	96 (100)	64 (100)

* Subtype of HPV-34. †Relative contribution to total number of infections (n=160). Because HPV types from different species may contribute to multiple infections, the sum of percentages exceeds 100%. ‡Contribution to total number of multiple infections (n=64). Because HPV types from different species may be involved in multiple infections, the sum of percentages exceeds 100%.

The age-specific prevalence of HPV infection (Figure 4.3) was highest among women less than 20 years (58%) and decreased with age until there was a second peak in prevalence of 28.1% amongst women aged 60-69 years. HR types were more commonly detected than LR types in all age groups except for women over 40 years, when LR-HPV was twice as prevalent as HR-HPV (Figure 4.4). Similarly to overall HPV infection, the age-specific HR-HPV prevalence showed a U-shaped curve, with the highest prevalance in women under 20 years (46.9%), decreasing prevalence with age, and a second peak in women aged 60-69 (12.5%). The age-specific LR-HPV prevalence pattern had a more pronounced U-shape than for overall HPV or HR-HPV prevalence.

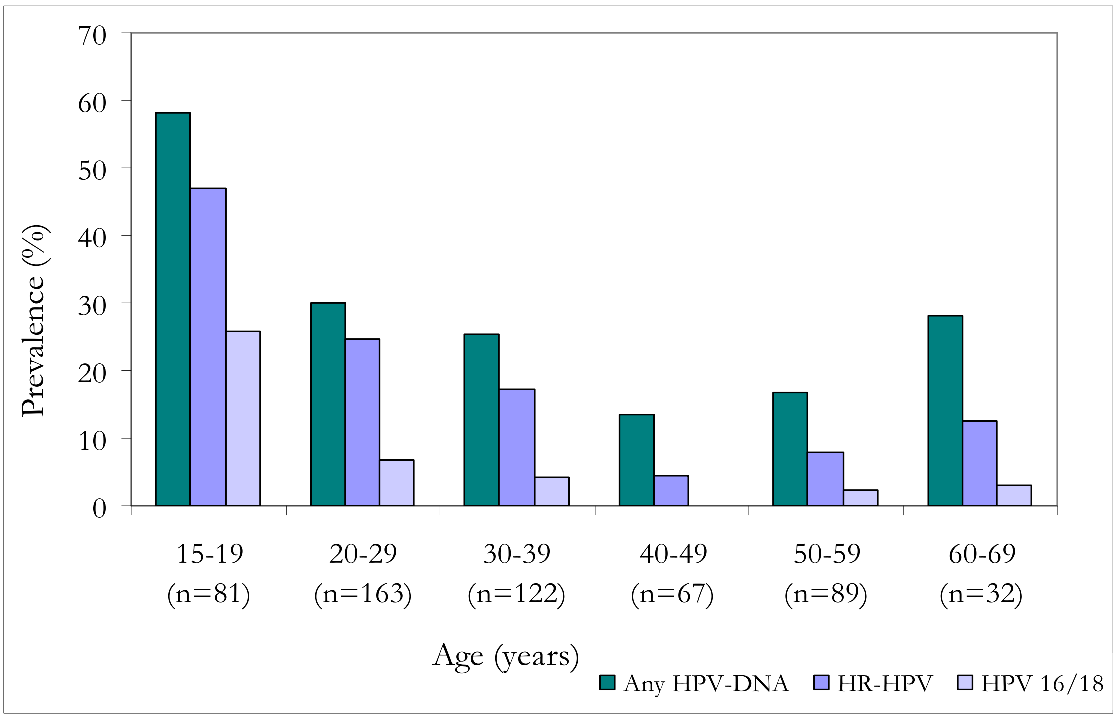

Figure 4.3: Age-specific prevalence of any HPV-DNA, HR-HPV, and HPV-16/18 (N=554)
HPV-DNA of any type was detected in 58% of women aged 15-19, 30.1% aged 20-29, 25.4% aged 30-39, 13.4% aged 40-49, 16.9% aged 50-59 and 28.1% aged 60-69. HR types were detected in 46.9% of women aged 15-19, 24.5% aged 20-29, 17.2% aged 30-39, 4.5% aged 40-45, 7.9% aged 50-59 and 12.5% aged 60-69. LR types were detected in 37% of women aged 15-19, 11.7% aged 20-29, 9.8% aged 30-39, 9% aged 40-49, 12.4% aged 50-59 and 25% aged 60-69. HPV-16 or -18 were detected in 25.9% of women aged 15-19, 6.7% aged 20-29, 4.1% aged 30-39, 0% aged 40-49, 2.2% aged 50-59 and 3.1% aged 60-69.

The prevalence of single type infections was 17.3% (n=96) in the overall study population and 60% amongst HPV-positive women. Among these women, the most common HR types were HPV-16 (n=13), HPV-52 (n=7) and HPV-31 (n=6) (Table 4.5). The most common LR types in single type infections were HPV-61, -70, -84 and -89 (n=6 for each). The prevalence curve for single type infections was U-shaped, with about 20% prevalence in the youngest and oldest age groups, but a decrease in prevalence amongst 40-49 year-olds (10.4%) and 50-59 year-olds (13.5%). HR-HPV was common in single type infections of women under 40 years of age (Figure 4.4), but made a smaller contribution to single type infections in older women.

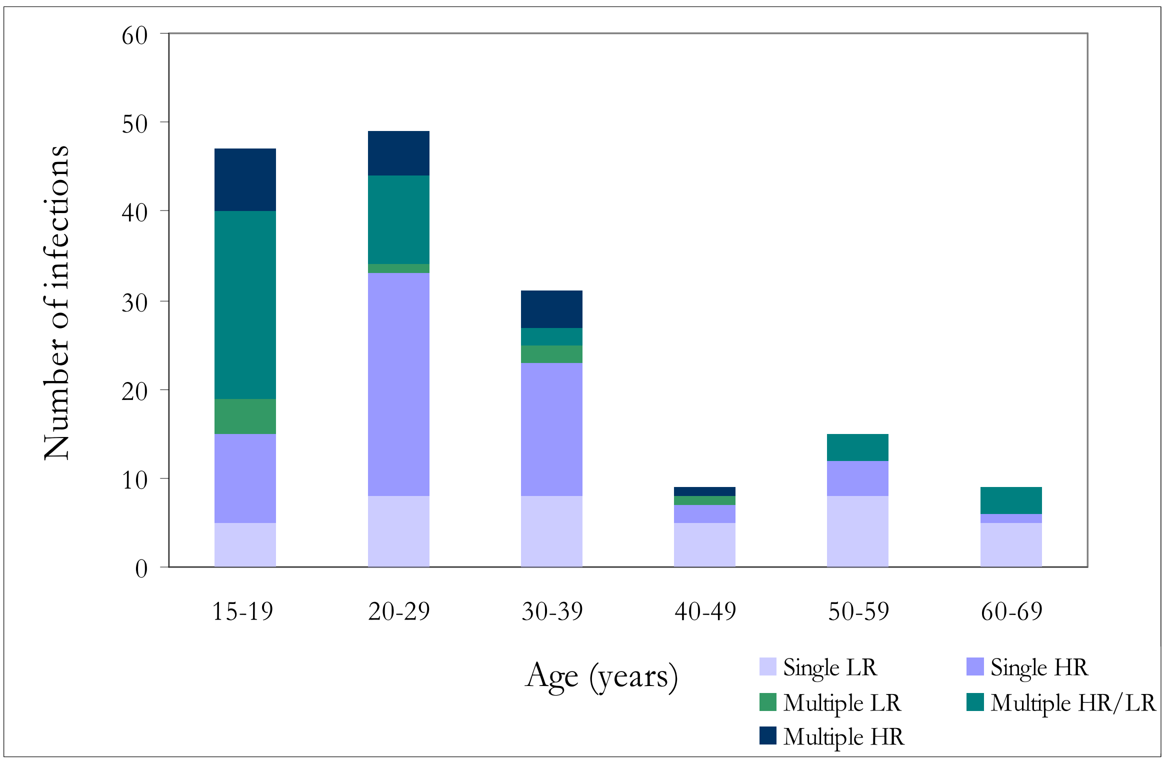

Figure 4.4: Distribution of single and multiple type infections by age (N = 554)

Multiple type infections were observed in 64 women (11.6% of the study population, 40% of HPV-positive women). Most multiple infections involved both HR and LR types (60.9%) and less frequently only HR types (26.6%) or only LR types (12.5%) (Figure 4.4). The prevalence of multiple infections was markedly higher among women under 20 years of age (39.5%) than in women of other age groups, and most of these infections involved at least one HR type. Overall, the most common HPV types involved in multiple infections (Table

4.5) were HPV-16 (n=18), HPV-31 (n=14), HPV-61 (n=14), and HPV-62 (n=12). The most common species involved in multiple infections were alpha-9 (n=61, 95% of multiple type infections), alpha-3 (n=45, 70%) and alpha-7 (n=30, 47%).

4.6 CLUSTERING OF HPV TYPES

In order to explore the tendency of particular HPV types to appear together in multiple type infections, the joint positivity of the thirteen most common HPV types (≥ 1.8 % baseline prevalence) was investigated. All pairwise frequency combinations at baseline are shown in Table 4.6. Each observed frequency was compared with the expected frequency under the assumption of no association between individual HPV types. With a total of 78 possible pairwise combinations, one would expect a few observed frequencies to depart significantly from expected frequencies by chance alone. If one assumes that the thirteen types analyzed are completely randomly distributed, we would expect about 5% (or four) of the associations to exceed the 5% significance level and about 1% (or one) to exceed the 1% significance level. We found a total of 25 pairs that exceeded the 5% level of significance, more than half of which also exceeded the 1% level. In all cases, the observed frequencies were greater than the corresponding expected value. The most common joint excesses involved the types HPV-58 (n=7), HPV-31 (n=6), HPV-54 (n=5), HPV-16 (n=4), HPV-62 (n=5) and HPV-67 (n=4), four of which are members of the alpha-9 species.

Table 4.6: Observed and expected frequencies of joint positivity for common HPV types (N=554)

HPV type	31	51	52	53	54	58	59	61	62	67	84	89
16	5** (1.1)	2 (0.6)	0 (0.7)	4** (0.7)	2 (0.6)	3* (0.8)	3* (0.7)	3 (1.1)	3 (0.9)	0 (0.7)	2 (1.0)	2 (0.6)
31		1 (0.4)	1 (0.5)	4** (0.5)	3** (0.4)	2 (0.5)	1 (0.4)	5** (0.7)	3* (0.6)	3** (0.4)	1 (0.6)	1 (0.4)
51			0 (0.2)	1 (0.2)	1 (0.2)	3** (0.3)	1 (0.2)	2* (0.4)	3** (0.3)	0 (0.2)	1 (0.3)	0 (0.2)
52				1 (0.3)	1 (0.2)	2* (0.4)	1 (0.3)	1 (0.5)	0 (0.4)	1 (0.3)	1 (0.4)	1 (0.3)
53					2* (0.2)	2* (0.4)	0 (0.3)	3** (0.5)	2 (0.4)	1 (0.3)	1 (0.4)	1 (0.3)
54						3** (0.3)	0 (0.2)	1 (0.4)	3** (0.3)	2* (0.2)	1 (0.3)	1 (0.2)
58							1 (0.3)	2 (0.5)	2 (0.4)	2* (0.3)	2* (0.5)	0 (0.3)
59								1 (0.4)	3** (0.3)	0 (0.3)	2* (0.4)	1 (0.2)
61									2 (0.6)	1 (0.4)	1 (0.6)	1 (0.4)
62										0 (0.3)	1 (0.5)	3** (0.3)
67											2* (0.4)	0 (0.2)
84												0 (0.3)

Expected frequencies appear in parentheses. Asterisks indicate significance level exceeded.
(* P<.05, ** P<.01)

4.7 PAP SCREENING HISTORY

Age-specific Pap screening history was examined amongst women for whom this information was available (n=460) (Figure 4.5). Non-coverage by Pap screening was most prevalent in the 15-19 year-old age group, 54% of whom had not had a Pap test in the previous three years. Pap screening coverage dramatically improved in women aged 20-29 years, with only 17% of women having a negative history. Of 30-39 year-old women, 25% had not had Pap test in the previous three years, which rose to 37% in 40-49 year-olds and 33% in 50-69 year-olds. When age-specific prevalence of HPV overall and HR-HPV were examined for these women, the highest prevalence (57% overall; 46% HR-HPV) was observed in women aged 15-19 years and prevalence decreased in women aged 20-29 years (30% overall; 24% HR-HPV). Further drops in HPV prevalence were observed across older age groups to a low in women aged 40-49 years (12% overall; 5% HR-HPV). Finally, a resurgence in HPV infection was detected in women aged 50-69 years (17% overall; 7% HR-

HPV), which was accompanied by only a slight improvement in Pap screening history when compared to 40-49 year-olds.

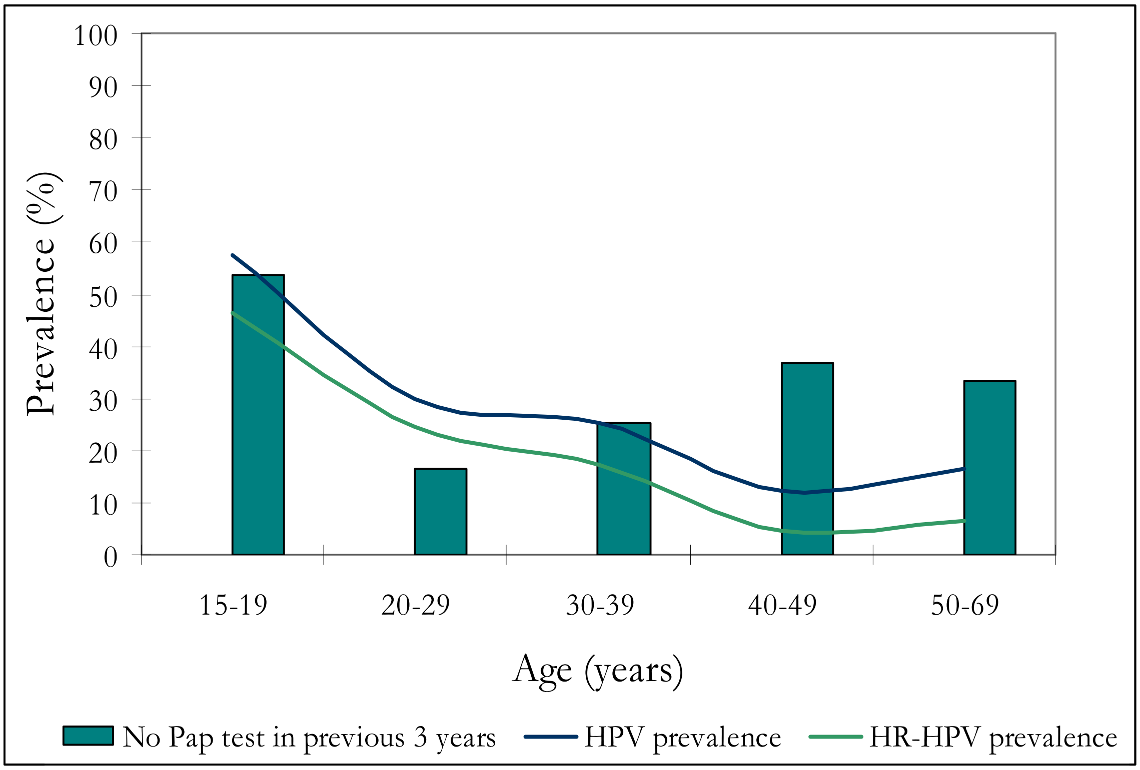

Figure 4.5: Pap screening history and HPV positivity (N=460)

4.8 DISTRIBUTION OF HR AND LR GENOTYPES BY CYTOLOGY

Of all subjects with a baseline cytology result (n=523), thirty-four women (6.5%) had an abnormal cytology that was classified as ASCUS, LSIL or HSIL. Seventeen women (3.3%) had LSIL or HSIL abnormalities detected at enrolment. Only 2.6% of specimens were inadequate, indicating good practices for specimen collection and preparation; 3.1% of the study population (n=17) had no baseline cytology result. The mean age of women with normal cytology was 35.9 years (SD=14.4); it was 33.7 years (SD=16.0) with ASCUS, 21.6 years (SD=6.6) with LSIL and 30.5 years (SD=8.5) with HSIL.

Table 4.7: Cytology results by HPV status (N=523)

Cytology	Overall N (%)	Any HPV n (%)	HR n (%)	LR n (%)	HPV 16/18 n (%)	Multiple HR/LR n (%)	Multiple HR n (%)
Normal	489 (93.5)	124 (25.4)	83 (17.0)	70 (14.3)	24 (4.9)	29 (5.9)	11 (2.2)
ASCUS	17 (3.3)	11 (64.7)	9 (52.9)	4 (23.5)	6 (35.3)	2 (11.8)	1 (5.9)
LSIL/HSIL	17 (3.3)	16 (94.1)	16 (94.1)	6 (35.3)	9 (52.9)	6 (35.3)	4 (23.5)
Total	523 (100)	151 (28.9)	108 (20.7)	80 (15.3)	39 (7.5)	37 (7.1)	16 (3.1)

The overall HPV prevalence in women with normal cytology was 25.4 %, with ASCUS it was 64.7% and with LSIL/HSIL it was 94.1% (Table 4.7). Similarly, there was increasing prevalence of HR-HPV across cytological class from 17% in normal cytology to 52.9% in ASCUS and 94.1% in LSIL/HSIL. More modest increases were observed for LR-HPV infection across cytological categories. The steepest increase in prevalence across cytological outcome categories was in HPV-16/18, which was detected in 4.9% of normal Pap smears, 35.3% of ASCUS specimens and 52.9% with LSIL/HSIL. Multiple HR infections also showed a high prevalence in women with ASCUS (5.9%) and LSIL/HSIL results (23.5%) compared to those with normal cytology (2.2%).

Women with normal cytology and ASCUS specimens had mostly single type infections (Table 4.8) whereas women with LSIL and HSIL results had predominantly multiple type infections. When analyzed separately, all women with LSIL cervical cytology were positive for HPV, while one woman with HSIL cervical cytology was HPV-DNA negative. It should be noted that a year later this woman tested positive for HPV-16 and it is possible that the initial test failed to detect an existing, and persistent, infection. The most common HPV type detected was HPV-16 for all cytological outcome groups (Table 4.9). The most common HPV types were from both HR and LR classes for women with normal cervical cytology but were mostly HR types for ASCUS, LSIL, and HSIL specimens. Overall, 52.9% (n=9) of women with ASCUS results were infected with HR-HPV. Considering women less than 30 years of age with ASCUS, 88.9% (n=9) had a HR infection, whereas only 12.5% (n=1) of women over 30 years were positive for HR-HPV. The most common HPV types in women with ASCUS specimens were HPV-16, -18 and -33. HPV-18 and -33 were less prevalent in

LSIL and neither was detected in subjects with HSIL. Women with LSIL and HSIL cervical cytology shared three of their most common HR types: HPV-16, -31 and -58.

Table 4.8: Frequency of detection of HPV in women with normal and abnormal (ASCUS, LSIL, HSIL) cervical cytology (N=523)

	Normal Cytology (n=489)				ASCUS Cytology (n=17)				LSIL Cytology (n=11)				HSIL Cytology (n=6)			
HPV Group and Type	**Single infection**	**Multiple infection**	**Total**	**%**	**Single infection**	**Multiple infection**	**Total**	**%**	**Single infection**	**Multiple infection**	**Total**	**%**	**Single infection**	**Multiple infection**	**Total**	**%**
Any HPV-DNA	76	48	124	25.4	8	3	11	64.7	4	7	11	100	2	3	5	83.3
HR Types																
16	9	10	19	3.9	2	2	4	23.5	2	3	5	45.4	0	3	3	50
18	1	4	5	1.0	1	1	2	11.8	0	1	1	9.1	0	0	0	0
26	1	0	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
31	4	10	14	2.9	0	0	0	0	1	3	4	36.4	1	0	1	16.7
33	0	0	0	0	0	2	2	11.8	0	1	1	9.1	0	0	0	0
35	1	1	2	0.4	0	0	0	0	0	0	0	0	0	0	0	0
39	3	4	7	1.4	1	0	1	5.9	0	1	1	9.1	0	0	0	0
45	2	2	4	0.8	0	0	0	0	0	0	0	0	0	0	0	0
51	3	4	7	1.4	1	0	1	5.9	0	2	2	18.2	0	0	0	0
52	5	5	10	2.0	1	0	1	5.9	0	1	1	9.1	0	0	0	0
53	4	7	11	2.2	0	1	1	5.9	0	1	1	9.1	0	0	0	0
56	0	4	4	0.8	0	0	0	0	0	2	2	18.2	0	0	0	0
58	2	7	9	1.8	0	0	0	0	0	2	2	18.2	1	1	2	33.3
59	3	6	9	1.8	0	1	1	0	0	1	1	9.1	0	1	1	16.7
66	2	3	5	1.0	0	0	0	0	1	1	2	18.2	0	0	0	0
68	2	3	5	1.0	0	0	0	0	0	0	0	0	0	1	1	16.7
73	0	4	4	0.8	0	0	0	0	0	1	1	9.1	0	0	0	0
82	1	2	3	0.6	0	1	1	5.9	0	0	0	0	0	0	0	0
LR Types																
6	0	1	1	0.2	0	0	0	0	0	1	1	9.1	0	0	0	0
11	0	0	0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	1	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
42	0	6	6	1.2	0	1	1	5.9	0	0	0	0	0	0	0	0
54	3	6	9	1.8	0	0	0	0	0	1	1	9.1	0	0	0	0
55	0	4	4	0.8	0	0	0	0	0	0	0	0	0	0	0	0
61	6	11	17	3.5	0	0	0	0	0	3	3	27.3	0	0	0	0
62	4	7	11	2.2	0	1	1	5.9	0	2	2	18.2	0	1	1	16.7
64	0	0	0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
67	2	7	9	1.8	0	0	0	0	0	1	1	9.1	0	0	0	0
69	0	0	0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
70	5	3	8	1.6	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
72	1	0	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
81	0	2	2	0.4	1	0	1	5.9	0	0	0	0	0	0	0	0
83	1	1	2	0.4	0	0	0	0	0	0	0	0	0	0	0	0
84	5	9	14	2.9	1	0	1	5.9	0	1	1	9.1	0	0	0	0
89 (CP6108)	6	3	9	1.8	0	0	0	0	0	1	1	9.1	0	1	1	16.7

Table 4.9: HPV types detected in study population, by cervical cytology (N=523)

Normal Cytology		**ASCUS**		**LSIL**		**HSIL**	
HPV type	**%**	**HPV type**	**%**	**HPV type**	**%**	**HPV type**	**%**
16	3.9	**16**	23.5	**16**	45.4	**16**	50.0
61	3.5	**18**/**33**	11.8	**31**	36.4	**58**	33.3
31/84	2.9	**51**/**52**/**53**/**82**	5.9	61	27.3	**31**/**59**/**68**	16.7
53/62	2.2	42/62/81/84	5.9	**51**/**56**/**58**/**66**	18.2	62/89	16.7
52	2.0			62	18.2		

HPV types highlighted in bold are classified as HR or probable HR types. The other HPV types are classified as LR or are unclassified with respect to their oncogenic potential

4.9 DETERMINANTS OF HR-HPV INFECTION

4.9.1 Univariate Analysis

Univariate analysis of sociodemographic and lifestyle variables showed several factors to be significantly associated with prevalent HR-HPV infection (Table 4.10). Results from multiple imputation and complete case analysis did not differ substantially and only results from the multiple imputation analysis are displayed here. As expected, age showed a significant association with HR-HPV infection, with older age being protective for HR-HPV. Being from a village other than one of the four main study communities had a protective effect when compared to being from Kuujjuaq, the largest community in the region. Both current smoking and alcohol use were risk factors for HR-HPV, showing an approximately two-fold higher risk of infection. Indicators of sexual activity were highly associated with the outcome of HR-HPV: having ten or more lifetime sexual partners, having two or more sexual partners in the previous month or the previous year, and being of single marital status were all associated with prevalent HR-HPV. Being older at first sexual intercourse and having a higher number of lifetime deliveries were protective factors. A higher educational attainment (grade nine or more) and current use of hormonal birth control were risk factors for HR-HPV.

Table 4.10: Univariate crude and age-adjusted estimates of association between independent variables and prevalent HR-HPV

	Crude		Age-adjusted	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
Community				
Kuujjuaq	1.00	Ref	1.00	Ref
Kangiqsujuaq	1.00	0.50 – 1.98	1.14	0.56 – 2.33
Kangiqsualujjuaq	0.74	0.42 – 1.31	0.77	0.43 – 1.37
Kangirsuk	1.03	0.53 – 2.00	1.01	0.50 – 2.02
Other	0.36	0.15 – 0.87	2.28	0.74 – 7.02
Age (per year)	0.94	0.93 – 0.96	-	-
Marital status				
Married or living with partner	1.00	Ref	1.00	Ref
Single	2.43	1.58 – 3.74	1.69	1.07 – 2.69
Educational attainment				
< Grade 9	1.00	Ref	1.00	Ref
≥ Grade 9	1.81	1.14 – 2.87	1.00	0.60 – 1.66
Employed				

No	1.00	Ref	1.00	Ref
Yes	0.66	0.42 – 1.02	0.70	0.44 – 1.11
Current smoker				
No	1.00	Ref	1.00	Ref
Yes	2.17	1.28 – 3.70	1.58	0.91 – 2.76
Current alcohol use				
No	1.00	Ref	1.00	Ref
Yes	2.12	1.28 – 3.50	1.48	0.87 – 2.50
History of STI				
No	1.00	Ref	1.00	Ref
Yes	1.26	0.80 – 2.00	1.17	0.72 – 1.90
Age at 1st sexual intercourse	0.84	0.76 – 0.93	0.97	0.86 – 1.09
Lifetime number of sexual partners				
< 10 partners	1.00	Ref	1.00	Ref
≥ 10 partners	2.11	1.37 – 3.25	2.39	1.51 – 3.77
Number of sexual partners in previous year				
0	1.00	Ref	1.00	Ref
1	2.14	0.74 – 6.20	1.03	0.34 – 3.16
≥ 2	5.49	1.90 – 15.85	1.83	0.58 – 5.79
Number of sexual partners in previous month				
0	1.00	Ref	1.00	Ref
1	1.12	0.65 – 1.94	0.82	0.46 – 1.47
≥ 2	2.55	1.14 – 5.73	1.44	0.62 – 3.35
Currently pregnant				
No	1.00	Ref	1.00	Ref
Yes	1.56	0.82 – 2.97	1.00	0.52 – 1.95
Lifetime deliveries				
0	1.00	Ref	1.00	Ref
1 – 3	0.41	0.24 – 0.69	0.63	0.35 – 1.11
≥ 4	0.22	0.12 – 0.40	0.61	0.28 – 1.31
Current use of any birth control				
No	1.00	Ref	1.00	Ref
Yes	1.35	0.88 – 2.08	0.82	0.51 – 1.30
Current use of hormonal birth control				
No	1.00	Ref	1.00	Ref
Yes	1.73	1.09 – 2.75	1.04	0.63 – 1.69
History of Pap test in previous 3 years				
No	1.00	Ref	1.00	Ref
Yes	1.31	0.81 – 2.14	1.37	0.81 – 2.32

4.9.2 Multivariate Analysis

Age-Adjusted Analysis

Most variables which showed a significant effect in crude (unadjusted) analyses did not retain an effect when adjusted for age (Table 4.10). The only variables that showed a sustained association with prevalent HR-HPV when adjusted for age were factors associated with sexual activity, including having ten or more lifetime sexual partners (OR: 2.39, 95% CI: 1.51 – 3.77) and being of single marital status (OR: 1.69, 95% CI: 1.07 – 2.69).

In addition to examining age-adjusted estimates, we studied the impact of adjusting for the number of years since first sexual intercourse. The adjusted ORs and 95% CIs did not differ substantially whether adjusted for age or for years since first sexual intercourse.

Multivariable Model

Variables were selected for inclusion into the multivariate analysis based on existing evidence in the literature. In particular, age and markers of sexual activity (single marital status, lifetime number of sexual partners) were included because they have consistently shown to be associated with prevalent HPV or HR-HPV in studies across different populations. Smoking and the number of lifetime deliveries were included in the model because there is some evidence in the literature suggesting that they may be associated with HPV infection, although the evidence is inconsistent. Educational attainment was included as a proxy for socioeconomic status.

In order to investigate the U-shaped HR-HPV prevalence pattern, age was categorized into three groups: age less than 30 years (n=164), age between 30-45 years (n=244) and age 45 years and older (n=146). The middle category was used as a reference (full model not shown). In this analysis, being in the youngest age group was significantly associated with prevalent HR-HPV (OR: 4.87, 95% CI: 1.96 – 12.08) but being in the oldest age group was not (OR: 0.68, 95% CI: 0.18 – 2.55). Age was included as a continuous variable in the final model. The linearity-in-logit assumption was tested and satisfied.

In the final multivariable model (Table 4.11), the only variables that showed a significant association with the outcome of prevalent HR-HPV infection were age and the lifetime number of sexual partners. Interaction terms for both age and number of lifetime sexual partners and age and single marital status were tested but neither was significantly associated with the outcome. Whether the outcome was defined as positive for HR-HPV infection versus the rest of the study population (reported here) or positive for HR-HPV infection versus negative for HR-HPV did not significantly alter the results.

Table 4.11: Multivariable model of association between independent variables and prevalent HR-HPV infection

	Crude		**Fully-adjusted**	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
Age (per year)	0.94	0.93 – 0.96	0.95	0.93 – 0.98
Lifetime number of sexual partners				
< 10 partners	1.00	Ref	1.00	Ref
≥ 10 partners	2.11	1.37 – 3.25	2.25	1.41 – 3.60
Educational attainment				
< Grade 9	1.00	Ref	1.00	Ref
≥ Grade 9	1.81	1.14 – 2.87	0.93	0.55 – 1.57
Lifetime deliveries				
0	1.00	Ref	1.00	Ref
1 – 3	0.41	0.24 – 0.69	0.65	0.34 – 1.22
≥ 4	0.22	0.12 – 0.40	0.73	0.32 – 1.68
Marital status				
Married or living with partner	1.00	Ref	1.00	Ref
Single	2.43	1.58 – 3.74	1.42	0.87 – 2.32
Current smoker				
No	1.00	Ref	1.00	Ref
Yes	2.17	1.28 – 3.70	1.44	0.81 – 2.58

5 DISCUSSION

The data presented here represent the cross-sectional analysis of an ongoing cohort study of HPV infection in Inuit women residing in Nunavik, Quebec. The primary results that are reported are: 1) type- and age-specific HPV-DNA prevalence and 2) sociodemographic and behavioural determinants of HR-HPV infection. To our knowledge, this work is the first published report of its kind for this population. The results of this study represent a starting point for understanding the burden of HPV infection amongst Quebec Inuit women and may be useful in assessing the public health impact of HPV vaccination and HPV-DNA testing as part of cervical cancer prevention and screening programs.

5.1 TYPE- AND AGE-SPECIFIC HPV PREVALENCE

Prevalence of HPV-DNA in this Quebec Inuit population was 28.9% overall and was 25.4% in cytologically normal women. This estimate of overall prevalence is similar to reports in other 'high-risk' screening populations in Canada such as Montreal university students[30] and attendees of an inner-city clinic in Winnipeg[34], and elevated when compared to a more representative screening population sampled from across health regions in Ontario[32]. The age composition of these first two populations was considerably younger than in our study which suggests that the Quebec Inuit may be at higher risk for HPV infection than other 'high-risk' populations in Canada. A comparison with the Ontario data suggests that our population may have as much as a two-fold higher burden of HPV infection than the general population overall, and close to a three-fold higher prevalence amongst women less than 20 years of age.

The most prevalent HPV types in the study population were HR types HPV-16 (5.6%) and HPV-31 (3.6%) and LR types HPV-61 (3.6%) and HPV-84 (3.1%). HPV-18 was detected in only 1.6% of the population, HPV-6 was detected in 0.4% and no HPV-11 was detected. The HPV-16 prevalence was lower than in all other Canadian studies[30, 32, 34], while HPV-18 was elevated only when compared to what Sellors et al.[32] found in their Ontario population. Interestingly, HPV-18 was reported as the most common HR type in Winnipeg Aboriginal women[34], detected in 14.7% of the population. A high prevalence of HPV-6 and -11 was also detected in Winnipeg Aboriginals[34] (9.1% and 7%, respectively) while a more modest, but still elevated, prevalence was observed in other studies[30, 32]. The prevalence of HPV-31

(3.6%) was higher in the study population than was reported in Montreal university students[30] (2.6%) and Ontario women[32] (0.6%), but lower than in both Aboriginal (6.6%) and non-Aboriginal (4.1%) women in Winnipeg[34].

The low prevalence of HPV-6 and -11 should be interpreted with the knowledge that healthcare providers in the region report seeing cases of condyloma and that a history of physician-reported condyloma was found in 15% of women whose medical charts were reviewed (n=447). Considering that these LR-HPV types are associated with genital warts that develop on the outer genitalia, samples from the endocervix may not have efficiently detected these infections. It remains unexplained why studies that used similar sample collection techniques found higher prevalence of HPV-6 and -11 than our study, however. The lower prevalence of HPV-16 and -18 that was observed among study participants when compared to other Canadian populations, including Aboriginal groups, suggests that there may be a unique pattern of HR types infecting our population. HPV-31, which shares the alpha-9 species categorization with HPV-16, appears to be an important HR type in the Quebec Inuit from our study and Winnipeg Aboriginals[34], but not other populations studied in Canada. HPV-52 and -58, which are also alpha-9 types, were not important in any Canadian populations that tested for them[30, 32], yet they were amongst the most common HR types in our study subjects.

The overall prevalence of HR types was 20.4% in our population which is comparable to other populations in Canada[30], although slightly lower than was observed in Inuit women residing in the Canadian territory of Nunavut (26%)[28]. HR prevalence reported in Ontario by Sellors et al.[32] was only 12.7% overall, however, and the age-specific prevalence in 15-19 year olds was 15.7%, almost a third of what we saw in our study. This suggests that HR types may be more prevalent in Quebec Inuit women than in the general population, particularly amongst young women.

Multiple type infections were more common in our population than in prevalence studies across geographically diverse regions[32, 38, 112]. The higher rate of detection of multiple infections could be related in part to the use of an assay that allowed the identification of nearly all genital genotypes, as compared to other studies that did not detect all of these genotypes. Infection with multiple HPV types seems to increase the risk of developing high-grade lesions and invasive cancer[45], likely through a synergistic effect of co-infecting types. Trottier et al.[49] found evidence that multiple infections involving HPV-16 and -58, in particular, might be associated with an elevated risk for SIL, as compared to infections with either of these types alone. The authors proposed that other oncogenic HPV types of the alpha-9 family may also be modulated by co-infection, since types within the same species tend to share some biological properties and may interact in similar ways to influence the development and progression of cytological abnormalities.

The clustering analysis presented in this report was not designed to formally test the significance of pairwise associations of co-infecting types. It is interesting to note, however, that four of the seven most common HPV types to be involved in joint excesses were in the alpha-9 family and, of twenty-five joint excesses that were flagged as significant, seventeen involved an alpha-9 type. If particular HPV types are involved in multiple infections in our population and are associated with the risk for development and progression of precancerous and cancerous lesions, this knowledge may be useful when integrating HPV-DNA testing into cervical cancer screening programs. It was reassuring to observe that of all pairwise combinations, none resulted in observed frequencies that were significantly lower than would be expected by chance alone. Such combinations would have suggested that the two putative types tend to compete for the same niche and exclude each other, a finding that could flag the possibility of type replacement if one of the types were to be eliminated by vaccination.

Several studies have reported a decrease in HPV prevalence with age that is accompanied by a 'resurgence' in older women. A study of Columbian women[38] and one in Costa Rica[26] reported an increased prevalence of HPV infection in women aged 55 years and older; in Mexico[78], this second peak in prevalence was observed in women 45 years and older. In our study, an increase in HPV prevalence was detected in women 50 years and older, amongst whom LR-HPV and single type infections were most common, but some multiple type infections with HR-HPV were still observed. Numerous hypotheses have been proposed for the U-shaped prevalence pattern observed in populations of women. If older women in the study population were comparatively more exposed to the HPV virus earlier in their lives than young women today, a cohort effect[26] rather than a true biological effect could explain this phenomenon. Some biological hypotheses have also been proposed to explain the increased prevalence in older women, including reactivation of latent infections due to (1) hormonal changes resulting from decline in ovarian function[78] and (2) decreased immune response with aging[113, 114]. Finally, age-related changes of the cervix may affect the sampling of endocervical cells and thus the detection of particular HPV types[115].

5.2 PAP SCREENING

When the history of Pap testing was examined amongst women in the study population, it was found that overall, 71% had attended Pap screening in the previous three years. When tabulation was restricted to women aged 20-69 years, coverage rose to 76%. These data represent only slightly lower coverage than was observed across Canada and Quebec in the 1998-1999 National Population Health Survey (NPHS)[54], in which 79% and 78% of women aged 20-69 years, respectively, reported having had a Pap test over the previous three years. A more concerning picture emerges, however, when age-specific Pap screening statistics are examined.

The highest rates of non-adherence to Pap screening were observed amongst 15-19 year olds (54%), 40-49 year olds (37%) and 50-69 year olds (33%). We would expect to see a large proportion of 15-19 year olds with a negative Pap history, considering that the self-reported mean age at first sexual intercourse was 15.5 years (SD=2.5, median=15) in this population. Thus, many young women will only be recently sexually active and beginning to initiate Pap testing. In women aged 40-69 years, there is no clear explanation for the low rates of Pap testing, however. It is possible that peri- and post-menopausal women ascribe less importance to their reproductive health and thus neglect to continue having Pap tests after their reproductive years, although no research exists in the literature to validate this hypothesis. Another explanation may be that of a cohort effect, by which young women today have a greater consciousness of the importance of Pap testing, an awareness which was not cultivated in older women during their youth.

When Pap screening history and HR-HPV prevalence are examined together, women aged 50-69 appear to represent an important risk group within the study population. Not only have 33% of these women not had a Pap test in the previous three years, but a resurgence in

HR-HPV is observed within the same group of study subjects. Coupled with the fact that the median age at diagnosis for cervical cancer is 48 years[116], this suggests that women aged 50-69 years may be at an elevated risk for living with undetected cervical cancer. That said, some experts have cautioned against annual screening in post-menopausal women after a normal Pap result, because of the limited benefit and risk of false positive results[117]. Thus, efforts to encourage participation in Pap screening should, in particular, target women aged 40 years and older who have not had a recent, normal Pap test. Any plans to develop formal cervical cancer screening and prevention programs should also take into consideration the specific characteristics of this subgroup.

5.3 HPV INFECTION AND CYTOLOGICAL OUTCOMES

Of women with available cytology results (n=523), 6.6% had an abnormal outcome (ASCUS, LSIL, HSIL) and 3.3% had a low-grade or high-grade lesion. Although mean age tends to increase with the severity of cervical cytological diagnosis[118, 119], the mean age of women with LSIL was younger (21.6 years) than for all other categories, while the mean age of women with normal cytology (35.6 years) was the oldest. It is unclear why this pattern was observed in the study population, although the relatively small number of abnormal Pap results may in part explain this irregularity.

Across all categorizations of infection, the prevalence of HPV increased with increasing abnormality of cytology results. This, of course, is expected because HPV infection is recognized as the biological precursor to cervical abnormalities leading to cancer. The steepest increases in prevalence were observed in categorizations of HPV infection that include HR types, which have demonstrated oncogenic potential. However, a substantial increase in the prevalence of LR types, which are not associated with cervical cancer, was also observed with increasing cytological abnormality. This can be explained by the fact that a large proportion of women who had an abnormal Pap result and were infected with LR-HPV were also co-infected with a HR type. Multiple type infections were more common in women with LSIL and HSIL results than in those with normal or ASCUS cytology, which is consistent with the hypothesis that the risk for precancerous and cancerous lesions is elevated when women are infected with multiple HPV types.

It is estimated that ten to fifteen percent of severe dysplasias are found in women with ASCUS cervical cytology[120]. Because performing immediate colposcopy on these women is considered overly costly and burdensome, HPV-DNA testing has emerged as an attractive means for triaging women with an ASCUS cytology result[121, 122]. Between 40-60% of ASCUS specimens are positive for HR-HPV, whereas HR-HPV is detected in as many as 83% of LSIL and 94% of HSIL cytologies[121]. Thus, triage by HPV-DNA testing may reduce the number of colposcopies required for ASCUS patients by up to 50%, but has less utility with respect to LSIL and HSIL results. Considering that in our study just over half of women with ASCUS results were positive for HR-HPV, triage by HPV-DNA testing may be both feasible and worthwhile in this population.

In general, women aged 30 years and older have been identified as the group most likely to benefit from triage by HPV-DNA testing since only 5 to 15% experience transient, clinically insignificant infections, as compared to 10-20% of women overall[123-125]. In our study population, only 13% of women over 30 years of age with ASCUS cytology were infected with HR types, whereas HR-HPV was detected in 89% of women aged 30 years and younger with ASCUS. It is expected that women aged 30 years and older experience more persistent HR infections than the younger portion of the population, and that HPV-DNA testing would be able to triage this group to further follow-up. This type of testing is also more appropriate than thin-prep Pap cytology for women who have irregular or extended screening intervals[123], and thus may be beneficial to such women in our population.

Worldwide, HPV-16 is the most common HPV type detected in women with both low-grade and high-grade lesions, present in 20% and 45% of specimens, respectively. HPV-31 and -51 (found in 8.3% of specimens each) are the next most commonly detected in LSIL cytology, while HPV-31 (8.7% of specimens) and HPV-33 (7.3% of specimens) are the next most commonly detected in HSIL cytology. In the study population, HPV-16 was the most common HPV type detected among women with LSIL/HSIL results, although the prevalence was elevated in LSIL cytology (45% of specimens) when compared to worldwide estimates. It is interesting to note that women with LSIL and HSIL cytology shared three of their most common HR types: HPV-16, HPV-31 and HPV-58. The relative importance of HPV-31 both in abnormal cytology and overall prevalence suggests that this population may benefit from cross-protection by vaccines that protect against HR types HPV-16 and -18[126, 127]. Vaccination is thought to confer a degree of cross-protection against HPV types that are members of the same species as one of the vaccine types and infection with HPV-31 and HPV-45 have shown to be particularly impacted. HPV-18 is an important contributor to cervical abnormalities on a global scale, but was only detected in one study subject with an LSIL result and no HPV-18 was detected amongst women with HSIL cytology. It is interesting to note, however, that two of the five HR types detected in HSIL specimens were members of the alpha-7 species, to which HPV-18 also belongs.

5.4 DETERMINANTS OF HR-HPV INFECTION

Although a number of variables showed a significant crude association with prevalent HR-HPV infection, most did not retain a significant effect when adjusted for age. The only variables that showed a significant association, even when adjusted for age, were having ten or more lifetime sexual partners and having single marital status. In the final multivariable model, the only variables that showed a significant association with the outcome were age and lifetime number of sexual partners. These results are consistent with the literature which, across many studies, shows age and markers of sexual activity to be the most consistent risk factors for HPV infection and HR-HPV infection[32, 34, 38, 39, 46, 128-130]. Oral contraceptive use has been associated with prevalent HR-HPV infection[32, 46, 128, 129] but neither overall use of birth control nor use of hormonal contraceptives showed a significant association in our study population when adjusted for age. Smoking has also been associated, if inconsistently, with prevalent HPV infection[32, 129] but was not associated with HR-HPV in our study, either when adjusted for age or in the final multivariable model.

We were particularly interested in investigating the U-shaped age-specific pattern of HR-HPV prevalence as well as exploring the possibility that certain risk factors showed an interaction with age. When age was categorized into three strata and the middle stratum was used as a reference, the youngest women showed a significantly higher risk for HR-HPV infection than women of middle-age, whereas a significant difference in risk was not found in the oldest age group. Thus, although there does seem to be an observed increase in HR-HPV prevalence amongst women 50-69 years of age in the study population, the increased risk is not significant when adjusted for other sociodemographic and lifestyle variables.

Interaction terms for both age and number of lifetime sexual partners and age and single marital status were entered into the model but neither was significantly associated with the outcome. In a study of Columbian women, Molano et al.[38] did not find any statistically significant interactions between age and other risk factors for HPV infection (both HR and LR), but did report some age-specific patterns. In particular, having two or more regular sexual partners had the greatest importance as a risk factor for HPV-DNA detection in women less than 25 years of age, it represented a less increased risk in women aged 25-34 years and it was not significantly associated with HPV infection in women 35 years and older. It was not possible to perform this type of stratified analysis for our study because of the limited size of the study population.

5.5 LIMITATIONS

It is important to examine limitations of this study while considering the implications of its results. The primary limitations include the fact that the study population was relatively small; a non-random recruitment strategy was used; the analysis was cross-sectional in nature; and there was a significant amount of missing data overall.

5.5.1 Non-Participation and Selection Bias

Women were recruited into the study when they presented for regularly scheduled Pap screening. Thus, not only are women who agreed to participate likely different from those who declined participation, but women who presented at the clinics may differ from the general population of the study communities on important characteristics. Unfortunately, it was not possible to formally assess participation rates, differences between participants and non-participants, or the degree to which selection bias influenced the makeup of the study population.

Although the number of patients who were approached and who agreed to participate in the study was not recorded, nurse practitioners estimated participation to be on the order of 80%. By comparing selected characteristics of our cohort to data for the general population of the communities in which they live, we were able to evaluate, in a limited way, the extent of selection bias in our study.

Data on the educational attainment of all female residents of Nunavik was available in the 2001 Census Community Profiles[96], although categorizations were different than in our study. Amongst women aged 20-34 in Nunavik, 24.6% had a high school education or more whilst in the study population, 20.4% had more than a high school education (>Grade 13). Amongst women aged 35-45 in Nunavik, 11.1% had graduated from high school, as compared to 6.7% who had more than a high school education in our study. Only 9.3% of women aged 45-64 years in Nunavik had graduated from high school according to the 2001 Census; in the study population, 6.4% of women in the same age range had more than a high school education. These data suggest that similar educational attainment was observed in the study population as in Nunavik in general.

According to the 2001 Community Profiles, the breakdown of the Nunavik population in terms of legal marital status was: 59% single, 32% married, 2% separated, 2% divorced, and 5% widowed. The breakdown for the study population closely mirrored this distribution: 63% single or living with partner (38% single, 25% living with partner), 31% married, 3% divorced or separated, and 4% widowed. The study population was also similar to Nunavik as a whole in terms of labour force participation: 70% of the study population self-identified as employed, while 64% of Nunavik women were active in the labour force, according to the 2001 Census.

In the 1992 Santé Québec Health Survey among the Inuit of Nunavik[131], 79.6% of females aged 15-24 years, 71.8% aged 25-44 years, and 55% aged 45 years and older were found to be regular smokers. In the same study, it was found that 61.3% of females aged 15-24 years, 58.7% aged 25-44 years, and 45.9% aged 45 and older were occasional or regular drinkers. In the study population, a similar pattern of smoking habits was observed: 82.1% of subjects aged 15-24 years, 75.3% aged 25-44 years, and 54.5% aged 45 years and older were current smokers. There were considerably higher rates of alcohol use in women aged 15-24 years (75.6% users) and women aged 25-44 years (79.5% users) in the study population, but lower rates were observed in women aged 45 years and older (38.6% users).

In the Nunavik Inuit Health Survey conducted in 2004[132], 82% of Inuit women aged 18 years and older reported having had a Pap test in the previous two years. In the study population, 60.2% of women aged 18 years and older were found to have a two-year Pap history by medical chart review. In the 2004 survey, compliance to Pap screening was relatively good across most age groups: 72.3 % of women aged 18-24 years, 84.7 % of 25-34 year-olds, 80.3 % of 35-44 year-olds, 80 % of 45-54 year-olds, 71% of 55-65 year-olds, and 46 % of women 65 years and older had a Pap test in the previous two years. Interestingly, when the history of Pap screening in the previous three years was examined amongst study participants for whom this information was available, compliance was lower than for two-year Pap history in the 'general population' across several age groups. We found that 76.5% of women aged 18-24 years, 84.3% of 24-34 year-olds, 62.4% of 35-44 year-olds, 70% of 45-54 year-olds, 66.7% of 55-64 year-olds and no women aged 65 years and older had attended Pap screening in the previous three years. Therefore, women in the study population may slightly over-represent some characteristics associated with non-compliance to Pap screening.

In the 2004 Nunavik Inuit Health Survey, 30% of women aged 15 years and older reported having no sexual partners in the previous 12 months, 51% reported having had one partner and 19% reported having had two or more. In our study, only 10% of women aged 15-69 years reported having had no sexual partners in the previous 12 months; 60% reported having had one partner and 30% reported having had two or more. These data may suggest that the women in our study population are, on average, more sexually active than the general population in Nunavik. The difference in the age ranges covered by the data and a misinterpretation by some participants in the 2004 Health Survey of the definition of 'sexual partner' as excluding a spouse make these data difficult to compare, however. In the 2004 Health Survey, 32% of women aged 15-29 years reported that they were using some form of birth control. In the study population, 52% of women within the same age range reported using birth control, a substantially elevated proportion compared to the general population.

Together, these comparisons suggest that our study population may be different than the general population of Nunavik on some important characteristics, such as use of alcohol, tobacco and birth control and Pap history, but that they are generally representative of the residents of these communities on variables such as education and marital status. Some comparisons such as for the number of sexual partners in the previous month were difficult to make because of the poor quality of data available for the general population.

5.5.2 Cross-Sectional Data

The cross-sectional nature of the data presented here implies that any observed patterns in age-specific HPV-DNA prevalence should be interpreted with caution. These patterns may either represent a biological phenomenon or a cohort effect. Further, the cross-sectional analysis of factors associated with prevalent HR-HPV infection does not in any way attempt to investigate causality and should not be interpreted to do so. It should be further noted that this study sought to detect HPV-DNA, not to differentially detect a latent, active or persistent infection. Thus, the nature of the infections that were detected cannot be characterized more specifically.

5.5.3 Missing Data

There was a large degree of missing data cumulatively across all variables of interest, with the extent of missing data for individual variables varying from 0.7% to 17%. It was necessary to use self-reported data for history of STI and number of lifetime deliveries since a significant proportion of data from medical chart review for these variables was missing. Only 471 of 554 (85%) of study subjects had complete information for all the variables included in the final multivariable model. For this reason, it was necessary to use multiple imputation to generate values for missing data. Since multiple imputation is widely accepted as a valid method for handling missing data when carried out appropriately[133-135], missing data was not considered a serious limitation of this study. Furthermore, our complete-case analysis yielded very similar results to the multiple imputation analysis, further confirming the robustness of our results.

5.6 STRENGTHS

Having discussed limitations of the study, it is important to recognize the specific strengths of this research. Despite limitations in assessing selection bias, it appears that the study population is reasonably representative of the target population. In addition, a reasonably adequate coverage was obtained overall for the four main communities (57%) and a similar age distribution was observed in the study population as in the target population. Finally, because recent surveys show relatively good compliance to Pap screening by women in Nunavik, we expect that our sampling procedure produced a study population that is roughly representative of the general population.

Although the cross-sectional nature of this analysis limits the way in which it can be interpreted, these prevalence data represent a rich 'baseline' picture of HPV infection in a population that is being followed up as part of an ongoing cohort study. A longitudinal analysis will allow us to evaluate other important questions in future work, including persistence of HPV infection. Perhaps the most important strength of this study, however, is the novelty of the research. To our knowledge, this is the first cohort study examining type-specific HPV infection amongst Quebec Inuit women, and this report represents the first analysis of HPV prevalence in the population. In addition, there is a clear relevance to studying this specific population and to the particular research questions that have been tackled. Inuit women have been recognized as a population at high risk for cervical cancer and for STIs other than HPV. Not only this, but the Quebec government will initiate a campaign for mass vaccination of young girls against HPV in the fall of 2008, which makes understanding the pre-vaccination burden of infection a particularly timely issue.

6 CONCLUSIONS

This study represents the first analysis of type-specific HPV prevalence and determinants of HR-HPV infection amongst Quebec Inuit women. These data provide a foundation on which to expand our understanding of the burden and patterns of HPV infection in a unique population at high risk for cervical cancer.

Our results show a high prevalence of HPV infection and, in particular, of multiple infections in a population of Inuit women undergoing routine screening in Nunavik, Quebec. Whereas the youngest women showed a large proportion of HR infections with multiple HPV types, the increase in HPV prevalence that was observed in the oldest age groups was characterized by mostly LR single type infections. Adherence to Pap screening appears reasonable overall but is suboptimal in women aged 40-69 years, who should be targeted when planning future cervical cancer screening and prevention programs. In addition to HPV-16, HPV types 31 and 58 appear to be important in this population, particularly in women with LSIL and HSIL cytology. HPV-6, -11 and -18 show lower prevalence than in other Canadian populations, although the reasons for this under-representation are unclear. Finally, age and number of lifetime sexual partners were shown to be the most important predictors of HR-HPV infection in Quebec Inuit women, which is consistent with what is reported in the literature for other populations.

The data from this research not only deepen our understanding of HPV infection as experienced by Quebec Inuit women, but may also be helpful in following the younger portion of this population post-HPV vaccination, which is poised to occur across Quebec. A baseline understanding of the burden of HPV infection will allow an informed evaluation of vaccination strategies, as well as the investigation of changes in the natural history of infection and phenomena such as type replacement. Finally, changes in HPV screening protocols may be informed by the data presented here, including the integration of HPV-DNA testing into cervical screening programs.

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APPENDIX 1: CONSENT FORM

McGill University Health Center
Division of Clinical Epidemiology of the Royal Victoria Hospital

Study on Human papillomavirus and cervical cancer among Inuit Women in Northern Quebec

Information Document

Researchers: Dr Paul Brassard, Division of Clinical Epidemiology, Royal Victoria Hospital, **Dr Eduardo L. Franco**, Department of Oncology, McGill University, **Dr François Coutlée** and **Dr Michel Roger**, Department of Microbiology, Notre-Dame Hospital, University of Montreal Hospital Center.

A) Purpose of this study

Human papillomavirus (HPV) is a virus that causes genital warts and is normally sexually transmitted. HPV infection is detected by collecting samples of cells from the cervix that can be obtained during a Pap test. The sample is then examined to determine the presence of HPV. If HPV is detected, further analysis is conducted to classify the type of HPV. Inuit Women seem to be more at risk for HPV infection. When HPV infection is present for a long time, it can cause in some women cervical cancer, a disease that can be prevented by early detection with the Pap test and treatment. We are doing this study to investigate how many women will acquire and stay with the infection over time. We also want to understand if there are human factors that predispose women to develop cervical disease. We call these host susceptibility factors (including HLA) for cancer and infection. The information found can then be used by Nunavik public health officials for developing effective cancer-screening program and prevention program for women in the area.

B) Procedure

If you agree to participate, you will be asked to complete a self-administered questionnaire after your Pap test at the Hygiene clinic. A nurse will accompany you in the process. If you feel uncomfortable to complete the questionnaire at once, you can always make arrangement with the nurse for another visit. Specimens of your Pap test will be collected for the study. It will be sent to the laboratory at Notre-Dame Hospital in Montreal to be tested for HPV infection. The Pap specimens will be taken and sent to Montreal each time you come back at the Hygiene clinic for your regular Pap test or for any other health condition that requires a cervical exam, over a 5 year period (60 months). Those specimens (including the host susceptibility factors) will be kept for the length of this study and the length of other studies that could come from this global project for a total of 10 years. We will also need to review your medical file to collect further information concerning your health status.

C) Risks and Benefits

There is no additional risk related to this study as the Pap smear is a safe examination. You will not have more visits to the clinic; you will only spend a little more time to fill out the questionnaire. Your participation will help in developing prevention for HPV infection. As well, if any lesions are detected, you may benefit directly by getting the proper treatment.

July 2003

Information Document

D) Participation

Your participation in this study is **of your own free will**. You can stop participating in any part of the project at any time and this will not affect your health care or treatment in any way. You will also get a copy of this consent form. You have the right to ask any question you want to the nurse about the study before accepting to participate.

E) Confidentiality

In order to ensure your privacy and confidentiality, your name will not appear on any study record or results presented by the research team. Instead a patient identification number will be assigned to you and will appear in all your records. Only the nurse and the researchers in Montreal will have access to the study number. You understand that all information about you and you Pap smear results and host susceptibility factors will be treated in the same confidential manner as other medical records and you will not be identified in any subsequent reporting of results.

F) Questions

If you have any specific questions, now or at any time about this study, please do not hesitate to contact the Director of Professional services of the Ungava Tulattavik Health center, Dr Nathalie Boulanger at (819) 964 2905 *or* make a collect call to the chief investigator, Dr Paul Brassard at (514) 842 1231 ext 36910.

July 2003

McGill University Health Care Center
Division of Clinical Epidemiology of the Royal Victoria Hospital

Study on human papillomavirus and cervical cancer among Inuit Women in Northern Quebec

Voluntary consent

By signing this form, I acknowledge having received and read a copy of the information paper concerning this study. I have had the opportunity to ask any questions I may have about this study, and they have been answered to my satisfaction. I agree to participate in this study and I understand that I may withdraw this agreement at any time. I understand that my decision whether or not to participate will not change any health care I might receive or my legal rights. I also understand that all information will be kept strictly confidential. My file will be coded and kept in place where only the research team will have access.

1) I agree to complete the questionnaire on risk factors for HPV infection

Yes: ___;   No: ___

2) I agree for my Pap specimens collected in the next 5 years be sent to Notre-Dame Hospital in Montreal for detection of HPV infection and host susceptibility factors for cancer and infection including HLA. Those specimens will be kept for the length of this study and the length of other studies that could come from this global project for a total of 10 years.

Yes: ___;   No: ___

3) I agree that my medical chart can be reviewed up to 5 years following my agreement to participate.

Yes: ___;   No: ___

Signature: ________________________

Write your name in block letters: ______________________________________

Date: _____________________________

Telephone number: _________________

Nurse section

I recognize having offered to the participant a copy of this consent form and a copy of the information document.

Participant ID number: IN- ___________________

Signature of the nurse: ____________________________
Name of the nurse in block letters: ______________________________________
Date: __________________________
July 2003

APPENDIX 2: QUESTIONNAIRE

HPV-INUIT

Research Coordinator Section

Date ____ dd/mm/yyyy	Chart number ____	Date of birth ____ dd/mm/yyyy

A. Participant Identification

2- What is your current marital status ?

Single (Not married and not living with partner) Married Divorced/Separated Widowed Living with partner

B. Socio economic status

3- Are you employed ? Yes No

4- What is your highest level of schooling ? Less than grade 9 Grade 9 to 13 More than grade 13

5- What is the current employement status of your husband or living partner ? Employed Unemployed

If employed, what type of work does he do ?

C. Life habits

6- Are you a current smoker ? Yes No

If yes, a) how many cigarettes do you smoke a day ? :

b) how long (in years) have you been smoking ? :

If no, a) have you ever smoked ? : Yes No

If yes, how long (in years) has it been since you stopped smoking ? :

7- Do you drink alcohol ? Yes No

If yes, a) how long (in years) have you been drinking ? :

b) how often do you drink :

Beer :	Never	Occasionally	Once a week	More than once a week	Every day
Wine :	Never	Occasionally	Once a week	More than once a week	Every day
Wisky/Gin/Vodka or any hard liquor	Never	Occasionally	Once a week	More than once a week	Every day

8- Are you currently using any birth control method ? Yes No

If yes, a) what kind of method do you currently use ? (you can put down more than one)

For how long in years	Birth control methods
	Birth control pills
	Latex safe (condom)
	Spermicides (gel)
	I.U.D (coil)
	Diaphragm
	Depo-Provera (injections)
	Rythm, calendar, natural meth od
	Other :

D. Sexual behavior

9- Have you ever had sex ? Yes No **If no,** go to question 17.

10- How old were you when you first had sex ? :

11- Throughout your life, what is the number of partners with whom you have had sex ? (appr oximately)

0-4 5-9 10 and more

12- How many sexual partners have you had in the last year ?

13- How many sexual partners have you had in the last month ?

14- Does your partner(s) have other sexual partner(s) cur rently ? Yes No Unknown

If yes, how many partners does he currently have (approximat ely) ?

E. Gynecological and obstetric events

15- Are you pregnant ? Yes No

16- Up to now :

How many times did you deliver a living baby ?

How many times did you have an abortion ?

How many times did you have a miscariage ?

17- Have you ever had a gynecological exam in the past (excluding the current one) ? Yes No Unknown

Year

If yes, what year did you have the first one (approximately) :

what year did you have the last one (approximately) :

18- Have you ever experienced sexually transmitted disease
(STD's or infection with herpes, chlamydia, gonorrhea, syphillis) in the past ? Yes No Unknown

If yes, Once 2-4 times 5 times or more

F. Health conditions

19- Are you experiencing one or more of the following health problems ?

HIV infection

Had an organ transplant

Use of cortisone (injection or pills) for more than 1 month

Other health problems:

G. Comments (please, write down any comment you want about a specific item or about the study in general) :

APPENDIX 3: MEDICAL CHART REVIEW FORM

CHARACTERIZATION OF THE HUMAN PAPILLOMAVIRUS INFECTION AMONG A POPULATION OF INUIT WOMEN IN QUEBEC

RÉVISION DE DOSSIER MÉDICAL

No d'identification de l'étude : [ ]

No de dossier médical : [ ] **Date de naissance :** [ ]

Historique médicale de la patiente :

Maladies chroniques : [ ]

Chirurgies : [ ]

Immunosupression : [ ]

Parité : G [ ] P [ ] A [ ]

A. Identification du participant

1- Quel est votre langue principale ? Inuktitut Anglais Francais Autre

2- Quel est votre état civil ? Célibataire Mariée Divorcée/Séparée Veuve Habitant avec un conjoint

B. Statut socio-économique

3- Avez-vous un emploi ? Oui Non

4- Quel est le plus haut degré de scolarisation que vous avez obtenu ? Moins que la 9e année Entre la 9e et la 13e année Plus que la 13e année

5- Quel est le statut de votre mari ou de votre conjoint ? Employé Sans emploi

Si employé, quel type de travail fait-il ?

C. Habitudes de vie

6- Fumez-vous présentement ? Oui Non

Si oui, a) Combien de cigarettes fumez-vous chaque jour ? :

b) Depuis combien de temps fumez-vous ? :

Si non, a) Avez-vous déjà fumé ? : Oui Non

Si oui, depuis combien de temps avez-vous arrêté de fumer ? :

7- Est-ce que vous buvez de l'alcool ? Oui Non

Si oui, a) Depuis combien de temps buvez-vous ? :

b) À quel fréquence buvez-vous de l'alcool :

Bière : Jamais Occasionellement Une fois par semaine Plus d'une fois par semaine Tous les jours

Vin : Jamais Occasionellement Une fois par semaine Plus d'une fois par semaine Tous les jours

Wisky/Gin/Vodka ou tout autre liqueur forte : Jamais Occasionellement Une fois par semaine Plus d'une fois par semaine Tous les jours

8- Est-ce que vous prenez de la drogue ? Oui Non

Si oui, a) Depuis combien de temps vous droguez-vous?

b) Quelle sorte de drogue prenez -vous ?

D. Historique Sexuel

9- Avez-vous déjà eu des relations sexuelles ? ☐ Oui ☐ Non

10- Quel âge aviez-vous lors de votre première relation sexuelle ? ☐

11- Durant votre vie, avec combien de partenaire(s) avez-vous eu des relations sexuelles ? (approximativement)

☐ 0-4 ☐ 5-9 ☐ 10 et plus

12- Avez-vous un partenaire présentement ? ☐ Stable ☐ Occasionnel

13- Combien de partenaires sexuels avez-vous eu dans la dernière année ? ☐

14- Combien de partenaires sexuels avez-vous eu dans le dernier mois ? ☐

15- Est-ce que votre(vos) partenaire(s) a(ont) d'autre(s) partenaire(s) sexuelle(s) ? ☐ Oui ☐ Non ☐ Inconnu

Si oui, combien de partenaires (approximativement) ? ☐

LISTE DES RÉSULTATS DE CYTOLOGIE OBTENUS

No.Étude:__________

Date	Normal	Inflammation	Atypie	LGSIL	HGSIL	Inadéquat	Autre

LISTE DES MTS   No.Étude:__________

Date	Source		MTS							
	Résultat de lab.	Note du médecin	Gonorrhée	Chlamydia	Syphylis	Lymphogranulome vénérien	Herpes	Condylome	Chancre mou	Autre

LISTE DES MÉTHODES CONTRACEPTIVES UTILISÉES

No.Étude:__________

	Date de début	Date de fin
Contraceptif oraux		
Condom		
Stérilet		
Diaphragme		
Injection Dépo-Provera		
Mousse-gel contraceptif		
Symptothermique		
Autre (spécifiez):		

APPENDIX 4: ETHICS

McGill

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

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September 11, 2007

Dr. Paul Brassard
Division of Clinical Epidemiology
Royal Victoria Hospital
687 Pine Avenue West, R4-29
Montreal, Quebec H3A 1A1

Dear Dr. Brassard:

We are writing in response to your request for continuing review by the Institutional Review Board of the study A09-M78-02A entitled ***"Characterization of the Human Papillomavirus Infection Among a Population of Inuit Women in Quebec".***

The progress report was reviewed and we are pleased to inform you that full board re-approval for the study was provided on ***September 10, 2007*** valid until ***September 09, 2008.*** The certification of annual review has been enclosed.

We ask you to take note of the investigator's responsibility to assure that the current protocol and consent document are deposited on an annual basis with the Research Ethics Board of each hospital where patient enrollment or data collection is conducted.

Should any modification or unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

Roberta Palmour

Roberta Palmour, PhD
Co-Chair
Institutional Review Board

cc: A09-M78-02A

UNGAVA TULATTAVIK HEALTH CENTER
CENTRE DE SANTÉ TULATTAVIK DE L'UNGAVA

Kuujjuaq, 12 Juin , 2000

Dr Paul Brassard
Hôpital Royal Victoria
Division Épidémiologie clinique
687, ave. des Pins
Montréal, Qc
H3A 1A1

Sujet : Projet de recherche sur le HPV

Dr Brassard,

Il me fait plaisir de vous confirmer que le conseil des médecins, dentistes et pharmacien de notre établissement a jugé « éthiquement acceptable » votre projet de recherche sur le HPV (« Detection of genital human papillomavirus and associated cytological abnormalities among the inuit population of Québec »).

Je vous prie d'accepter, Dr Brassard, mes salutations cordiales.

Dr Luc Farrier
Président du CMDP

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