Distribution of human papillomavirus types, cervical cancer screening history, and risk factors for infection in Manitoba

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Abstract

Objectives: We conducted a study to investigate the prevalence of human papillomavirus (HPV) infections in an opportunistic sample of women in Manitoba, Canada. We inquired about risk factors associated with HPV infections and linked the HPV typing results with the cervical cancer screening history of the participants.

Methods: The study population included 592 women attending Papanicolaou (Pap) test clinics. After signing a consent form, participants were given a self-administered questionnaire on risk factors and received a conventional Pap test. Residual cells from the Pap tests were collected and sent for HPV typing.

Results: The mean age of the population was 43 years. A total of 115 participants (19.4%) had an HPV infection, 89 of whom had a normal Pap test. Of those who were HPV-positive, 61 (10.3%) had high-risk (Group 1) HPV. HPV-16 was the most prevalent type (15/115: 13.0% of infections). The most consistent risk factors for HPV infection were young age, Aboriginal ethnicity, higher lifetime number of sexual partners and higher number of sexual partners in the previous year.

Conclusion: The prevalence of HPV types in Manitoba is consistent with the distributions reported in other jurisdictions. These data provide baseline information on type-specific HPV prevalence in an unvaccinated population and can be useful in evaluating the effectiveness of the HPV immunization program. An added benefit is in the validation of a proof of concept which links a population-based Pap registry to laboratory test results and a risk behaviour survey to assess early and late outcomes of HPV infection. This methodology could be applied to other jurisdictions across Canada where such capacities exist.

Keywords: *papillomavirus infections, prevalence, risk factors, uterine cervical dysplasia, early detection of cancer*

Introduction

The publicly funded human papillomavirus (HPV) immunization programs implemented across Canada between 2007 and 2009 have the potential to prevent a large proportion of anogenital warts, high-grade cervical lesions and HPV-related invasive cancers.[1-6] They also have the potential to influence cervical cancer screening as currently practiced because of the changes in prevalence of cervical abnormalities they can bring about.[1,7] The extent of this impact, however, will depend on the distribution of HPV types, the type-specific infection rates among females and the vaccine uptake.

The objective of this study was to determine the baseline type-specific prevalence of and risk factor for HPV infection in an opportunistic sample of women attending walk-in, no-appointment Papanicolaou (Pap) test clinics in Manitoba (Canada) during an annual cervical cancer awareness week. The survey information and HPV typing results were linked to the Manitoba Cervical Cancer Screening Program (MCCSP) database. Manitoba is well positioned to host and conduct this kind of surveillance projects because of the availability of linkable population-based databases on cancer,

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cervical screening, medical procedures provided by physicians and immunization.[8] These resources provide a robust environment to evaluate the impact of the HPV immunization program impact, the utilization of cervical screening among vaccinated and non-vaccinated females, and the resulting disease distribution and outcomes.

Methods

Study environment

Since 2003 the MCCSP has conducted an annual Pap Week in October. During this week women are encouraged to attend Pap test clinics without appointment. The objective is to reach those who have never attended or do not regularly attend cervical screening. In 2008, 123 clinics participated in Pap Week across Manitoba. Of these, 52 consented to take part in this study. In addition to performing conventional Pap tests, these clinics took the residual cells from the Pap tests, put them in a liquid-based cytology medium, and sent the samples to Cadham Provincial Laboratory in Winnipeg, Manitoba, for HPV typing. The participating clinics also supervised the administration of a consent form and a self-administered survey on risk factors for HPV infections.

Population

The study population was composed of an opportunistic sample of women aged 18 years and older from different ethnic backgrounds. Pregnant women were excluded. Women interested in participating in the study discussed the objectives with clinic staff and, upon agreement, signed a consent form and completed a risk factor questionnaire. Women who decided not to complete the questionnaire were still eligible for HPV testing, and their HPV results were included in the analysis.

The study was publicized on posters in the clinics, and staff told potential participants about it. Overall, 1182 women underwent cervical screening in the 52 participating clinics, and 642 (54%) consented to participate in the study.

Follow-up of participants

Health care providers received the Pap test results and the HPV typing results. Medical management of participants diagnosed with cervical abnormalities followed the MCCSP cervical cancer screening management guidelines in effect at the time of the study. Women who tested positive for high-risk HPV and negative for cytology were recalled by the clinics for further investigation according to the MCCSP guidelines.

Risk factor survey

The survey included questions on socio-demographic characteristics and relevant risk factors for cervical neoplasia including smoking, oral contraceptive use, recent sexual activity, previous diagnosis with sexually transmitted infections and HPV immunization status. The questionnaire was tested to a grade four reading level before use.

Cervical specimen processing and HPV detection and typing

The Luminex assay is a method developed at the National Microbiology Laboratory that detects 45 HPV types. These include 23 of the 25 high-risk (as defined by the International Agency for Research on Cancer) types found in groups 1, 2a and 2b: HPV types 16, 18, 26, 30, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and 85.[9] Also included are 22 types considered of low risk or unknown risk: HPV types 6, 11, 13, 32, 40, 42, 43, 44, 54, 61, 62, 71, 72, 74, 81, 83, 84, 86, 87, 89, 90 and 91. In brief, samples in viral transport medium were centrifuged and their DNA extracted from the resulting pellet using a MagnaZorb DNA extraction kit.[10,11] The DNA was amplified with a nested polymerase chain reaction (PCR) method using the general PGMY primer set for the first round[12] and the GP5+/GP6+ primer set for the second.[13] This method amplifies a fragment of the L1 region of the HPV genome (about 150 base pairs in length). The quality of the DNA sample for PCR was checked by co-amplification of the human beta-globin gene. PCR products were visually detected by gel electrophoresis.[13-16]

HPV DNA was detected and typed by hybridization to microspheres coupled to specific probes for the 45 HPV types according to the xMAP Luminex technology*. Specificity and sensitivity of this method for all the 45 types of HPV was measured using cloned HPV DNAs. Comparison against the LinearArray (Roche)[17] and other HPV genotyping kits showed that this Luminex assay is comparable to other commercial genotyping methods.[18]

Data analysis

HPV typing results and survey results were linked to the MCCSP database using a unique identifier in order to get the results of the Pap tests performed during Pap Week 2008 and the cervical cancer screening history of the consenting participants. Univariate and multivariate logistic regression analyses was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) as estimates of the relative risk of HPV detection associated with the various predictor variables. Because of the higher prevalence of HPV in women aged less than 30 years, results were tabulated for women aged less than 30 years and for those aged 30 years plus. HPV types were grouped according to Bouvard et al. and de Villiers et al.[9,19]

The protocol was approved by the research ethics boards of Health Canada/Public Health Agency of Canada and the University of Manitoba.

Results

Tissue samples collected from the 642 women who consented to participate in the study were sent for HPV infection testing. Of these, 33 women did not complete the consent form and were excluded from the analyses. A further 17 were excluded because of inadequate samples. The final study population included 592 participants, of which 527 completed the questionnaire. The

* http://www.luminexcorp.com/

mean age of the study population was 43 years (median: 44). The mean age of infected women was 35 years (median: 31 years), and the mean age of non-infected women was 45 years (median: 46 years). The majority of participants came from rural areas (66.3%), and the remainder came from Winnipeg and Brandon.

Survey results

Variables associated with the HPV infection using univariate analysis are reported in Table 1. Results are presented for women aged less than 30 years (referred to as "younger") and for women aged 30 years and older (referred to as "older") to reflect the higher prevalence of HPV infections in younger women. In older women, HPV infection was associated with Aboriginal ethnicity and a self-described difficult financial situation. Compared with non-smokers, participants who smoked were at greater risk of being HPV-positive, regardless of age. Not having a history of

TABLE 1
Survey results by age and HPV infection status

Variables[a]	Categories	Age < 30 years					Age ≥ 30 years				
		HPV− (n = 75)		HPV+ (n = 56)		OR (95% CI)	HPV− (n = 402)		HPV+ (n = 59)		OR (95% CI)
		n	%	n	%		n	%	n	%	
Ethnic identity	Aboriginal	18	(24.0)	19	(33.9)	1.7 (0.7, 3.8)	58	(14.4)	18	(30.5)	**3.3 (1.7, 6.4)**
	Caucasian	38	(50.7)	24	(42.9)	Reference	276	(68.7)	26	(44.1)	Reference
	Other	10	(13.3)	3	(5.4)	0.5 (0.1, 1.9)	26	(6.5)	5	(8.5)	2.0 (0.7, 5.8)
	Not stated	9	(12.0)	10	(17.9)	1.8 (0.6, 5.0)	42	(10.4)	10	(16.9)	**2.5 (1.1, 5.6)**
Financial situation	Difficult	5	(6.7)	5	(8.9)	1.3 (0.3, 4.9)	19	(4.7)	6	(10.2)	**3.3 (1.2, 9.4)**
	Moderate	20	(26.7)	14	(25.0)	0.9 (0.4, 2.1)	110	(27.4)	18	(30.5)	1.7 (0.9, 3.4)
	Comfortable	32	(42.7)	25	(44.6)	Reference	201	(50.0)	19	(32.2)	Reference
	Very comfortable	9	(12.0)	2	(3.6)	0.3 (0.1, 1.4)	32	(8.0)	5	(8.5)	1.7 (0.6, 4.7)
	Not stated	9	(12.0)	10	(17.9)	1.4 (0.5, 4.0)	40	(10.0)	11	(18.6)	**2.9 (1.3, 6.5)**
Education	High school or less	28	(37.3)	19	(33.9)	Reference	139	(34.6)	18	(30.5)	Reference
	College	14	(18.7)	8	(14.3)	0.8 (0.3, 2.4)	114	(28.4)	15	(25.4)	1.0 (0.5, 2.1)
	University	25	(33.3)	19	(33.9)	1.1 (0.5, 2.6)	110	(27.4)	16	(27.1)	1.1 (0.5, 2.3)
	Not stated	8	(10.7)	10	(17.9)	1.8 (0.6, 5.5)	39	(9.7)	10	(16.9)	2.0 (0.8, 4.6)
Currently smoking	Yes	21	(28.0)	23	(41.1)	2.5 (1.1, 5.7)	101	(25.1)	24	(40.7)	**2.5 (1.3, 5.0)**
	Former smoker	7	(9.3)	6	(10.7)	2.0 (0.6, 6.7)	103	(25.6)	11	(18.6)	1.1 (0.5, 2.6)
	Never	39	(52.0)	17	(30.4)	Reference	159	(39.6)	15	(25.4)	Reference
	Not stated	8	(10.7)	10	(17.9)	**2.9 (1.0, 8.5)**	39	(9.7)	9	(15.3)	**2.5 (1.0, 6.0)**
Currently use oral contraceptive	Yes	24	(32.0)	15	(26.8)	0.7 (0.3, 1.6)	18	(4.5)	3	(5.1)	1.3 (0.4, 4.6)
	No	31	(41.3)	27	(48.2)	Reference	292	(72.6)	38	(64.4)	Reference
	Don't know	2	(2.7)	1	(1.8)	—	1	(0.2)	1	(1.7)	—
	Not stated	18	(24.0)	13	(23.2)	0.8 (0.3, 2.0)	91	(22.6)	17	(28.8)	1.4 (0.8, 2.7)
Ever had a Pap test	Yes	47	(62.7)	41	(73.2)	Reference	357	(88.8)	46	(78.0)	Reference
	No	20	(26.7)	5	(8.9)	**0.3 (0.1, 0.8)**	7	(1.7)	2	(3.4)	2.2 (0.4, 11.0)
	Don't know	0	(0.0)	0	(0.0)	—	0	(0.0)	2	(3.4)	—
	Not stated	8	(10.7)	10	(17.9)	1.4 (0.5, 4.0)	38	(9.5)	9	(15.3)	1.8 (0.8, 4.0)
Ever had an STI	Yes	15	(20.0)	18	(32.1)	Reference	55	(13.7)	15	(25.4)	Reference
	No	52	(69.3)	24	(42.9)	**0.4 (0.2, 0.9)**	286	(71.1)	29	(49.2)	**0.4 (0.2, 0.7)**
	Don't know	0	(0.0)	4	(7.1)	—	20	(5.0)	6	(10.2)	—
	Not stated	8	(10.7)	10	(17.9)	1.0 (0.3, 3.3)	41	(10.2)	9	(15.3)	0.8 (0.3, 2.0)
Number of children	None	44	(58.7)	29	(51.8)	Reference	48	(11.9)	10	(16.9)	Reference
	1	10	(13.3)	9	(16.1)	1.4 (0.5, 3.8)	33	(8.2)	10	(16.9)	1.5 (0.5, 3.9)
	2	7	(9.3)	5	(8.9)	1.1 (0.3, 3.7)	113	(28.1)	9	(15.3)	**0.4 (0.2, 1.0)**
	≥ 3	4	(5.3)	2	(3.6)	0.8 (0.1, 4.4)	167	(41.5)	21	(35.6)	0.6 (0.3, 1.4)
	Not stated	10	(13.3)	11	(19.6)	1.7 (0.6, 4.4)	41	(10.2)	9	(15.3)	1.1 (0.4, 2.8)
Number of sexual partners over last year	0	7	(9.3)	2	(3.6)	0.7 (0.1, 3.8)	74	(18.4)	11	(18.6)	1.4 (0.7, 2.9)
	> 0[b]	0	(0.0)	1	(1.8)	—	7	(1.7)	1	(1.7)	—
	1	48	(64.0)	19	(33.9)	Reference	280	(69.7)	30	(50.8)	Reference
	2 or more	15	(20.0)	26	(46.4)	**4.4 (1.9, 10.0)**	12	(3.0)	11	(18.6)	**8.6 (3.5, 21.1)**
	Not stated	5	(6.7)	8	(14.3)	**4.0 (1.2, 13.9)**	29	(7.2)	6	(10.2)	**1.9 (0.7, 5.0)**

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TABLE 1 (continued)
Survey results by age and HPV infection status

Variables[a]	Categories	Age < 30 years					Age ≥ 30 years				
		HPV− (n = 75)		HPV+ (n = 56)		OR (95% CI)	HPV− (n = 402)		HPV+ (n = 59)		OR (95% CI)
		n	%	n	%		n	%	n	%	
Lifetime number of sexual partners	0	6	(8.0)	2	(3.6)	1.0 (0.2, 5.6)	12	(3.0)	3	(5.1)	3.3 (0.9, 13.0)
	> 0[b]	5	(6.7)	1	(1.8)	—	23	(5.7)	4	(6.8)	—
	1–4	36	(48.0)	12	(21.4)	Reference	227	(56.5)	17	(28.8)	Reference
	≥ 5	24	(32.0)	33	(58.9)	**4.1 (1.8, 9.5)**	111	(27.6)	28	(47.5)	**3.4 (1.8, 6.4)**
	Not stated	4	(5.3)	8	(14.3)	**6.0 (1.5, 23.5)**	29	(7.2)	7	(11.9)	**3.2 (1.2, 8.4)**
Had unprotected anal sex over last year	Yes	14	(18.7)	10	(17.9)	0.9 (0.4, 2.4)	38	(9.5)	7	(11.9)	0.8 (0.3, 1.8)
	No	52	(69.3)	35	(62.5)	Reference	307	(76.4)	43	(72.9)	Reference
	Don't know	0	(0.0)	1	(1.8)	—	3	(0.7)	0	(0.0)	—
	Not stated	9	(12.0)	10	(17.9)	1.6 (0.5, 5.2)	54	(13.4)	9	(15.3)	0.9 (0.3, 2.6)
Currently in a stable relationship	Yes	54	(72.0)	23	(41.1)	Reference	289	(71.9)	35	(59.3)	Reference
	No	11	(14.7)	19	(33.9)	**4.1 (1.7, 9.9)**	64	(15.9)	12	(20.3)	1.5 (0.8, 3.1)
	Not sure	1	(1.3)	4	(7.1)	—	4	(1.0)	3	(5.1)	—
	Not stated	9	(12.0)	10	(17.9)	2.6 (0.9, 7.3)	45	(11.2)	9	(15.3)	1.7 (0.7, 3.7)
Total number of Pap tests[c]	0	22	(29.3)	14	(25.0)	0.8 (0.3, 1.8)	43	(10.7)	11	(18.6)	**2.1 (1.0, 4.5)**
	1–4	30	(40.0)	25	(44.6)	Reference	270	(67.2)	33	(55.9)	Reference
	5+	23	(30.7)	17	(30.4)	0.9 (0.4, 2.0)	89	(22.1)	15	(25.4)	1.4 (0.7, 2.7)
Total number of colposcopies[c]	0	64	(85.3)	49	(87.5)	Reference	379	(94.3)	55	(93.2)	Reference
	1+	11	(14.7)	7	(12.5)	0.8 (0.3, 2.3)	23	(5.7)	4	(6.8)	1.2 (0.4, 3.6)
Worst cytology[c]	No history	22	(29.3)	14	(25.0)	1.0 (0.5, 2.4)	43	(10.7)	11	(18.6)	**2.0 (1.0, 4.3)**
	Normal	42	(56.0)	26	(46.4)	Reference	323	(80.3)	40	(67.8)	Reference
	Other than normal	11	(14.7)	16	(28.6)	2.4 (0.9, 5.8)	36	(9.0)	8	(13.6)	1.8 (0.8, 4.1)
Worst histology[c]	No history	64	(85.3)	49	(87.5)	Reference	379	(94.3)	55	(93.2)	Reference
	Normal	3	(4.0)	2	(3.6)	0.9 (0.1, 5.4)	12	(3.0)	1	(1.7)	0.6 (0.1, 4.5)
	Other than normal	8	(10.7)	5	(8.9)	0.8 (0.3, 2.7)	11	(2.7)	3	(5.1)	1.9 (0.5, 6.9)

Abbreviations: ASC-H, atypical squamous cells–cannot rule out high-grade lesion; ASC-US, atypical squamous cells of unknown significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HPV−, HPV-negative; HPV+, HPV-positive; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions; OR, odds ratio; Pap, Papanicolaou; STI, sexually transmitted infection.

Note: Bolded values are significant.

[a] Variables are all self-reported.

[b] Value obtained by combining information on the number of children and sexual activity questions.

[c] Manitoba Cervical Cancer Screening Program data; other cytology: ASC-US, LSIL, ASC-H, HSIL; other histology: CIN I, CIN II, CIN III; all the other variables are self-reported by the participants.

sexually transmitted infections (STIs) was protective for HPV infection for both age groups. Women with a higher number of lifetime sexual partners or a higher number of sexual partners over the previous year were more likely to be HPV-positive. Younger women who were not in a stable relationship were more likely to be HPV-positive than those in a stable relationship or older women.

In the multivariate logistic regression analysis, being younger (OR = 0.97; 95% CI: 0.95–0.99; age was treated as a continuous variable), Aboriginal (OR = 4.83; 95% CI: 2.70–8.65; compared to non-Aboriginal), and having two or more sexual partners in the previous year (OR = 2.04; 95% CI: 1.20–3.47 compared to one or no sexual partner) were significant predictors for testing HPV-positive. The variables that were not significant predictors of HPV infection in the multivariate model were currently smoking (yes/no), Pap test history (yes/no), history of cervical abnormality (yes/no) and having had at least two consecutive screening events within a year (yes/no).

Reported and registry-based Pap test history

Older women who had had zero Pap tests between 2001 (the year the MCCSP database was started) and October 2008 were at higher risk of being HPV-positive (Table 1; data from the MCCSP). A similar trend was observed with Pap test history, although the number of respondents who had had no Pap test was small. Younger women who self-reported not ever having a Pap test were at lower risk of having an HPV infection, although this was not observed when the analyses were performed with the MCCSP data.

HPV infections and cytological outcomes

A total of 115 participants (19%) were found to be HPV-positive (Table 2). Overall, 33% (38/115) of these infections were among participants aged less than 25 years. The participants aged less than 25 years were also more likely to be infected with Group 1 HPV types

TABLE 2
Age distribution of women by infection status and HPV type (person-based)

Age, years	HPV−		HPV+[a]		Group 1[b]		HPV 16 or 18[c]		Group 2[d]		HPV 6 or 11[c]		Low-risk[e]		Multiple infections		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
< 25	40	(8.4)	38	(33.0)	27	(44.3)	8	(40.0)	4	(21.1)	3	(75.0)	13	(29.5)	15	(50.0)	78	(13.2)
25–29	35	(7.3)	18	(15.7)	9	(14.8)	4	(20.0)	3	(15.8)	1	(25.0)	7	(15.9)	4	(13.3)	53	(9.0)
30–34	46	(9.6)	5	(4.3)	5	(8.2)	2	(10.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(3.3)	51	(8.6)
35–39	41	(8.6)	9	(7.8)	2	(3.3)	0	(0.0)	3	(15.8)	0	(0.0)	4	(9.1)	1	(3.3)	50	(8.4)
40–44	66	(13.8)	11	(9.6)	6	(9.8)	1	(5.0)	1	(5.3)	0	(0.0)	5	(11.4)	2	(6.7)	77	(13.0)
45–49	56	(11.7)	14	(12.2)	4	(6.6)	1	(5.0)	3	(15.8)	0	(0.0)	7	(15.9)	3	(10.0)	70	(11.8)
50–54	62	(13.0)	10	(8.7)	5	(8.2)	2	(10.0)	2	(10.5)	0	(0.0)	4	(9.1)	3	(10.0)	72	(12.2)
55–59	51	(10.7)	5	(4.3)	2	(3.3)	2	(10.0)	1	(5.3)	0	(0.0)	2	(4.5)	0	(0.0)	56	(9.5)
60–64	36	(7.5)	4	(3.5)	1	(1.6)	0	(0.0)	2	(10.5)	0	(0.0)	1	(2.3)	1	(3.3)	40	(6.8)
65+	44	(9.2)	1	(0.9)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.3)	0	(0.0)	45	(7.6)
Total	477		115		61		20		19		4		44		30		592	

Abbreviation: HPV, human papillomavirus.

[a] Any HPV type included in Group 1, Group 2, and low-risk (see text); note that HPV 34 and 97, which belong to Group 2,[9] are not included in the HPV types covered by the methodology used in this study.

[b] Group 1: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.

[c] Either one type or both present at the same time.

[d] Group 2: HPV 26, 30, 53, 66, 67, 68, 69, 70, 73, 82, 85.

[e] Low-risk: HPV 6, 11, 13, 32, 40, 42, 43, 44, 54, 61, 62, 71, 72, 74, 81, 83, 84, 86, 87, 89, 90, 91.

(44%; 27/61) than Group 2 types (21%; 4/19). While HPV types 6 and 11 were not detected in women aged 30 years plus, HPV types 16 and 18 (but mostly 16) were detected over a wider age range. One-quarter of the infected women (26%; 30/115) had multiple HPV infections, that is, more than one HPV of any type.

Of the study population with a normal Pap test, 17% (89/517) tested positive for an HPV infection and 9% (46/517) were infected with Group 1 HPV (Table 3). Overall, 7% (41/592) of all participants had an abnormal Pap test result. An HPV infection (any type) was found in 11% of unsatisfactory Pap tests (2/18), 32% of atypical squamous cells of unknown significance (ASC-US; 6/19), 63% of low-grade squamous intraepithelial lesions (LSIL; 10/16) and 75% of high-grade squamous intraepithelial lesions (HSIL; 3/4). Group 1 HPV type was found in 6% of unsatisfactory Pap tests (1/18), 11% of ASC-US (2/19), 38% of LSIL (6/16) and 75% of HSIL (3/4). Group 1 HPV types (overall: 10.3% [61/592]; among HPV-infected participants: 53.0% [61/115]) were detected more frequently than Group 2 (overall: 3.2% [19/592]; among HPV-infected participants: 16.5% [19/115]) and low-risk HPV types (overall: 7.4% [44/592]; among HPV-infected participants: 38.2% [44/115]). Pap test results were not available for 3% (16/592) of the HPV samples tested.

It is not clear why some Pap tests were not sent to the lab for evaluation. We suspect that the clinicians that performed these tests understood that taking a tissue sample for HPV typing

TABLE 3
Person-based HPV prevalence by cytological outcome

HPV types	Missing	Normal	Unsatis-factory	ASC-US	LSIL	ASC-H	HSIL	Total	
	n	n	n	n	n	n	n	n	%
Negative	11	428	16	13	6	2	1	477	80.6
Any[a]	5	89	2	6	10	0	3	115	19.4
6 or 11[b]	0	3	1	0	0	0	0	4	0.7
16	2	10	0	1	2	0	0	15	2.5
16 or 18[b]	2	14	0	1	2	0	1	20	3.4
Group 1[c]	3	46	1	2	6	0	3	61	10.3
Group 2[d]	1	13	0	2	2	0	1	19	3.2
Low-risk[e]	1	36	1	3	3	0	0	44	7.4
Multiple[f]	1	22	0	2	4	0	1	30	5.1
Total	16	517	18	19	16	2	4	592	

Abbreviations: ASC-H, atypical squamous cells–cannot rule out high-grade lesion; ASC-US, atypical squamous cells of unknown significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

[a] Any HPV type included in Group 1, Group 2, and low-risk (see following text); note that HPV 34 and 97, which belong to Group 2,[9] are not included in the HPV types covered by the methodology that was used in this study.

[b] One type or the other or both can be present at the same time.

[c] Group 1: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.

[d] Group 2: HPV 26, 30, 53, 66, 67, 68, 69, 70, 73, 82, 85.

[e] Low-risk: HPV 6, 11, 13, 32, 40, 42, 43, 44, 54, 61, 62, 71, 72, 74, 81, 83, 84, 86, 87, 89, 90, 91.

[f] Multiple HPV infections.

was their only task for this study and did not request a regular cytological testing.

Among Group 1 types, HPV-16 (10%) was the most frequently detected followed by HPV-39 (5%), 58 (5%), 18 (4%), 35 (4%), 51 (4%), 52 (4%), 59 (4%) and 33 (3%) (Table 4). Within the genus alpha, species 9 (29%; 45/157), 3 (19%; 29/157) and 7 (17%; 26/157) were the most frequently detected. Species 9 includes viruses related to HPV-16, while species 7 includes those related to HPV-18, and species 3 includes low-risk HPV types.

Discussion

Comparing the prevalence of HPV infections across studies is difficult because typing technologies, sampled populations and sampling strategies are often different. In addition, prevalence rates are rarely age-standardized. With this in mind, a meta-analysis reported HPV infection rates as varying from 7% to 8% in Europe and Asia, 14% in North America, and 23% in Africa in women with normal cytology.[20] In the United States, rates have been estimated as 27% in females aged 14 to 59 years.[21] Our study found an HPV prevalence of 19% (17% among those with a normal Pap test). HPV-16 was the most prevalent cervical type detected, while other common high-risk types included types 18, 33, 35, 39, 51, 52, 58 and 59. These results are consistent with other findings where HPV types 16, 18, 31, 39, 51, 52, 56 and 58 were found to be among the most frequent types worldwide in women with normal cytological findings;[22] HPV types 16, 18, 31, 33, 45, 51, 52, 56 and 58 in women diagnosed with low-grade cervical lesions;[23] and HPV types 16, 18, 31, 33, 35, 45, 52 and 58 in women diagnosed with high-grade abnormalities.[24] HPV type-specific prevalence rankings, however, varied regionally and by country.[22-24] For example, a Belgium population-based study reported that the most common high-risk type was HPV-16 (3.7%), followed by types 31, 51 and 53, which were identified in at least 2% of the population (HPV-18 was found in 1.5% of the population).[25] A Swedish population-based study reported infection prevalence for HPV-16 of 2.5%, followed by HPV-31 (1.4%), HPV-45 (0.9%)

TABLE 4
Infection-based prevalence of the HPV genital species of the alpha genus

	HPV types	Missing	Negative	Unsatisfactory	ASC-US	LSIL	HSIL	Total	
		n	n	n	n	n	n	n	%
A1	32	0	4	0	0	0	0	4	2.5
	42	0	6	0	0	0	0	6	3.8
	Total	0	10	0	0	0	0	10	6.4
A3	62	0	5	0	1	0	0	6	3.8
	72	0	2	0	0	0	0	2	1.3
	81	0	5	0	0	1	0	6	3.8
	83	0	3	0	0	1	0	4	2.5
	84	0	2	0	0	0	0	2	1.3
	86	0	2	0	0	0	0	2	1.3
	89	0	6	0	0	1	0	7	4.5
	Total	0	25	0	1	3	0	29	18.5
A5	51	0	4	0	0	1	1	6	3.8
	69	0	1	0	0	0	0	1	0.6
	82	0	1	0	0	0	0	1	0.6
	Total	0	6	0	0	1	1	8	5.1
A6	30	0	2	0	0	1	0	3	1.9
	53	0	0	0	0	1	1	2	1.3
	56	0	3	0	0	0	0	3	1.9
	66	0	4	0	0	0	0	4	2.5
	Total	0	9	0	0	2	1	12	7.6
A7	18	0	5	0	0	0	1	6	3.8
	39	1	4	0	0	1	1	7	4.5
	45	0	1	0	0	0	0	1	0.6
	59	0	5	0	0	1	0	6	3.8
	70	0	4	0	1	0	0	5	3.2
	85	0	1	0	0	0	0	1	0.6
	Total	1	20	0	1	2	2	26	16.6
A8	7	0	1	0	0	0	0	1	0.6
	40	0	1	0	2	0	0	3	1.9
	91	0	0	0	1	0	0	1	0.6
	Total	0	2	0	3	0	0	5	3.2
A9	16	2	10	0	1	2	0	15	9.6
	31	0	2	0	1	0	0	3	1.9
	33	0	5	0	0	0	0	5	3.2
	35	0	4	0	0	1	1	6	3.8
	52	0	6	0	0	0	0	6	3.8
	58	1	4	1	0	1	0	7	4.5
	67	1	1	0	0	1	0	3	1.9
	Total	4	32	1	2	5	1	45	28.7
A10	6	0	2	0	0	0	0	2	1.3
	11	0	1	1	0	0	0	2	1.3
	44	1	3	0	1	0	0	5	3.2
	74	0	5	0	0	0	0	5	3.2
	Total	1	11	1	1	0	0	14	8.9
A11	73	0	0	0	1	0	0	1	0.6
A13	54	0	4	0	0	1	0	5	3.2
Other	8	0	1	0	0	0	0	1	0.6
	38	0	0	0	0	1	0	1	0.6
	Total	0	1	0	0	1	0	2	1.3
Total		6	120	2	9	15	5	157	

Abbreviations: ASC-H, atypical squamous cells–cannot rule out high-grade lesion; ASC-US, atypical squamous cells of unknown significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

and HPV-18 (0.7%); 13.0% of women had multiple infections.[26] Many studies have reported an increase in HPV infections in women 60 years of age and older.[27] There were insufficient cases to confirm that trend in Manitoba.

A few studies have investigated the prevalence of HPV in Canada. A British Columbia study found an overall HPV prevalence rate of 16.8% (high-risk HPV: 13.9%; HPV-16: 10.7%);[28] an Ontario study found an overall infection rate of 13.3% (high-risk HPV: 9.6%, HPV-16: 7.3%);[29] A New Brunswick study found a prevalence of 28% (high-risk HPV: 21%).[30] A study conducted between 1992 and 1995 recruited a large proportion of Aboriginal women (42%) from a clinic located in a low-income inner-city area of Winnipeg, Manitoba, and found that HPV infections rates in Aboriginal and non-Aboriginal women were comparable (33.6% and 31.8%, respectively).[31] However, because of the different populations included in our present study and this earlier one, comparison of results is difficult.

The prevalence of high-risk HPV has been consistently reported to increase with the severity of lesions. For example, a meta-analysis reported high-risk HPV in 71.9% (95% CI: 62.8%–80.9%) of LSIL cases[23] and 88.3% (95% CI: 85.8%–90.8%) of HSIL cases.[24] Moore et al.[28] reported that 52.3% of LSIL and 79.4% of HSIL contained high-risk HPV. They also found that HPV positivity increased from normal (12.3%) to benign (19.6%) to low-grade (69.3%) to high-grade (81.0%).[28] We found 37.5% of LSIL were high-risk (Group 1) HPV-positive, as were 75% of HSIL.

A number of cofactors are associated with risk of having an HPV infection and different grades of cervical abnormalities, many of which are related to sexual behaviours. The factors that have been the most consistently associated with higher rates of HPV infections include younger age and having a greater number of lifetime and recent sex partners.[32,33] Other cofactors for HPV infection, including age at sexual debut, smoking, oral contraceptive use, ethnicity, alcohol consumption, history of STI, income, and condom use have also been reported, but not consistently.[33-41] The multivariate analysis showed that age, ethnicity, and the number of sexual partners in the last year were independent predictors. Our present study also suggests that some of these risk factors are common for all age groups while other factors are found only in either younger or older women.

Women with no history of cervical cancer screening and those who were under-screened have been reported to have higher incidence rates of cervical cancer than women who regularly received screening.[42-45] In the present study, women 30 years of age and older with no Pap test history were found to be HPV-positive more often.

Limitations of the study

The present study has several limitations. As with almost all seroprevalence studies, our study relied on opportunistic samples and was not population-based. Consequently, the results do not necessarily represent the rate of HPV infections in the general female population. The publicity made around Pap Week in Manitoba and the clinics dedicated to one-day screening could also create a selection bias by encouraging symptomatic women who have delayed screening to finally get a Pap test. It is difficult to predict the outcome of such bias on the current risk factor analysis, but if it is differential, it may explain why the risk of infection was higher in some groups of people. The cervical screening participation rate in Manitoba between 2007 and 2009 in women aged 20 to 69 years was 65.9%. The breakdown of their cytological results was normal cytology, 95.5%; ASC-US 3.1%; LSIL 2.1%; atypical glandular cells (AGC) 0.1%; ASC-H 0.3%; and HSIL 0.9%. Among study participants, the cervical screening participation rate since 2001 was 84.8% (502/592), with a breakdown of cytology results of normal cytology 87% (517/592); ASC-US 3% (19/592); LSIL 3% (16/592); ASC-H 0.3% (2/592); and HSIL 1% (4/592).

This comparison suggests that most of the study participants attend cervical screening regularly and that their cytological outcomes were comparable to the women who attended cervical screening in Manitoba between 2007 and 2009. The cross-sectional nature of the study design does not allow for establishing a causal relationship between HPV infection and the cofactors investigated. In addition, self-administered questionnaires can be subject to biases. Nevertheless, findings are consistent with current knowledge on risk factors for HPV infections. Due to the high sensitivity of the HPV detection method, the clinical significance of the present study is limited. The PCR amplification can detect as little as one copy of the targeted genes (L1 DNA), and this sensitivity does not necessarily translate into infection of clinical significance. Depuydt et al. showed that below a critical viral load, detection of visually detectable lesions is very rare.[46] A highly sensitive test has the potential to limit the triaging of people with HPV infections.

Conclusion

The results from our study suggest that the distribution of oncogenic HPV types in Manitoba is in accordance with what has been reported in Canada and in other countries. These data provide a baseline of HPV prevalence in an unvaccinated population in Manitoba. In addition, the use of data linkage provides a proof of concept for the applicability of population-based registry linkage to evaluate HPV immunization programs in those jurisdictions where the capacity to conduct such linkages exist.

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