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Assessment of herd effects among women and heterosexual men after girls-only HPV16/18 vaccination in the Netherlands: A repeated cross-sectional study

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Data on the impact of human papillomavirus (HPV) vaccination on the population HPV prevalence are largely obtained from women. We assessed the impact of the girls-only HPV16/18 vaccination program in the Netherlands that started in 2009, on trends in HPV prevalence among women and heterosexual men, using data from the PASSYON study. In this cross-sectional study, the HPV prevalence among 16- to 24-year-old visitors to sexually transmitted infection clinics was assessed in 2009, 2011, 2013, and 2015. We compared the genital postvaccination HPV prevalence with the prevaccination prevalence (2009) using Poisson GEE models. In total, we included 4,996 women and 1,901 heterosexual men. The percentage of women who reported to be vaccinated increased from 2.3% in 2009 to 37% in 2015. Among all women, the HPV16/18 prevalence decreased from 23% prevaccination to 15% in 2015 (adjusted prevalence ratio [aPR] 0.62, $p_{trend} < 0.01$). Among heterosexual men, the HPV16/18 prevalence decreased from 17% prevaccination to 11% in 2015 (aPR 0.52, $p_{trend} < 0.01$). Of the heterosexual men with a steady partner, HPV16/18 prevalence was lower among those whose steady partner had been vaccine-eligible in the national immunization program (aPR 0.13). Among unvaccinated women, the HPV16/18 prevalence in 2015 was not different from prevaccination. The decreasing HPV16/18 prevalence among heterosexual men and the reduced HPV16/18 prevalence among heterosexual men with a vaccine-eligible steady partner strongly suggests herd protection from girls-only vaccination. Absence of notable herd effects among unvaccinated women 6 years postvaccination may be due to the moderate vaccine uptake among girls in the Netherlands.

Key words: human papillomavirus, vaccination, herd protection, population effects, public health

Abbreviations: 95% CI: 95% confidence interval; aPR: adjusted prevalence ratio; GEE: generalized estimating equation; HPV: human papillomavirus; hrHPV: high-risk human papillomavirus; NIP: National Immunization Program; PR: prevalence ratio; STI: sexually transmitted infection

Additional Supporting Information may be found in the online version of this article.

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What's new?

Human papillomavirus (HPV) is a sexually transmitted virus that plays a causal role in the development of anogenital and oropharyngeal cancers in both men and women. The population-level impact of HPV vaccination programs on the HPV prevalence has however mainly been studied in women. This study shows decreasing trends in the HPV16 and HPV18 prevalence among both women and heterosexual men after the introduction of a girls-only HPV16/18 vaccination program in the Netherlands. The findings provide compelling evidence for herd protection in men. Because HPV16/18 are the most oncogenic types, HPV-related cancers are expected to decline in both sexes after girls-only HPV vaccination.

Introduction

Human papillomavirus (HPV) is a sexually transmitted virus that plays a causal role in the development of anogenital and oropharyngeal cancers in both men and women.[1] To prevent HPV-related cancers, many countries have included HPV vaccination in their national immunization program (NIP), using one of the available vaccines that provide direct protection against two, four, or nine HPV types, all including HPV16 and HPV18.[2]

In the Netherlands, the bivalent vaccine (Cervarix®, GSK) is used in the NIP; to date, this has been a girls-only program.[3] In 2009, there was a catch-up campaign for girls born from 1993 to 1996 with 52% that completed the 3-dose schedule.[4] Routine HPV vaccination was introduced in 2010 for girls in the year they turn 13, with an initial 3-dose uptake of 56% (birth cohort 1997). The uptake increased to 61% for birth cohort 2001, but decreased again to 53% for birth cohort 2002. In 2014, routine HPV vaccination changed to a 2-dose schedule.[5]

We previously reported on direct bivalent HPV vaccine effectiveness using cross-sectional data from female sexually transmitted infection (STI) clinic visitors. We showed high effectiveness against the vaccine types HPV16 and HPV18 and cross-protection against other oncogenic HPV types.[6] These findings were reiterated in a longitudinal cohort study among vaccine-eligible girls.[7]

The population-level impact of HPV vaccination programs also includes possible indirect effects, such as herd protection. So far, the population-level impact of HPV vaccination programs on the HPV prevalence has mainly been studied among women. Surveillance studies have shown a decrease in the HPV16/18 prevalence since the introduction of vaccination.[8] Some studies have also shown decreases in the HPV16/18 prevalence among unvaccinated women.[9–11] This decrease among unvaccinated women is attributed to herd protection in men, yet there is limited information about trends in HPV prevalence among men, especially after bivalent HPV vaccination. One study has shown that the HPV16/18 prevalence in urine samples from men decreased from 5.0% prevaccination to 1.1% 2–4 years post girls-only bivalent vaccination.[12] However, since the method of sample collection had changed, the authors were cautious in drawing conclusions. Because HPV16/18 are associated with the majority of HPV-related cancers in men,[1] demonstrating herd protection for these types in heterosexual men is important for assessing the overall health gain from a girls-only HPV vaccination program.[13,14]

We assessed the population-level impact of the girls-only bivalent HPV vaccination program in the Netherlands by studying trends in the prevalence of HPV vaccine and cross protective types from prevaccination up to 6 years postvaccination. We included women as well as heterosexual men, and focused on unvaccinated women and heterosexual men with vaccine-eligible partners to study herd protection. We used data from the PASSYON (PApillomavirus Surveillance among STI clinic YOungsters in the Netherlands) study, a biennial cross-sectional study among visitors to STI clinics that had been designed to monitor the HPV vaccination program in the Netherlands.

Materials and Methods

Study design and population

The PASSYON study started in 2009 when HPV vaccination was implemented in the Netherlands. Young (16- to 24-year-old) people who visited STI clinics throughout the Netherlands were asked to participate in the study. In addition to the routine STI consultation, participants were asked to fill out a questionnaire regarding demographics, sexual behavior and vaccination status. Moreover, they were asked to provide a self-collected genital swab for HPV testing. Women were instructed to insert a swab (Copan Diagnostics, Italy) about 4 cm into the vagina until resistance was felt and to turn it around along the walls of the vagina. Men were instructed to firmly move the swab up and down the entire shaft, the glans, the coronal sulcus and under the foreskin of the penis. More details about the PASSYON study have been published previously.[15] To explore trends in the HPV prevalence after implementation of HPV vaccination, the PASSYON study was repeated in 2011, 2013 and 2015 using the same study protocol. Participants could be included in multiple study rounds, but the probability of repeated consultations is low as we sampled for only 2 months in the same period every other year (Fig. 1). The Dutch Medical Research Involving Human Subjects Act (Dutch acronym: WMO) does not apply for our study, because only for-the-researchers-anonymized-data were used and there were no (medical) interventions other than routine care. The Medical Ethical Committee of the University of Utrecht, the Netherlands, provided a waiver for full medical ethical review (protocol number 08/397). Data were obtained using a unique code per person and all participants gave informed consent.

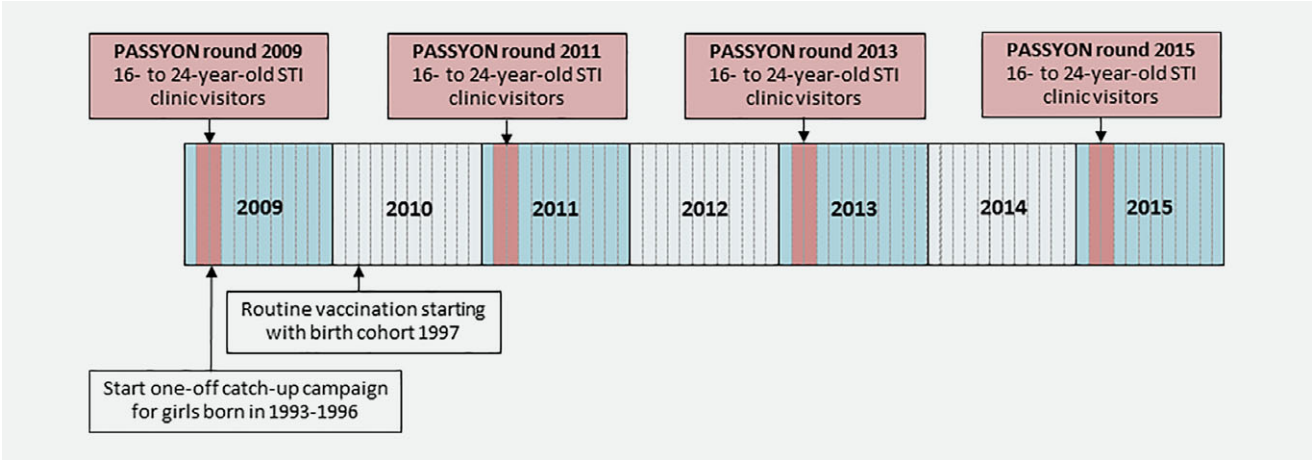

Figure 1. Human papillomavirus (HPV) vaccination in the Netherlands and the PASSYON study design. Abbreviation: STI: sexually transmitted infection. [Color figure can be viewed at wileyonlinelibrary.com]

Laboratory methods

HPV testing protocols were constant across all years and described in detail elsewere.[15] Briefly, DNA was extracted using the MagnaPure platform (Total Nucleic Acid Isolation Kit, Roche, the Netherlands) and HPV-DNA was amplified using the SPF10 primer set and detected using the DNA enzyme-linked immunoassay (HPV-DEIA, DDL Diagnostics Laboratory, the Netherlands). Positive samples were genotyped with line-probe assay (HPV-LiPA25, DDL Diagnostics Laboratory, the Netherlands), which is able to detect 25 HPV types, including HPV16 and HPV18.

Statistical analyses

Only participants with a genital swab were included in the analyses. All analyses were performed separately for all women (irrespective of vaccination status), heterosexual men (based on self-identified sexual preference) and unvaccinated women (based on self-reported vaccination status).

We calculated the prevalence and Wilson score 95% confidence interval (95% CI) of HPV16 and HPV18 (combined and separately) for each PASSYON year and performed a crude Cochran-Armitage Trend Test. Next, we compared the HPV prevalence of the postvaccination periods (2011, 2013 and 2015) with the prevaccination period (2009) and calculated prevalence ratios (PRs) using a Poisson model with robust error variance. This results in comparable estimates as compared to log-binomial regression, and improves numerical convergence.[16] Additionally, because we assumed identical effects of covariates on the prevalence of HPV types included in the analyses, we made use of a generalized estimating equation (GEE) model with an exchangeable correlation structure. This allows efficient estimation of coefficients and calculation of the population-averaged effect of study year on the HPV prevalence, either type-specific or pooled (as a weighted average).[17] Linear trends over time were assessed by including PASSYON year as a continuous variable in the model. These analyses were adjusted for age (16–20 and 21–24 years) and possible confounders, presented in Table 1. The variables age at sexual debut and number of sex partners in the past 6 months and lifetime were categorized for analyses purposes based on knowledge about the HPV risk and size of each category. The selection of confounders was based on the following procedure. First, we explored the association with PASSYON year and high-risk HPV (hrHPV) positivity (being positive for HPV16/18/31/33/35/39/45/51/52/56/58/59), using Chi-square tests. Using hrHPV instead of HPV16/18 positivity for the selection of confounders gave more power to detect possible associations. Variables associated with PASSYON year and hrHPV positivity ($p < 0.05$) in univariable analyses were selected. Second, because sexual risk behavior variables were highly correlated, we used computerized selection models (stepwise with $p < 0.05$ as entry and stay criteria) with hrHPV positivity as an outcome and the sexual risk behavior variables that were selected as independent variables. Variables that were included in the final selection model were evaluated as possible confounders to adjust for in the Poisson GEE models for comparing HPV prevalence between study rounds. For all women, we also adjusted for self-reported vaccination status to assess if possible trends in HPV prevalence over time was explained by an increasing proportion of vaccinated women in our study population. Although HPV vaccination was not offered to men in the Dutch NIP, it is possible that men were vaccinated elsewhere. In sensitivity analyses, we excluded heterosexual men who reported to have been HPV vaccinated. To study the population-level impact and herd protection for the cross-protective types HPV31/33/45[9], we repeated the analyses by also including these types.

Because women who were offered vaccination in the Netherlands (women born in 1993 or later), were aging over the span of the PASSYON study, the vaccination coverage by age category differed over the years. We assessed for effect modification by age category by including an interaction term

Table 1. Characteristics of the study population of all PASSYON years combined for all women, heterosexual men and unvaccinated women

	All women	Heterosexual men	Unvaccinated women
	N (%)	*N* (%)	*N* (%)
Total	4,996	1,901	3,594
Age			
16–20 years	2,012 (40.3)	557 (29.3)	1,186 (33.0)
21–24 years	2,984 (59.7)	1,344 (70.7)	2,408 (67.0)
Self-defined ethnicity			
Dutch	4,319 (86.5)	1,522 (80.1)	3,127 (87.1)
Not Dutch	675 (13.5)	377 (19.9)	465 (12.9)
Education level[1]			
Low/middle	1,246 (25.1)	591 (31.2)	835 (23.3)
High	3,719 (74.9)	1,303 (68.8)	2,745 (76.7)
Sexual preference			
Heterosexual	4,804 (96.2)	1,901 (100)	3,457 (96.2)
Gay or bisexual	192 (3.8)	-	137 (3.8)
Age sexual debut[2]			
≤14 years	647 (13.1)	322 (17.1)	439 (12.3)
15–16 years	2,396 (48.5)	762 (40.5)	1,697 (47.7)
≥17 years	1,898 (38.4)	799 (42.4)	1,423 (40.0)
Sex partners past 6 months[3]			
0–1 partner	1,627 (32.6)	418 (22.0)	1,203 (33.5)
2–3 partners	2,412 (48.3)	715 (37.6)	1,721 (47.9)
4–5 partners	687 (13.8)	390 (20.5)	499 (13.9)
≥6 partners	265 (5.3)	378 (19.9)	169 (4.7)
Lifetime sex partners[3]			
≤2 partners	570 (11.6)	105 (5.8)	396 (11.2)
3–4 partners	973 (19.8)	202 (11.1)	688 (19.4)
5–6 partners	966 (19.7)	240 (13.2)	694 (19.6)
7–14 partners	1,702 (34.7)	585 (32.2)	1,245 (35.2)
≥15 partners	693 (14.1)	687 (37.8)	516 (14.6)
Anal sex past 6 months			
No	4,351 (87.6)	1,590 (84.8)	3,122 (87.3)
Yes	614 (12.4)	284 (15.2)	455 (12.7)
Notified for STI[4]			
No	4,511 (90.6)	1,608 (85.0)	3,263 (91.1)
Yes	467 (9.4)	284 (15.0)	319 (8.9)
STI related symptoms[4]			
No	3,799 (76.5)	1,367 (72.4)	2,721 (76.1)
Yes	1,170 (23.5)	521 (27.6)	853 (23.9)
Self-reported history of any STI			
No	2,852 (57.4)	1,055 (55.7)	2,101 (58.7)
Yes	1,266 (25.5)	377 (19.9)	920 (25.7)
Never tested	851 (17.1)	462 (24.4)	558 (15.6)
Genital chlamydia infection[4]			
No	4,283 (86.1)	1,594 (84.4)	3,098 (86.5)
Yes	694 (13.9)	294 (15.6)	482 (13.5)
Steady partner			
No	2,961 (60.7)	1,037 (56.5)	2,127 (60.6)
Yes, for 0–6 months	1,102 (22.6)	475 (25.9)	801 (22.8)
Yes, for ≥6 months	813 (16.7)	324 (17.6)	583 (16.6)

(Continues)

Table 1. Characteristics of the study population of all PASSYON years combined for all women, heterosexual men and unvaccinated women (Continued)

	All women	Heterosexual men	Unvaccinated women
	N (%)	*N* (%)	*N* (%)
Condom use past 6 months, casual partner[5]			
Inconsistent	1,950 (39.2)	851 (44.9)	1,344 (37.5)
Consistent	1,806 (36.3)	658 (34.7)	1,361 (37.9)
No casual partners	1,224 (24.6)	385 (20.3)	882 (24.6)

Abbreviations: STI: sexually transmitted infection.
Numbers vary because of missing values.
[1]High educational level included school of higher general secondary education, pre-university education, university of applied sciences and university. Low/middle educational level included all other levels of education.
[2]Categorized for analyses purposes. Minimum-maximum age reported: 9–24 years among (unvaccinated) women and heterosexual men.
[3]Categorized for analyses purposes. Maximum partners reported: 540 past 6 months and 900 lifetime partners among (unvaccinated) women; 50 past 6 months and 400 lifetime partners among heterosexual men.
[4]Based on information of the STI clinic visit.
[5]Inconsistent included reporting never, rarely and "sometimes I do, sometimes I do not" condom use. Consistent included reporting often or always condom use.

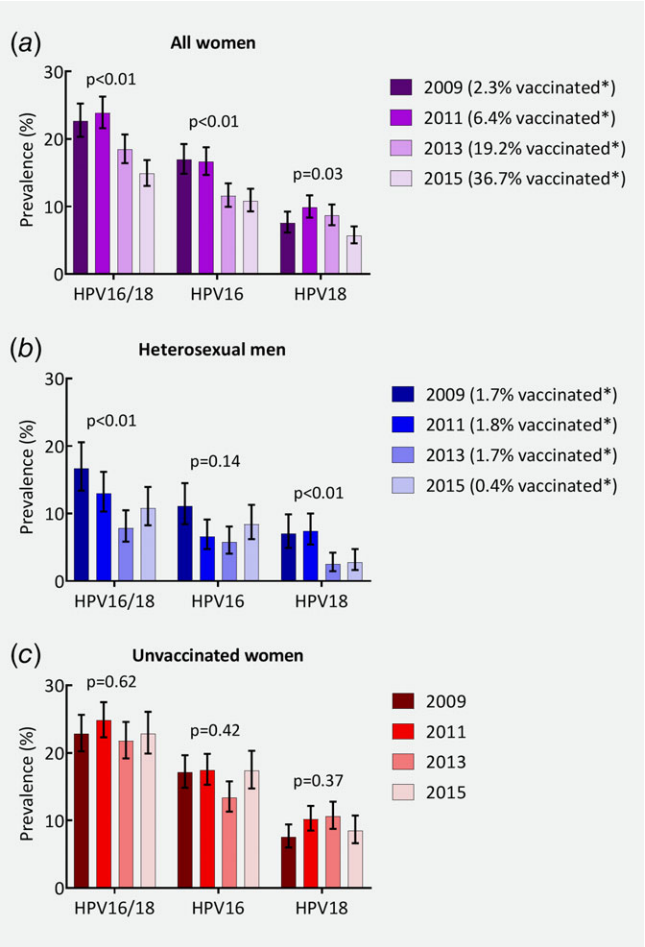

Figure 2. Prevalence of human papillomavirus (HPV) types 16 or 18, HPV16 and HPV18 over time and *p* values for the crude trend test, among (*a*) all women; (*b*) heterosexual men; (*c*) unvaccinated women. Note: the *p* value presents the Cochran-Armitage Trend Test. *Percentage of women and heterosexual men who reported to be vaccinated at least once. [Color figure can be viewed at wileyonlinelibrary.com]

between PASSYON year and age category. For all women, we again additionally adjusted for self-reported vaccination status to assess if the possible difference in trends by age category were explained by differences in vaccination coverage. We also calculated the adjusted PRs (aPRs) and the trend for the age categories separately.

If participants reported being in a relationship, the age of the steady partner was asked. For heterosexual men, we assessed if the steady partner had been eligible for HPV vaccination in the Dutch NIP based on the reported age of the steady partner. We assumed the steady partner had been eligible for vaccination if she was ≤17 years in PASSYON round 2011, ≤19 years in PASSYON round 2013 and ≤ 21 years in PASSYON round 2015. If the steady partner was older is a specific PASSYON round, we assumed she had not been eligible for HPV vaccination in the NIP. Also for all heterosexual men included in PASSYON round 2009, we assumed the steady partner had not been eligible for HPV vaccination. To consider herd effects, we calculated the combined HPV16/18 prevalence among heterosexual men by age of the steady partner and vaccine-eligibility of the steady partner. Next, we assessed the difference in HPV16/18 prevalence between heterosexual men with or without a vaccine-eligible steady partner by using a GEE model with HPV16/18 as an outcome and vaccine-eligibility of the steady partner as an independent variable. This analysis was adjusted for age of the men and age of the steady partner.

All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). We used a significance level of $p < 0.05$. We did complete case analyses, as none of the variables had more than 5% missing.

Results

Study population

A total of 7,108 women and heterosexual men participated in the PASSYON study, of whom 6,897 (4,996 women and 1,901

heterosexual men) delivered a genital swab and were included in the current analysis; 1,524 in 2009, 1,775 in 2011, 1,816 in 2013 and 1,782 in 2015. The proportion of women who had been eligible for vaccination increased from 0.4% in 2009 to 5.3% in 2011, 27% in 2013 and to 57% in 2015. Of the women who had been eligible for vaccination, 55% (n = 650) reported to be vaccinated at least once (30 reported to be vaccinated with 1 dose, 42 with 2 doses, 456 with 3 doses and 122 did not know the number of doses). The proportion of all women reporting to be vaccinated at least once increased from 2.3% in 2009 to 37% in 2015. In total 27 heterosexual men (1.4%) reported to be HPV vaccinated.

Characteristics of the study population of all PASSYON years combined are presented in Table 1. In general, the indicators for sexual risk behavior increased over the years among women, heterosexual men and unvaccinated women (Supporting Information Tables 1–3). For example, we observed an association between lifetime sex partners and PASSYON year; with proportions reporting ≥15 lifetime sex partners of 12% in 2009 and 18% in 2015 among all women; 31% in 2009 and 44% in 2015 among heterosexual men; and 12% in 2009 and 19% in 2015 among unvaccinated women. The genital chlamydia prevalence was associated with PASSYON study year among heterosexual men only. Supporting Information Tables 1–3 also show the association between the characteristics and hrHPV positivity. In general, people with higher sexual risk behavior were more often hrHPV positive.

HPV prevalence over time

Figure 2 presents the HPV16 and HPV18 prevalence over time and the crude trend test among all women, heterosexual men and unvaccinated women. Among all women, the HPV16/18 prevalence decreased from 23% in 2009 to 15% in 2015 (aPR 0.62, Table 2). Also for HPV16 and HPV18 separately, there was a significant decrease over time (aPR 0.59 and 0.69 respectively). When we additionally adjusted for vaccination status, the prevalences in 2015 were no longer significantly different from 2009. Among heterosexual men, the combined HPV16/18 prevalence decreased from 17% in 2009 to 11% in 2015 (aPR 0.52). Also separately, HPV16 and HPV18 prevalences were significantly lower in 2015 compared to 2009 (aPR 0.64 and 0.33 respectively). Excluding the 27 heterosexual men who reported to be vaccinated did not lead to different results (Supporting Information Table 4). Among unvaccinated women, we observed no trends in the HPV16 or HPV18 prevalence.

For HPV31, HPV33 and HPV45, we only observed a declining trend in the HPV31 prevalence among all women

Table 2. Comparing postvaccination human papillomavirus (HPV) prevalence with prevaccination prevalence (2009) and assessing the trend among all women, heterosexual men and unvaccinated women

	All women		Heterosexual men		Unvaccinated women	
	% positive (95% CI)	aPR (95% CI)[1]	% positive (95% CI)	aPR (95% CI)[2]	% positive (95% CI)	aPR (95% CI)[3]
HPV16/18[4]						
2009	22.7 (20.3–25.3)	Reference	16.7 (13.4–20.6)	Reference	22.8 (20.2–25.6)	Reference
2011	23.9 (21.6–26.3)	1.08 (0.92–1.26)	13.0 (10.3–16.2)	0.75 (0.54–1.05)	24.8 (22.3–27.5)	1.11 (0.94–1.31)
2013	18.5 (16.4–20.7)	0.79 (0.67–0.94)	7.9 (5.8–10.5)	0.44 (0.30–0.64)	21.8 (19.2–24.6)	0.90 (0.76–1.08)
2015	14.9 (13.1–16.9)	0.62 (0.52–0.74)	10.8 (8.3–13.9)	0.52 (0.36–0.75)	22.8 (19.9–26.1)	0.94 (0.78–1.14)
p_{trend} value[5]	<0.01	<0.01	<0.01	<0.01	0.62	0.16
HPV16						
2009	16.9 (14.8–19.3)	Reference	11.1 (8.4–14.5)	Reference	17.1 (14.8–19.7)	Reference
2011	16.6 (14.7–18.8)	0.98 (0.81–1.17)	6.6 (4.7–9.1)	0.57 (0.36–0.89)	17.5 (15.3–19.9)	1.01 (0.83–1.23)
2013	11.6 (10.0–13.5)	0.66 (0.54–0.81)	5.7 (4.1–8.1)	0.49 (0.31–0.78)	13.4 (11.3–15.8)	0.73 (0.59–0.91)
2015	10.8 (9.3–12.6)	0.59 (0.48–0.73)	8.4 (6.2–11.3)	0.64 (0.42–0.98)	17.3 (14.7–20.3)	0.92 (0.74–1.14)
p_{trend} value[5]	<0.01	<0.01	0.14	0.06	0.42	0.08
HPV18						
2009	7.6 (6.2–9.3)	Reference	7.0 (4.9–9.9)	Reference	7.5 (6.0–9.4)	Reference
2011	9.9 (8.4–11.7)	1.31 (1.00–1.71)	7.4 (5.4–10.0)	1.04 (0.64–1.67)	10.2 (8.5–12.2)	1.35 (1.01–1.79)
2013	8.7 (7.2–10.3)	1.09 (0.83–1.43)	2.5 (1.5–4.2)	0.35 (0.18–0.66)	10.6 (8.8–12.8)	1.31 (0.97–1.75)
2015	5.7 (4.6–7.1)	0.69 (0.51–0.94)	2.8 (1.6–4.7)	0.33 (0.17–0.65)	8.5 (6.6–10.7)	0.98 (0.70–1.37)
p_{trend} value[5]	0.03	<0.01	<0.01	<0.01	0.37	0.95

Abbreviations: aPR: adjusted prevalence ratio; 95% CI: 95% confidence interval.
[1]Adjusted for: age, lifetime sex partners, history of any sexually transmitted infection, steady partner and condom use with casual partners.
[2]Adjusted for: age, lifetime sex partners, history of any sexually transmitted infection and steady partner.
[3]Adjusted for: age, lifetime sex partners, history of any sexually transmitted infection, and condom use with casual partners.
[4]Defined as positive for HPV16 or HPV18 in the percentage positive, and as a pooled estimate to calculate the aPR.
[5]The crude p_{trend} values were calculated using the Cochran-Armitage Trend Test. The adjusted p_{trend} values were calculated by including PASSYON year as a continuous variable.

(adjusted p_{trend} 0.01), but not among heterosexual men or unvaccinated women. For the other HPV types, no trends were observed except for an increasing trend of HPV45 among unvaccinated women (Supporting Information Table 5).

The vaccination coverage over time differed by age category; for example in 2013, 40% of the 16- to 20-year-old women reported to be vaccinated (≥1 dose), while 4.6% of the 21- to 24-year-old women reported to be vaccinated. We observed that the HPV16/18 prevalence among 16- to 20-year-old women decreased faster as compared to 21- to 24-year-old women (aPR 0.41 and 0.74 respectively for 2015 compared to 2009, Supporting Information Fig. 1 and Supporting Information Table 6). The difference in the effect of year by age group was statistically significant ($p < 0.01$). After additional adjustment for vaccination status, the difference between ages was no longer statistically significant. Among heterosexual men and unvaccinated women, there was no statistically significant interaction with age. Among unvaccinated women, there were no statistically significant trends in the HPV16 or HPV18 prevalence for both age categories (Supporting Information Table 6).

Vaccine-eligible steady partner

The proportion of heterosexual men reporting a steady partner who had been eligible for HPV vaccination increased from 2.2% in 2011 to 15% in 2013 and 19% in 2015. Figure 3 shows the combined HPV16/18 prevalence among heterosexual men according to the age of the steady partner and vaccine-eligibility of the steady partner. Overall, heterosexual men whose steady partner had been vaccine-eligible were less often HPV16/18 positive compared to heterosexual men whose steady partner had not been vaccine-eligible in the NIP (aPR 0.13 [95% CI 0.04–0.41]).

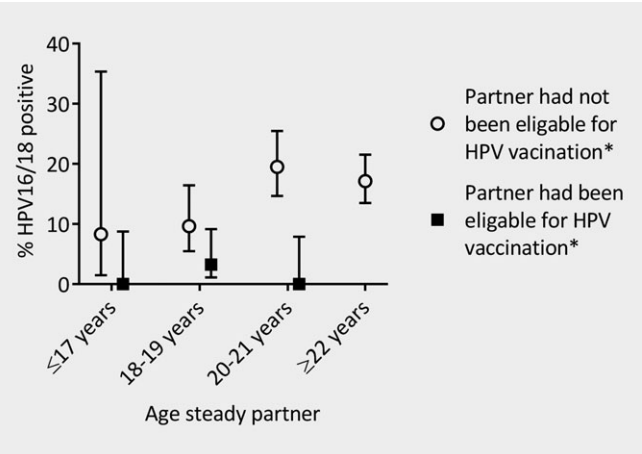

Figure 3. Prevalence of human papillomavirus (HPV) types 16 or 18 among heterosexual men who reported to have a steady partner, by age of the steady partner and vaccine-eligibility of the steady partner. *Vaccine-eligibility of the steady partner was based on the reported age of the steady partner in a specific PASSYON study round and the Dutch national immunization program. None of the steady partners of ≥22 years had been eligible according to the national immunization program in the Netherlands.

Discussion

We estimated the population-level impact of the national girls-only bivalent HPV vaccination program in the Netherlands by comparing HPV prevalence from prevaccination to postvaccination periods among male and female visitors to STI clinics. We showed decreasing trends in the HPV16 and HPV18 prevalence among all women and heterosexual men, but not among unvaccinated women separately. Of the heterosexual men who reported to have a steady partner, HPV16/18 prevalence was lower among those whose steady partner had been vaccine-eligible.

Our results provide compelling evidence for herd protection for the vaccine-types among men in the aftermath of girls-only HPV16/18 vaccination and show that herd effects among heterosexual men will likely precede those among unvaccinated women. Our data offer empirical support for the population-level impact of vaccination against the two most oncogenic HPV types as previously predicted by transmission-dynamic models.[18]

We do acknowledge some limitations. First, STI clinics became stricter in prioritizing high-risk individuals especially since 2015, when the funding of the STI clinics had changed.[19] We indeed observed increased sexual risk behavior over time possibly related to changes in the access policy of STI clinics. Although we adjusted for known changes, unknown changes in the study population may have resulted in changes in the HPV prevalence unrelated to HPV vaccination. This could for instance explain the observed increase in HPV16 prevalence in 2015 compared to 2013 among heterosexual men. If participants in the postvaccination study periods were at higher HPV risk, we may have underestimated the impact of vaccination, including declines in HPV16/18 prevalence among unvaccinated women. However, the chlamydia prevalence did not increase among (unvaccinated) women, suggesting that unrecorded sexual risk behavior likely did not chance that much among female study participants. Analyses restricted to chlamydia positive unvaccinated women did not lead to different results (results not shown). Second, the use of self-reported vaccination status may have induced bias. Among women, we believe the bias will be minimal as we previously showed that the HPV16 and HPV18 antibody concentrations agreed well with the self-reported vaccination status.[6] Of the heterosexual men 1.4% reported to be HPV vaccinated. If these men were truly vaccinated against HPV, this would lead to an overestimation of the herd effects. However, excluding the heterosexual men who reported to be HPV vaccinated did not lead to different results. We also used self-reported sexual preference to identify heterosexual men. It might be that some men did (also) have sex with men. Such bias would have underestimated the impact of vaccination. Third, only one

prevaccination measurement was available. If there were natural fluctuations in the HPV prevalence in the absence of vaccination, multiple prevaccination measurements would have been preferred to obtain an accurate estimate of the average prevaccination prevalence and to assess possible prevaccination trends. Last, we used a population of visitors to STI clinics who are at higher HPV risk as compared to the general population. The results are therefore not representative of the general Dutch population, probably underestimating the impact of vaccination.[20]

The decrease in the HPV16/18 prevalence among women in our study, coincided with an increase in the percentage of women who reported to be vaccinated. After adjustment for vaccination status, the HPV16/18 prevalence did not differ in 2015 as compared to 2009, indicating that the increasing proportion of vaccinated women explained the decreasing HPV prevalence. In other countries, also a decline in the HPV16/18 prevalence among women was observed after introduction of bivalent HPV vaccination. In England, the HPV16/18 prevalence decreased from 18% prevaccination to 4.0% 4–5 years postvaccination among 16 to 18-year-old sexually active women.[21] In Scotland, the HPV16/18 prevalence decreased from 30% prevaccination to 4.5% 7 years postvaccination among 20 to 21-year-old women who underwent their first cervical screening.[9] The larger declines in these countries as compared to our study could be explained by an overall lower percentage of women vaccinated in our study (37% ≥1 dose in 2015). This reflects both a lower percentage of women who had been eligible for vaccination (57% in 2015) and a lower vaccination uptake among vaccine-eligible women (55% ≥1 dose). Among 16- to 20-year-old women, with a higher percentage vaccinated, we observed larger declines in the HPV16/18 prevalence.

We also observed a decrease in the HPV16/18 prevalence among heterosexual men since the introduction of girls-only bivalent HPV vaccination. Our results are comparable to Australia where a declining trend in the HPV16/18 prevalence among heterosexual men was observed after girls-only quadrivalent HPV vaccination.[22] In Australia, also a decline in the HPV16/18 prevalence was observed among foreign-born heterosexual men who had arrived from countries with a bivalent HPV vaccination program within 2 years of study inclusion. However, effects of exposure within those countries might have been negligible because the majority of the HPV16/18 infections clear within 2 years.[23]

While the decreasing HPV16/18 prevalence among heterosexual men in our study strongly suggests herd protection, causality cannot be concluded based on ecological analyses. Nonetheless, the decreasing prevalence in combination with a lower HPV16/18 prevalence among men whose steady partner had been vaccine-eligible strongly indicates that heterosexual men receive indirect protection. With most HPV-related penile cancers attributed to HPV16,[24] cancer reductions are also expected to occur for heterosexual men in the aftermath of girls-only HPV vaccination. Among men who have sex with men, large reductions in HPV-related cancers are not expected, because they benefit less from herd protection after girls-only vaccination.[25] While it is anticipated that HPV prevalence will also decline at other anatomical sites among heterosexual men, this has not yet been demonstrated. Given that oropharyngeal cancers constitute the largest HPV-related burden in men,[26] showing herd effects against oral HPV is valuable to acknowledge the ultimate impact of HPV vaccination.

Among heterosexual men, the decline in HPV prevalence was larger for HPV18 than for HPV16, which is in line with data from Finland where also larger herd effects were observed for HPV18.[10] Higher herd effects for HPV18 could be explained by a lower basic reproduction number (as a consequence of a higher clearance relative to HPV16).[23,27,28] Even though HPV31/33/45 could also be expected to have a lower basic reproduction number than HPV16, there were no signs of herd effects for these types. This could be related to a relatively low background prevalence in combination with reduced vaccine effectiveness, resulting in limited power to detect herd effects against cross-protective types compared to the vaccine types.

Because the vaccination coverage of completed schedule among vaccine-eligible women in the Netherlands is 50% to 60%,[5] herd protection will not have reached its full potential.[13,14] This is particularly true for herd protection in unvaccinated heterosexual women, which is derived from herd protection in heterosexual men, and thus constitutes a second-order effect. With suboptimal girls-only vaccination coverage, vaccinating boys along with girls will not only protect boys themselves, but could also increase herd protection to unvaccinated women.[29,30] Based on modeling studies, 80% vaccination coverage in both men and women, but not in either sex, could eradicate the vaccine types.[18]

We did not find signs of herd effects among unvaccinated women, also not when stratified by age. Nonetheless, other studies have observed a declining prevalence of the HPV vaccine types among unvaccinated women suggestive of herd protection. In Scotland, unvaccinated women born in 1995 were less often HPV16/18 positive at their first cervical screening compared to women born in 1988 who were not eligible for vaccination (5.3% *versus* 30%).[9] Also in Australia and the United States, decreases in the vaccine type prevalence have been recorded among unvaccinated women.[31,32] There are several possible explanations for the absence of a declining trend among unvaccinated women in our study. First, in high-risk populations with frequent changes in sex partners, people are more likely to encounter a HPV-positive man or women, limiting herd effects.[20] However, in Australia declines in vaccine-type prevalence were also observed among the high-risk group of chlamydia-positive unvaccinated women.[33] Second, in Australia and Scotland, the vaccination initiation rate was much higher: over 80% in Australia and over 90% in

Scotland.[2] Third, the time horizon of our study (6 years post-vaccination) might be too short to observe second-order herd effects. In the United States, where vaccination coverage was also limited, decreases in vaccine type prevalence among unvaccinated women were noted 5–8 years after vaccine introduction and not yet after 3–6 years.[32,34]

In conclusion, the declining HPV16/18 prevalence among women is consistent with previous studies, but our findings also provide evidence for herd protection in heterosexual men after girls-only HPV16/18 vaccination. Due to the reduction in the HPV16/18 prevalence among women and heterosexual men, HPV-related cancers are expected to decline in both sexes after girls-only HPV vaccination. The absence of measurable herd effects among unvaccinated women 6 years post-vaccination highlights once again the importance of high vaccination coverage to optimally reduce HPV-related cancer morbidity.

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Author Contribution

MS developed the PASSYON study design. The Public Health Services collected the data. PW coordinated the data collection. SL and the Medical Microbiological Laboratories obtained, identified, stored the samples and performed the laboratory analyses. AK coordinated the laboratory analyses. PW led on the statistical analyses and data interpretation with oversight from JB. BB and CH assisted with data interpretation. PW drafted the paper. All authors contributed to drafting and revision of the paper and all authors read and approved the final version. The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and final responsibility to submit for publication.

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