Changes in HPV Seroprevalence from an Unvaccinated toward a Girls-Only Vaccinated Population in the Netherlands

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ABSTRACT

Background: In the Netherlands, bivalent human papillomavirus (HPV) vaccination was included in the National Immunization Program for 12-year-old girls in 2010 (vaccination coverage, 45%–60%). We examined possible changes in HPV seroprevalence in the HPV-unvaccinated Dutch population aged 0–89 years, comparing prevaccination data with data of approximately 6 years after implementation of national vaccination.

Methods: Serum samples of men and women were used from two cross-sectional population-based serosurveillance studies performed before (2006–07, $n = 6{,}384$) and after (2016–17, $n = 5{,}645$) implementation of HPV vaccination in the Netherlands. Seven high-risk HPV-specific antibodies (HPV16, 18, 31, 33, 45, 52, and 58) were tested in a virus-like particle-based multiplex immunoassay.

Results: Type-specific HPV seroprevalence increased in women between 2006–07 and 2016–17. Also, a higher seroprevalence for at least one type in women >15 years was found in 2016–17 (31.7%) compared with 2006–07 (25.2%). In men, overall HPV seroprevalence remained similar; however, a lower seroprevalence was found for HPV16 in 2016–17 (7.5%) compared with 2006–07 (10.6%).

Conclusions: Our results indicate an increase in high-risk HPV types in women and a rather stable exposure in men. No clear effects of the strategy of girls-only vaccination were observed in men, probably because of the short time after introduction combined with suboptimal coverage.

Impact: No herd immunity has been observed yet in a population with suboptimal HPV vaccination coverage.

Introduction

Human papillomavirus (HPV), a virus capable of infecting the epithelial cells of the mucosa, is the cause of anogenital warts and cervical cancer (1). Besides cervical cancer, HPV is also linked to various other cancers in the anogenital tract and oral cavity (2). By routine HPV vaccination and effective cervical cancer screening programs, countries can reduce the burden of HPV-related disease.

All current globally available vaccines provide protection against HPV types 16 and 18, for example, and are included in the current bivalent vaccine. HPV types 6 and 11 are added in the quadrivalent vaccine and the nonavalent vaccine included additional HPV types 31, 33, 45, 52, and 58. Vaccination against HPV has been implemented in many countries, with the primary aim to protect women against cervical cancer. In the Netherlands, the bivalent HPV vaccine was implemented in the Dutch National Immunization Program as a girls-only vaccine for 12-year olds in a three-dose schedule in 2010, and is currently still being used, protecting them against HPV types 16 and 18. In addition, a catch-up campaign was initiated for girls from the birth cohorts 1993–1996 (i.e., 13–16 year olds) in 2009. From 2014 onward, the Netherlands shifted to a two-dose schedule (starting from birth cohort 2001 onward). The HPV vaccination coverage in girls in the Netherlands varied from 2009–2017 from 45% to 62% (3).

To gain information about previous HPV exposure, HPV serology is established as an important tool for population-based studies (4). This provides a view on type-specific cumulative lifetime exposure to HPV. Antibodies against HPV L1 virus-like particles (VLP) remain stable over time, and therefore reflect past infection and cumulative exposure. However, not everyone who contracted HPV will seroconvert, and the rate of seroconversion is known to be sex dependent (5). After HPV vaccination, HPV-specific antibodies are 10–100 times higher than (natural) infection-induced antibodies in serum (6), and therefore could be used to monitor vaccine uptake.

We assessed the (natural) infection-induced HPV seroprevalence for seven high-risk (hr) HPV types in the Dutch population in 2006/2007 (i.e., 4 years before the introduction of HPV) and 2016/2017 (i.e., 6 years postvaccination implementation). In addition, we investigated the effects of the introduction of the HPV vaccination on the seroprevalence of HPV types in our population.

Materials and Methods

Study design

Serum samples from two cross-sectional population-based serosurveillance studies performed from February 2006 to June 2007 and from September 2016 to October 2017 in the Netherlands were used for this study. Participants were 0–79 years of age in the 2006–2007 survey ($n = 6{,}384$), and 0–89 years of age for the 2016–17 cohort ($n = 5{,}645$). Study designs have been previously described in detail (7, 8). Briefly, the randomly invited participants were asked to fill in a questionnaire and to provide a blood sample. Questionnaires of both surveys included data on demographic characteristics, ethnicity (first- and second-generation migrants), vaccination history, and sexual behavior. Vaccination history was determined via the individuals' registration booklet and the Dutch vaccination registration

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Praeventis (9). The questionnaire used in 2006–7 was extended in the 2016–17 survey with more questions regarding sexual behavior. Information related to sexual behavior was only available from participants older than 14 years of age in both the 2006–07 study and the 2016–17 study.

We obtained written informed consent from all participants or their guardians before participation. The studies were conducted in accordance with recognized ethical guidelines (the Declaration of Helsinki) and were approved by an institutional review board "The Medical Ethics Committee Noord-Holland" in the Netherlands (METC number: ISRCTN 20164309 and M015–022).

Serologic measurement

Serum samples of both surveys were stored at −80°C until analysis, samples were measured at random for age and sex. For the measurement of HPV-specific IgG serum antibodies against L1 VLP of HPV16, 18, 31, 33, 45, 52, and 58, a VLP-based multiplex immunoassay was used as described previously (10). GSK (2006–07 survey) and MSD (Merck Sharp & Dohme; 2016–17 survey) produced the HPV-VLPs in used these studies. Briefly, VLPs were conjugated to seven distinct fluorescent microspheres via amine coupling. Serum samples were 1/50, 1/100, or 1/10,000 diluted and incubated with the VLP-coupled microspheres. HPV-specific IgG serum antibodies were detected using a secondary goat anti-human phycoerythrin-labeled antibody. Four in-house control sera and an in-house standard were used on each plate. The in-house standard (IVIG, lot LE12H227AF, Baxter) was calibrated against reference serum of GSK for all the seven HPV types. HPV-specific IgG antibodies were analyzed using the Bioplex system 200 with Bioplex software (Bio-Rad Laboratories). Samples were assumed to be seropositive above cutoffs according to the 99% Frey method (with 99% one-sided t values, based on concentrations measured in children of 1–10 years old ($n = 859$; ref. 11) and found to be 9, 13, 27, 11, 19, 14, and 31 Luminex units/mL (LU/mL; ref. 10) for HPV16, 18, 31, 33, 45, 52, and 58, respectively. As samples from 2016–17 were measured using a different batch of VLPs than those used in 2006–07, a correction formula was applied on the data of the 2016–17 survey. This correction formula was based on retesting of a random subset of 160 samples of the 2006–07 samples with the new VLPs. The correction formula was applied to the 2016–17 antibody measurements to align them with the 2006–07 measurements.

Statistical analysis

Data analyses were conducted using SAS version 9.4 and GraphPad Prism version 8.0.2. Women who were vaccinated against HPV according to the vaccination registry ($n = 228$) were excluded from analysis. In addition, women under 31 years of age and with arbitrary antibody concentration cutoff of >100 LU/mL for HPV16 and >50 LU/mL for HPV18 were considered to be "highly likely to have been vaccinated" and were excluded from the analyses ($n = 18$). Characteristics of the study population were compared among the 2006–2007 cohort and the 2016–2017 cohort using χ^2 tests. Seroprevalence for "any" or "all" hr-HPV-type(s) refer to the seven hr-serotypes that have been measured in this study. The study design (i.e., a two-stage cluster sampling method including specific regions and municipalities from which participants were invited) was taken into account in the analyses, as well as weights determined proportional to the reference population (Dutch population, January 1, 2007 and January 1, 2017, respectively) taking into account sex, age, ethnic origin, and urbanization degree. Seroprevalences were calculated per age-cohort and as large differences already have been observed between men and women (10), analyses were stratified for men and women. Crude seroprevalences of the different cohorts, age groups, and/or sexes were compared using Monte Carlo simulations. Parameters of the beta distribution for both seroprevalences were estimated and used in the simulations to obtain P values. Geometric mean concentrations (GMC) were calculated among HPV16 and HPV18 seropositive individuals from both cohorts, taking the study design into account. P values of <0.05 were considered statistically significant.

The associations between HPV seropositivity (positive for at least one out of the seven HPV types) in sexually active individuals older than 14 years of age who were not vaccinated and demographic characteristics (age, ethnic origin, degree of urbanization, education level, and socioeconomic status), was examined for the 2016–2017 cohort for men and women separately. In addition, associations with (sexual) behavior characteristics were taken into account, including: body mass index (BMI), alcohol consumption, smoking, having a steady partner, age of sexual debut (being defined as the first time of vaginal and/or penile intercourse), condom use at last sex act, number of partners in the last 6 months, lifetime number of partners, and reported history of STI (note: participants with missing values for a specific variable were allocated to a unknown category). We used generalized estimation equation (GEE) logistic regression models with a log link function and robust error variance. The incorporation of a GEE with exchangeable correlation structure accounted for dependency of multiple HPV types within an individual. First, univariate logistic regression analyses were conducted to study characteristics associated with HPV seropositivity. Variables that had $P < 0.1$ in univariate analyses were included in the multivariate analysis and backward selection (dropping variables one-by-one) was then applied. Hence, a multivariate model only including independently associated risk factors ($P < 0.05$) remained.

To study the differences in seroprevalence between the 2006–2007 and 2016–2017 cohort more closely, a pooled dataset was created including all HPV-unvaccinated participants from both cohorts. Again, the association between HPV seropositivity in sexually active individuals older than 14 years of age and demographics and sexual behavior characteristics was studied, in addition to the variable defining the cohort. Only characteristics available from both surveys were considered for inclusion in the model. Using a Poisson regression with robust error variance, we first calculated the crude prevalence ratio (PR). Next, we included the variables of interest to adjust for differences between the two surveys resulting in an adjusted PR (aPR). The analyses were performed for seroprevalence of any HPV type as well as type-specific. In addition, we stratified the analyses for men and women; we assumed that if herd effects on seroprevalence were to be observed this short after HPV vaccine introduction, this would be among men (first-order effect), in particular, younger males. Therefore, we looked also into the aPR for younger males (15–39 years of age).

Results

Study and participant characteristics

We tested 5,645 serum samples, with corresponding response rates of 13.2% for men and 18.4% for women from the 2016–17 survey, and 6,384 serum samples, with corresponding response rates 28.9% for men and 34.7% for women from the 2006–07, which were tested previously (10, 12). Study characteristics were stratified for sex. In the 2016–17 survey, for both men and women, participants of 15 years and older were higher educated and had a higher net monthly income in comparison with both men and women in the 2006–07 survey. The mean age of sexual debut for people under 25 years of age was similar between the different surveys and sexes. However, age of sexual

Table 1. Sociodemographic and sexual behavior characteristics of participants aged 15 years and older without vaccination, with a blood sample for HPV IgG antibody determination in the Netherlands, by sex and survey.

Sociodemographic characteristic	Men 2006–07 % (*n*) *N* = 1,937	Men 2016–17 % (*n*) *N* = 1,911	*P*	Women 2006–07 % (*n*) *N* = 2,535	Women 2016–17 % (*n*) *N* = 2,415	*P*
Age group, years						
15–19	6.87 (133)	5.67 (110)		6.51 (165)	1.98 (43)	
20–24	7.12 (138)	10.36 (198)		8.92 (226)	4.93 (107)	
25–29	6.50 (126)	9.00 (172)		8.76 (222)	9.36 (203)	
30–39	14.97 (290)	15.07 (288)		16.76 (425)	20.2 (438)	
40–49	14.51 (281)	13.55 (259)		14.20 (360)	18.44 (400)	
50–59	15.07 (292)	14.49 (277)		16.65 (422)	16.74 (363)	
60–69	18.79 (364)	16.27 (311)		17.16 (435)	16.04 (348)	
70–79	16.15 (313)	12.55 (240)		11.05 (280)	10.01 (217)	
80–89		2.93 (56)			2.31 (50)	
Educational level[a]			<0.0001			<0.0001
High	29.17 (565)	40.24 (769)		23.59 (598)	35.04 (760)	
Middle	29.94 (580)	28.78 (550)		31.52 (799)	29.74 (645)	
Low	39.34 (762)	25.64 (490)		43.35 (1,099)	28.82 (625)	
Unknown	1.55 (30)	5.34 (102)		1.54 (39)	6.41 (139)	
Net monthly income			<0.0001			<0.0001
<850/<970	5.94 (115)	502 (96)		8.72 (221)	5.3 (115)	
851–1,150/971–1,335	7.80 (151)	6.65 (127)		9.47 (240)	9.04 (196)	
1,151–1,750/1,356–1,969	19.00 (368)	11.93 (228)		17.87 (453)	14.66 (318)	
1,751–3,050/1,970–3,314	32.27 (625)	28.52 (545)		25.68 (651)	29.28 (635)	
3,051–3,500/3,315–3,500	7.02 (136)	8.16 (156)		6.11 (155)	6.69 (145)	
>3,501	11.31 (219)	26.95 (515)		8.36 (212)	20.89 (453)	
Unknown	16.68 (323)	12.77 (244)		23.79 (603)	14.15 (307)	
Ethnicity			0.0307			0.0018
Dutch	81.78 (1,584)	79.96 (1,528)		81.85 (2,075)	78.98 (1,713)	
First-generation migrant	10.84 (210)	13.50 (258)		11.76 (298)	15.26 (331)	
Second-generation migrant	7.38 (143)	6.54 (125)		6.39 (162)	5.76 (125)	
Smoking						
Yes		48.77 (932)			43.11 (935)	
No		42.96 (821)			47.76 (1,036)	
Unknown		8.27 (158)			9.13 (198)	
Alcohol						
Yes		76.35 (1,459)			64.04 (1,389)	
No		14.70 (281)			26.09 (566)	
Unknown		8.95 (171)			9.87 (214)	
BMI						
<18.5		1.99 (38)			1.89 (41)	
18.5–25		43.22 (826)			43.52 (944)	
25–30		33.18 (634)			26.97 (585)	
≥30		10.52 (201)			14.98 (325)	
Unknown		11.09 (212)			12.63 (274)	
Current steady partner			0.0005			<0.0001
Yes	79.50 (1,540)	77.60 (1,483)		75.31 (1,909)	77.04 (1,671)	
No	19.05 (369)	19.05 (364)		22.72 (139)	18.86 (409)	
Unknown	1.45 (28)	3.35 (64)		1.97 (50)	4.1 (89)	
Ever had sexual intercourse			<0.0001			<0.0001
Yes	89.93 (1,742)	91.63 (1,751)		90.49 (2,294)	95.67 (2,075)	
No	6.87 (133)	8.37 (160)		5.48 (139)	4.33 (409)	
Unknown	3.20 (62)	0.00 (0)		4.02 (102)	0.00	
Median age at sexual debut (<26 years of age)	16.9	16.8		16.7	16.7	
Age at sexual debut			<0.0001			<0.0001
<17 years	12.80 (248)	18.32 (350)		17.32 (439)	24.57(533)	
17–19 years	24.37 (472)	31.08 (594)		30.73 (779)	34.76 (754)	
≥20 years	31.96 (619)	28.57 (546)		27.14 (688)	24.85 (539)	
Unknown	30.87 (598)	22.03 (421)		24.81 (629)	15.81 (343)	

(Continued on the following page)

Table 1. Sociodemographic and sexual behavior characteristics of participants aged 15 years and older without vaccination, with a blood sample for HPV IgG antibody determination in the Netherlands, by sex and survey. (Cont'd)

Sociodemographic characteristic	Men 2006–07 % (*n*) *N* = 1,937	Men 2016–17 % (*n*) *N* = 1,911	*P*	Women 2006–07 % (*n*) *N* = 2,535	Women 2016–17 % (*n*) *N* = 2,415	*P*
Number of partners last 6 months			<0.0001			<0.0001
0	14.09 (273)	11.15 (213)		15.35 (389)	16.32 (354)	
1–2 partners	62.36 (1,208)	68.13 (1,302)		60.79 (1,541)	67.31 (1,460)	
>2 partners	0.98 (19)	2.30 (44)		0.79 (20)	0.83 (18)	
Unknown	22.56 (437)	18.42 (352)		23.08 (585)	15.54 (337)	
Lifetime sexual partners						
1–2 partners		38.72 (740)			47.63 (1,033)	
3–5 partners		21.04 (402)			23.19 (503)	
6–9 partners		10.57 (202)			8.21 (178)	
≥10 partners		12.87 (246)			8.85 (192)	
Unknown		16.80 (321)			12.13 (263)	
Condom use last time sex			<0.0001			<0.0001
Yes	8.00 (155)	14.08 (269)		7.65 (991)	13.05 (283)	
No	56.01 (1,085)	69.65 (1,331)		53.25 (1,350)	73.95 (1,604)	
Unknown	35.98 (697)	16.27 (311)		39.09 (991)	13 (282)	
Ever had sexually transmitted disease			0.0016			<0.0001
Yes	3.82 (74)	6.17 (118)		5.44 (138)	8.16 (177)	
No	89.00 (1,724)	82.42 (1,575)		86.04 (2181)	79.07 (1,715)	
Unknown	7.18 (139)	11.41 (218)		8.52 (216)	12.77 (277)	

[a]Educational level was used for participants 0–11 years, active education was used for participants 12–25 years, and highest accomplished educational level was used for participants >25 years. Low, no education, primary school, prevocational education (VMBO), lower vocational education (LBO/MBO-1), lower general secondary education (MAVO/VMBO); Middle, intermediate/secondary vocational education (MBO-2–4), higher/senior vocational education (HAVO), preuniversity education (VWO/Gymnasium); High, higher professional education (HBO), University BSc., University MSc., Doctorate; Missing, ethnicity $n = 13$.

debut across all ages was lower in the 2016–17 survey compared with 2006–07 survey, for both men and women. In addition, the percentage of participants reporting to have a current steady partner was lower in 2016–2017, while "the number of sex partners in the last 6 months" and "ever having been diagnosed with a sexually transmitted disease (STD)" were higher in the 2016–17 survey compared with 2006–07 (**Table 1**).

HPV Seroprevalence

Age-specific seroprevalence and GMC in an unvaccinated population, by sex and survey

An increase in seroprevalence for any hr-HPV type was observed in women in the age cohort from 15–19 years old, which reflects the median age of sexual debut. In the 2016–17 survey, seroprevalence for any type increased from 3.0% (10–14 years old) to 30.5% (20–24 years old) and 33.7% (25–29 years old) and peaked at 37.0% in the 30–39 year old. The greatest rise was seen for HPV16 and HPV18. This increase in seroprevalence was much more gradual for men, and mainly in the 2006–07 survey was most pronounced for any hr-HPV type and HPV16 (**Fig. 1**). Samples sizes of age cohorts can be found in **Table 1** and Supplementary Table S1.

Low seroprevalences were observed in children 0–14 years of age in both sexes and surveys. In the 2016–17 survey, the highest seroprevalences in children (0–14) were detected for HPV16 and HPV18 (**Fig. 1**).

In the older female age groups, overall seroprevalence decreased from the age of 49 years onward in the 2006–07 survey and from 60–69 years onward in the 2016–17 survey. Age-specific higher seroprevalence for any hr-HPV type was observed in 2016–17 compared with 2006–07, being significant in age groups 30–39 ($P = 0.0108$), 50–59 ($P = 0.0406$), and 60–69 ($P = 0.0056$) years of age. A lower seroprevalence for any hr-HPV type was observed in the age group 10–14 ($P = 0.0118$) in 2016–17 compared with 2006–07. In men, a lower age-specific seroprevalence for any hr-HPV type was observed in 2016–17 compared with 2006–07, being only significant in the age groups 10–14 ($P = 0.0056$). No significant difference was found for the age-specific seroprevalence for any hr-HPV type excluding 16 and 18 in the age groups 10–14 and 15–19 years of age between the two surveys, $P = 0.091$ and $P = 0.1206$, respectively.

Overall HPV seroprevalence from unvaccinated individuals 15 years and onward

Unvaccinated female participants older than 15 years of age showed significantly higher seroprevalence for any hr-HPV type in 2016–17 compared with 2006–07; 31.4% [95% confidence interval (CI), 29.1–33.7] and 25.2% (95% CI, 23.1–2.3), respectively. For men from 15 years of age and older, seroprevalence for any hr-HPV type was similar between the 2006–07 and 2016–17 surveys; 19.7% (95% CI, 17.9–21.6) and 20.3% (95% CI, 18.4–22.1), respectively. In women, also seropositivity for one up to all seven types was significantly higher in 2016–17 and hr-type specific. Type-specific HPV16, HPV18, HPV31, and HPV58 were higher in 2016–17 compared with 2006–07, which was also true for the combinations HPV16 and 18, HPV16 or 18, and HPV16 and/or 18 (**Table 2**).

For men, this was true for the combination HPV16 or 18, positivity for more than two hr-HPV types and type-specific HPV18, 31, 33, 45, 52, and 58. For HPV16 a lower seroprevalence was seen in 2016–17 (7.5%; 95% CI, 6.5–8.5) compared with 2006–07 (10.6%; 95% CI, 9.2–12.0). Just as for the combination HPV16 and 18, HPV16 and/or HPV18 and positivity for more than one hr-HPV type (**Table 2**).

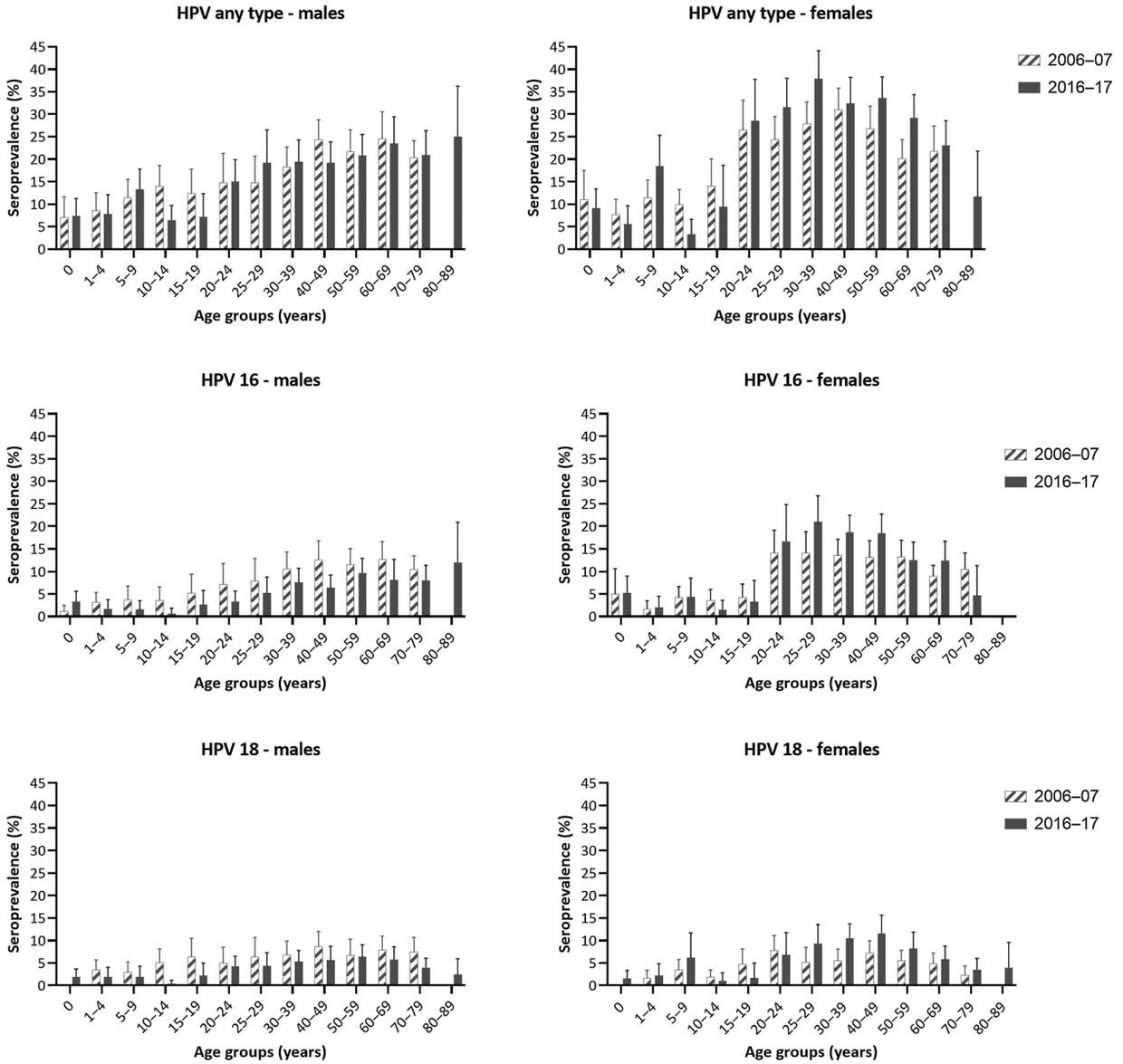

Figure 1.

Age-specific seroprevalence (%; with 95% CIs) of any high-risk type HPV IgG antibodies for men (**A**) and women (**B**), HPV 16 for men (**C**) and women (**D**), and HPV18 for men (**E**) and women (**F**) in the unvaccinated general population of the Netherlands.

HPV16 was also most prevalent in both surveys, followed by HPV18, HPV45, and the rest of the types (**Table 2**). Only a very small percentage of the males were seropositive for all seven hr-HPV types, 0.6% and 0.3% for 2006–07 and 2016–17, respectively.

HPV type-specific antibody concentrations among seropositive individuals

The age-specific HPV16 GMCs of (natural) infection-induced seropositive women as well as of seropositive men were comparable in all age cohorts between both studies. No differences were found between the GMCs of the HPV16 and HPV18 seropositive individuals between 2006–07 and 2016–17 (Supplementary Fig. S3).

Risk factors for hr-HPV seropositivity

For women, the univariate analysis showed an association for HPV seropositivity for any hr-HPV type with middle educational level, being a first- or second-generation migrant, having a lower income, ever used alcohol, not having a steady partner, lower age of sexual debut, having more than two sexual partners last 6 months, history of reported STD, and having more than two sexual partners during lifetime. In the backward selection model, low and middle educational level, first-generation migrants, more than two sexual partners during lifetime, and history of self-reported STDs remained and were independently associated with seropositivity for any hr-HPV type (**Table 3A** and **B**).

Table 2. Weighted seroprevalence, and corresponding 95% CIs, for seven high-risk HPV types and combinations in the total population of the Netherlands from 15 years of age without vaccination, stratified by sex and survey.

Total population from 15 years of age, without vaccination	Men (2006–07) (n = 1,937)	Men (2016–17) (n = 1,916)	P	Women (2006–07) (n = 2,535)	Women (2016–17) (n = 2,177)	P
High-risk HPV types						
HPV16	10.6 (9.2–12.0)	7.2 (6.2–8.2)	0.0000	11.9 (10.3–13.6)	15.8 (13.9–17.7)	0.0006
HPV18	7.2 (6.1–8.2)	5.0 (3.8–6.2)	0.0078	5.6 (4.7–6.4)	7.9 (6.7–9.1)	0.0006
HPV31	2.5 (1.7–3.4)	1.4 (0.9–1.9)	0.0158	3.3 (2.4–4.2)	5.2 (4.1–6.3)	0.009
HPV33	6.0 (4.7–7.2)	5.4 (4.4–6.3)	0.4338	6.5 (5.5–7.6)	8.0 (6.3–9.7)	0.116
HPV45	6.8 (5.4–8.1)	9.6 (8.2–10.9)	0.0022	7.5 (6.2–8.9)	9.6 (8.2–10.9)	0.0304
HPV52	5.4 (4.4–6.3)	5.1 (4.1–6.0)	0.6576	6.9 (5.9–8.0)	8.7 (7.1–10.2)	0.0610
HPV58	3.7 (2.8–4.5)	3.0 (2.1–3.9)	0.2914	4.5 (3.6–5.3)	6.4 (5.2–7.5)	0.0052
HPV combinations						
HPV16 and 18	5.7 (4.9–6.6)	2.8 (2.0–3.6)	0.0000	3.6 (2.9–4.4)	4.8 (3.8–5.9)	0.0572
HPV16 or 18	6.3 (5.1–7.5)	6.6 (5.5–7.7)	0.7376	10.2 (8.8–11.6)	14.1 (12.4–15.7)	0.00040
HPV16 and/or 18	12.0 (10.6–13.5)	9.4 (8.2–10.5)	0.0026	13.8 (12.1–15.5)	18.9 (17.0–20.7)	0.0000
Positive for at least 1 hr-HPV type	20.3 (18.4–22.1)	19.3 (17.7–21.0)	0.4378	25.2 (23.1–27.3)	30.1 (27.7–32.4)	0.0052
Positive for at least 1 hr-HPV type, excluding HPV16 and 18	13.6 (11.7–15.5)	15.3 (13.7–16.8)	0.1826	17.9 (16.0–19.8)	21.0 (18.9–23.2)	0.0234
Positive for more than 1 hr-HPV type	9.8 (8.8–10.8)	7.3 (6.0–8.7)	0.0066	10.4 (9.1–11.7)	14.4 (12.3–16.4)	0.0004
Positive for more than 2 hr-HPV types	5.2 (4.0–6.3)	4.3 (3.4–5.2)	0.2078	4.9 (3.9–6.0)	7.8 (6.4–9.3)	0.001
Positive for 7 hr-HPV types	0.6 (0.2–0.9)	0.3 (0.0–0.5)	0.1686	0.3 (0.1–0.5)	0.7 (0.2–1.1)	0.1146

For men, the univariate analyses showed only an association of seropositivity for any hr-HPV type with history of self-reported STDs (**Table 3A** and **B**).

Pooled risk factor analysis associated with HPV seropositivity for the 2006–07 and 2016–17 surveys

HPV seropositivity for any hr-HPV type for women from 15 years onward was 25.2% in 2006–07 and 31.4% in 2016–17. After pooling both surveys, and adjusting for demographic characteristics (age, sex, urbanization, education, income, ethnicity) and sexual risk factors (age of sexual debut, number of partners during the last 6 months, history of STDs), this resulted in a smaller, but still significant, difference between 2006–07 and 2016–17, (aPR 1.16; 95% CI, 1.02–1.32). Before adjustment of any variables, all seven hr-HPV types were significantly higher in 2016–17 compared with 2006–07. However, after adjustment for the demographic characteristics and sexual risk factors, the differences remained only significant for HPV16 (aPR 1.29; 95% CI, 1.07–1.55), HPV18 (aPR 1.31; 95% CI, 1.01–1.70), HPV31 (aPR 1.54; 95% CI, 1.111–2.14), and HPV52 (aPR 1.27; 95% CI, 1.00–1.62; **Table 4**).

For men from 15 years onward, HPV seropositivity for any hr-HPV type was significantly lower in 2016–17(18.2%) compared with 2006–07 (20.3%). After adjustment for the demographic characteristics and sexual risk factors, this did not remain significant (aPR 0.99; 95% CI, 0.83–1.17). Before adjustment, HPV16, HPV18, and HPV31 were significantly lower in 2016–17 compared with 2006–07 and HPV45 was significantly higher in 2016–17 compared with 2006–07. This difference only remained significant for HPV16 after adjustment (aPR 0.71; 95% CI, 0.55–0.91; **Table 4**) and HPV45 (aPR 1.47; 95% CI, 1.13–1.92). Zooming in on men in the age cohort of 15–39, a nonsignificant decrease for HPV16 between 2006–07 and 2016–17 was observed (aPR 0.84; 95% CI, 0.52–1.37).

Discussion

In this study, we assessed the (natural) infection-induced seroprevalence of seven hr-HPV types in the Dutch population before and 6 to 7 years after the introduction of a girls-only bivalent HPV vaccination program, with an uptake varying over the years between 42% and 61%. Surprisingly, HPV seroprevalence in female age cohorts of 15 years and older has increased in a 10-year time period, mainly due to a significant increase in HPV16, 18, 31, and 58. In men, however, seroprevalence for any hr-HPV type remained similar with a decreasing trend found for HPV16 and increasing trend for HPV45.

We restricted the analyses to unvaccinated individuals thereby estimating naturally acquired and cumulative type-specific HPV exposure. The increase in seroprevalence with age for women was in line with the age of sexual debut. The peak in HPV seropositivity was highest in women ages 30–39, which has been reported in other publications (13–15). This peak in seroprevalence around 10 to 20 years after sexual debut might reflect repeated exposures resulting in a subsequent increase of the seroconversion rate to induce a detectable antibody response (16).

In the 2006–07 survey, the seroprevalence in middle-aged and older women declined at an earlier age than in the 2016–17 survey, where levels started to decrease from 70 years and onward. In males, this decline in HPV seropositivity is not seen. The slight decrease of seropositivity observed in older women could be explained by waning of antibodies which was suggested by af Geijersstam and colleagues (4). This would mean that seroprevalence is underestimating lifetime cumulative exposure. Alternatively, it could reflect a cohort effect, which is more likely as this effect is not seen in both sexes. Indeed, although age of sexual debut is similar, the sexual behavior pattern differs in the younger women having more lifetime sexual partners than the older women in this cohort (17).

Hr-HPV antibodies, albeit at very low concentrations, could be detected in children, which confirm other population studies (10, 16). These antibodies might be derived from vertical or horizontal transmission (18).

In both surveys, a lower seroprevalence is observed in males compared with women, which conform other population studies (10, 13, 15, 16, 19). It is unlikely that the overall lower seroprevalence seen in males is due to lower infection rates, because males reported a significantly higher number of lifetime sexual partners compared with females in the 2016–17 cohort. HPV DNA

Table 3A. Risk factor analysis for any high-risk type HPV IgG seropositivity among sexually active and unvaccinated participants from 15 years of age in the Netherlands, by sex.

Males ($n = 1{,}751$)	Univariate model		Multivariate model	
Risk factor	**OR**	**95% CI limits**	**OR**	**95% CI limits**
Age				
15–19	Ref		Ref	
20–24	0.60	0.18–2.01		
25–29	0.68	0.21–2.26		
30–39	0.98	0.31–3.08		
40–49	0.94	0.30–2.99		
50–59	1.04	0.33–3.29		
60–69	1.01	0.32–3.16		
70–79	0.94	0.30–3.00		
80–89	0.93	0.25–3.46		
Education[a]				
High	Ref		Ref	
Middle	0.86	0.61–1.20		
Low	1.11	0.81–1.54		
Unknown	0.81	0.44–1.46		
Net monthly income[b]				
<850/<970	Ref		Ref	
851–1,150/971–1,335	0.68	0.31–1.48		
1,151–1,750/1,970–3,314	1.59	0.77–3.30		
1,751–3,050/1,970–3,314	1.18	0.59–2.35		
3,051–3,500/3,315–3,500	1.82	0.84–3.95		
>3,501	1.17	0.59–2.32		
Unknown	1.01	0.48–2.11		
Ethnicity[c]				
Dutch	Ref		Ref	
First-generation migrant	1.00	0.69–1.44		
Second-generation migrant	0.78	0.42–1.45		
Smoking ever				
No	Ref		Ref	
Yes	0.95	0.72–1.25		
Unknown	0.98	0.61–1.59		
Alcohol use				
No	Ref		Ref	
Yes	1.04	0.70–1.56		
Unknown	0.92	0.53–1.61		
BMI				
<18.5	Ref		Ref	
18.5–25	1.06	0.34–3.34		
25–30	1.23	0.39–3.87		
>30	0.88	0.26–2.93		
Unknown	1.03	0.32–3.41		
Current steady partner				
No	Ref		Ref	
Yes	1.12	0.74–1.71		
Unknown	1.08	0.47–2.49		
Age of sexual debut				
<17 years	Ref		Ref	
17–19 years	1.21	0.84–1.73		
≥20 years	0.86	0.58–1.27		
Unknown	1.18	0.76–1.84		
History STD				
No	Ref		Ref	
Yes	**1.70**	**1.11–2.61**		
Unknown	0.88	0.58–1.34		
Condom use				
No	Ref		Ref	
Yes	0.77	0.50–1.17		
Unknown	0.76	0.46–1.26		

(Continued on the following page)

Table 3A. Risk factor analysis for any high-risk type HPV IgG seropositivity among sexually active and unvaccinated participants from 15 years of age in the Netherlands, by sex. (Cont'd)

Males ($n = 1,751$)	Univariate model		Multivariate model	
Risk factor	**OR**	**95% CI limits**	**OR**	**95% CI limits**
Partners last 6 months (sexual)				
0	Ref		Ref	
1–2	0.95	0.62–1.45		
>2	0.84	0.37–1.91		
Unknown	1.03	0.58–1.83		
Partners lifetime (sexual)				
1–2	Ref		Ref	
3–5	0.83	0.57- 1.21		
6–9	0.97	0.62–1.50		
>10	1.34	0.94–1.91		
Unknown	1.45	0.92–2.28		

Note: Boldface text indicates that the OR is significant.
[a]According to definition of CBS in 2018.
[b]Left 2006–07 survey; right 2016–17 survey.
[c]Country of birth or country of birth of parents.

Table 3B. Risk factor analysis for any high-risk type HPV IgG seropositivity among sexually active and unvaccinated participants from 15 years of age in the Netherlands, by sex.

Females ($n = 2,075$)	Univariate model		Multivariate model	
Risk factor	**OR**	**95% CI limits**	**OR**	**95% CI limits**
Age				
15–19	Ref		Ref	
20–24	1.90	0.50–7.23		
25–29	2.18	0.59–8.05		
30–39	2.16	0.60–7.78		
40–49	2.12	0.58–7.68		
50–59	2.19	0.60–7.91		
60–69	1.83	0.51–6.65		
70–79	1.19	0.32–4.36		
80–89	0.69	0.15–3.10		
Education[a]				
High	Ref		Ref	
Middle	**1.36**	**1.08–1.70**	**1.47**	**1.17–1.85**
Low	1.10	0.88–1.38	**1.40**	**1.09–1.78**
Unknown	1.17	0.78–1.75	1.22	0.83–1.80
Net monthly income[b]				
<850/<970	Ref		Ref	
851–1,150/971–1,335	1.14	0.75–1.73		
1,151–1,750/1,970–3,314	0.93	0.62–1.39		
1,751–3,050/1,970–3,314	**0.68**	**0.46–0.98**		
3,051–3,500/3,315–3,500	**0.45**	**0.28–0.73**		
>3,501	**0.61**	**0.41–0.90**		
Unknown	0.73	0.48–1.10		
Ethnicity[c]				
Dutch	Ref		Ref	
First-generation migrant	**2.27**	**1.83–2.81**	**2.47**	**1.97–3.10**
Second-generation migrant	**1.57**	**1.04–2.36**	1.27	0.85–1.91
Smoking ever				
No	Ref		Ref	
Yes	1.20	0.99–1.45		
Unknown	**1.49**	**1.07–2.06**		
Alcohol use				
No	Ref		Ref	
Yes	**1.29**	**1.03–1.62**		
Unknown	**1.61**	**1.15–2.27**		

(Continued on the following page)

Table 3B. Risk factor analysis for any high-risk type HPV IgG seropositivity among sexually active and unvaccinated participants from 15 years of age in the Netherlands, by sex. (Cont'd)

Females ($n = 2{,}075$)	Univariate model		Multivariate model	
Risk factor	**OR**	**95% CI limits**	**OR**	**95% CI limits**
BMI				
<18.5	Ref		Ref	
18.5–25	1.07	0.52–2.20		
25–30	1.16	0.56–2.40		
>30	1.29	0.61–2.72		
Unknown	1.38	0.65–2.91		
Current steady partner				
No	Ref		Ref	
Yes	**0.71**	**0.59–0.90**		
Unknown	0.96	0.58–1.57		
Age of sexual debut				
<17 years	Ref		Ref	
17–19 years	**0.77**	**0.61–0.95**		
≥20 years	**0.52**	**0.0–0.67**		
Unknown	0.79	0.58–1.07		
History STD				
No	Ref		Ref	
Yes	**2.92**	**2.27–3.77**	**1.54**	**1.18–2.01**
Unknown	1.22	0.93–1.60	1.04	0.74–1.47
Condom use				
No	Ref		Ref	
Yes	1.07	0.82–1.40		
Unknown	0.88	0.62–1.26		
Partners last 6 months (sexual)				
0	Ref		Ref	
1–2	0.92	0.73–1.17		
>2	**3.03**	**1.58–5.78**		
Unknown	0.91	0.64–1.29		
Partners lifetime (sexual)				
1–2	Ref		Ref	
3–5	**2.22**	**1.75–2.81**	**2.03**	**1.60–2.59**
6–9	**3.71**	**2.80–4.90**	**3.47**	**2.58–4.67**
>10	**6.93**	**5.30–9.06**	**6.23**	**4.68–8.29**
Unknown	**2.72**	**1.93–3.83**	**2.55**	**1.66–3.93**

Note: Boldface text indicates that the OR is significant.
[a]According to definition of CBS in 2018.
[b]Left 2006–07 survey, right 2016–17 survey.
[c]Country of birth or country of birth of parents.

prevalence studies showed similar results among both sexes (20, 21). The fact that women display a higher seroprevalence then men is likely to be explained by the anatomic site of the HPV infection, influencing its immune response. Infections at the epithelium of the cervix and anal tract induce higher immune response in comparison with infections that occur at the keratinized epithelia, such as genital skin (22–25).

Seroprevalence for HPV16 was highest of all HPV types in both surveys, which is in accordance with other population studies (15, 16, 26–29). In a 10-year time period, HPV type-specific seroprevalence for HPV16, 18, 31, and 58 has increased in the female population of 15 years and older in the Netherlands. In addition, being seropositive for one up to all seven types increased over the years. This is possibly explained by the observed change toward a higher number of sexual partners in the last 6 months and history of self-reported STDs in the 2016–17 survey compared with the 2006–07 survey.

The risk factor analysis was restricted to the HPV unvaccinated, sexually active population from 15 years onward and stratified for sex. For women several behavioral factors, such as number of lifetime partners, history of STDs, and ethnicity, were independently associated with HPV seropositivity, which was also found in other studies (10, 15, 30, 31). Alcohol use and smoking were only associated with HPV seropositivity in the univariate model. This is especially interesting with respect to the current increased risk in HPV-associated head and neck cancers (32, 33). In these studies, alcohol use is often allied together with tobacco use; however, in our study, we could not find an association with smoking or alcohol use in the multivariate model. Other studies find varying results, showing a negative association (29), a positive association (14, 34), or no association at all (35). Thereby leaving the relation between smoking and HPV seropositivity unclear.

In the male part of our study, we only found history of self-reported STDs to be significantly associated with HPV seropositivity in the univariate analyses. In the backward selection model, this factor did not remain independently associated with HPV seropositivity anymore. Comparison with other studies is challenging as most population studies combine men and women in their risk factor

Table 4. Pooled analysis of the 2006–7 and 2016–17 survey after adjustments for sociodemographic characteristics.

	Men $N = 3,493$		Women $N = 4,369$	
	HPV seropositive, *n* (%)	**aPR (95% CI)**	**HPV seropositive, *n* (%)**	**aPR (95% CI)**
Any HPV type				
2006–2007	366 (21.0)	Ref	596 (26.0)	**Ref**
2016–2017	361 (20.6)	0.99 (0.83–1.17)	989 (33.1)	**1.16 (1.02–1.32)**
HPV16				
2006–2007	192 (11.0)	**Ref**	279 (12.2)	**Ref**
2016–2017	137 (7.8)	**0.71 (0.55–0.91)**	363 (17.5)	**1.29 (1.07–1.55)**
HPV18				
2006–2007	130 (7.5)	Ref	128 (5.6)	**Ref**
2016–2017	98 (5.6)	0.77 (0.57–1.05)	187 (9.0)	**1.31 (1.01–1.70)**
HPV31				
2006–2007	49 (2.8)	Ref	80 (3.5)	**Ref**
2016–2017	29 (1.7)	0.66 (0.38–1.12)	128 (6.2)	**1.54 (1.11–2.14)**
HPV33				
2006–2007	111 (6.4)	Ref	151 (6.6)	Ref
2016–2017	96 (5.5)	0.89 (0.65–1.22)	186 (9.0)	1.08 (0.84–1.38)
HPV45				
2006–2007	123 (7.1)	**Ref**	184 (8.0)	Ref
2016–2017	180 (10.3)	**1.47 (1.13–1.92)**	222 (10.7)	1.16 (0.92–1.45)
HPV52				
2006–2007	103 (5.9)	Ref	167 (7.3)	**Ref**
2016–2017	92 (5.3)	0.95 (0.68–1.32)	212 (10.2)	**1.27 (1.00–1.62)**
HPV58				
2006–2007	70 (4.0)	Ref	113 (4.9)	**Ref**
2016–2017	54 (3.1)	0.71 (0.47–1.06)	152 (7.3)	1.28 (0.96–1.70)

Note: Boldface text indicates that the OR is significant.

analysis. Studies including separate male analysis reported a variety of associated factors. Most consistent findings were associations related to age (13, 31, 36–42), number of male anal sexual partners (30, 40, 41, 43), and (self-reported) circumcision (44). Number of male anal sexual partners and (self-reported) circumcision were unfortunately not included in our questionnaire. In addition, some of these studies were performed among men who have sex with men, which is considered a high-risk population with specific behavior, complicating direct comparison.

Also after pooling both surveys and adjusting for demographic and sexual risk factors, the increase in HPV seroprevalence in women in the years after the introduction of the HPV vaccine remained significant.

An interesting finding is the decreased HPV16 seroprevalence between the two surveys for the Dutch male population. Although this might be due to herd immunity of the girls-only vaccination program, among 15–39 year old men, we observed a not statistically significant decline, while they seem likely to be the first age groups benefitting from the girls-only vaccination. In the even younger age group of 10–14 year old boys, we did find a difference between the two surveys for any hr-HPV type, but when excluding the vaccine types this difference was not significant anymore. This indicates that the. Albeit minor, difference in seroprevalence in the 10-year period was mainly attributable to the vaccine types. Please note that in this age group, the seroprevalences could not be adjusted as questionnaires including sexual behavior were only filled in by people above 15 years of age. In Australia, with high vaccine uptake percentages, herd protection impacts on seroprevalence in males (15–39 years of age) from a girls-only vaccination program were clearly shown 5 years after introduction of HPV vaccination (45). Moreover, even a benefit for the nonvaccinated females was observed (46). In contrast, no reduction was found in HPV seropositivity in males followed by a girls-only vaccination program in a study in the United States with comparable vaccination coverage in a girls-only program as in the Netherlands (47). With a vaccination coverage of approximately 50% among vaccine-eligible girls in the Netherlands in 2016–17, herd effects on seroprevalence in the male part of the population might therefore be less pronounced. Nevertheless, in recent analysis among STI clinic visitors in the Netherlands, both first-order herd immunity effects among unvaccinated males as well as second-order herd immunity effects in unvaccinated women were found (48). However, this was measured through infection rates where effects can be detected earlier than by seroprevalence.

A strength of this study is that we compared two surveys with a broad age range, one before and one 6 to 7 years after introduction of the HPV vaccination program, thereby enabling us to evaluate this program at a population level. An additional strength of this study is that an identical methodology and antibody assay is used between the two surveys. It must be kept in mind that the VLP sources have changed over time and could possibly cause some variance, for which we corrected by using QC and bridging. Furthermore, this study used two-stage cluster sampling strategy, including oversampling of minorities (7, 8), therefore being representative of the total Dutch population.

The use of different techniques and associated cutoff levels hinders the comparison with other (population) studies. International standardization for all hr-HPV types, in addition to HPV16 and 18 which are applied in this study, could help to overcome

this difficulty in future studies. Besides this, it could be argued that HPV seropositivity is not a conclusive marker for cumulative exposure. HPV has the capability to evade the host immune system and as a consequence, detectable HPV-specific antibodies in serum are only developed in approximately 50%–70% of HPV-infected individuals (22). Thus, seroprevalence will underestimate the actual lifetime HPV exposure and infection rate. Moreover, it should be noted that questions regarding sexual behavior were among the least well completed. Self-reporting of sexual behavior could lead to bias due to social desirability and this was also illustrated by our risk factor analysis for some variables (e.g., a high unknown category).

HPV prophylactic vaccination programs are most effective when offered to nonsexually active preadolescents. In the Netherlands, the age of receiving the HPV vaccine is approximately 12 years old and recently the Health Council has advised to lower the age to 9 years. Our data support this change as HPV seropositivity begins to increase markedly after 10 years of age. In addition, the Health Council also advised to implement a sex-neutral HPV program which will be effective from 2021. On top of that, a catch-up vaccination will be offered to all young adults up to 26 years of age (49).

To conclude, our data showed that HPV infection-specific seroprevalence in women has increased in the Netherlands in a 10-year period. In men, however, seroprevalence for any hr-HPV type remained similar, a decrease was found for HPV16 and an increase for HPV45. Whether the decline in HPV16 is a first sign of a herd effect remains uncertain because a less pronounced effect was observed in men ages 15–39 years of age, where we would have expected that a herd effect would be visible first. Future seroprevalence studies will be interesting to capture the effect of a longer follow-up period after introduction of the girls-only program and possibly effects of the sex-neutral vaccination.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

H. Pasmans: Conceptualization, formal analysis, investigation, methodology, writing–original draft. **J. Hoes:** Formal analysis, writing–review and editing. **L. Tymchenko:** Data curation, investigation. **H.E. de Melker:** Conceptualization, supervision, writing–review and editing. **F.R.M. van der Klis:** Conceptualization, supervision, funding acquisition, project administration, writing–review and editing.

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References

1. Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis 2009;9:347–56.
2. Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjose S, Hammouda D, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111:278–85.
3. van Lier EA, Oomen PJ, Giesbers H, van Vliet JA, Drijfhout IH, Zonnenberg-Hoff IF, et al. Vaccination Coverage and Annual Report National Immunisation Programme Netherlands 2018 [in Dutch: Vaccinatiegraad en Jaarverslag Rijksvaccinatieprogramma Nederland 2018]. Bilthoven (the Netherlands): RIVM; 2019.
4. af Geijersstam V, Kibur M, Wang Z, Koskela P, la Eero P, Schiller J, et al. Stability over time of serum antibody levels to human papillomavirus type 16. J Infect Dis 1998;177:1710–4.
5. Mollers M, Vossen JM, Scherpenisse M, van der Klis FRM, Meijer CJLM, de Melker HE. Review: current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. J Med Virol 2013;85:1379–85.
6. Hoes J, Pasmans H, Knol MJ, Donken R, van Marm-Wattimena N, Schepp RM, et al. Persisting antibody response nine years after bivalent HPV vaccination in a cohort of Dutch women: immune response and the relation with genital HPV infections. J Infect Dis 2020;221:1884–94.
7. van der Klis FR, Mollema L, Berbers GAM, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. Neth J Med 2009;67:301–8.
8. Verberk JDM, Vos Regnerus A, Mollema L, van Vliet J, van Weert JWM, de Melker HE, et al. Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. BMC Infect Dis 2019;19:470.
9. van Lier A, Oomen P, de Hoogh P, Drijfhout I, Elsinghorst B, Kemmeren J, et al. Praeventis, the immunisation register of the Netherlands: a tool to evaluate the National Immunisation Programme. Euro Surveill 2012;17:20153.
10. Scherpenisse M, Mollers M, Schepp RM, Boot HJ, de Melker HE, Meijer CJLM, et al. Seroprevalence of seven high-risk HPV types in the Netherlands. Vaccine 2012;30:6686–93.
11. Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. J Immunol Methods 1998;221:35–41.
12. Scherpenisse M, Mollers M, Schepp RM, Boot HJ, Meijer Chris JLM, Berbers GAM, et al. Changes in antibody seroprevalence of seven high-risk HPV types between nationwide surveillance studies from 1995–96 and 2006–07 in The Netherlands. PLoS One 2012;7:e48807.
13. Newall AT, Brotherton JML, Quinn HE, McIntyre PB, Backhouse J, Gilbert L, et al. Population seroprevalence of human papillomavirus types 6, 11, 16, and 18 in men, women, and children in Australia. Clin Infect Dis 2008;46:1647–55.
14. Wang SS, Schiffman M, Shields TS, Herrero R, Hildesheim A, Bratti MC, et al. Seroprevalence of human papillomavirus-16, -18, -31, and -45 in a population-based cohort of 10000 women in Costa Rica. Br J Cancer 2003;89:1248–54.
15. Loenenbach AD, Poethko-Müller C, Pawlita M, Thamm M, Harder T, Waterboer T, et al. Mucosal and cutaneous human papillomavirus seroprevalence among adults in the prevaccine era in Germany - results from a nationwide population-based survey. Int J Infect Dis 2019;83:3–11.
16. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, et al. Seroprevalence of 34 human papillomavirus types in the German general population. PLoS Pathog 2008;4:e1000091.
17. Lindau ST, Schumm LP, Laumann EO, Levinson W, O'Muircheartaigh CA, Waite LJ. A study of sexuality and health among older adults in the United States. N Engl J Med 2007;357:762–74.
18. Syrjanen S. Current concepts on human papillomavirus infections in children. APMIS 2010;118:494–509.

19. Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. Prevalence of HPV after introduction of the vaccination program in the United States. Pediatrics 2016;137:e20151968.
20. Giuliano AR, Lu B, Nielson CM, Flores R, Papenfuss MR, Lee Ji-Hyun, et al. Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. J Infect Dis 2008;198: 827–35.
21. Kjaer SK, Munk C, Winther JF, Jørgensen HO, Meijer CJLM, van den Brule AJC. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. Cancer Epidemiol Biomarkers Prev 2005;14:1528–33.
22. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis 2000;181:1911–9.
23. Heiligenberg M, Alberts CJ, Waterboer T, Speksnijder AGCL, De Vries Henry JC, Pawlita M, et al. Route of sexual exposure is independently associated with seropositivity to HPV-16 and HPV-18 among clients of an STI clinic in the Netherlands. J Infect Dis 2013;208:1081–5.
24. Lu B, Viscidi RP, Wu Y, Nyitray AG, Villa LL, Lazcano-Ponce E, et al. Seroprevalence of human papillomavirus (HPV) type 6 and 16 vary by anatomic site of HPV infection in men. Cancer Epidemiol Biomarkers Prev 2012;21: 1542–6.
25. Vriend HJ, Bogaards JA, van der Klis FRM, Scherpenisse M, Boot HJ, King AJ, et al. Patterns of human papillomavirus DNA and antibody positivity in young males and females, suggesting a site-specific natural course of infection. PLoS One 2013;8:e60696.
26. Castro FA, Dominguez A, Puschel K, Van De Wyngard V, Snijders PJF, Franceschi S, et al. Serological prevalence and persistence of high-risk human papillomavirus infection among women in Santiago, Chile. BMC Infect Dis 2014; 14:361.
27. Desai S, Chapman R, Jit M, Nichols T, Borrow R, Wilding M, et al. Prevalence of human papillomavirus antibodies in males and females in England. Sex Transm Dis 2011;38:622–9.
28. Sudenga SL, Torres BN, Botha MH, Zeier M, Abrahamsen ME, Glashoff RH, et al. HPV serostatus pre- and post-vaccination in a randomized phase II preparedness trial among Young Western Cape, South African women: the Evri Trial. Papillomavirus Res 2017;3:50–6.
29. Wilson LE, Pawlita M, Castle PE, Waterboer T, Sahasrabuddhe V, Gravitt PE, et al. Natural immune responses against eight oncogenic human papillomaviruses in the ASCUS-LSIL Triage study. Int J Cancer 2013;133:2172–81.
30. Stone KM, Karem KL, Sternberg MR, McQuillan GM, Poon AD, Unger ER, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396–402.
31. Thompson DL, Douglas JM Jr, Foster M, Hagensee ME, DiGuiseppi C, Barón AE, et al. Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. J Infect Dis 2004;190:1563–74.
32. Guha N, Boffetta P, Filho VW, Neto JE, Shangina O, Zaridze D, et al. Oral health and risk of squamous cell carcinoma of the head and neck and esophagus: results of two multicentric case-control studies. Am J Epidemiol 2007;166:1159–73.
33. Hashibe M, Brennan P, Chuang S-C, Boccia S, Castellsague X, Chen C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev 2009;18:541–50.
34. Coseo S, Porras C, Hildesheim A, Rodriguez AC, Schiffman M, Herrero R, et al. Seroprevalence and correlates of human papillomavirus 16/18 seropositivity among young women in Costa Rica. Sex Transm Dis 2010;37:706–14.
35. Bedoya AM, Gaviria AM, Baena A, Borrero M, Duarte DF, Combita AL, et al. Age-specific seroprevalence of human papillomavirus 16, 18, 31, and 58 in women of a rural town of Colombia. Int J Gynecol Cancer 2012;22:303–10.
36. Hagensee ME, Kiviat N, Critchlow CW, Hawes SE, Kuypers J, Holte S, et al. Seroprevalence of human papillomavirus types 6 and 16 capsid antibodies in homosexual men. J Infect Dis 1997;176:625–31.
37. Slavinsky J 3rd, Kissinger P, Burger L, Boley A, DiCarlo RP, Hagensee ME. Seroepidemiology of low and high oncogenic risk types of human papillomavirus in a predominantly male cohort of STD clinic patients. Int J STD AIDS 2001;12: 516–23.
38. Strickler HD, Kirk GD, Figueroa JP, Ward E, Braithwaite AR, Escoffery C, et al. HPV 16 antibody prevalence in Jamaica and the United States reflects differences in cervical cancer rates. Int J Cancer 1999;80:339–44.
39. Dunne EF, Nielson CM, Hagensee ME, Papenfuss MR, Harris RB, Herrel N, et al. HPV 6/11, 16, 18 seroprevalence in men in two US cities. Sex Transm Dis 2009; 36:671–4.
40. Goldstone S, Palefsky JM, Giuliano AR, Moreira ED, Aranda C, Jessen H, et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. J Infect Dis 2011;203:66–74.
41. Lu B, Viscidi RP, Lee J-H, Wu Y, Villa LL, Lazcano-Ponce E, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 seroprevalence is associated with sexual practice and age: results from the multinational HPV Infection in Men Study (HIM Study). Cancer Epidemiol Biomarkers Prev 2011;20:990–1002.
42. Zhang C, Liu F, Deng Q, He Z, Xi L, Liu Y, et al. Seroprevalence of 10 human papillomavirus types in the general rural population of Anyang, China: a cross-sectional study. Sex Transm Infect 2015;91:506–9.
43. Rahman S, Pierce Campbell CM, Rollison DE, Wang W, Waterboer T, Michel A, et al. Seroprevalence and associated factors of 9-valent human papillomavirus (HPV) types among men in the multinational HIM Study. PLoS One 2016;11: e0167173.
44. Castellsagué X, Bosch FX, Muñoz N, Meijer CJLM, Shah KV, de Sanjosé S, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 2002;346:1105–12.
45. Pillsbury AJ, Quinn HE, Evans TD, McIntyre PB, Brotherton JML. Population-level herd protection of males from a female human papillomavirus vaccination program: evidence from Australian serosurveillance. Clin Infect Dis 2017;65: 827–32.
46. Tabrizi SN, Brotherton JML, Kaldor JM, Skinner SR, Liu B, Bateson D, et al. Assessment of herd immunity and cross-protection after a human papillomavirus vaccination programme in Australia: a repeat cross-sectional study. Lancet Infect Dis 2014;14:958–66.
47. Brouwer AF, Eisenberg MC, Carey TE, Meza R. Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES 2003–2012. BMC Infect Dis 2015;15:575.
48. Woestenberg PJ, Bogaards JA, King AJ, Leussink S, Ab van der Sande M, Jpa Hoebe C, et al. Assessment of herd effects among women and heterosexual men after girls-only HPV16/18 vaccination in the Netherlands: a repeated cross-sectional study. Int J Cancer 2019;144:2718–27.
49. Health Council of the Netherlands. Vaccination against HPV [in Dutch: Vaccinatie tegen HPV]. The Hague (the Netherlands): Health Council of the Netherlands; 2019.