



# No evidence for cross-protection of the HPV-16/18 vaccine against HPV-6/11 positivity in female STI clinic visitors

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## KEYWORDS

Human papillomavirus 6;  
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**Summary** *Objectives:* Data from a vaccine trial and from post-vaccine surveillance in the United Kingdom have suggested that the bivalent HPV-16/18 vaccine offers cross-protection against HPV-6/11 and protection against anogenital warts (AGW). We studied the effect of the bivalent vaccine on genital HPV-6/11 positivity and AGW in the Netherlands.

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Condylomata acuminata;  
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**Methods:** We included all vaccine-eligible women from the PASSYON study, a biennial cross-sectional study among 16- to 24-year-old sexually transmitted infection (STI) clinic attendants. Vaginal self-swabs were analyzed for type specific HPV and AGW were diagnosed at the STI-clinic. Prevalence of HPV-6 and/or HPV-11 and AGW were compared between self-reported vaccinated and unvaccinated women by log-binomial regression analysis, adjusted for demographics and risk behavior.

**Results:** Of the 1198 women included, 56% reported to be vaccinated at least once. Relative to unvaccinated women, the adjusted prevalence ratio (PR) for HPV-6/11 was 1.03 (95% confidence interval [CI] 0.74–1.43) for women vaccinated at least once. The crude PR for AGW was 0.67 (95% CI 0.22–2.07) for women vaccinated at least once. Adjustment did not change these results.

**Conclusions:** We observed no cross-protective effect of the bivalent vaccine on genital HPV-6/11 positivity and a non-significant partially protective effect on AGW.

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## Introduction

Human papillomavirus (HPV) is a sexually transmitted virus of which many different types exist. Types 16 and 18 are responsible for approximately 70% of all cervical cancers<sup>1</sup> and types 6 and 11 are responsible for approximately 90% of all anogenital warts (AGW).<sup>2</sup>

Currently, three different vaccines against HPV are available: (A) the bivalent vaccine (Cervarix<sup>®</sup>) which protects against HPV types 16 and 18<sup>3</sup>; (B) the quadrivalent vaccine (Gardasil<sup>®</sup>) which protects against HPV types 16, 18, 6 and 11<sup>4</sup>; (C) the nonavalent vaccine (Gardasil9<sup>®</sup>) which protects against HPV types 16, 18, 6, 11, 31, 33, 45, 52 and 58.<sup>5</sup>

Although the bivalent vaccine is not indicated to prevent HPV-6/11, some studies have suggested a cross-protective effect against HPV-6/11 and a protective effect against AGW. In post-hoc analyses of a bivalent vaccine trial (PATRICIA trial) a vaccine efficacy of 35% (95% confidence interval [CI] 11–52) against 6-month persistent HPV-6 and/or HPV-11 infection was estimated among women who were HPV seronegative at baseline.<sup>6</sup> Moreover, data from post-vaccine surveillance in the United Kingdom suggested that the bivalent vaccine offers protection against AGW. They observed a decline in AGW among young girls and heterosexual men after introduction of the bivalent vaccine in 2008, while such a decline was not observed among older women or men who have sex with men.<sup>7,8</sup> The vaccine effectiveness (VE) against AGW was calculated to be 34% (95% CI 29–38).<sup>7</sup> In 2012, the United Kingdom switched to the quadrivalent vaccine in their National immunization program (NIP) and a further drop in AGW is expected due to direct protection against HPV-6/11.

The Netherlands is one of the few Western countries using the bivalent vaccine in the NIP. In 2009, there was a one-off catch-up campaign for girls born in 1993–1996 (12- to 16-years-old). From 2010 onwards, girls are offered vaccination in the year they turn 13, starting with birth cohort 1997 in 2010.<sup>9</sup> The vaccination coverage for the catch-up cohorts was 52%.<sup>10</sup> The vaccination coverage of the routine vaccination was 56% for birth cohort 1997 and increased to 61% for birth cohort 2001.<sup>11</sup>

From a public health perspective, monitoring of the impact of the HPV vaccination program includes its possible

effect on non-vaccine HPV types. For this reason, a biennial cross-sectional study among young sexually transmitted infection (STI) clinic attendants was initiated in the Netherlands in 2009, the year of HPV vaccine introduction in the Netherlands.

In this paper, we explore the effects of the bivalent HPV-16/18 vaccine on genital HPV-6 and/or HPV-11 positivity and AGW by comparing vaccinated and unvaccinated women with similar exposure, using data from STI clinics from 2011 to 2015.

## Materials and methods

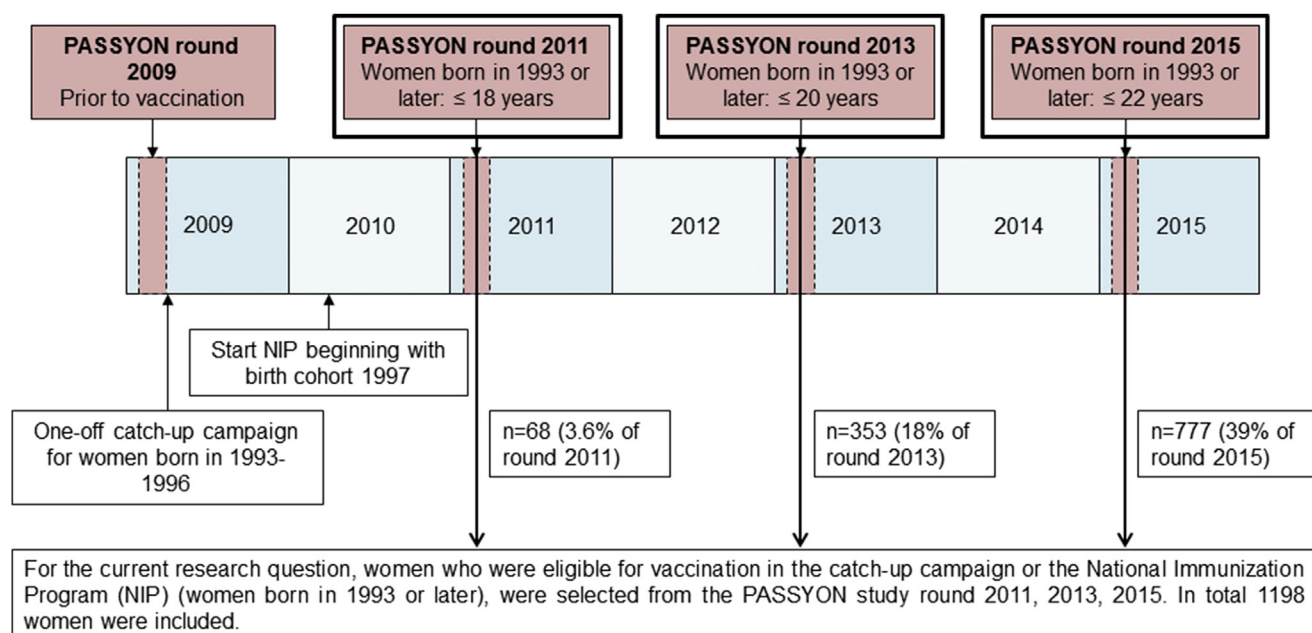
### Study design and population

We used data from the PASSYON (PApillomavirus Surveil-lance among STI clinic YOUNgsters in the Netherlands) study. The study design is described in detail elsewhere.<sup>12</sup> Briefly, the PASSYON study is a biennial cross-sectional study among 16- to 24-year-old attendants of STI clinics located throughout the Netherlands. It started in 2009 and was repeated in 2011, 2013 and 2015 (Fig. 1). Additional to routine STI consultation, participants were asked to provide a genital self-swab for HPV testing and to fill-in a questionnaire including self-reported vaccination status. From participants who provided blood for routine STI diagnostics, serum was collected for HPV serology. AGW were diagnosed during routine STI consultation, based on clinical presentation. The Medical Ethical Committee of the University of Utrecht, the Netherlands approved this study (protocol number 08/397). Data was obtained anonymously and all participants gave informed consent.

To study the effect of the bivalent vaccine on HPV-6/11 and AGW, we included from the PASSYON study years 2011, 2013 and 2015 all women born in 1993 or later, i.e. those who were eligible for vaccination in the catch-up campaign or the NIP (Fig. 1).

### Laboratory methods

Swabs were stored at –20 °C until analyses.<sup>12</sup> DNA was extracted using the MagnaPure platform (Total Nucleic Acid



**Figure 1** PASSYON study design and the study population selection.

Isolation Kit, Roche, the Netherlands) and eluted in 100-microliter elution buffer. HPV-DNA was amplified using the SPF10 primer set. Subsequently, HPV specific amplicons were detected using the DNA enzyme-linked immunoassay (HPV-DEIA, DDL Diagnostics Laboratory, the Netherlands). Amplicons of positive samples were genotyped with Line probe assay (HPV-LiPA, DDL Diagnostics Laboratory, the Netherlands) which is able to detect 25 HPV types including types 6 and 11.<sup>12</sup>

Serum samples were stored at  $-80^{\circ}\text{C}$  until analyses.<sup>13</sup> HPV antibodies against L1 virus-like particles (VLPs) for seven serotypes including types 16 and 18 were assessed using an in house multiplex immunoassay.<sup>14</sup> Cut-off levels for seropositivity were previously determined and were 9 Lumindex Units (LU)/ml for HPV-16 and 13 LU/ml for HPV-18.<sup>14</sup>

## Statistical analyses

First, we checked for differences in demographics and risk behavior between vaccinated and unvaccinated women. We compared women who reported to be vaccinated at least once with unvaccinated women and women who reported to be fully vaccinated (three doses according guidelines prevailing at the time of vaccination for women eligible for this study<sup>15</sup>) with unvaccinated women. Women with an unknown vaccination status were analyzed separately.

We considered the demographic variables age, ethnicity and education level. Ethnicity was based on (parental) country of birth. A woman was defined as native Dutch if both parents were born in the Netherlands.<sup>16</sup> Education level was self-reported and categorized as high and low/middle. High educational level included university of science and university of professional education, low/middle educational level included all other forms of education.

For risk behavior, we considered number of sex partners in the past six months, number of lifetime sex partners, age at sexual debut, history of STIs, condom use with casual partners in the past six months, hormonal contraceptives use and current genital chlamydia or gonorrhea infection. The latter was based on diagnoses during the routine STI consultation. The other variables were self-reported and categorized (Table 1).

Prevalence of genital HPV-6 and/or HPV-11 positivity and AGW were calculated by self-reported vaccination status. We calculated prevalence ratios (PR) using log-binomial regression analyses.

For the outcome genital HPV-6 and/or HPV-11 positivity, we first calculated the crude PR. Second, we adjusted for age and demographic variables that were associated with vaccination status. Third, we further adjusted for risk behavior variables that were associated with vaccination status to measure the adjusted effect of vaccination on HPV-6/11.

For the outcome AGW, we calculated the crude PR. Due to low numbers of AGW in our study, we could not adjust for potential confounders all at once, so we adjusted for potential confounders in bivariable analyses only. Moreover, we described the genital HPV types that were found among women diagnosed with AGW.

All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary NC, USA) with a significance level of  $p < 0.05$ . The records with missing data were excluded from the analyses, as these represented less than 5% of the study population.

## Sensitivity analyses

HPV-16 and HPV-18 antibody concentrations were compared between self-reported vaccinated and unvaccinated women with serum available. Since all women

vaccinated with the bivalent vaccine have an antibody response against HPV-16 and HPV-18 up to 9.4 years after vaccination,<sup>17</sup> it is unlikely that vaccinated women are seronegative for HPV-16/18. We repeated the analyses excluding women who reported to be vaccinated, but were seronegative for HPV-16 or HPV-18.

## Results

### Study population

In the PASSYON study, 1198 women were eligible for HPV vaccination (born in 1993 or later) and included in the current study (Fig. 1). Of these women, 666 (56%) reported

to be vaccinated at least once and 450 (38%) reported not to be vaccinated. The remaining did not know or did not report their vaccination status. Four hundred sixty seven women (39%) reported to be fully vaccinated. Of the women who reported to be vaccinated at least once, the majority (94%) was vaccinated during the catch-up campaign (birth cohort 1993–1996). Of the total 1198 women included, 1168 women (97.5%) had a genital swab taken and 1193 women (99.6%) provided information about the STI consult including diagnoses of AGW.

In Table 1, the characteristics of the study population are presented, stratified by vaccination status. There were some differences between vaccinated and unvaccinated women. Vaccinated women were more often native Dutch and highly educated. They less often had a history

**Table 1** Characteristics of the study population and a comparison between vaccinated and unvaccinated women.

	Total	Unvaccinated	Vaccinated at least once		Fully vaccinated <sup>a</sup>	Unknown
	N (%)	N (%)	N (%)	p-Value <sup>b</sup>	N (%)	N (%)
<b>Total</b>	1198	450	666		467	82
<b>Age</b>				0.47		0.69
16–18 years	353 (29.5)	138 (30.7)	191 (28.7)		149 (31.9)	24 (29.3)
19–22 years	845 (70.5)	312 (69.3)	475 (71.3)		318 (68.1)	58 (70.7)
<b>Ethnicity</b>				<0.001		<0.001
Native Dutch	938 (78.3)	323 (71.8)	557 (83.6)		397 (85.0)	58 (70.7)
Not native Dutch	255 (21.3)	125 (27.8)	107 (16.1)		69 (14.8)	23 (28.0)
<b>Education level</b>				<0.01		<0.01
Low/middle	386 (32.2)	176 (39.1)	174 (26.1)		126 (27.0)	36 (43.9)
High	809 (67.5)	274 (60.9)	491 (73.7)		341 (73.0)	44 (53.7)
<b>Recent sex partners<sup>c</sup></b>				0.01		0.08
0–1 partner	349 (29.1)	151 (33.6)	169 (25.4)		127 (27.2)	29 (35.4)
2–3 partners	578 (48.2)	207 (46.0)	339 (50.9)		245 (52.5)	32 (39.0)
≥4 partners	271 (22.6)	92 (20.4)	158 (23.7)		95 (20.3)	21 (25.6)
<b>Lifetime sex partners</b>				0.23		0.95
0–3 partners	317 (26.5)	130 (28.9)	164 (24.6)		132 (28.3)	23 (28.0)
4–6 partners	370 (30.9)	140 (31.1)	215 (32.3)		150 (32.1)	15 (18.3)
≥7 partners	486 (40.6)	172 (38.2)	280 (42.0)		180 (38.5)	34 (41.5)
<b>Age sexual debut</b>				0.04		0.15
≤14 years	210 (17.5)	93 (20.7)	102 (15.3)		76 (16.3)	15 (18.3)
15–16 years	616 (51.4)	227 (50.4)	346 (52.0)		240 (51.4)	43 (52.4)
≥17 years	353 (29.5)	124 (27.6)	213 (32.0)		148 (31.7)	16 (19.5)
<b>History of sexually transmitted infections</b>				0.02		<0.01
No	625 (52.2)	221 (49.1)	373 (56.0)		273 (58.5)	31 (37.8)
Yes	269 (22.5)	116 (25.8)	129 (19.4)		78 (16.7)	24 (29.3)
Never tested	296 (24.7)	111 (24.7)	162 (24.3)		114 (24.4)	23 (28.0)
<b>Current genital chlamydia/gonorrhea</b>				0.93		0.57
No	980 (81.8)	366 (81.3)	547 (82.1)		389 (83.3)	67 (81.7)
Yes	212 (17.7)	80 (17.8)	118 (17.7)		77 (16.5)	14 (17.1)
<b>Condom use with casual partners<sup>c</sup></b>				0.22		0.47
(Usually) not	567 (47.3)	204 (45.3)	322 (48.3)		221 (47.3)	41 (50.0)
(Usually) yes	356 (29.7)	134 (29.8)	207 (31.1)		145 (31.0)	15 (18.3)
No casual partners	267 (22.3)	111 (24.7)	135 (20.3)		99 (21.2)	21 (25.6)
<b>Ever used hormonal contraceptives</b>				0.01		0.01
No	52 (4.3)	26 (5.8)	18 (2.7)		12 (2.6)	8 (9.8)
Yes	1125 (93.9)	416 (92.4)	641 (96.2)		448 (95.9)	68 (82.9)

Numbers do not always add up to 100% because of missing values.

<sup>a</sup> Fully vaccinated women reported to be vaccinated three times.

<sup>b</sup> Comparing women vaccinated at least once and women fully vaccinated with unvaccinated women.

<sup>c</sup> In the past 6 months.

of STIs and used hormonal contraceptives more often. Women vaccinated at least once had more partners in the past six months and were older at sexual debut, but these factors did not differ statistically significant between fully vaccinated and unvaccinated women.

## HPV-6/11

In total, 122 women (10%) were positive for HPV-6, 18 women (1.5%) for HPV-11 and 2 women (0.2%) for both. Among unvaccinated women, 12% was positive for genital HPV-6 and/or HPV-11. Among women who reported to be vaccinated at least once, this was 13% (Fig. 2). After adjustment for demographics and risk behavior, the PR of women vaccinated at least once was 1.03 (95% CI 0.74–1.43) relative to unvaccinated women (Table 2).

Of the fully vaccinated women, 11% was positive for genital HPV-6 and/or HPV-11 (Fig. 2). Comparing fully

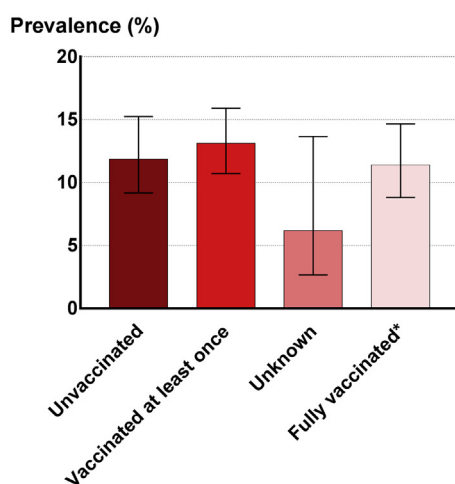
vaccinated women with unvaccinated women, the PR was 0.91 (95% CI 0.63–1.31) adjusted for demographics and risk behavior (Table 2).

## Anogenital warts

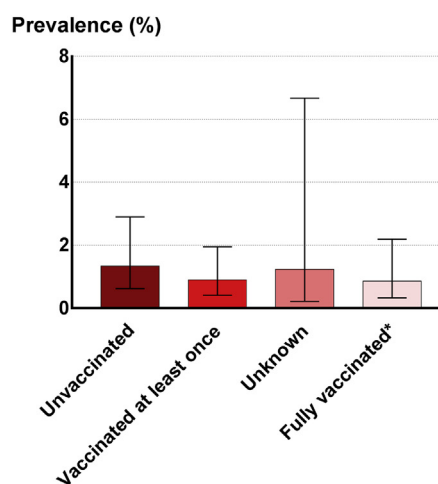
In total, only 13 (1.1%) out of 1193 vaccine-eligible women with information of the STI consult were diagnosed with AGW. Among unvaccinated women, 1.3% was diagnosed with AGW and among women vaccinated at least once this was 0.9% (Fig. 2), resulting in a PR of 0.67 (95% CI 0.22–2.07) (Table 2). Adjustment for demographics or risk behavior in bivariable analyses did not lead to other PRs; ranging from 0.66 adjusted for education level to 0.69 adjusted for number of recent partners.

Among fully vaccinated women, 0.9% was diagnosed with AGW (Fig. 2), giving a PR of 0.64 (95% CI 0.18–2.25) relative to unvaccinated women (Table 2). Adjustment for

A) HPV-6/11



B) Anogenital warts



**Figure 2** Prevalence of genital HPV-6 and/or HPV-11 positivity (A) and anogenital warts (B) by vaccination status. \* Fully vaccinated women reported to be vaccinated three times. Note the different scale of the y-axes.

**Table 2** Prevalence and prevalence ratios of genital HPV-6 and/or HPV-11 positivity and anogenital warts by vaccination status.

	N	n Events (%)	Crude PR [95% CI]	aPR [95% CI] <sup>b</sup>	aPR [95% CI] <sup>c</sup>
<b>Genital HPV-6 and/or HPV-11</b>					
Unvaccinated	438	52 (11.9)	Reference	Reference	Reference
Vaccinated at least once	649	85 (13.1)	1.10 [0.80–1.52]	1.04 [0.75–1.45]	1.03 [0.74–1.43]
Fully vaccinated <sup>a</sup>	456	52 (11.4)	0.96 [0.67–1.38]	0.91 [0.63–1.31]	0.91 [0.63–1.31]
Unknown	81	5 (6.2)	0.52 [0.21–1.26]	0.43 [0.16–1.16]	0.45 [0.17–1.21]
<b>Anogenital warts</b>					
Unvaccinated	447	6 (1.3)	Reference	—	—
Vaccinated at least once	665	6 (0.9)	0.67 [0.22–2.07]	—	—
Fully vaccinated <sup>a</sup>	466	4 (0.9)	0.64 [0.18–2.25]	—	—
Unknown	81	1 (1.2)	0.92 [0.11–7.54]	—	—

PR = prevalence ratio; aPR = adjusted prevalence ratio.

<sup>a</sup> Fully vaccinated women reported to be vaccinated three times.

<sup>b</sup> Corrected for demographic variables (age, ethnicity and education level).

<sup>c</sup> Corrected for demographic variables (age, ethnicity and education level) and risk behavior (number of partners in the past 6 months, age at sexual debut, history of STI, ever use of hormonal contraceptives).



demographics or risk behavior resulted in PRs ranging from 0.63 adjusted for hormonal contraceptives use to 0.65 adjusted for number of recent partners.

Table 3 shows the genital HPV types that were found among the women diagnosed with AGW, by vaccination status. All women with AGW were HPV positive and all six vaccinated women were positive for HPV-6. Of the six unvaccinated women, three were positive for HPV-6. HPV-11 was not found among women diagnosed with AGW.

### Sensitivity analyses

Of the total study population, 43% had serum available for HPV antibody testing. Of the 268 women who reported to be vaccinated at least once with serum available, only 11 (4.1%) were seronegative for either HPV-16 or HPV-18. Excluding these 11 women from the analyses did not change the results. Of the 194 women who reported to be fully vaccinated with serum available, 8 (4.1%) were seronegative for HPV-16 or HPV-18. Excluding these 8 women from the analyses comparing fully vaccinated with unvaccinated women did also not change the results (Supplementary file 1).

### Discussion

We estimated the effect of the bivalent HPV-16/18 vaccine on genital HPV-6 and/or HPV-11 positivity and AGW among vaccine-eligible women attending STI clinics in the Netherlands. We observed no cross-protective effect of the bivalent vaccine on genital HPV-6/11 positivity and a non-significant partially protective effect on AGW.

To our knowledge, this is the first study to directly estimate the protective effect of the bivalent HPV vaccine on both genital HPV-6/11 positivity and AGW among vaccine-eligible women. However, we do acknowledge some limitations.

First, we had very low numbers of AGW in our study population leading to insufficient power to robustly assess an effect of vaccination on AGW diagnoses. The low number of AGW is partly because most AGW in the Netherlands are diagnosed by the general practitioner.<sup>18</sup> Another reason is that among women, physical examination and thus diagnosis of AGW is performed in case of reported symptoms only. Likely, some diagnoses of AGW were missed in our study and the true prevalence of AGW was probably higher. While it is unlikely that the effect estimate is biased for this reason, as the rate of underdiagnoses is probably not related to vaccination status, the low occurrence of AGW influenced the precision of our effect estimate.

Second, vaccination status was self-reported, which is prone to recall bias. However, only 4% of the women who reported to be vaccinated were seronegative for HPV-16 or HPV-18. We performed a sensitivity analyses, excluding the women who likely incorrectly reported to be vaccinated, but this did not change the results.

Third, there were some differences between vaccinated and unvaccinated women in our study. Vaccinated women were often native Dutch and highly educated, which is in line with earlier findings.<sup>19</sup> Moreover, we observed some differences in sexual behavior between vaccinated and unvaccinated women. Although there is no evidence that HPV vaccination leads to other/higher sexual risk behavior,<sup>20</sup> it is possible that sexual behavior was indirectly associated with vaccination, meaning that girls who chose to get vaccinated were a different population than girls who chose not to get vaccinated and therefore developed another sexual risk profile. While we adjusted for demographics and risk behavior, we cannot rule out residual confounding.

Last, our study population consisted of women who visited the STI clinic (suggesting high sexual risk behavior) and who were primarily vaccinated during the catch-up campaign. Based on the reported age at sexual debut in the questionnaire and the scheduled vaccinations by birth cohort,<sup>19</sup> about 7.7–25% of the vaccinated women in our study were possibly already exposed to HPV before vaccination.

There is a possibility that the bivalent vaccine offers protection against AGW, whilst not providing cross-protective effectiveness against HPV-6/11. In many AGW, multiple HPV types have been found, including types 16 and 18.<sup>21,22</sup> HPV-16/18 could play a role in the development of AGW, either indirectly by interaction with HPV-6/11 or directly. Indeed, in some AGW HPV-16 was pointed out to be the probable type causing the wart.<sup>23,24</sup> Although surface swabbing does not necessarily indicate the causative HPV type,<sup>23</sup> in our study we found one woman with AGW who was positive for HPV-16 only.

HPV-6 and HPV-11 (both from the alpha-10 species), are phylogenetically not closely related to the vaccine types HPV-16 (alpha-9 species) and HPV-18 (alpha-7 species).<sup>25</sup> Nevertheless, in the PATRICIA trial, a vaccine efficacy of the bivalent vaccine of 35% against 6-month persistent HPV-6/11 infection was reported among women who were HPV seronegative at baseline.<sup>6</sup> Adjusted for demographics and risk behavior, we calculated a PR of 0.91 (95% CI 0.63–1.31) against HPV-6/11 positivity for fully vaccinated women. This estimate corresponds to a VE of 9% (95% CI 31% to 37%).<sup>26</sup> Although this suggests no effect, the confidence

**Table 3** Genital HPV types of women with anogenital warts, by vaccination status.

Sample	Self-reported vaccination status	Genital HPV types isolated
1	Unvaccinated	6, 18, 31, 53, 56, 59
2	Unvaccinated	6, 31, 33, 52
3	Unvaccinated	6, 39, 51, 56
4	Unvaccinated	16
5	Unvaccinated	31
6	Unvaccinated	51
7	Fully vaccinated <sup>a</sup>	6, 51, 66
8	Fully vaccinated <sup>a</sup>	6, 51
9	Fully vaccinated <sup>a</sup>	6
10	Fully vaccinated <sup>a</sup>	6
11	Vaccinated at least once	6
12	Vaccinated at least once	6
13	Unknown	31, 52, 58

<sup>a</sup> Fully vaccinated women reported to be vaccinated three times.

interval includes the 35% efficacy reported from the vaccine trial. In our cross-sectional study, we did not have information on duration of infection hampering a direct comparison.

The cross-protective effect of the bivalent vaccine on HPV-6/11 positivity has so far not been replicated in post-vaccine surveillance studies. In England, where the bivalent vaccine was used until 2012, the prevalence of HPV-6 and/or HPV-11 among 16- to 18-year-old women increased from 5.8% in the pre-vaccination period to 8.3% in the post-vaccination period.<sup>27</sup> In Scotland, where the bivalent vaccine was also used until 2012, there were no differences in HPV-6/11 positivity among vaccinated and unvaccinated women who underwent their first cervical screening.<sup>28</sup> However, these analyses were unadjusted for confounders and not all women were eligible for vaccination.

Interestingly, we calculated a crude PR of 0.64 for AGW for fully vaccinated women, corresponding to a VE of 36%. Although non-significant, this point estimate is almost identical to the VE against AGW calculated by Howell-Jones and colleagues in an ecological study in the United Kingdom (34%).<sup>7</sup> However, in a study conducted in the Czech Republic, no effect of the bivalent vaccine on AGW prevalence was observed among 16- to 40-year-old women.<sup>29</sup>

In conclusion, our study neither supports nor excludes cross-protection from the bivalent HPV vaccine against HPV-6/11. We also could not confirm a protective effect of the bivalent vaccine against AGW, 6 years after introduction of vaccination. For a more definite outcome, larger and longer duration studies would be needed. We are currently working on a research proposal to investigate the effect of the bivalent vaccine on AGW using data from a large general practice network in the Netherlands where more diagnoses of AGW are anticipated.

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## Conflicts of interest

None.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jinf.2017.01.007>.

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