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Prevalence of human papillomavirus in teenage heterosexual males following the implementation of female and male school-based vaccination in Australia: 2014–2017

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ABSTRACT

Background: Australia introduced a school-based human papillomavirus (HPV) vaccination program for females aged 12–13 years in 2007, with a three-year catch-up to age 26; and for boys aged 12–13 from 2013, with a two-year catch-up to age 15. This study aimed to compare the prevalence of penile HPV between teenage heterosexual males in cohorts eligible or non-eligible for the school-based male vaccination program.

Methods: Between 2014 and 2017, sexually active heterosexual males aged 17–19 were recruited from sexual health centres and community sources across Australia. Males provided a self-collected penile swab for 37 HPV genotypes using Roche Linear Array and completed a questionnaire. We calculated adjusted prevalence ratios (aPR) of HPV between males in two periods: 2014–2015 (preceding implementation of school-based male vaccination) and 2016–2017 (eligible for school-based male vaccination). Self-reported vaccine doses were confirmed with doses reported to the National HPV Vaccination Program Register.

Results: Overall, 152 males were recruited in 2014–2015 and 146 in 2016–2017. Numbers of female sex partners and condom use did not differ between the two periods. The prevalence of quadrivalent vaccine-preventable [4vHPV] genotypes (6/11/16/18) was low in both periods (2.6% [2014–15] *versus* 0.7% [2016–17]; $p = 0.371$; aPR 0.28 [95% CI: 0.03–2.62]). Compared with men in 2014–2015, men in 2016–2017 had a lower prevalence of any of the 37 HPV genotypes tested (21.7% *versus* 11.6%; aPR 0.62 [95% CI: 0.36–1.07]) and any of the 13 high-risk genotypes tested (15.8% *versus* 7.5%; aPR 0.59

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[95% CI: 0.30–1.19]). Prevalence of low-risk HPV genotypes did not differ between the two periods. Of the males recruited in 2016–2017, 55% had received ≥1 vaccine dose.
Conclusion: The prevalence of 4vHPV genotypes among teenage heterosexual males in both cohorts was low, presumably due to herd protection from the female-only vaccination program. Further studies are required to determine the impact of universal HPV vaccination on HPV prevalence in males.

1. Introduction

In April 2007, Australia introduced a national school-based human papillomavirus (HPV) vaccination program for girls aged 12–13 years with a three-year catch-up program for females aged up to 26 years until the end of 2009. In 2013, the program was extended to include boys aged 12–13 years with a two-year catch-up for boys aged up to 15 years until the end of 2014 [1,2]. A three-dose schedule of quadrivalent HPV vaccination covering HPV genotypes 6, 11, 16 and 18 was recommended for the school-based HPV vacciantion program. From 2018, this was replaced by a two-dose schedule of nonavalent HPV vaccination covering HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58 in the school-based HPV vacciantion prorgam.

Australia has had high HPV vaccination coverage of females compared to most other countries [3]. It is estimated that in Australia, the 3-dose HPV vaccination coverage was 80% of females and 76% of males turning 15 years of age in 2017 [4,5]. In addition, it is estimated that 55% of females aged 18–26 years received at least one dose of HPV vaccine during the catch-up program [6]. The female-only vaccination program has resulted in marked reductions in genital warts, quadrivalent HPV vaccine-preventable (4vHPV) genotypes, and high-grade cervical lesions among young Australian females [7–10]. Similar reductions in genital warts and 4vHPV genotypes have also been observed among heterosexual males as a result of herd protection [7,11,12]. Results from several mathematical models and a community-randomised clinical trial have suggested that the introduction of the universal (gender-neutral) HPV vaccination program would provide incremental benefit, including a near elimination of genital warts and HPV vaccine-preventable genotypes in both females and males [13–18]. Previous studies have examined HPV prevalence among unvaccinated heterosexual males after the introduction of female-only vaccination programs [17]; however, to date, there have been no epidemiological studies from any country comparing HPV prevalence among heterosexual males before and after the implementation of a school-based male vaccination program in addition to a female vaccination program.

The aim of this study was to examine the impact of universal HPV vaccination on penile HPV detection in young heterosexual males, by comparing the penile HPV prevalence in two independent successive cohorts of heterosexual males, before and after the implementation of the school-based male HPV vaccination program in Australia.

2. Methods

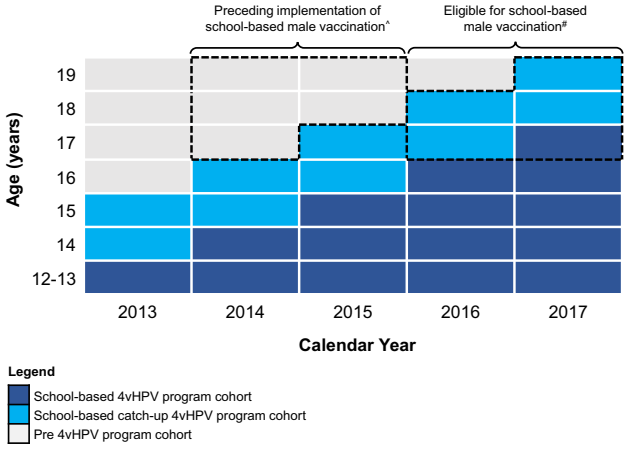
2.1. Study design and setting

The IMPact of human papillomavirus vaccination RESearch Study (IMPRESS) was a four-year cross-sectional study conducted between 2014 and 2017. Data on HPV prevalence from the first phase of the IMPRESS study (2014–2015) have been published previously [12]. Participants were recruited from clinical and community sources across Australia. Clinic-based recruitment included publicly funded sexual health clinics in Melbourne, Sydney, Perth, Adelaide, Hobart, and Cairns together with family planning and youth clinics in Melbourne. Community based recruitment included Universities and Technical And Further Education (TAFE) campuses with advertising via online student message boards and orientation weeks. Individuals could register their interest in the study via the study website with registered individuals contacted by a research nurse to confirm their eligibility before enrolment.

This study was approved by the Human Research and Ethics Committees governing recruitment sites: Alfred Hospital Ethics Committee (503/13), South Eastern Sydney Local Health District Ethics Human Research Ethics Committee (14/253), South Metropolitan Health Services Human Research Ethics Committee (14/76), Royal Adelaide Hospital Committee (HREC/14/RAH/441), Tasmania Health & Medical Human Research Ethics Committee (H0014507) and Far North Queensland Human Research Ethics Committee (HREC/14/QCH/119–940). Written informed consent was obtained from all participants. The study was registered with the U.S. National Institutes of Health ClinicalTrials.gov (NCT02003508).

2.2. Study population

Participants were eligible if they fulfilled the following criteria: (1) male gender; (2) had never had any sexual contact with another men; (3) resided in Australia from the age of 12 years or younger; (4) had any kind of sexual contact (oral, vaginal or anal) sex with a woman; and (5) were aged 17–19 years at the time of recruitment. Fig. 1 illustrates the age criteria for each study year used to group men: (1) males aged 17–19 years in 2014 or 18–19 years in 2015, a cohort preceding the introduction of the school-based male 4vHPV vaccination program and (2) males aged 17–18 years in 2016 or 17–19 years in 2017, who were age-eligible



^Males aged 17-19 years in 2014 and aged 18-19 years in 2015 within the dashed lines were eligible for the IMPRESS study [i.e. pre school-based male 4vHPV vaccination program cohort]. Males aged 17 years in 2015 were excluded because they were eligible for the school-based vaccination program.

#Males aged 17-18 years in 2016 and aged 17-19 years in 2017 within the dashed lines were eligible for the IMPRESS study [i.e. school-based male 4vHPV vaccination program cohorts]. Males aged 19 years in 2016 were excluded because they were not eligible for the school-based vaccination program.

Fig. 1. Age cohort for the national school-based quadrivalent HPV vaccination program and eligibility criteria for the IMPRESS study.

Table 1
Demographic characteristics and sexual behaviours among 345 heterosexual males by study period.

	2014–2015 (N = 152)	2016–2017 (N = 146)	P value*
Demographic characteristics			
Age (years)			<0.001
17	8 (5.3%)	16 (11.0%)	
18	53 (34.9%)	75 (51.4%)	
19	91 (59.9%)	55 (37.7%)	
Country of birth†			0.391
Australia	135 (88.8%)	124 (84.9%)	
Overseas	17 (11.2%)	22 (15.1%)	
Education			0.022
Has not completed Year 12	14 (9.2%)	11 (7.5%)	
Completing Year 12	20 (13.2%)	21 (14.4%)	
Completed Year 12	43 (28.3%)	21 (14.4%)	
Current tertiary study	75 (49.3%)	92 (63.0%)	
Others	0 (0%)	1 (0.7%)	
Circumcision†			0.743
Yes	21 (13.9%)	22 (15.5%)	
No	130 (86.1%)	120 (84.5%)	
Source of recruitment			<0.001
Clinic	120 (78.9%)	82 (56.2%)	
Community	32 (21.1%)	64 (43.8%)	
Vaccination status			<0.001
Fully vaccinated	5 (3.3%)	57 (39.0%)	
Partly vaccinated	4 (2.6%)	24 (16.4%)	
Not vaccinated	140 (92.1%)	60 (41.1%)	
Unknown	3 (2.0%)	5 (3.4%)	
Sexual behaviours in lifetime and in the previous 12 months			
Oral sex‡ in lifetime			1.000
Yes	148 (97.4%)	142 (97.3%)	
No	4 (2.6%)	4 (2.7%)	
Oral sex‡ in the previous 12 months†			1.000
Yes	143 (94.1%)	137 (94.5%)	
No	9 (5.9%)	8 (5.5%)	
Vaginal sex in lifetime			0.328
Yes	149 (98.0%)	140 (95.9%)	
No	3 (2.0%)	6 (4.1%)	
Vaginal sex in the previous 12 months†			0.190
Yes	147 (96.7%)	135 (93.1%)	
No	5 (3.3%)	10 (6.9%)	
Anal sex with female in lifetime†			0.888
Yes	34 (22.7%)	31 (21.4%)	
No	116 (77.3%)	114 (78.6%)	
Anal sex with female in the previous 12 months†			0.359
Yes	29 (19.3%)	22 (15.1%)	
No	121 (80.7%)	124 (84.9%)	
Number of female sexual partners in lifetime, median [IQR]			
Oral sex‡	4 [2–8]	3 [2–8]	0.156
Vaginal sex	4 [2–9]	3 [1–8]	0.069
Anal sex§	1 [1–2]	1 [1–1]	0.203
Number of female sexual partners in the previous 12 months, median [IQR]			
Oral sex‡	2 [1–4]	2 [1–4]	0.685
Vaginal sex	2 [1–4]	2 [1–4]	0.558
Anal sex§	1 [1–1]	1 [0–1]	0.325
Consistent condom use in the previous 12 months ¶			
Oral sex‡			0.361
Yes	4 (2.9%)	7 (5.5%)	
No	135 (97.1%)	120 (94.5%)	
Vaginal sex			0.654
Yes	27 (18.4%)	28 (20.7%)	
No	120 (81.6%)	107 (79.3%)	
Anal sex			0.380
Yes	12 (41.4%)	6 (27.3%)	
No	17 (58.6%)	16 (72.7%)	
Heath and lifestyle			
Has genital warts currently†			0.067
*Yes*δ	7 (4.9%)	1 (0.7%)	
No	136 (95.1%)	139 (99.3%)	

(*continued on next page*)

Table 1 (*continued*)

	2014–2015 (N = 152)	2016–2017 (N = 146)	P value*
Smoke cigarettes currently†			0.090
Yes	48 (31.8%)	33 (22.6%)	
No	103 (68.2%)	113 (77.4%)	

* Fisher's exact test was used for categorical variables. *T*-test was used to compare age between the two periods. Mann-Whitney *U* test was used to compare the number of sexual partners between the two periods.
† Numbers do not add up to the total due to missing data.
‡ Oral sex means fellatio where participant's penis in the female partner's mouth.
¶ Consistent condom use was defined as condom use at all sexual acts in the previous 12 months. Men who did not report the particular sex act were excluded.
§ Due to small number of men that had engaged in anal sex with females, the median number of anal sex partners in the previous 12 months was calculated among men who had had anal sex with females in the previous 12 months.
δ Eight men (Study participants A to H) self-reported with current genital warts. Study participants A to G were in 2014–2015 and participant H was in 2016–2017. Study participants C, E, G and H were HPV negative. The HPV genotypes for participant A were 42, 52, 61, 66, 84; participant B were 6, 81, 89; participant D were 6, 89; participant F were 52, 73, 84.

for the school-based or catch-up male 4vHPV vaccination program following its introduction. Males aged 17 years in 2015 and aged 19 years in 2016 were not eligible for this study (Fig. 1).

2.3. Study procedures

All eligible participants were required to attend one of the recruiting clinics to complete the study procedures. No follow-up visit was required. Enrolled participants were required to complete a self-administered questionnaire. This questionnaire included questions on demographic characteristics (age, country of birth, educational level), sexual practices (number of female partners and condom use in the previous 12 months and lifetime), sexual health and lifestyle habits (diagnosis of genital warts and smoking status) and self-reported HPV vaccination status.

Participants were also required to provide a self-collected penile swab for HPV testing. Penile samples were collected using a flocked swab (Copan Diagnostics, Inc., Brescia, Italy) moistened by sterile water. Participants were instructed to use the moistened swab to firmly rub the entire surface of the penis, including the coronal sulcus, glans penis and, if uncircumcised, retracted prepuce. An illustrated instruction sheet on penile sample self-collection was provided to all participants.

A separate consent was obtained from participants for validation of their self-reported HPV vaccination status using the National HPV Vaccination Program Register (NHVPR) [10]. The NHVPR collects vaccination data (number of doses and date of vaccine administered) delivered via the school-based program, general practices and other immunisation providers. Vaccination status was categorised into four groups: (1) fully vaccinated; (2) partly vaccinated; (3) unvaccinated; and (4) unknown. Fully vaccinated was defined as participants who received all three doses of HPV vaccines as recorded on the NHVPR, regardless of self-reported HPV vaccination status. Partly vaccinated was defined as participants who either: (1) had one or two doses recorded by the NHVPR or (2) self-reported receiving the vaccine but had no record in the NHVPR, or the NHVPR was unable to confirm the receipt of the vaccine with the immunisation provider due to lack of information. Unvaccinated was defined as self-reportedly not receiving the vaccine and had no record in the NHVPR or was unable to be confirmed via the NHVPR. Unknown was defined as participants who self-reported not knowing their vaccination status and were unable to be confirmed via the NHVPR. These definitions have been used elsewhere [19].

2.4. HPV testing

All penile swabs were vigorously agitated in 500 μL of phosphate-buffered saline after sample collection. Once received in the laboratory (molecular microbiology of the Centre for Women's Infectious Diseases, The Royal Women's Hospital), DNA was extracted from a 200 μL of the sample aliquot by MagNA Pure 96 System (DNA and Viral Nucleic Acid Small Volume Kit; Roche Molecular Diagnostics; Mannheim, Germany) as per the manufacturer's Pathogen Universal 200 protocol and eluted in 100 μL. Extracted DNA was assessed for integrity by quantitative polymerase chain reaction (PCR) amplification of a 260 base-pair product of the human beta-globin gene [20]. HPV triage testing was performed using a PCR ELISA system (Roche Molecular Diagnostics, Mannheim, Germany) after amplification of the HPV L1 gene using consensus primers PGMY-09-PGMY11 [20–22]. HPV genotyping was performed on all PCR ELISA positive samples using Linear Array HPV genotyping test (Roche Molecular Diagnostics, Mannheim, Germany), with modifications as previously published [23]. Linear Array is capable of detecting 37 HPV genotypes were detected (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–56, 58, 59, 61, 62, 64, 66–73, 81–84, 82v, and 89). Due to possible cross-reactivity of the HPV 52 probe with genotypes 33, 35, and 58 amplicons, samples positive for the HPV52 probe in the presence of one or more of these three probes were further tested for HPV 52 using a type-specific PCR assay [24]. Samples with a negative result for both beta-globin and PCR ELISA were considered not assessable and were excluded from the analysis.

2.5. Statistical analysis

The study was stratified into two periods: (1) 2014–2015 (cohort where men aged 16–19 years were not eligible for school-based male vaccination program as such a program did not exist); and (2) 2016–2017 (cohort where men aged 16–19 years were eligible for the recently introduced school-based male vaccination program). The prevalence of HPV was calculated according to the following groups: any of the 37 HPV genotypes tested; any 13 high-risk genotype (16/18/31/33/35/39/45/51/52/56/58/59/68); any 4vHPV vaccine-preventable genotype (6/11/16/18); any 9vHPV vaccine-preventable genotype (6/11/16/18/31/33/45/52/58); any 24 low-risk genotype (6/11/26/40/42/53/54/55/61/62/64/66/67/69/70/71/72/73/80/81/ 82/82v/83/84/89); any low-risk 4vHPV vaccine-preventable genotype (6/11); and any high-risk 4vHPV vaccine-preventable genotype (16/18). The prevalence of genotypes 31/33/45 was also calculated as data suggest there may be cross-protection against these genotypes from quadrivalent HPV vaccination [19]. HPV prevalence was compared between the two periods using Fisher's exact test. Log-linear regression models were performed to calculate the crude and adjusted prevalence ratios (PRs) of HPV genotypes for men recruited in 2016–2017 compared to men recruited in 2014–2015. Age and source of recruitment were considered as potential

confounding factors and were included in the adjusted analyses [25–27]. A sensitivity analysis was also performed to compare the HPV prevalence between vaccinated and unvaccinated men in 2014–2017. All statistical analyses were performed using Stata (version 14.2, Stata, College Station, Texas, USA).

3. Results

There were 345 age-eligible men recruited between 2014 and 2017. Thirteen men were excluded because they self-reported never having any penetrative (i.e. oral, vaginal or anal) sex with a woman. Of the 332 eligible men, 34 men (10.2%) were excluded because they provided samples that could not be assessed for HPV DNA and this proportion did not differ between the two periods ($p = 0.831$). The remaining 298 men were included and analysed: 152 men recruited in 2014–2015 and 146 in 2016–2017. A small proportion of males (5.9%; $n = 9$) in 2014–2015 were vaccinated against HPV outside the school-based program, while 55.5% ($n = 81$) of males in 2016–2017 received at least one dose of HPV vaccine.

The demographic and sexual behavioural characteristics of men recruited during each period are shown in Table 1. The mean age of men recruited in 2014–2015 was slightly higher than men recruited in 2016–2017 (18.5 *versus* 18.3; $p < 0.001$). A higher proportion of males were recruited from community sources in 2016–2017, compared to 2014–2015 (43.8% *versus* 21.1%; $p < 0.001$). More males were currently in tertiary study in 2016–2017, than in 2014–2015 (63.0% *versus* 49.3%; $p = 0.022$).

There was no significant difference in any of the sexual practices reported by men between the two periods (Table 1). Overall, almost all males had engaged in oral (94.3%) or vaginal (94.9%) sex and 17.2% in insertive anal sex with females in the previous 12 months.

The prevalence of penile HPV genotypes detected during the two periods is shown in Table 2. The prevalence of 4vHPV genotypes (6/11/16/18) was lower in 2016–2017 (0.7%; 95% CI: 0–3.8%) compared with the 2014–15 period (2.6%; 95% CI: 0.7–6.6%); however, the difference was not statistically significant ($p = 0.371$). Only one man in 2016–2017 had a 4vHPV genotype

Table 2
Prevalence, crude and adjusted prevalence ratio of penile HPV among 345 heterosexual males by study period.

	2014–2015 (N = 152)		2016–2017 (N = 146)		P value*	Crude PR (95% CI)	P value	Adjusted PR (95% CI)‡	P value
	n	*% (95% CI)*	*n*	*% (95% CI)*					
Any of 37 HPV genotypes	33	21.7% (15.4–29.1%)	17	11.6% (6.9–18.0%)	0.021	0.54 (0.31–0.92)	0.024	0.62 (0.36–1.07)	0.088
Any of 13 high-risk genotypes	24	15.8% (10.4–22.6%)	11	7.5% (3.8–13.1%)	0.031	0.48 (0.24–0.94)	0.032	0.59 (0.30–1.19)	0.142
Any of 13 high-risk genotypes, other than 16 and 18	23	15.1% (9.8–21.8%)	11	7.5% (3.8–13.1%)	0.045	0.50 (0.25–0.98)	0.045	0.61 (0.30–1.24)	0.172
Genotypes 31, 33 or 45†	4	2.6% (0.7–6.6%)	0	0% (0–2.5%)	0.123	–	–	–	–
Any of 24 low-risk genotypes	24	15.8% (10.4–22.6%)	14	9.6% (5.3–15.6%)	0.120	0.61 (0.33–1.13)	0.114	0.71 (0.38–1.35)	0.297
Any 4vHPV vaccine genotype	4	2.6% (0.7–6.6%)	1	0.7% (0–3.8%)	0.371	0.26 (0.03–2.30)	0.226	0.28 (0.03–2.62)	0.265
Any 9vHPV vaccine genotype	14	9.2% (5.1–15.0%)	6	4.1% (1.5–8.7%)	0.105	0.45 (0.18–1.13)	0.089	0.58 (0.22–1.51)	0.266
Genotypes 6 or 11	3	2.0% (0.4–5.7%)	1	0.7% (0–3.8%)	0.623	0.35 (0.04–3.30)	0.357	0.32 (0.03–3.24)	0.338
Genotypes 16 or 18	1	0.7% (0–3.6%)	0	0% (0–2.5%)	1.000	–	–	–	–

PR: Prevalence ratio; CI: confidence intervals.
* Fisher's exact test was used.
† Genotypes 31, 33 and 45 have been grouped as previous studies suggest quadrivalent HPV vaccination may confer cross protection against these genotypes.
‡ Adjusted for age and source of recruitment.

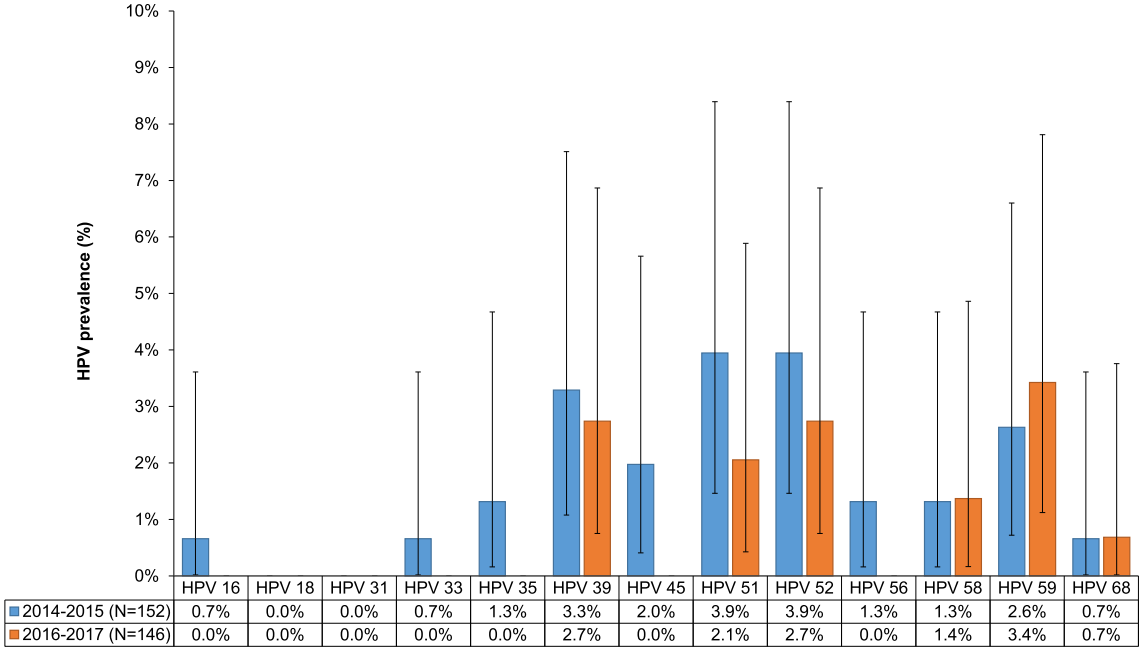


Fig. 2. The prevalence of high-risk HPV genotypes, stratified by study period. *Note. There was no significant difference for any type-specific prevalence between the two periods.*

Table 3
Comparison of the prevalence of penile HPV between vaccinated and unvaccinated heterosexual males in 2014–2017.

	Unvaccinated (*N* = 200)	Partly/fully vaccinated (*N* = 90)	P value*
Any of 37 HPV genotypes	40 (20.0%)	10 (11.1%)	0.067
Any of 13 high-risk genotypes	29 (14.5%)	6 (6.7%)	0.078
Any of 13 high-risk genotypes, other than 16 and 18	28 (14.0%)	6 (6.7%)	0.079
HPV 31, 33 or 45†	3 (1.5%)	1 (1.1%)	1.000
Any of 24 low-risk genotypes	28 (14.0%)	10 (11.1%)	0.576
Any 4vHPV vaccine genotype	5 (2.5%)	0 (0%)	0.329
Any 9vHPV vaccine genotype	17 (8.5%)	3 (3.3%)	0.136
HPV 6 or 11	4 (2.0%)	0 (0%)	0.314
HPV 16 or 18	1 (0.5%)	0 (0%)	1.000

* Fisher's exact test was used.
† HPV 31, 33 and 45 have been grouped as previous studies suggest quadrivalent HPV vaccination may confer cross protection against these genotypes.

detected – HPV 6 – and he was unvaccinated (Fig. 2). The prevalence of any of the 37 HPV genotypes tested was significantly lower in 2016–2017 (11.6%) than in 2014–15 (21.7%; $p = 0.021$). There was a significantly lower prevalence of any of the 13 high-risk genotypes tested in 2016–17 (7.5%) compared with the 2014–15 period (15.8%; $p = 0.031$). This included a significantly lower prevalence of any of the 13 high-risk genotypes tested excluding HPV 16 and 18: 15.1% in 2014–15 and 7.5% in 2016–17 ($p = 0.045$). There was no significant difference in the prevalence of any of the 24 low-risk genotypes tested between the two periods ($p = 0.120$). The prevalence of genotypes 31/33/45 was 2.6% in 2014–2015 and 0% in 2016–2017 (0%) ($p = 0.123$). However, after adjusting for age and source of recruitment, there was no statistically difference in HPV prevalence across all groups among men recruited in 2016–2017 compared to those recruited in 2014–2015 (Table 2). There was no difference in HPV prevalence between the 200 unvaccinated and 90 partly/fully vaccinated males recruited (Table 3).

4. Discussion

Several studies have demonstrated a significant reduction in HPV prevalence among unvaccinated heterosexual males in populations where HPV vaccination of females has been established. While some mathematical models and a community-randomised clinical trial have predicted incremental benefits from the addition of male vaccination to existing female vaccination programs, to date, there have not been any studies comparing the prevalence among young heterosexual men before and after the introduction of a universal school-based HPV vaccination program. This repeated cross-sectional study provides data comparing the prevalence of penile HPV between sexually-active teenage heterosexual males before and after the implementation of school-based male HPV vaccination, which was added to a pre-existing school-based female vaccination program in Australia. The prevalence of 4vHPV vaccine-preventable genotypes among teenage heterosexual males was low both before and after the introduction of male vaccination compared to the period before the introduction of female vaccination: the prevalence of 4vHPV among heterosexual males aged $\leq$ 21 years was 22% in 2006–2007 [11].

The low prevalence of 4vHPV genotypes (2.6%) among males prior to male vaccination is likely to be attributable to herd protection resulting from high coverage of females from the existing female-only HPV vaccination program. It is also important to note that the combined prevalence of genotypes 16/18 was low in both periods (0.7% in 2014–2015 and 0% in 2016–2017) compared to the period before the introduction of the female HPV vaccination program. The prevalence of HPV16/18 among heterosexual males aged $\leq$21 years was 11% in 2006–2007 [11]. As of 2015, all Australian-born females aged $\leq$ 34 years would have been eligible for HPV vaccination [28]. Ostensibly, the majority of female partners of the 17–19 year-old heterosexual men were vaccinated against HPV [29,30]. This is consistent with a previous study that demonstrated a 78% reduction of 4vHPV genotypes among young, unvaccinated Australian-born heterosexual males eight years after the introduction of the female-only vaccination program [11,12]. In the current study, the prevalence of 4vHPV genotypes was lower (0.7%) four years following the start of male vaccination, but this was not significantly different from the prevalence preceding the introduction of male HPV vaccination program. Of the males recruited into the study following the introduction of male vaccination, 39% were fully vaccinated with three doses of the 4vHPV vaccine (confirmed with the National HPV Vaccination Program Register). This is similar to national data for Australia where 42% of males aged 18–19 years in 2017 had received three doses of HPV vaccine [31].

Compared with the prevalence of HPV in males preceding the male vaccination program, there was a 50% lower prevalence of any of the 13 high-risk HPV genotypes tested among males eligible for the school-based male vaccination program that was significant with univariate analysis; however, this association was not significant after adjusting for age and source of recruitment. This also applied if genotypes 16 and 18 were excluded. One possible explanation for this observation is that cross-protection from the addition of male vaccination contributed to a fall in prevalence of high-risk genotypes. Evidence of cross-protection has been shown in other populations: in a meta-analysis of nine studies and a recent large Norwegian study of 17,749 participants [32,33]. HPV 31 and 33 are phylogenetically related to HPV 16; while HPV 45 is phylogenetically related to HPV 18 [34,35]. While there was a lower prevalence of HPV 31/33/45 following male vaccination (2.6% *versus* 0%) this was not statistically significant. The sample size in this study was limited, which also limits its power to detect differences in HPV prevalence between the two periods. It is important to note that the study was not powered to detect the difference in prevalence of 4vHPV genotypes even if it was 0% in 2016–2017. This reflected difficulty in recruiting males of this age group into research into sexual health [36]. Because differences in the prevalence of high risk genotypes were not significant after adjustment for age and source of recruitment it is possible that differences in prevalence were due to some extent from sampling bias. Other studies with larger sample sizes should be conducted in countries where HPV vaccination of males has been added to pre-existing female programs to determine whether these falls are real.

Other limitations should be noted when interpreting the findings from this study. Firstly, because this was a before-and-after study, it is possible the lower prevalence of penile HPV genotypes in the second study period may reflect factors other than a true fall in the community prevalence of penile HPV among males. More males were recruited from the community in the period following male vaccination, and although there were no significant difference in behavioural risks reported by participants, it is possible differences in sexual behaviours between the two periods may have contributed to lower HPV prevalence in the second phase of the study. Although there was a significant difference in age between the two periods, this difference is unlikely to have influenced the prevalence of HPV due to small age range in this study (from 17 to 19 years). Secondly, the results of this study may not apply to other populations where there are different levels of HPV vaccine coverage among females or males. Thirdly, 10% of penile HPV samples among males in the study were not assessable; however, this

is comparable to a previous Canadian study among heterosexual males [37]. Studies have shown that the HPV detection rate varies across the male genital area and it has been suggested that penile sampling for HPV should include the entire surface of the penile shaft and glans penis among heterosexual males [38], which was the sampling method employed in our study. Further research is required to improve the penile sampling method in heterosexual males. Digital media platforms such as social media or smartphone dating applications may be considered in reaching young heterosexual males [39]. Fourthly, we categorised HPV vaccination status based on self-report and NHVPR records. There were 19 men (5.5%) who self-reported they were HPV vaccinated but who had no record of vaccination on the NHVPR. These men were categorised into the 'partly vaccinated' group; however, it is possible they received all three doses as their vaccination status may not have been reported to the NHVPR [40].

Evaluating the impact of the universal HPV vaccination program among heterosexual males in Australia proved challenging because of the pre-existing high level of herd protection from the female-only vaccination program and relatively low prevalence of HPV among males even before male vaccination was introduced. Interpreted with caution, our data suggest the universal HPV vaccination program may have contributed to incremental reductions in HPV prevalence to Australian heterosexual males and this may reduce the costs of cervical cancer screening remarkably if the prevalence of oncogenic HPV remains low in both females and heterosexual males. This study focuses on the impact of HPV vaccination on heterosexual transmission of HPV. To help ascertain whether male HPV vaccination is cost-effective and warranted, studies examining the impact of universal HPV vaccination programs on gay and other men who have sex with men are required because they are a major risk group for anal cancer [41–43] but may not receive herd protection from female-only vaccination programs.

Author contribution

The IMPRESS Study investigators were Eric Chow, Marcus Chen, Christopher Fairley, Catriona Bradshaw, John Kaldor, Sepehr Tabrizi, Suzanne Garland, Jane Hocking, David Regan, Julia Brotherton, Anna McNulty, Darren Russell, Lewis Marshall and Louise Owen. Eric Chow and Rebecca Wigan performed the data analyses. Rebecca Wigan coordinated the study across all sites. Steph Atchison performed the HPV testing. Dorothy A Machalek, Alyssa Cornall and Sepehr Tabrizi contributed to HPV sampling methodology and involved in the interpretation of laboratory testing results. Eric Chow, Rebecca Wigan, Dorothy Machalek and Marcus Chen contributed to the first draft of the manuscript. All authors were involved in data interpretation, reviewed the manuscript for intellectual content and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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CKF has received research funding from CSL Biotherapies and Merck & Co. CKF owns shares in CSL Biotherapies.

DGR has received honoraria from CSL Limited. JSH has received honoraria from CSL Limited.

CSB has received institutional funding from Speedx Pty Ltd for investigator-initiated research on Mycoplasma genitalium.

SMG and JMLB were investigators on a national HPV typing study of cervical cancer that received unrestricted funding from CSL Biotherapies and on a recurrent respiratory papillomatosis typing study that received unrestricted funding from Merck. JMLB has never received any personal financial benefits.

SMG has received advisory board fees and grant support from Merck & Co. and lecture fees for work in private time. SMG has received funding through her institution (Royal Women's Hospital) to do studies of HPV vaccines for Merck & Co. and is a member of the Merck Global Advisory Board for the HPV vaccine.

DAM reports educational grants from Seqirus to assist with education, training and academic purposes in the area of HPV, and travel funding and honoraria to her institute from Merck & Co., outside of the submitted work.

All other authors have no conflicts of interest to declare.

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